THALASSEMIA AND HEMOGLOBINOPATHIES

DIAGNOSIS, THERAPY AND PREVENTION

Editor

Prof. Dr. Duran Canatan

Editor Assistants

Dr. Özlem Zümrüt, Dr. Zekiye Özdemir

nobel

THALASSEMIA AND HEMOGLOBINOPATHIES: DIAGNOSIS, THERAPY AND PREVENTION

Editor: Prof. Dr. Duran Canatan

Editor Assistants: Dr. Özlem Zümrüt, Dr. Zekiye Özdemir

Publishing No.: 5231
Health Sciences No.: 250
ISBN: 978-625-371-028-6
E-ISBN: 978-625-371-029-3
Edition: 1. Edition, February 2024

Executive Editor: Nevzat Argun -nargun@nobelyayin.com-
Publishing Coordinator: Gülfem Dursun -gulfem@nobelyayin.com-

Interior Designer: Ahmet Baydar -ahmet@nobelyayin.com-
Proofreader: Seval Sezer -seval@nobelyayin.com-
Cover Designer: Mervin Selda Adal -mervin@nobelyayin.com-

Library Information Card

Canatan, Duran.
THALASSEMIA AND HEMOGLOBINOPATHIES: DIAGNOSIS, THERAPY AND PREVENTION / Editor: Duran Canatan
1. Edition, xviii + 502 p., 21x29,7 cm. Includes references and index.
ISBN: 978-625-371-028-6
E-ISBN: 978-625-371-029-3
1. Thalassememia 2. Hemoglobinopathie 3. Diagnosis, Therapy and Prevention

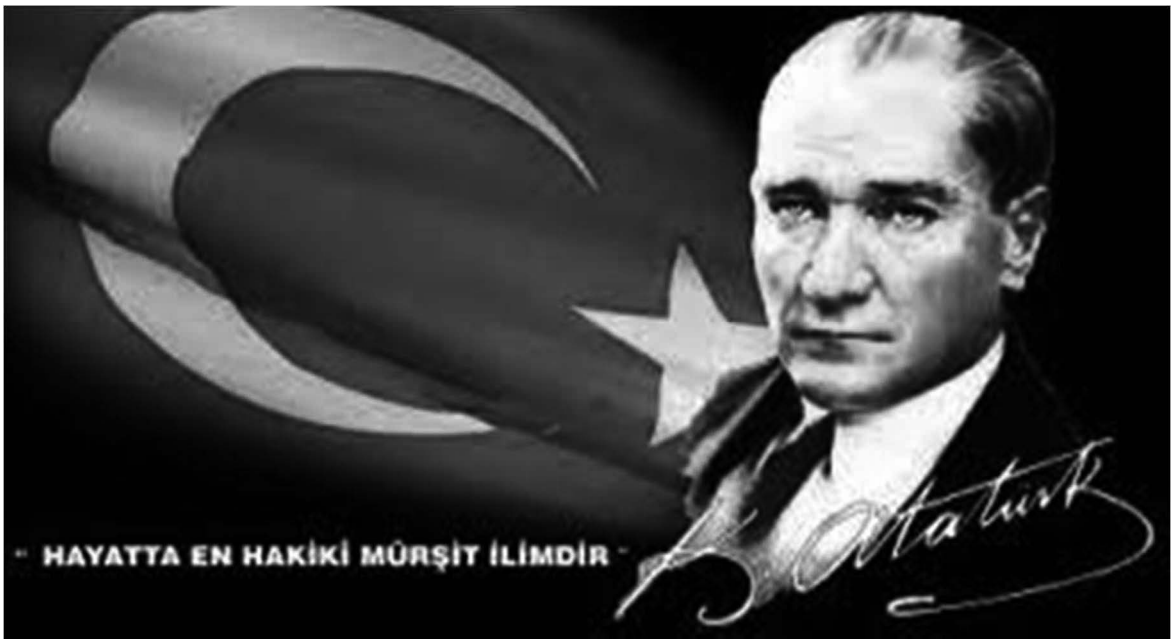
General Distribution

ATLAS AKADEMİK BASIM YAYIN DAĞITIM TİC. LTD. ŞTİ.
Address: Bahçekapı Mh. 2465 Sk. Oto Sanayi Sitesi No:7 Bodrum Kat, Şaşmaz/ANKARA
Phone: +90 312 278 50 77 - **Fax:** 0 312 278 21 65

Printing and Binding: Ada Matbaacılık Yayın San. Tic. Ltdi. Şti. Sertifika No.: 44093
Ostim OSB Mah. 1578. Cad. No.:21 Yenimahalle/Ankara

THE MOST TRUE GUIDE IN LIFE IS SCIENCE

MUSTAFA KEMAL ATATÜRK



Foreword by Dr Fahrettin Koca, Minister of Health

As a ministry, we perform our duties within the framework of our Constitution and the Law on Public Health and Hygiene in order to regulate the health conditions of our country, to combat all factors that harm health and to raise future generations in a healthy way. In addition to maintaining and improving all service programmes, our Ministry also plans and implements its activities in areas where new needs arise. After controlling child mortality in our country through nutritional disorders, infectious diseases and newborn screenings, we continue to work on the prevention of hereditary diseases.

As in the world, inherited blood diseases, especially thalassemia and sickle cell anaemia, are an important public health problem in our country. The high number of consanguineous marriages in our country increases the incidence of Thalassemia, which is a genetically inherited disease, hundreds of children with the disease are born every year, and families and society suffer material and moral damage.

In order to prevent thalassaemia and haemoglobinopathies and to prolong the life expectancy of patients, the Law No. 3960 on Combating Hereditary Blood Diseases was published on 30.12.1993. Within this framework, thalassaemia centres were established in Antalya, Antakya, Mersin and Muğla under the Ministry in 1994. The "Regulation on Hemoglobinopathy Control Programme and Diagnosis and Treatment Centres" prepared on the basis of the Law on Combating Hereditary Blood Diseases was published in the Official Gazette dated 24 October 2002 and numbered 24916.

The National Haemoglobinopapathy Council, which was established on 23.06.2002 by bringing together the General Directorate of Maternal and Child Health and Family Planning and the General Directorate of Treatment Services in the previous structure of our Ministry and all non-governmental organisations related to the subject in our country, was established and the patient profile of the country was revealed. Since the carrier frequency is 2.1% in our country, it was recorded that there are 1500.000 carriers and 4500 patients.

In cooperation with our Ministry and the National Hemoglobinopathy Council, a National Hemoglobinopathy Prevention Programme was initiated on World Thalassemia Day on 08.05.2003 in Mersin with 33 Provincial Health Directors. These risky provinces were Adana, Ankara, Antalya, Aydın, Batman, Bilecik, Burdur, Bursa, Çanakkale, Denizli, Diyarbakır, Düzce, Edirne, Erzurum, Eskişehir, Gaziantep, Hatay, İçel, Isparta, İstanbul, İzmir, Kahramanmaraş, Karaman, Kayseri, Kırklareli, Kocaeli, Konya, Kütahya, Manisa, Muğla, Sakarya, Şanlıurfa, Tekirdağ. With the National Haemoglobinopathy Prevention Programme, ninety percent of new patient births were prevented in 33 provinces in 2010. In 2013, 8 more provinces (Afyonkarahisar, Kilis, Mardin, Osmaniye, Siirt, Şırnak, Uşak, Yalova) with high incidence of the disease were added to the programme.

With the Presidential decree, the Hemoglobinopathy Control Programme was started to be implemented in 81 provinces under the name of "Pre-Marital Hemoglobinopathy Screening Programme" as of 1 November 2018. Thus, the birth of new patients has been zeroed with prevention efforts covering the whole country.

Due to the immigrants coming to our country, there have been changes in thalassemia and haemoglobinopathies as in all health data. In this context, trainings for migrants and migrant family physicians are continuing in migrant health centres.

I would like to thank the Mediterranean Blood Diseases Foundation for publishing such a textbook on Thalassemia and Haemoglobinopathies in Turkish and English on the 100th anniversary of the Republic of Türkiye, and all the scientists who supported the book.

Dr Fahrettin Koca
Republic of Türkiye, Minister of Health

29 October 2023

Foreword by Prof. Dr. İsmail Yüksek
Rector of Antalya Bilim University

Antalya Bilim University contributes to Türkiye's higher education life with its structure that perceives differences as richness, qualified academic staff, administrative staff who adopt the principle of supporting all the needs of students and academic staff.

Since the day we were founded, we aim to raise young people who are critical, original and scientific thinkers who protect the values of society by following scientific and social developments, providing education and research services that contribute to the development of society.

Antalya Bilim University is open to all kinds of projects that will contribute to our society and the world in the field of science and technology in accordance with its mission and vision. With new and different projects that have not yet been realised, it aims to be among the leading universities of our country and among the top 500 universities in the world. In order to achieve this goal, we are working with valuable scientists with high scientific level in their fields.

Thalassemia and haemoglobinopathies are an important public health problem in our country, and we have been following the efforts of the Ministry of Health and non-governmental organisations working on this problem for years with appreciation.

As Antalya Bilim University, I would like to thank our faculty member Prof. Dr. Duran Canatan, who edited the textbook "Thalassemia and Hemoglobinopathies" on the 100th anniversary of the Republic of Türkiye, the Mediterranean Blood Diseases Foundation, which has been working in this field for thirty years, and hundred scientists from twenty two countries who supported the book with their valuable articles.

We hope that the book will be useful to scientists working in this field in our country and all over the world.

Prof. Dr. İsmail Yüksek

Rector of Antalya Bilim University

29 October 2023

Foreword by Prof. Dr. Şükrü Cin and Prof. Dr. Nejat Akar representing our professors who have worked on Thalassemia and Haemoglobinopathies in Türkiye

It is an honour for us to write a foreword to the book on behalf of our professors in this book prepared with the contributions of scientists who have done research and served in the international arena and in our country on Thalassemia and Hemoglobinopathies **in 100th anniversary of the Republicof Türkiye,**

We would like to take this opportunity to congratulate Prof. Dr. Duran Canatan, who started this journey in 1977 with the suggestion of our teacher Prof. Dr. Ayten Arcasoy, who was our student at Ankara University Faculty of Medicine, Department of Pediatrics and Department of Pediatric Hematology, and with whom we have been together for 46 years in research, service, scientific meetings and social activities in the journey of Thalassemia.

We would also like to congratulate the board of directors of the Mediterranean Blood Diseases Foundation, which has been working not only in the Antalya region but also in Türkiye and Europe for 27 years, for the work they have done and the support they have given to the writing and printing of this book.

Prof. Dr. Muzaffer Aksoy is the first person who comes to mind when it comes to Thalassemia in Türkiye and who contributed to Thalassemia in the international arena. Prof. Aksoy's studies, which started in the population he defined as Eti Turks, were a first in this field. Prof. Dr. Günçay Dinçol from Istanbul U. Çapa Medical Faculty and Prof. Dr. Lamia Ulukutlu from Istanbul U. Cerrahpaşa Medical Faculty are our professors working in this field.

Prof. Nazlı Başak from Boğaziçi University is a pioneer in this field with her work covering the whole country in the molecular field.

Prof. Dr. Ayten Arcasoy and Prof. Dr. Ayhan Çavdar, our professors at Ankara University Faculty of Medicine, have conducted researches and scans in this field for many years. They trained us and many other scientists in the field of haematology. Especially Prof Arcasoy pioneered the screening studies carried out throughout the country. In the 1970s, they wrote together that the incidence of thalassaemia in Türkiye was 2.1%, which is still up to date.

In the 1980s, we carried out thalassaemia prevention activities together in the Turkish Republic of Northern Cyprus. Dr Gülsen Bozkurt, who is still actively working in the Turkish Republic of Northern Cyprus, was trained in this field.

Prof. Dr. Çiğdem Altay and Prof. Dr. Aytemiz Gürgey from Hacettepe University Faculty of Medicine have made very important contributions to the world of science, especially their molecular studies as well as their screening studies in this field. They have trained scientists working actively in this field.

Prof. Dr. Güngör Nişli from Ege University Faculty of Medicine pioneered the studies in the Aegean region and trained valuable scientists.

Prof. Dr. Güneş Yüreğir from Çukurova University Faculty of Medicine conducted thalassemia and haemoglobinopathy screening in the

Çukurova region on the one hand and molecular studies with Prof. Dr. Kıymet Aksoy and Prof. Dr. Yurdanur Kılınç from the same team on the other. He has trained many scientists in this field.

In this book, we would like to thank the esteemed scientists from Europe, America, Asia and Africa and many universities in our country who have written scientific articles summarising the current situation of thalassemia and haemoglobinopathies in the countries, diagnosis of the disease, treatment, complications, stem cell transplantation, gene therapy, community education on prevention, screening methods, genetic counselling, prenatal diagnosis and preimplantation diagnosis.

We think that the book, written in both English and Turkish, will be very useful for scientists and physicians working in this field both in our country and all over the world.

We also congratulate Prof. Dr. Duran Canatan who edited the book.

Prof. Dr Şükrü Cin
Ankara University Faculty of Medicine, Department of Pediatrics, Department of Paediatrics, Department of Paediatric Haematology, Faculty Member Emeritus- Ankara -Türkiye

Prof. Dr Nejat Akar
Ankara University Faculty of Medicine
Department of Child Health and Diseases,
Division of Paediatric Haematology
Retired Faculty Member-
TOBB University Faculty of Medicine
Ankara -Türkiye

29 October 2023

From the Editor

It has been a great honour at the peak of my professional life to write and edit this book with 100 scientists from the Turkish Republic of Northern Cyprus, Azerbaijan, USA, Italy, Spain, UK, Greece, Bulgaria, Pakistan, Iran, Syria, Iraq, Iraq, Israel, Saudi Arabia, United Arab Emirates, Qatar, Oman, Egypt, Sudan and Morocco, as well as Türkiye, in order to address the 100-year status of thalassemia and haemoglobinopathies in our country and in the world, the genetics, clinic, diagnosis, treatment and prevention programmes **on the 100th anniversary of the Republic of Türkiye**.

When we look at world history, the first "Sickle Cell Anaemia" patient was identified by Herrick in 1910.

In 1925, Prof. Dr Thomas B Cooley described his first patient as "Cooley's Anaemia".

In 1932 Whipple and Bardford described it as "Thalassanameia", which means sea anaemia.

In 1948, Cominopetros described the Mendelian genetic inheritance of the disease.

In 1945, Silvestroni and Bianco in Italy described it as "Constitutional Microcytic Anemia".

In 1948, Vezzoso in Italy published that thalassaemia and malaria are of the same distribution.

In 1949, Chini and Valeri reported that bone changes in the skull bones of patients with thalassaemia were found in many parts of the world.

In 1950, Neel and Itano published for the first time abnormal haemoglobins in haemoglobin electrophoresis.

Between 1950 and 1960, Aksoy, Chatterja, Chernof, Le-Injo, Minich, Vella, Vong and Whetherall described and published THALASSEMIA in our country and other countries.

Between 1960 and 1980, it was published that alpha, beta, delta and gamma globins were from different genes, thus thalassaemia syndromes were found due to the genetic heterogeneity of thalassaemia.

In 1960, Wolman gave the first blood transfusion to a thalassaemia patient.

In the 1970s, the first iron-removing drug "Desferrioxamine" came into use.

In 1972, Kan et al. performed the first prenatal diagnosis of sickle cell anaemia.

In the 1980s, molecular genetic studies of thalassaemia began to be published.

In 1981, Edward Thomas performed the first bone marrow transplantation and received the Nobel Prize for this work.

In the 1990s, the first oral chelator "Deferiprone" came into use.

In the 2000s, the second oral chelator "Deferasirox" came into use.

Since 2000 years, complete cure has been achieved with Stem Cell transplantation in patients.

Since 2010, gene transplantation has been on the agenda and has been successfully applied in patients.

The World Health Organization initiated the "Thalassaemia Prevention Programme" in the Mediterranean Countries in the 1970s for the prevention of the disease and although it has been successfully implemented in these countries, Thalassaemia continues to be a worldwide problem due to migration.

There are currently around 300 million thalassaemia carriers in the world, especially in developing countries, and around 300,000 children with thalassaemia are born every year.

When we look at the history of Türkiye, haemoglobinopathies have a history of eighty-one years. Because the first patient with thalassemia major was described in 1942 and the first patient with sickle cell anaemia was described in 1946 by Egeli and Ergun.

In 1950, Prof. Dr. Muzaffer Aksoy started nationwide studies and scans were carried out in Antalya, Mersin, Antakya and Western Thrace. In Antakya, sickle cell anaemia was detected in people of African origin, who were described as Eti Turks with white skin, and it was published that they had similar structures with patients of African origin.

In the 1970s, Arcasoy and Çavdar published the prevalence of beta-thalassaemia in healthy population as 2.1% nationwide.

In 1983, Altay and colleagues performed the first prenatal diagnosis procedure at Hacettepe University Faculty of Medicine.

In 1987, Akar et al. published the first molecular studies on thalassaemia in the Turkish population.

In 1991, Özerkan and his team performed the first bone marrow transplantation at Hacettepe University Faculty of Medicine.

In 1992, Başak et al. published the molecular spectrum of thalassaemia in the Turkish population.

In 1993, with the initiative of Arcasoy and Canatan, the Law No. 3960 on Combating Hereditary Blood Diseases was adopted by the Turkish Grand National Assembly and published in the official gazette.

In 1994, the first thalassemia centre affiliated to the Ministry of Health was established by Canatan and colleagues at Antalya State Hospital.

Between 1994-1997, under the leadership of Altay and Cin, meetings were organised with scientists working on thalassemia in Türkiye in the UNION.

In 2000, under the auspices of our 9th President Süleyman Demirel, the first International Thalassaemia Summer School held in Antalya by the Mediterranean Blood Diseases Foundation under the presidency of Canatan, brought together foreign and local scientists and patients from all over the world.

In 2000, National Thalassemia Youth camps were organised for the first time.

The year 2000 was a milestone year in the lives of patients in our country.

In 2000, the Ministry of Health, universities, associations and foundations came together and established the National Haemoglobinopathy Council under the chairmanship of Canatan.

In 2002, in cooperation with the Ministry of Health and National Haemoglobinopathy, a regulation was published.

In 2002, a thalassaemia map including the status of patients in 81 provinces of our country was published.

In 2002, Altay published studies of abnormal haemoglobin over the last 40 years.

In 2003, the Ministry of Health and the National Hemoglobinopathy Council and 33 Provincial Health Directors initiated the Hemoglobinopathy Control Programme in 33 provinces in Mersin.

In 2004, Kahraman et al. reported for the first time that HLA-matched preimplantation genetics diagnosis has been made.

In 2005, under the chairmanship of Canatan, the Thalassaemia Federation was established instead of the National Haemoglobinopathy Council. Between 2005 and 2013, the Ministry of Health and the Thalassaemia Federation organised five International Thalassaemia Summer Schools and Congresses, educators and health workers were trained through the Talotır training project and the Formator Teacher project in 2007, and health workers were trained in 18 provinces between 2009 and 2010.

In 2010, the Haemoglobinopathy Control Programme prevented ninety percent of new sick child births.

In 2011, the 12th World Thalassemia Congress was held in Antalya under the chairmanship of Canatan and Panos Englezos in cooperation with the Ministry of Health, the World Thalassemia Federation and the Turkish Thalassemia Federation.

In 2013, the Ministry of Health expanded the Haemoglobinopathy Control Programme to 41 provinces.

In 2018, by presidential decree, the Haemoglobinopathy Control Programme was extended to 81 provinces.

In 2022, the number of newborn patients was zeroed throughout the country, but in order to control the birth rate and the number of patients in migrants, haemoglobinopathy control studies were intensified in provinces where migrants are concentrated with European Union projects.

I owe my school life to the Republic of Türkiye and its founder Gazi Mustafa Kemal ATATÜRK and his friends, who enabled me to study with the support of the state, starting from primary school, secondary school, high school and university, and to reach this summit as a physician. Our Great Leader Gazi Mustafa Kemal ATATÜRK's saying "Entrust me to Turkish Physicians" has guided and empowered us to become the best physicians.

I would like to thank my late grandfather, who called me to be a doctor when I was born, and my late mother, father and family who educated me with all kinds of devotion and hardship.

I would like to thank my beloved wife Ayfer and my children Selim and Simge, who have provided me with all kinds of support throughout my professional life and who have always and everywhere supported all my work in the field of thalassaemia.

I remember with mercy and gratitude my esteemed teacher Prof. Dr. Ayten Arcasoy, who guided my professional life towards thalassemia when I was an intern student at Ankara University Faculty of Medicine in 1977.

As a thalassemia scientist on the path shown by my teacher, I have been working as a thalassemia scientist for forty-six years at Ankara, Akdeniz and Süleyman Demirel University Faculties of Medicine, Antalya State Hospital and Azerbaijan Haydar Aliyeva Thalassemia Centre, and I am currently working at the Mediterranean Blood Diseases Foundation Thalassemia Diagnosis Centre, Antalya Genetic Diseases Assessment Centre and Antalya Bilim University.

I would also like to thank Dr Özlem Zümrüt and Dr Zekiye Özdemir, with whom we have been working together at the Mediterranean Blood Diseases Foundation for 27 years, who served as assistant editors of the book, and our foundation board members Av. Ali Rıza Tıraş, Mustafa Ülgüt, Hayri Oruç and Gürsel Kaya, who supported the printing of the book.

I would like to thank hundred distinguished scientists from twenty-two countries of the world for their valuable contributions to this book.

I hope that this book will be useful to all scientists, physicians and students working in the field of thalassaemia and haemoglobinopathies.

Yours sincerely

Prof. Dr Duran Canatan
Paediatric Blood and Genetic Diseases Specialist
President of the Mediterranean Blood Disorders Foundation
Antalya Genetic Diseases Evaluation Centre Responsible Manager
Antalya Bilim University Faculty Member

29 October 2023

CHAPTERS

CHAPTER 1

THALASSEMIA AND HEMOGLOBINOPATHIES IN THE WORLD

THALASSEMIA AND HEMOGLOBINOPATHIES IN TÜRKİYE

Prof. Dr Duran Canatan
Antalya Bilim University Faculty Member
President of the Mediterranean Blood Diseases Foundation-Antalya
Medical Director of Antalya Genetic Diseases Assessment Center-Antalya-Türkiye

ABSTRACT

Hemoglobinopathies have a seventy-one year history in Türkiye. Although the first patient with thalassemia major was described by Egeli and Ergun in 1942 and the first patient with sickle cell anaemia (SCA) in 1946, the first studies on haemoglobinopathies were initiated by Aksoy in the 1950s. Arcasoy and Çavdar published the prevalence of beta-thalassaemia in healthy population as 2.1% in 1970s. Akar et al. 1987 conducted the first molecular studies on thalassaemia in the Turkish population and IVS1.110 was defined as the most common mutation. Altay et al. 1983 performed prenatal diagnosis for the first time. Başak et al. 1992 studied the molecular spectrum of beta thalassaemia in the Turkish population and reported that the six most common mutations were IVS-I-110 (G>A), IVS-I-6(T>C), FSC-8 (-AA), IVS-I-1(G>A), -30(T>A) and FSC-5 (-CT), which accounted for 70% of the total mutations. In 2002, Altay reviewed the abnormal haemoglobin studies conducted in the last 40 years and published that the most common abnormal haemoglobin in our country was Hb S, followed by Hb D, Hb E and Hb O Arab, and 42 other abnormal Hb's were found in our country.

In order to prevent thalassaemia and abnormal haemoglobins, the Law No. 3960 on Combating Inherited Blood Diseases was published on 30.12.1993. Representatives of universities, SSK, state hospitals, foundations and associations working on thalassaemia and haemoglobinopathy in Türkiye established the National Hemoglobinopathy Council (NHC) on 23.06.2000. The Regulation on Hereditary Blood Diseases prepared by the Ministry of Health and NHC was published on 24.10.2002 and the Haemoglobinopathy Prevention Programme was initiated in 33 provinces in a meeting held in Mersin on 08.05.2003. With this programme, the number of new sick child births decreased from 272 in 2002 to 25 in 2010, and ninety percent was prevented. With the arrival of immigrants, the number of newborn patients started to increase, and therefore, in 2018, pre-marital thalassaemia testing was made compulsory in Türkiye. As a result, the screening studies carried out by the Ministry of Health in all provinces in Türkiye for 20 years have shown that the carrier frequency is a total of 1,700,000 carriers with an average of 2%. The number of newborn patients is 99% prevented and the total number of patients is around 6000. Transfusion, chelation and other treatments of all patients are covered free of charge. The quality and duration of life of patients have improved significantly since 2000.

The aim of this review is to update epidemiological studies, prevention studies and the status of patients in thalassemia and haemoglobinopathies in our country.

Keywords: Türkiye, thalassaemia, haemoglobinopathies

IMPORTANT CORNERSTONES IN TÜRKİYE

Hemoglobinopathies have a history of eighty-one years in our country. When we look at the important cornerstones, although the first patient with thalassemia major was described in 1942 and the first patient with sickle cell anaemia (SCA) was described by Egeli and Ergun in 1946, the first studies on haemoglobinopathies were initiated by Prof. Dr. Muzaffer Aksoy in the 1950s. In his studies starting from the 1950s, Aksoy found sickle cell anaemia in people who were of African origin but were described as Eti Turks with white skin and published

that they had similar structures with patients of African origin **(1, 2).**

Arcasoy and Çavdar reported the prevalence of beta-thalassaemia as 2.1%, alpha-thalassaemia as 0.25%, Hb S as 0.26%, Hb D as 0.12% and HbE as 0.03% among abnormal haemoglobins in the healthy population throughout the country in 1970s **(3, 4).**

The first prenatal diagnosis procedure was performed by Altay et al. at Hacettepe University Medical Faculty in 1983 **(5)**.

Akar et al. performed the first molecular studies on thalassaemia in the Turkish population in 1987 and published that the IVS 1-110 mutation was the most common mutation **(6).**

The molecular spectrum of beta thalassaemia in the Turkish population was published by Başak et al. in 1992. In this study, IVS-I-110 (G>A), IVS-I-6(T>C), FSC-8 (-AA), IVS-I-1(G>A), -30(T>A) and FSC-5 (-CT) constituted 70% of the total mutations **(7).**

The first bone marrow transplantation in thalassaemia was performed by Özerkan et. al. at Hacettepe University, Medical Faculty in 1991 **(8).**

In 2002, Altay reviewed the abnormal haemoglobin studies conducted in the last 40 years and found that Hb S, then Hb D, HbE and Hb O Arabs were the most common in our country, and 42 published abnormal Hb was found in our country **(9)**.

In 2004, HLA-matched preimplantation genetics diagnosis by Kahraman et al. was made **(10).**

With the initiatives of Arcasoy and Canatan, the draft law, which was given to a Hatay deputy to prevent thalassemia and abnormal haemoglobins, was adopted by the Turkish Grand National Assembly on the last day of the year on **30.12.1993 and the** Law No. 3960 on Combating Hereditary Blood Diseases was published in the official gazette. Following the law, it was decided to establish thalassemia centres in Antalya, Antakya, Mersin and Muğla state hospitals affiliated to the Ministry of Health **(11)**.

The first thalassemia centre affiliated to the Ministry of Health was established in Antalya State Hospital by Canatan et al. **(12).**

Between 1994 and 1997, under the leadership of Altay and Cin, UNION formation meetings were held with employees of universities, hospitals, foundations and associations working on thalassemia and hemoglobinopathies in Türkiye.

The National Hemoglobinopathy Council (NHC) was established on **23.06.2000** by the Ministry of Health, General Directorate of Mother-Child Health-Family Planning and Treatment Services and representatives of universities, SSK, State Hospitals, foundations and associations working on thalassemia and haemoglobinopathy in Türkiye.

The Regulation on Hereditary Blood Diseases prepared by the Ministry of Health and the NMC was published on **24.10.2002**. On **08.05.2003,** on the 10th World Thalassaemia Day in Mersin, the Ministry of Health, NHC and 33 Provincial Health Directors initiated the Haemoglobinopathy Control Programme (HCP) in 33 provinces **(13)**.

While the number of new sick child births was 272 in 2002, it decreased to 25 in 2010 and was prevented by ninety per cent (**14, 15)**.

With the arrival of immigrants, the number of newborn patients started to increase, so in 2018, thalassaemia test before marriage was made compulsory all over Türkiye.

The year 2000 was a milestone for patients with thalassaemia in Türkiye. Because the organisation of the first Thalassemia Summer School in 2000 with all patients and scientists together, the start of Thalassemia Youth camps in the same year, and the introduction of oral chelators since 2000 have improved the chelation compliance of patients and improved the quality of life.

EPIDEMIOLOGICAL STUDIES

The first large thalassaemia prevalence study in our country was performed by Çavdar and Aracasoy and the prevalence of beta thalassaemia was found to be 2.1% and the prevalence of alpha thalassaemia was found to be 0.25% and published **(3, 4)**.

The Ministry of Health and NHC collected and evaluated the screening studies conducted by 16 centres in the Marmara, Aegean and Mediterranean regions between 1995 and 2000. According to the results of this study, a total of 377,399 healthy peo-

ple were screened and the mean prevalence of thalassaemia and abnormal haemoglobin was found to be 4.3%. Beta thalassaemia was found most frequently in Antalya (13%), and SCA was found most frequently in Mersin (13.6%), Hatay (10.6%) and Adana (10%).

Epidemiological studies have increased with premarital screenings from all regions when premarital screening programmes were started to be implemented all over the country with the HCP successfully carried out by the Ministry of Health. According to the latest data of the General Directorate of Public Health of the Ministry of Health, 409,654 people have been screened in premarital screening programmes conducted for twenty years since 2003 and the carrier frequency was found to be 2.05%.

A map of Türkiye has been prepared with epidemiological studies conducted in seven regions of our country from past to present.

BETA THALASSEMIA AND HAEMOGLOBINOPATHY PREVALENCE STUDIES

Studies carried out in the Mediterranean Region:

Antalya: The first studies in Antalya were performed by Aksoy and the prevalence of β-thalassemia was found to be 6.7% and the prevalence of Hb S was found to be 2.5%. Bircan et al. found a prevalence of 10.7% and Hb S prevalence of 0.8% **(16,17).** A total 89.981 individuals who applied to the haemoglobinopathy centre of our foundation in Antalya between 2003 and 2012 for premarital testing; β-thalassemia was found to be 6.57%, α-thalassemia was found to be 3.56% and abnormal haemoglobin frequency was found to be 0.54%; Hb S 0.31%, Hb D-Los Angeles 0.15%, Hb G-Coushatta 0.06% and Hb E 0.02%, respectively **(18).** Between 2013 and 2022, a total 49,600 people applied to our foundation for the pre-marital screening programme. Since the majority of the applicants were foreigners, the rate of β-thalassemia was found to be 4.66%, α-thalassemia 1.21% and abnormal haemoglobin frequency 1.02%. In Antalya, villages along the Köprüçay river were surveyed by our foundation to support the malaria theory in thalassaemia. While the frequency of beta-thalassaemia was found to be 3-4% in the villages in the mountainous region of the Köprüçay River where malaria was absent, it was found to be 12-13% in the villages by the sea where malaria was intense. In this study, which supports the malaria theory, it was observed that the frequency of thalassaemia decreased as you go from the coast to the mountains in a region with migration routes **(19).** Another study of our foundation was carried out in İbradı, where Prof. Dr. Muzaffer Aksoy was born, with the project **"Commemorating our professors in their birthplaces".** In this study, the prevalence of beta-thalassaemia was found to be 3.2% in Ibradı, which is a mountainous region **(20).**

Isparta: A screening study in 6054 school studentswas performed by **Tunç** et al. the prevalence of β-thalassaemia was found to be 2.5% **(21).**

Burdur: A total 3515 8th grade primary school students' study was conducted by Çatak et al. in 2011, the prevalence of βthalassaemia was found to be 5.2% and abnormal haemoglobin was found to be 0.9% **(22).**

Mersin: Altay and Gürgey A reported a β-thalassemia frequency of 1.7% **(23).** Tosun et al. found beta thalassaemia 2.04%, Hb S 1.21%, Hb D 0.17% and Hb E 0.04% in a premarital screening study performed in 79.000 individuals **(24).**

Antakya: The first studies on β-thalassaemia in our country were conducted by Aksoy et al. in Eti Turks in Antakya, which is the cultural mosaic of our country, and then by Özsoylu, Altay and Dinçol et al. in the same province and found a frequency of 0.8-1.4%.

In the studies conducted by Aksoy, Özsoylu and Altay et al. The frequency of Hb S varies between 15.3-37.6% **(25-28).**

Çukurova: Yüreğir et al. found the frequency of beta thalassaemia to be 3% in Çukurova **(29).**

In a nine-year study conducted in the Çukurova region and neighboring provinces (Adana, Hatay, Mersin, Konya and Kayseri), 1382 abnormal hemoglobin cases (17%) were found in 8135 samples, the frequency of Hb S was 10.1%, the frequency of β-thalassemia was 5.1% **(30).**

Adana: Güvenç et al. found the frequency of β-thalassemia to be 13.46% and the frequency of

haemoglobinopathy to be 6.83% in 3000 individuals who applied to Seyhan Thalassemia Centre and reported that the most common haemoglobinopathies were Hb S, Hb D Los Angeles and Hb E (**31).** In Kadirli district, β-thalassaemia trait was found in 98 (4.91%) of 1994 individuals who underwent premarital screening **(32)**.

Kahramanmaraş: Yüreğir et al. found β-thalassaemia at a frequency of 0.68%, Hb D at 0.28% and Hb O Arab at 0.013% **(33).** Güler et al. found a prevalence of 2.8% for β-thalassaemia and 0.4% for HbS in 48.126 individuals in premarital screening **(34).** In Elbistan**,** Canatan et al. found the prevalence of β-thalassemia to be 1% and Hb D to be 0.3% **(35).**

Epidemiological studies conducted in the Aegean Region:

Izmir: In a study conducted by Aydınok et al. in students, the frequency of β-thalassemia was found to be 3% **(36).** Irken et al. found Hb D 0.37%, Hb S 0.32%, Hb E 0.18%, Hb O-Arab 0.12%, Hb G-Copenhagen 0.09%, Hb D-Iran 0.06%, Hb Lepore 0.06% and Hb Hasharon 0.03% in the analysis of patients admitted to hospital **(37).** Uysal et al. found the prevalence of β-thalassaemia to be 4.96%, the prevalence of abnormal haemoglobin to be 0.53%, and the distribution of the most common abnormal haemoglobins to be HbS 0.33%, HbD 0.13%, HbE 0.06%, and HbC 0.01% in a premarital screening of 19,277 couples (**38).**

Aydın: The frequency of β-thalassaemia was found to be 4.6% in a screening study conducted by Yenice in Nazilli **(39).**

Denizli: In a study conducted by Sözmen et al., the prevalence of β-thalassaemia was found to be 3.6% **(40)**.

Keskin et al. found a β-thalassaemia frequency of 2.6% in 19.804 individuals screened before marriage **(41).** Bolaman et al. found a β-thalassaemia prevalence of 2.2% in 14,200 individuals **(42)**.

Mugla: Arcasoy et al. conducted a screening study on 734 individuals in Muğla and its villages and found the frequency of β-thalassemia to be 8.7%, HbS 0.41%, HbD 0.27% and Hb D Los Angeles 0.14%. **(43**).

In the centre of Muğla, Topal et al. screened a total of 164.814 primary school students with complete blood count and HPLC in a six-year period between 1997 and 2013, and abnormal results were found in 5861 students (3.8%), among which β-thalassemia was found in 3.2%, HbS in 0.15% and other abnormal haemoglobins in 0.4% **(44).**

Epidemiological studies conducted in the Marmara Region:

Bursa: In Mustafa Kemal Pasha town, Akar et al. found the frequency of β-thalassaemia to be 2.6% **(45)**.

Çanakkale: Uludag et al. found a prevalence of 1.4% for β-thalassaemia in a premarital screening of 8904 individuals **(46).**

Koceli: Sarper et al. found 0.89% prevalence of β-thalassaemia and 0.05% prevalence of HbS in 88,888 individuals in premarital screening **(47).**

Western Thrace: The prevalence of β -thalassaemia was found to be 10.8%, Hb S 2.9% and O Arab 3.9% in 102 healthy immigrants by Aksoy et al **(48)**.

Epidemiological studies conducted in Central Anatolia Region:

Konya: In the study conducted by Turan et al. the prevalence of β -thalassaemia was found to be 3% **(49).** (**50).** The premarital screening of hemoglobinopathies was evaluated retrospectively in 72,918 subjects by Güler et.al., the thalassemia trait was detected in 1465 subjects (2%), and the SCA trait was detected in 37 subjects (0.05%) **(50).**

Kayseri: In a premarital screening performed by Karakükçü et al., the prevalence of beta thalassaemia was found to be 1.71%, Hb D 0.36% and Hb O Arab 0.09% in 10,261 individuals **(51).**

Nigde: The prevalence of β-thalassaemia was found to be 2.63% in a premarital screening study conducted by Sevdel et al. in 2013 subjects **(52).**

Epidemiological studies conducted in South East Anatolia Region:

Gaziantep: Gürbaş et al. performed in 2439 students, the frequency of β-thalassaemia was found to be 1.84% **(53).**

Şanlıurfa: İncebıyık et al. performed in 37.962 individuals, the prevalence of β-thalassaemia was 2.4% and the prevalence of abnormal haemoglobin was 1.7% (Hb S, Hb C and Hb D-Punjab 0.50, 0.38 and 0.69%) **(54).**

Adıyaman: Genç et.al. performed screening in 3571 school students, the frequency of β-thalassaemia was found to be 1.06% and the frequency of abnormal haemoglobin was found to be 0.20% **(55).**

Epidemiological studies in the Eastern Anatolia region:

In a screening study conducted by Kürkçüoğlu et al. in Eastern Anatolia, the prevalence of β-thalassaemia was found to be 0.6% **(56)**.

Erzurum: Acemoğlu et al screened 1,610 individuals and the prevalence of β-thalassaemia was found to be 0.68% **(57).**

Van: The prevalence of β -thalassaemia was found to be 2.6% in the screening conducted by Aksoy et al. **(58).**

Epidemiological studies in the Black Sea region:

In a study which has not been published yet, 52.338 people were screened in a three-year premarital screening conducted in one of our provinces and β-thalassaemia was found with a frequency of 1.37% and Hb S with a frequency of 0.04%.

ALPHA THALASSEMIA FREQUENCY STUDIES

Ankara: Regarding the frequency of alpha thalassaemia, Fei et al. found 3.6% in cord blood by gene mapping method and Özsoylu et al. found 4.1% by chromatographic method **(59,60).**

Antalya: In α thalassaemia screening performed in 205 cord blood samples of 205 babies in Antalya State Hospital Thalassaemia Centre, the frequency of α thalassaemia was found to be 6.3% **(61)**.

In a nine-year study conducted in Çukurova region and neighbouring provinces (Adana, Hatay, Mersin, Konya and Kayseri), α thalassaemia was found to be 1.6% in 8135 samples **(30).**

Adana: Kılınç et al. reported a frequency of 2.9% in α thalassaemia screening performed in cord blood **(62).** Güvenç et al. detected α thalassaemia (7.5%) in 225 individuals in α thalassaemia screening performed in 3000 individuals **(31).**

As a result, in the first epidemiological studies conducted in our country, 2.1% thalassemia trait was detected throughout the country. In the screenings carried out by the Ministry of Health in the provinces for 20 years, the average rate was found to be around 2.05%.

According to the screening conducted in the regions, the average carrier frequency in the regions is as follows: 5.2% in the Mediterranean region, 4.1% in the Aegean Region, 3.9% in the Marmara Region, 2.3% in the Central Anatolia Region, 1.7% in the Southeastern Anatolia Region, it was found to be 1.4% in the Eastern Anatolia Region and 1.3% in the Black Sea Region. Especially in recent years, there have been changes in the country map due to immigrants coming to our country **(Table 1).**

GENETIC STUDIES

The first studies on Beta thalassemia genetics in our country were conducted by Akar et al. Haplotypes in IVS-I-110 (G>A), Codon 39 (C>T), IVS-I-6 (T>C) mutations were examined by haplotype analysis and oligonucleotide hybridization method in the blood samples of the thalassemia major patient and 13 out of 20 alleles were detected. IVS-I-110 (G>A) (65%) **(6).**

In a study conducted by Tadmouri et al. to investigate haplotypes of thalassaemia, they published that the oldest mutation in Anatolia between 6500-2000 BC may be IVS-I-110 (G>A), based on the malaria natural selection theory **(63)**.

Başak et al. detected 18 different mutations in a total of 268 individuals (161 beta-thalassaemia homozygotes and 107 beta-thalassaemia heterozygotes) from Türkiye. It was reported that the six most common mutations, IVS-I-110 (G>A), IVS-I-6(T>C), FSC-8 (-AA), IVS-I-1(G>A), -30(T>A) and FSC-5 (-CT), constituted 69.3% of the total mutations and represented 85.8% of the 26 mutations tested **(7).**

In another analysis study, Tadmori et al. analysed the molecular pathology and frequency distribution of 795 cases including 754 beta-thalassaemia and 42 abnormal haemoglobin cases in 6 different regions of Türkiye. When the regional results were compared with the country-wide mutation frequencies, they reported that the frequencies in the western and southern parts of Türkiye were compatible with the general distribution, whereas the northern and eastern parts had more regional distribution and rare mutations **(64).**

Başak conducted the first large study on beta thalassaemia by analysing the DNA of 1500 homozygous patients between 1987 and 2006 and revealed that the most common mutation in Türkiye was IVS-I-110 (G>A), followed by IVS-I-6 (T>C) and FSC-8 (-AA). She published that the total frequency of the six most common mutations is approximately 70.3%, and the overall frequency of the top 12 mutations is 83.3%. Türkiye's great molecular heterogeneity is explained by its unique geographical location and rich history, which has been an important crossroads between cultures, civilizations and continents for centuries. **65).**

In a study conducted at Boğaziçi University on α thalassaemia, α3.7 and -α4.2 mutations were investigated in the cord blood of 116 babies born in Antalya State Hospital; α3.7 was found in six samples (5.7%), whereas -α4.2 mutation was not found (**66)**.

In Antalya, IVS-I-110 (G>A) (44.4%) was found to be the most common mutation among 16 different mutations in a study of 459 samples, 377 postnatal and 82 prenatal, conducted by Keser et al. between 2000 and 2003. A new Hb variant, Hb Antalya, and a new mutation, Cod 3 (+T), were found, and it was published that HbS constitutes 10.3% of all mutations. **(67).**

In another study conducted in Antalya with SNP haplotypes, beta globin gene haplotyping was analysed in 197 patients by direct sequencing of the entire beta globin gene and using five common intragenic SNPs and 25 different beta globin gene point mutations were detected. Turkish type inv/del mutation was also detected by MLPA and Gap-PCR. The seven most common mutations with a frequency higher than 5% were IVS-I-110 (G>A) (35.6%), Hb S (10.6%), IVS-I-6 (T>C) (7.4%), IVS-I-1 (G>C) (7.4%)A) (6.9%), IVS-II-1 (G>A) (6.9%), Cod8(-AA) (6%), IVS-II-745 (C>G) (5.1%) **(68).**

In Antalya, Kurtoğlu et al. published the most common mutation among 14 different mutations was IVS-I-110 (G > A) with a rate of 52.7%, in the mutation analysis of a total of 146 β-thalassemia patients, followed by IVS-I-6 (T > C)] (14.4%). -30 (T > A) (8.2%), IVS-II-1 (G) > A)] (8.2%) and that these constitute 83.1% of the existing mutations. **(69).**

In the molecular analysis of carrier individuals performed in healthy high school students in Isparta province and region, the most common mutations found were IVS-I-110 (G>A)), Codon 39 (C> T) and IVSII-745 (C>G), respectively **(21).**

In a seven-year retrospective study conducted in the Mediterranean region by Arpacı et al., 73 different mutations were found and IVS I-110 G>A) mutation was found to be the most common mutation among the existing mutations. Mutations in the UTR region [HBB:C*62 A>G (3'UTR+1536 A>G) and HBB:C*1 G>A (3'UTR+1475 G>A)], which were not defined before, were defined **(70).**

In the screening of 8135 individuals conducted by Çukurova University in the Çukurova region and nearby provinces, 826 mutations were analyzed, the most common in Hb S [β6(A3) Glu→Val (GTG > GAG), and IVS-I-110 (G > A) and in alpha thalassemia. α 3.7 kb deletion detected **(30).**

Guzelgul et al. conducted the study in the Çukurova region. In the mutation distribution of 52 beta thalassemia patients, IVS-I-110 (G>A) (58.3%) comes first, followed by codon 8 (-AA) (5.6%), -30 (T>A) (5%). 6), IVS-I-6 (T>C) (5.6%) and IVS-II-1 (G>A) (5.6%). They published that the most common β-thal mutation in the Çukurova region, where various types of hemoglobinopathies are observed, is the IVS-I-110 mutation. **(71).**

Güvenç et al. By screening 3000 people, they detected β-thalassemia and hemoglobinopathy in 609 people, and published 18 different β-thalassemia mutations and three abnormal HbS, HbD Los Angeles and HbE in Adana. Beta mutations in order of frequency; IVSI.110 (G > A), codon 8 (-AA), IVSI.1 (G > A), IVS I.6 (T > C), -30 (T > A), IVS II.1 (G > A), codon 39 (C > T), codon 44 (-C), IVS I.5 (G > C), codon 5 (-CT), codon 8/9 (+G), IVSII.745 (C > G), codon 22 (7bp del), -101(C >

T), codon 36/37 (-T), IVSI.15 (T > G), codon 6 (-A), -88 (G > A) They determined. They also published the mutations detected in the alpha gene as α 3.7, α4.2 --(MED), --(20.5), α(PA-2), α anti-3.7), and α(PA-1) in order of frequency. **(31).**

Çürük et al. published Hb Adana or alpha 2(59) (E8) Gly-->Asp beta 2, a highly unstable alpha 1-globin variant, in combination with -(alpha) 20.5 Kb alpha-tal-1 deletion in two Turkish patients. **(72).**

In Hatay the mutation analysis of 93 patients with β-thalassaemia in a study conducted by Aldemir et al. They found 16 different mutations and the most common mutations were IVS-I-110 (G>A), IVS-I-6 (T>C), IVS-I-1 (G>A), (FSC) 8 (-AA), codon 39 (C>T) and IVS-II-745 (C>G), respectively. Although there are many Syrians and Iraqis in the region, IVS-I-110 (G>A) was found in the first place **(73).**

In Kahramanmaraş, Kurutaş et al. found seven different mutations in 245 individuals with β-thalassaemia and published the most common mutation as IVS1-110 with 57.1% **(74)**.

In Siirt, Yılmaz et al.34 found 13 different mutations in β-thalassaemia, and it was published that the most common mutations were IVS-I-110 (G>A) (38.9%), IVS-II-1 (G>A) (11.1%), -30 (T>A) (9.25%) and IVS-I-1 (G>A) (9.25%) which constituted 68.5% **(75).**

In the meta-analysis study of the Turkish Paediatric Haematology Association, in the molecular analysis of 1988 patients from 27 thalassaemia centres, 90% of patients had 11 mutations, 47% of which were IVS 1-110 (G >A), the rest are IVS I-1(G>A), IVSI-6(T>C), Codon 39(C>T) IVSII-745(C>G), IVSII-1(G>G)A), Codon 8(-AA), Codon 44(-C), Codon 5(-CT), -30 (T>A) and IVSI-5(G>C) **(76).**

Unal and Gümrük from Hacettepe University conducted a comprehensive analysis of α thalassemia on a total of 78 patients with α thalassemia, 35 of whom were HbH patients. They identified α3.7 as the most common mutation in 62.8% of Hb H patients and 39.7% of α thalassemias. In 10 (28.6%) of the patients with Hb H disease, the most common genotypes were -α3.7/--20.5, -α3.7/--26.5 and -α3.7/--17, 5 detected **(77).**

Karakaş et al. from Istanbul University investigated 260 patients with hypochromic and microcytic anaemia in terms of α thalassaemia and found 14 different mutations in 95 (46.1%) patients. The most common mutation was α 3.7 single gene deletion (39%). Other common mutations were 20.5 kb double gene deletion (21%), MED double gene deletion (17.9%), α2 IVS1 (10.5%), α2 cd142 Hb Koya Dora (6.3%), α2 polyA1 (Saudi type) (6.3%), 4.2 single gene deletion (4%),2%), α1 cd14 (2.1%) and -FIL mutation (2.1%), Hb Adana, Hb Icaria, α2 init cd and α2 polyA2 (Turkish type) were found in 1% of the patients, and α-thalassemia triplication was found in seven patients (7.4%). In the study, 3 mutations (Hb Icaria, α1 cd14, α2 init.cd) were published for the first time in Türkiye **(78)**.

In conclusion, in all regions of our country, IVS-I-110 (G>A), the first mutation found in the history of Anatolian civilisation, is the most common mutation in β-thalassemia. The others are IVS-I-6(T>C), FSC-8 (-AA), IVS-I-1(G>A), -30(T>A) and FSC-5 (-CT), with partial differences between regions. In alpha thalassaemia, α3.7 is the most common mutation in all regions.

ABNORMAL HEMOGLOBINS IN TÜRKİYE

Arcasoy published the geographical distribution of abnormal haemoglobins in our country in 1992 **(79).** Altay, in 2002, scanned the abnormal haemoglobin studies performed in the last 40 years and published that Hb S was the most common abnormal haemoglobin in our country, followed by Hb D, Hb E and Hb O Arab, and 42 others abnormal Hb's were found in our country **(9).** Akar E&N published an update with newly found abnormal haemoglobins in 2007 **(80).** Akar N published the update of abnormal haemoglobins again in 2014 **(81).** Canatan published 42 different abnormal haemoglobins among a total of 745 abnormal haemoglobins in the last ten years at AKHAV Haemoglobinopathy Centre in Antalya **(18).** Akar updated the latest abnormal haemoglobins and published a table of the abnormal haemoglobins found in the Turkish population to date according to globins **(82).** According to this table, 98 abnormal haemoglobins including thirty-three alpha, fifty-three beta, two gamma, five delta, two hybrid and one insertion variant were defined **(Table 2).**

In the distribution of the four most frequently detected abnormal hemoglobins in our country, Hb S

in the Çukurova and Hatay regions of the Mediterranean region, Hb D in the Eastern and Southeastern Anatolia of the Mediterranean region, Hb E in the Western Mediterranean region, and Hb O Arap in the Thrace region.

PATIENTS' CONDITION

In order to determine the number of thalassemia and haemoglobinopathy patients in the provinces and to start prevention activities accordingly, the Ministry of Health, in cooperation with the General Directorate of Mother and Child Health and the National Haemoglobinopathy Council, sent a form to 81 provinces in 2002 and requested information on haemoglobinopathy patients in the provinces. Provincial health directorates sent the standard form to all health institutions in their provinces and sent the results to the General Directorate. Newly detected patients are reported to the General Directorate every month. Out of a total of 4512 patients, 57% were reported as Thalassaemia Major, 23% as Sickle Cell Anaemia, 11% as Thalassaemia Intermedia and 9% as other haemoglobinopathies. Thus, a thalassaemia map of Türkiye was drawn (**83). (Figure 1).**

WHITE:0, YELLOW:1-10, PURPLE:10-50, GREY:50-100, GREEN:100-200, CLARET RED: over 200

Figure 1: *Thalassaemia map of Türkiye*

Ministry of Health and the National Haemoglobinopathy Council started Haemoglobinopathy Control Programme in 33 provinces in 2003, the number of newborn patients decreased from 272 in 2002 to 30 in 2010, thus preventing ninety percent of newborn patients in seven years (**Figure 2.)**.

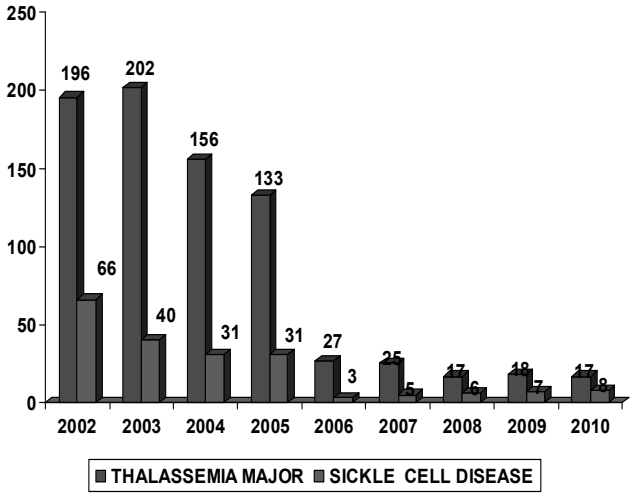


Figure 2: *Number of newborns with thalassemia and hemoglobinopathy between 2002-2010*

While the annual expected number of patients in our country is around 300 and 2400 sick children are expected to be born in eight years, with the Hemoglobinopathy Control Program, the total number of newborn patients was 700, so the number of patients remained at 5200 in 2010. **(14,15).**

When around 4 million immigrants came to our country due to the war in Syria, the data in our country suddenly started to change. In 2018, the average number of newborns increased to 100 and reached a total of 6000 patients.

For this reason, by Presidential decree, pre-marital thalassaemia screening became compulsory throughout the country in 2018.

In 2022, within the framework of the European Union Erasmus Project, under the coordination of the Mediterranean Blood Diseases Foundation and in cooperation with Italy, Spain and the General Directorate of Public Health, trainings were carried out for Syrian physicians working in migrant health centres in Adana, Mersin, Hatay, Gaziantep, Kilis and Şanlıurfa provinces **(84).**

A study on the status of migrant patients was conducted with the participation of 18 Turkish Paediatric Haematology Oncology Centres (PHOC). A total of 318 patients participated in the study, with a mean age of 8.1 ± 4.8 years (range 0.5-21 years). The mean time since immigration to Türkiye was 2.5 ± 1.5 years (range, 0.1-7 years). Of these, 72 (22.6%) were born in Türkiye and diagnosed with TBT. The most common mutation was IVSI-110 (G>A) (31%). Only 177 patients (55.6%) were reported to use chelators before migration, but this number increased to 268 (84.3%) after migration. Communication difficulties, finding an interpreter who has a good command of medical terminology, not using medications regularly and insensitivity to prenatal diagnosis were found to be the main problems **(85)**.

In the meta-analysis study of the Turkish Paediatric Haematology Society, in the evaluation of 1988 patients (1658 TDT and 215 NTDT) from 27 thalassaemia centres, it was found that while the probability of splenectomy in the first 10 years of life was 20%, this rate had not changed since the 1980s. Iron chelators were administered as a monotherapy regimen to 95% of patients and 81.3% of these patients received deferasirox. The highest rate of deferasirox administration (93.6%) was seen in patients <10 years of age. Haematopoietic stem cell transplantation was performed in 5.8% of thalassaemia major patients and the success rate was 77%. Heart disease was found to be an important cause of death and it was reported that it did not show a decreasing trend in 5-year cohorts since 1999 (**76).**

The situation of patients in our country has changed since 2000. Because with the 1st International Thalassaemia Summer School held in Antalya on 22-26 April 2000, the patients in our country were together with the leading scientists of our country and the world for the first time, and with the Thalassaemia Youth camps started in the same year, they held on to life with the trainings they received, and the use of new oral chelators prolonged their quality and duration of life.

In our country, transfusion and chelation treatments of patients with thalassaemia and haemoglobinopathy are provided free of charge (**14,15).**

CONCLUSION

1. In epidemiological studies conducted in our country, it has continued at an average rate of 2.1% throughout the country and 2.05 % in the scans conducted by the Ministry of Health in the provinces for 20 years. When we look at the distribution according to regions, it was found to be 5.2% in the Mediterranean region, 4.1% in the Aegean region, 3.9% in the Marmara region, 2.3% in the Central Anatolia region, 1.7% in the South Eastern Anatolia region, 1.4% in the Eastern Anatolia region and 1.3% in the Black Sea region, especially in recent years, there have been changes in the map of the country due to the immigrants coming to our country.

2. The number of abnormal haemoglobins changes every day. Of the four most common abnormal haemoglobins, Hb S has the highest frequency in the Çukurova and Hatay regions of the Mediterranean region of Türkiye, Hb D in the Eastern Mediterranean and South Eastern Anatolia, Hb E in the Western Mediterranean Western region and Hb O Arab in the Thrace region.

3. Genetically, IVS-I-110 (G>A), the first mutation found in the history of Anatolian civilisation, is the most common mutation found in beta thalassaemia in all regions of our country. The others are IVS-I-6 (T>C), FSC-8 (-AA), IVS-I-1 (G>A), -30 (T>A) and FSC-5 (-CT), with some interregional differences. In alpha thalassaemia, α3.7 is the most common mutation in all regions.

4. The year 2000 has been a milestone for the life status of patients in our country. Because the International Thalassaemia Summer Schools and National Thalassaemia Youth Camps that started in 2000 have increased education and the introduction of new oral chelators has prolonged the quality and duration of life. Transfusion, chelation and all other treatments of patients with thalassaemia and haemoglobinopathy in our country are provided free of charge.

REFERENCES

1. Aksoy M. Sickle-cell anaemia in South Türkiye; a study of fifteen cases in twelve white families. Blood 1956; 11:460-467.
2. Aksoy M. The history of beta-thalassemia in Türkiye. Turk J Pediatr 1991; 33:195-197.
3. Çavdar AO, Arcasoy A. The incidence of beta-thalassemia and abnormal haemoglobins in Türkiye. Acta Haematol 1971; 45:313-318.
4. Arcasoy A, Çavdar A, Cin Ş, et al. Thalassemia and incidence of abnormal haemoglobin in Türkiye. Nuray Matbaası. Ankara 1978,19-26.
5. Beksac MS, Gumruk F, Gurgey A, et.al.Prenatal diagnosis of haemoglobinopathies in Hacettepe University, Türkiye. Pediatr Hematol Oncol 2011; 28:51-55.
6. Akar N, Çavdar AO, Dessi E, et.al. Beta thalassemia mutations in the Turkish population. J Med Genet 1987; 24:378-379.
7. Basak AN, Ozcelik H, Ozer A et.al. The molecular basis of beta-thalassemia in Türkiye. Hum Genet 1992; 89:315-318.
8. Canatan D, Arcasoy A. Bone marrow transplantation in thalassaemia. Ankara U. TıpFakültesi Mecmuası 1993; 46:173-186.
9. Altay Ç. Abnormal haemoglobins in Türkiye. Turk J Haematol 2002; 19:63-74.
10. Kahraman S, Karlikaya G, Sertyel S, et.al. Clinical aspects of preimplantation genetic diagnosis for single gene disorders combined with HLA typing. Reprod Biomed Online 2004; 9:529-532.
11. Arcasoy A, Canatan D. Thalassaemia and haemoglobinopathies in the world and Türkiye.Editors: Arcasoy A, Canatan D, Köse R, Üstündağ. Haemoglobinopathy and Thalassemia Prevention-Diagnosis-Treatment. National Haemoglobinopathy Council-Ministry of Health, Antalya 2002;13-17.
12. Canatan D. The thalassemia centre of Antalya State Hospital: 15 years of experience(1994 to 2008). J Pediatr Hematol Oncol 2013; 35:24-27.
13. Canatan D, Köse MR, Üstundağ M, et.al. Haemoglobinopathy control programme in Türkiye. Community Genet 2006; 9:124-126.
14. T.C. Ministry of Health AÇSAP General Directorate.Hemoglobinopathy Control Programme. (Editor: Canatan D) Thalassemia Special Issue Türkiye Klinikleri J Hem Onc.Special Topics 2010;3(1):5-8.
15. Canatan D. Thalassemias and Haemoglobinopathies in Türkiye. Haemoglobin. 2014;38(5):305-7. doi: 10.3109/03630269.2014.938163.
16. Aksoy M, Dinçol G, Erdem S. Survey on Hb variants, beta-thalassemia, G6PD deficiency and hain haptoglobin types in Turkish people living in Manavgat, Serik, Boztepe (Antalya). Human Heredity 1980; 30:3.
17. Bircan I, Sişli S, Güven A, et al. Haemoglobinopathies in the district of Antalya,Türkiye. Pediatr Hematol Oncol 1993; 10:289-291.
18. Canatan D, Delibas S. Report on Ten Years' Experience of Premarital Haemoglobinopathy Screening at a Centre in Antalya, Southern Türkiye. Haemoglobin. 2016 Aug;40(4):273-6. doi: 10.3109/03630269.2016.1170030. Epub 2016 May 20.
19. Canatan D. Thalassemia Screening from Highland to Seaside: Malaria Hypothesis in South of Türkiye. J Haematol Transfus. 2017, 5(1): 1057.
20. Canatan D, Keser İ, Delibaş S,et.al. Thalassemia and haemoglobinopathy education and screening in Antalya Ibradı in memory of Prof. Dr. Muzaffer Aksoy. Canatan D. Kılınç Y (Eds): 6th International Thalassaemia Summer School.18-23 April 2013;17-21.
21. Tunç B, Çetin H, Gümrük F, et al. The prevalence and molecular basis of beta thalassemia in Isparta province and region. Turk J Pediatr 2002; 44:18-20.
22. Çatak B, Sütlü S Selçuk Kılınç S et.al. Prevalence of β thalassaemia carriage in the 8th grade of primary school in Burdur. Cumhuriyet Tıp Derg. 2013; 35: 193-198.doi.org/10.7197/1305-0028.1827
23. Altay Ç, Gürgey A. A distribution of haemoglobinopathies in Türkiye. Turkish J Pediatr 1986; 28:219.
24. Tosun F, Bilgin A, Kizilok A et.al. Five-year evaluation of premarital screening programme for haemoglobinopathies in the province of Mersin. Turk J Hematol 2006; 23:84-89.
25. Aksoy M, Erdem Ş. Abnormal haemoglobin and thalassemia in Eti Turks living in Antakya. Istanbul Med Bull 1968; 1:296.

26. Özsoylu Ş and Şahinoğlu M. Haemoglobinopathy survey in Eti Turk village. Human Heredity 1975; 25:50-59.
27. Altay Ç, Yetkin S, Özsoylu Ş, Kutsal A. Haemoglobin S and some other haemoglobinopathies in Eti Turks. Human Heredity 1978; 28:56-61.
28. Dinçol G, Aksoy M, Kazancıoğlu R, et.al Abnormal haemoglobins and frequency of A2 high Beta thalassaemia in two Eti Turkish villages of Antakya. Doga 1984; 8:14-17.
29. Yüreğir G, Pauline A, Refik B, et.al. A survey of high A2, beta thalassemia, haemoglobin variants, deficiency and iron deficiency anemia in Karakaş, Çukurova, Southern Türkiye. Doga 1989; 13:3.
30. Ariyurek SY, Yildiz SM,Yalin AE, Hemoglobinopathies in the Çukurova Region and Neighboring Provinces. Hemoglobin.2016 Jun;40(3):168-72. doi: 10.3109/03630269.2016.1155156. Epub 2016 Mar 17.
31. Guvenc B, Canataroglu A, Unsal C et.al. β-thalassemia mutations and haemoglobinopathies in Adana, Türkiye:results from a single centre study. Arch Med Sci 2012; 8:411-414.
32. Ulutas KT, Sahpaz F, Sarici İŞ et.al. Premarital haemoglobinopathy screening: Is Kadirli, Türkiye a risk area for beta-thalassaemia? Turk J Biochem. 2014; 39(3):357-361doi: 10.5505/tjb.2014.90217.
33. Yüreğir GT, Kılınç M, Ekerbiçer H, et.al Screening of haemoglobinopathies in Kahramanmaraş, Türkiye. J Haematol 2001; 18:79-83.
34. Güler E, Garipardic M, Dalkıran T et.al. Premarital screening test resultsfor β-thalassemia and sickle cell anemia trait in east Mediterranean region of Türkiye. Pediatr Hematol Oncol 2010; 27:608-613.
35. Canatan D, Arcasoy A, Bor S et.al. Screening of B-thalassemia carriers characterised by abnormal Hb and HbA2 levels in Elbistan region. Doga Bilim Der 1990; 14:555-561.
36. Aydinok Y, Oztop S, Nisli G, et.al. Prevalence of beta-thalassaemia trait in
1124 students from Aegean region of Türkiye. J Trop Pediatr 1997; 43:184-185.
37. Irken G, Ören H, Ündar B, et al. Analysis of thalassemia syndromes and abnormal
haemoglobins in patients from the Aegean region of Türkiye. Turk J Pediatr2002; 44:21-24.
38. Uysal A, Genc A, Tasyurek N et.al. Prevalence of β-thalassemia trait and abnormal haemoglobin in premarital screening in the province of Izmir, Türkiye. Pediatr Hematol Oncol. 2013 Feb;30(1):46-50. doi: 10.3109/08880018.2012.742604. Epub 2012 Nov 15.
39. Yenice, S. Frequencies of some blood disorders in Nazilli, Abstract, I, National
Conference of Hematology, Antalya, Türkiye, February 25-26, 1999.
40. Sözmen M, Uysal Z, Yesil N et.al. Abnormal haemoglobins in Denizli and Hb A2 elevation characterised beta-thalassemia carriage investigation. Ankara Medicine Faculty Mecmuası 1990; 43:959-964.
41. Keskin A, Türk T, Polat A et.al.Premarital screening of beta-thalassemia trait in the province of Denizli, Türkiye Acta Haematol.2000;104(1):31-3. doi: 10.1159/000041066.
42. Bolaman B, Enli Y, Köseoğlu M et.al. Prevalence of Beta Thalassemia Trait in Denizli. Turk J Haematol.2001 Jun 5;18(2):85-8.
43. Arcasoy A, Turan F, Yesil N, et.al. Screening of the frequency of thalassemia and abnormal haemoglobin in Muğla province and its surroundings. Pediatride Yönelişler 1994; 1:78-81.
44. Topal Y, Topal H, Ceyhan MN et.al.The Prevalence of Haemoglobinopathies in Young Adolescents in the Province of Muğla in Türkiye: Results of a Screening Programme. Haemoglobin. 2015;39(4):247-50. doi: 10.3109/03630269.2015.
45. Akar N, Uysal Z, Yesil N, et.al. Investigation of beta-thalassaemia carriage characterised by abnormal haemoglobins and elevated Hb A2 in Mustafakemal Pasha and its villages. Doga 1990; 14:551-554.
46. Uludağ A, Uysal A, Uludağ A et.al.Prevalence and mutations of β-thalassemia trait and abnormal haemoglobins in premarital screening in Çanakkale province, Türkiye Balkan J Med Genet.. 2016 Aug 2;19(1):29-34. doi: 10.1515/bjmg-2016-0004. eCollection 2016 Jul 1.
47. Sarper N, Şenkal V, Güray F, et.al.Premarital haemoglobinopathy screening in Kocaeli, Türkiye: a crowded industrial centre on the north coast of Marmara Sea Turk J Haematol. 2009 Jun 5;26(2):62-6.
48. Aksoy M, Kutlar A, Kutlar F.Survey on haemoglobin variants beta-thalassemia, G6PD deficiency and haptoglobulin types in Turks from Western Thrace. J Med Genet 1985; 22:288-290.
49. Turan C, Topal B, Gürgey A, Altay Ç. Frequency of beta thalassaemia in Konya and Denizli region. Journal of Paediatrics 1991; 34:9-11.
50. Guler E, Caliskan U, Ucar Albayrak C et al.Prevalence of beta-thalassemia and sickle cell anemia trait in premarital screening in Konya urban area, TürkiyeJ Pediatr Hematol Oncol. 2007 Nov;29(11):783-5. doi: 10.1097/MPH.0b013e318159a570.
51. Karakukcu C, Kocer D, Torun YA et.al.Premarital haemoglobinopathy screening in Kayseri: a city in Middle Anatolia region of Türkiye. J Pediatr Hematol Oncol.2012 Mar;34(2):e49-52. doi: 10.1097/MPH.0b013e3182370bdf.
52. Seydel GS, Ayan D, Balci T. et.al. Prevalence of Haemoglobinopathies in Premarital Screening in the Province of Nigde, Türkiye Hemoglobin.2023

Nov;47(2):36-41. doi: 10.1080/03630269.2023.2206971. Epub 2023 May 15.
53. Gürbak M, Sivaslı E, Çoskun Y, et.al. Prevalence and haematologicalcharacteristics of beta-thalassemia trait in Gaziantep urban area, Türkiye. Pediatr.Hematol Oncol 2006;23:419-425.
54. Incebiyik A, Genc A, Gul Hilali N et.al. Prevalence of β-thalassemia trait and abnormal haemoglobins in Sanliurfa Province in southeast Türkiye. Hemoglobin.2014;38(6):402-4. doi: 10.3109/03630269.2014.978008.
55. Genc A, Korkmaz TD, Kucuk MU et.al. Prevalence of beta-thalassemia trait and abnormal haemoglobins in the province of Adıyaman, Türkiye . Pediatr Hematol Oncol. 2012 Oct;29(7):620-3. doi: 10.3109/08880018.2012.713085. Epub 2012 Aug 16.
56. Kürkçüoğlu M, Dağcı A, Y, Arcasoy A, et.al. Beta thalassaemia screening in Eastern Anatolia. Doga Bilim Der 1986; 8:319-325.
57. Acemoglu H, Beyhun NE, Vancelik S et.al. Thalassaemia screening in a non-prevalent region of a prevalent country (Türkiye): is it necessary? PublicHealth 2008; 122:620-624.
58. Aksoy H, Bayraktar Y, Yüzbaşıoğlu T, et al. Frequencies of abnormal haemoglobins high A2 beta thalassemia and distribution of haplotypes in Turks living in Van and its vicinities, Eastern Türkiye. Doğa 1986; 10:1-4.
59. Fei YJ, Kutlar F, Harris HF et al. A search for anomalies in the zeta, alpha, beta, and gamma globin gene arrangements in normal black, Italian, Turkish, and Spanish newborns.Hemoglobin 1989;13:45-65.
60. Özsoylu Ş, Malik SA. Incidence of alpha thalassemia in Türkiye. Turk J Pediatr 1982; 24:235-244.
61. Canatan D, Oguz N, Guvendik I et.al. The incidence of alpha-thalassemia in Antalya. Türkiye. Turk J Haematol 2002; 19:433-434.
62. Kılınç Y, Kümi M, Gürgey A, Altay Ç. Investigation of the frequency of alpha thalassaemia, G6PD enzyme deficiency and HbS in babies born in Adana region by cord blood study. Doğa Tıp ve Ecz Der 1986; 10:162.
63. Tadmouri GO, Garguier N, Demont J et.al. History and origin of beta-thalassemia in Türkiye: sequence haplotype diversity of beta-globin genes. Hum Biol. 2001 Oct;73(5):661-74. doi: 10.1353/hub.2001.0075.
64. Tadmouri GO, Tüzmen S, Ozçelik H. Molecular and population genetic analyses of beta-thalassemia in Türkiye. Am J Haematol. 1998 Mar;57(3):215-20. doi: 10.1002/(sici)1096-8652(199803)57:3<215::aid-ajh6>3.0.co;2-
65. Başak AN.The molecular pathology of beta-thalassemia in Türkiye: the Boğaziçi university experience. Haemoglobin. 2007;31(2):233-41. doi: 10.1080/03 6302 6070 12 96 735.
66. Tastan AO, Canatan D, Basak AN. A search for the α-thal-2 determinants -α3.7 and -α4.2 in the newborns from Antalya district of Türkiye: Cord blood study using the PCR method. B Balkan Journal of Medical Genetics 1999; 2:23-5.
67. Keser I, A D Sanlioglu AD, E Manguoglu E et al. Molecular analysis of beta-thalassemia and sickle cell anemia in Antalya. Acta Haematol. 2004;111(4):205-10. doi: 10.1159/000077567.
68. Bilgen T, Arikan Y, Canatan D et.al. The association between intragenic SNP haplotypes and mutations of the beta globin gene in a Turkish population. Blood Cells Mol Dis. 2011 Mar 15;46(3):226-9. doi: 10.1016/j.bcmd.2011.01.004. Epub 2011 Feb 18.
69. Kurtoğlu A, Karakuş V, Erkal Ö, et.al.β-Thalassemia gene mutations in Antalya, Türkiye: results from a single centre study. Haemoglobin.2016 Nov;40(6):392-395. doi: 10.1080/03630269.2016.1256818. Epub 2017 Mar 3.
70. Arpaci A, Gul BU, Ozcan O et.al. Presentation of two new mutations in the 3'untranslated region of the β-globin gene and evaluating the molecular spectrum of thalassemia mutations in the Mediterranean region of Türkiye. Ann Haematol. 2021 Jun;100(6):1429-1438. doi: 10.1007/s00277-021-04509-9. Epub 2021 Apr 13.
71. Guzelgul F, Seydel S,Aksoy K. β-Globin Gene Mutations in Pediatric Patients with β-Thalassemia in the Region of Çukurova, Türkiye Hemoglobin.2020 Jul;44(4):249-253. doi: 10.1080/03630269.2020.1792489. Epub 2020 Jul 14.
72. Cürük MA, Dimovski AJ, Baysal E et.al. Hb Adana or alpha 2(59)(E8)Gly-->Asp beta 2, a severely unstable alpha 1-globin variant, observed in combination with the -(alpha)20.5 Kb alpha-thal-1 deletion in two Turkish patients Am J Hematol1993 Dec;44(4):270-5. doi: 10.1002/ajh.2830440410.
73. Aldemir O, Izmirli M, Kaya H.The spectrum of β-thalassemia mutations in Hatay, Türkiye: reporting three new mutations. Haemoglobin. 2014;38(5):325-8. doi: 10.3109/03630269.2014.944915. Epub 2014 Aug 26.
74. Yilmaz S. The Spectrum of β-Thalassemia Mutations in Siirt Province, Southeastern TürkiyeHemoglobin.2019 May;43(3):174-181. doi: 10.1080/03630269.2019.1647852. Epub 2019 Aug 14.
75. Yilmaz S. The Spectrum of β-Thalassemia Mutations in Siirt Province, Southeastern TürkiyeHemoglobin.2019 May;43(3):174-181. doi: 10.1080/03630269.2019.1647852. Epub 2019 Aug 14.
76. Aydinok Y, Oymak Y, Berna Atabay B et.al. A National Registry of Thalassemia in Türkiye: Demographic and Disease Characteristics of Patients, Achievements, and Challenges in Prevention. Turk J Haematol.2018 Mar 1;35(1):12-18. doi: 10.4274/tjh.2017.0039. Epub 2017 Apr 13.

77. Ünal Ş, Gümrük F. The Haematological and Molecular Spectrum of α-Thalassemias in Türkiye: The Hacettepe Experience. Turk J Haematol. 2015 Jun;32(2):136-43. doi: 10.4274/tjh.2014.0200.
78. Karakaş Z, Koç B, Temurhan S, et.al.Evaluation of Alpha-Thalassemia Mutations in Cases with Hypochromic Microcytic Anemia: The Istanbul Perspective Turk J Haematol. 2015 Dec;32(4):344-50. doi: 10.4274/tjh.2014.0204. Epub 2015 Aug 6.
79. Arcasoy A. Haemoglobinopathies in Türkiye. Haematology Reviews 1992; 6:61-67.
80. Akar E&N. A review of abnormal haemoglobins in Türkiye Turk J Hematol 2007; 24:143-145
81. Akar N: An Update Review of Abnormal Haemoglobins in Turkish Population Turk J Hematol 2014; 31:97-98.
82. Akar N, Abnormal haemoglobins defined in Turkish Population. Türkiye Clinics, J Med Genet 2017: (2(1), 27-30.
83. Arcasoy A, Canatan D, Köse MR, Üstündağ M: Haemoglobinopathy and Thalassemia Prevention-Diagnosis-Treatment. National Haemoglobinopathy Council-Ministry of Health, 2nd Edition, Antalya, 2003.
84. Canatan D, Corrons JVL, Giorgio G, et.al. What health care professionals need to know about haemoglobinopathies in migrants and refugees? (5th Part) ENDOTHAL Rivista Italiana di Medicina dell 'Adolescenza - 2022; 20, n. 2:1-9.
85. Erdem AY, Yenigürbüz FD, Pekpak E.et.al. Refugee children with beta-thalassemia in Türkiye: Overview of demographic, socioeconomic, and medical characteristicsPediatr Blood Cancer. 2019 May;66(5):e27636. doi: 10.1002/pbc.27636. Epub 2019 Feb 1.

Correspondence Address:
Prof. Dr Duran Canatan
Paediatric Haematology and Genetics Specialist
Antalya Bilim University - Antalya - Türkiye
Mail. duran.canatan@Antalya.edu.tr/
durancanatan@gmail.com
Tel: +90.532.3641373

Table 1: *Regional thalassemia and hemoglobinopathy studies in Türkiye*

REGION AND CITY	BETA THALASSEMIA %	HEMOGLOBINOPATHY %	AUTHOR
MEDITERRANEAN Average	**5,2**	**Hb S: 7.2**	
ANTALYA	6,7-13 Sahil 12,3 Mountainous Region 3.4 Alpha: 2nd,-6,3	Hb S 0.8-2.5	Aksoy, Bircan, Canatan
ISPARTA	2,5	Hb S 0.8	Tunç
BURDUR	5,2	HbS 0.9	Çatak
MERSIN	1,7-2,04	HbS 1.21	Altai-Georgia Tosun
ADANA	1,7-4,-13,4 Alpha: 7.5	Hb S 6.8	Guvenc, Ulutas
CUKUROVA	3.0-5.1	**HbS 10.1**	Yuregir, Ariyürek
ANTAKYA	0,8-1,4	HbS 15.3-37.6	Aksoy, Özsoyly Altay, Dinçol
KAHRAMANMARAS	0,68-0,9-2,8	HbD: 0.28 HbS:0.4	Yüreğir Guler, Canatan
Aegean Average	**4,1**	**Hb S:0.2**	
IZMIR	3,0-4,9	HbD: 0.37 HbS: 0.32 HbE0.18	Aydinok , Irken, Guler
AYDIN	4,6		Yenice
DENIZLI	2,2-2,6-3,6		Bolaman Sözmen Keskin
MUĞLA	3,2-8,7	HbS:0.15-0.41	Lame Arcasoy
MARMARA Average	**3,9**	**Hb O Arab:3.9** **Hb S: 1.4**	
BURSA	2,6		Akar
ÇANAKKALE	1,4		Uludag
KOCELI	0,89	HbS:0,05	Sarper
TRAKYA	10,8	HbS : 2.9 Hb O Arab:3.9	Aksoy
MIDDLE ANATOLIA Average	**2,3**	**Hb D 0.36**	
KONYA	2,0-3,0		Guler, Topal
KAYSERI	1,71	Hb D 0.36 Hb O Arab 0.09	Karakükçü
NIGDE	2.63		Sevdel
SOUTHEAST ANATOLIA Average	**1,7**	**Hb D 0.69**	
GAZIANTEP	1,84		Gürbak
SANLIURFA	2,4	Hb S: 0.5 Hb C: 0.38 Hb D-Punjab 0.69	Incebıyık
ADIYAMAN	1,06	Hbs:0.02	Young
EAST ANATOLIA Average	**1,4**		
EAST ANATOLIA	0,9		Arcasoy Kürkçüoğlu
ERZURUM	0,68		Acemoglu
VAN	2,6		Aksoy

Table 2: *Abnormal hemoglobins identified in the Turkish population (82)*

a. Alpha chain abnormalities

Abnormal Hb	Change	Author
1. J-Toronto	5 (A3) Ala > Asp (17C>A)	(Canatan)
2. Handsworth	18 (A16) Gly>Arg (55G>C)	(Canatan)
3. Fontainebleau	21 (B2) Ala>Pro (64G>C)	(Canatan)
4. Hb Memphis	23 (B4) Glu>Gln (70G > C)	(Lubrano)
5. O-Padova	30 (B11) Glu->Lys (91G->A)	(Sword)
6. Hb Erzeroum	42 (C7) Tyr>Ser (128 A>G) (*)	(Hbvar)
7. Hasharon	47 (CE5) Asp>His (142G>C)	(Irken)
8. Montgomery	48 (CE6) Leu>Arg (146T>G)	(Irken)
9. Adana	59 (E8) Gly>Asp (179G>A)	(Rotten) (*)
10. J-Anatolia	61 (E10) Lys>Thr (185A>C)	(Giardano) (*)
11. G-Waimanalo	64 (E13) Asp>Asn (193G>A)	(Canatan)
12. Q-India	64 Asp-His (193G>C)	(Canatan)
13. Ube-2	68 (E17) Asn>Asp (205A>G)	(Bilginer)
14. Q-Thailand	74(EF3) Asp>His (223G>C)	(Canatan)
15. Q-Iran	75 (EF4) Asp>His (226G>C)	(Aksoy)
16. Toulon	77(EF6) Pro>His (233C>A)	(Canatan)
17. Moabit	86 (F7) Leu>Arg (260T>G)	(Knuth) (*)
18. M-Iwate	87 (F8) His>Tyr (263A>G)	(Ozsoylu)
19. Hb Lansing (A)	87 (His>Glu) (264C>A)	(Akar) (*)
20. Anchor	94 (G1) Asp>Gly (284A>G)	(Dinçol) (*)
21. Hb Setif	94 (G1) Asp>Tyr 283 G>T	(Dinçol)
22. G-Georgia	95 (G2) Pro>Leu (287C>T)	(Van Houte)
23. Dallas	97(G4)Asn>Lys (294C>A)	(Canatan)
24. Hb Bronovo	103 His> Leu (311A>T)	(Harteveld) (*)
25. Strumica	112 (G19) His->Arg (338A>G)	(Mite)
26. Grady	119 Thr>Pro120insGluPheThr	(Canatan)
27. J-Meerut	120 (H3) Ala>Glu (362C>A)	(Yalcin)
28. Westmead	122(H5) His>Gln (369C>G)	(Canatan)
29. Hb Westeinde	125(H8) Leu>Gln (377T>A)	(Kaufmann)
30. Tarrant	126(H9) Asp>Asn (379G>A)	(Canatan)
31. Hb Wayne	elongation Asn or Asp (420delA)	(Canatan)
32. Constant Spring	142 (H19) (+ 31)	(Irken)
33. Hb GNorfolkA2	:c256G>A	(Unal)

b. Beta chain variants

1. South Florida	1(NA1) Val>Met (4G>A)	(Kaufmann)
2. Raleigh	1(NA1) Val>Ala (5T>C)	(Canatan)
3. Jabalpur	3 (NA3) Leu>Pro (11T>C)	(Colak)
4. Tyne	5 Pro>Ser (16C>T)	(with strap)
5. S	6 (A3) Glu>Val (20A>T)	(Aksoy)
6. C	6 (A3) Glu>Lys (19G>A)	(Celestial)
7. Ankara	10 (A7) Ala>Asp (32C>A)	(Arcasoy) (*)
8. J-Baltimore	16(A13) Gly>Asp (50G>A)	(Canatan)
9. D-Quled Rabah	19 Asn>Lys (60C>A)	(Köseler)
10. E-Saskatoon	22 (B4) Glu>Lys (67G>A)	(Gurgey)
11. G-Coushatta	22 (B4) Glu>Ala (68A>C)	(Dinçol)
12. D-Iran	22 (B4) Glu>Gln (67G>C)	(Irken)
13. E	26 (B8) Glu->Lys (79G>A)	(Aksoy)

14.	Knossos	27 (B9) Ala>Ser (82G>T)	(Kutlar)
15.	Volga	27 (B9) Ala-Asp (83C>A)	(Sözen)
16.	Siirt	27 (B9) Ala-Gly (83C>G)	(Bianco) (*)
17.	Hakkari	31 (B13) Leu>Arg (95T>G)	(Gurgey) (*)
18.	G-Galveston	43 (G19)His>Arg.(131A>C)	(Canatan)
19.	G-Copenhagen	47 (CD6) Asp->Asn (142G>A)	(Irken)
20.	Willamette	51(D2) Pro >Arg (155C>G)	(Canatan)
21.	Summer Hill	52 (D3) Asp>His (157G>C)	(Gin)
22.	Akron	52 (D3) Asp>Val (158A>T	(Canatan)
23.	Osu Christiansborg	52(D3) Asp>Asn (157G>A)	(Canatan)
24.	Hamadan	56 (D7) Gly>Arg (169G>C)	(Dinçol)
25.	J-Daloa	57 (E1) Asn>Asp (172A>G)	(Canatan)
26.	High Wycombe	59(E3) Lys>Glu (178A>G)	(Canatan)
27.	M Saskatoon	63 (E7) His>Tyr (190C>T)	(Mite)
28.	J-Antakya	65 (E9) Lys>Met (197A>T)	(Huisman) (*)
29.	City of Hope	69 (E13) Gly>Ser (208G>A)	(Kutlar)
30.	J-Chicago	76(E20)Ala>Asp (.230C>A)	(Canatan)
31.	J-Iran	77 (EF1) His>Asp (232C>G)	(Arcasoy)
32.	Hb Yaizu	79(EF3) Asp>Asn (238G>A)	(Atalay)
33.	G-Szuhu	80 (EF4) Asn>Lys (243C>A or 243C>G)	(Kaufman)
34.	Hb Pyrgos	83 (EF7) Gly>Asp (251G>A)	(Mite)
35.	Hb Izmir	86(F2)Ala>Val, (260C>T)	(Çelebiler) (*)
36.	D-Ibadan	87(F3)Th->Lys (263C>A)	(Canatan)
37.	Istanbul/Saint Etienne	92 (F8) His->Gln ([279C>A or 279C>G)	(Aksoy)
38.	N-Baltimore	95% (FG2) Lys>Glu (286A>G)	(Bircan)
39.	Cologne	98 (FG5) Val->Met (295G>A)	(Gurgey)
40.	Richmond	102 Asn>Lys c(309C>A or 309C>G)	(Canatan)
41.	Rhode Island	116 (G18) His>Tyr (349C>T)	(Canatan)
42.	Minneapolis	118 (GH1) Phe>Tyr 356 (356T>A)	(Aykut)
43.	D-Punjab	121 (GH4) Glu->Gln (364G>C)	(Ozsoylu)
44.	O-Arab	121 (GH4) Glu->Lys (364G>A)	(Aksoy)
45.	Beograd	121 (GH4) Glu->Val (365A>T)	(Aksoy)
46.	Hb Ernz	123(H1) Thr>Asn (371C>A)	(Sunachar)
47.	Tunis	124 (H2) Pro>Ser (373C>T)	(Köseler)
48.	Hb Crete	129(H7) Ala>Pro 388G>C	(Duwig)
49.	Sarrebourg	131 (H9) Gln->Arg (395A>G)	(Duwig)
50.	Hope	136(H14)Gly > Asp (410G>A)	(Canatan)
51.	Brockton	138 (H16) Ala->Pro (415G>C)	(Ulukutlu)
52.	Hinsdale	139 (H17) Asn-Lys (420T>C or 420T>A)	(Aykut)
53.	Hb Andrew Minosta	144(HC1)Lys>Asn)	(Aykut)

Gamma chain variants

1.	F-Capital	128 (H6) Ala->Thr (385G>A)	(Altai) (*)
2.	F-Istambul	146 (HC3) His > Arg (440A>G)	(Hbvar) (*)

Delta chain variants

1.	Yokoshima	25(B7)Gly >Asp) (77G>A)	(Köseler)
2.	Yialousa	28 Ala>Ser)(82 C-T)	(Köseler)
3.	Edirne	53 Asp>His (160G>C)	(Tabakcioglu) (*)
4.	Turkish	79 (EF3) Asp>Gly (239 A>G)	(Hbvar) (*)
5.	Hb Noah Mehmet Oeztuerk	143 (H21) His>Tyr .(430C>T)	(Bisse) (*)

Hybrid Haemoglobins

1.	Lepore-Boston-Washington		(Rye)
2.	P-Nilotic	75	(Altai)

Haemoglobin variant s defined by insertion

1. Antalya Unstable B 2-5 His-Leu-Thr-Pro> His-Ser-Asp-Ser (Keser) (*)

(*) Abnormal hemoglobin variants identified for the first time in Turkish population

OVERVIEV OF THALASSEMIAS AND SICKLE CELL DISEASE IN ITALY

Vincenzo De Sanctis[1], Maria Concetta Galati[2], Giuseppe Raiola[3], Bernadette Fiscina[4]
[1]Coordinator of ICET-A Network (International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine) and Pediatric and Adolescent Outpatient Clinic, Quisisana Hospital, Ferrara, Italy, [2]Department of Oncohematology, Pugliese-Ciaccio Hospital, Catanzaro, Italy, [3]Department of Pediatrics, Pugliese-Ciaccio Hospital, Catanzaro, Italy, [4] Department of Pediatrics, NYU School of Medicine, New York, NY, USA

ABSTRACT

More than five decades ago, β-thalassemia major (β-TM) was fatal in the first decade of life. This poor prognosis changed and the survival rates started to increase progressively, thanks to the significant improvement in diagnostic and therapeutic methods, consisting mainly of an intensive transfusion program combined with iron chelation therapy and improved imaging methods. However, iron overload and disease-associated complications remain a challenge in this population. The implementation of prevention and screening programs has lowered the prevalence and incidence of β-TM in regions with a historically high number of carriers and patients. In addition to reducing incidence, research improving the overall standard of care in Italy has allowed patients with thalassemia to live longer into adulthood. However, despite important improvements in the management of β-TM, there are still many challenges to overcome before global disease control is achievable. Significant progress has been made in increasing the knowledge regarding subjects with sickle cell disease (SCD). Nevertheless, its treatment is still unsatisfactory for both acute and chronic clinical complications. Multidisciplinary centers specialized in caring for patients with hemoglobinopathies are needed, along with improved understanding of the optimal structure and function of such facilities.

Keywords: Hemoglobinopathies, healthcare organization, National Registry, prevalence, prevention programmes, immigrants, medical costs

BACKGROUND

Hemoglobinopathies are the most common genetic disorder worldwide, and include the thalassemia syndromes, which are disorders of globin chain expression, and sickle cell disease (SCD) caused by structural changes in the globin chains of hemoglobin (Hb) **(1).**

Thalassemia syndromes are characterized by the absence or reduced synthesis of β-globin chains, are heterogeneous at the molecular level with almost 300-point mutations and deletions classified as severe, mild and silent, that can produce clinical and hematological phenotypes of variable severity ranging from the asymptomatic carrier to the severe transfusion-dependent type **(2).**

Clinically, β-thalassemias can be classified as transfusion-dependent thalassemia (TDT or β-thal major - β-TM) and non-transfusion-dependent thalassemia (NTDT) according to the severity of the phenotype, which is caused by a wide spectrum of mutations in a homozygous or compound heterozygous state **(4, 5).** Current conventional treatment of TDT consists of regular transfusions that lead to iron overload, requiring iron chelation to prevent iron-related organ toxicity **(3-5).**

SCD is a disorder of hemoglobin synthesis characterized by the production of an altered form of hemoglobin, hemoglobin S (HbS). The most severe form is homozygous HbSS (sickle cell anemia, SCA), but there are other compound heterozygous conditions such as HbS and β-thalassemia, or HbS and other Hb variants. Chronic anemia, hemolysis, and recurrent acute vaso-occlusive crises, characterized by pain and systemic inflammatory response, are the main clinical features **(4).**

Hematopoietic stem cell transplantation (HSCT) remains the only established definitive cure for

severe hemoglobinopathies, such as β-TM and sickle cell disease (SCD) **(6).** Gene therapy is a promising alternative treatment modality, but long-term outcome data are currently lacking **(7).**

The main objective of this article is to examine the current status of thalassemia, focusing on data from Italy where the history of thalassemia management and its associated complications is well-documented, based on the published literature and our own clinical experience, and to provide a useful opportunity for reviewing the emerging public health burden posed by the increasing number of subjects with SCD.

A BIT OF HISTORY NOT TO GORGET

In 1925 Cooley and Lee, in the United States, described some children affected by anemia, which later took the name of Cooley's anemia **(8).** In the same year in Ferrara, Rietti described some patients with milder haemolytic anemia with increased globular resistance **(9).** Cooley's paper was the first description of thalassemia major and Rietti's of the intermediate form. In the early forties, Silvestroni and Bianco hypothesized that Cooley's anemia was the result of the presence in the homozygous state of an abnormal gene which in the heterozygous state was responsible for "thalassaemia", and demonstrated that thalassaemia was particularly frequent in southern Italy, in the area of the Po Delta and in the major islands **(10, 11).**

Some years later Haldane **(12)** hypothesized that this particular distribution of thalassaemia was related to malaria endemic areas and therefore could be a factor of resistance against that disease.

In 1949, Silvestroni and Bianco founded in Rome the "Center of Microcythemia" aimed at the study of hemoglobinopathies and their prevention. In the following years, similar centers were founded in Ferrara and many other cities. Rational transfusion therapy programs were only proposed at the end of the 1960s by US authors, based on the maintenance of almost normal hemoglobin levels **(13).** This program proved to be able to ensure good tissue oxygenation, promote growth, prevent or reduce bone tissue alterations, enlargement and hyperactivity of the spleen. The program was adopted all over the world only after a few years, perhaps due to inertia and scepticism, but in Ferrara it was introduced by Prof. Marino Ortolani at the end of the sixties (13), albeit with some initial uncertainty. This program, however, had the drawback of causing a significant iron overload in various organs and tissues, particularly in the heart, liver and endocrine glands. In fact, the leading cause of death in these thalassemia patients was heart disease. In 1962, the use of the first iron chelator, desferrioxamine, in thalassemia was proposed. This chelator was found to be able to keep iron stores under control, if injected regularly subcutaneously over a long period of hours, usually at night **(14, 15).** The first successful bone marrow transplantation was done in the 1980s by Prof. Guido Lucarelli. In low-risk young patients, the thalassemia free survival was 87% and mortality was 3% **(16).**

SCREENING FOR THE IDENTIFICATION OF CARRIERS OF B THALASSEMIA AND OTHER HEMOGLOBINOPATHIES

β-TM is considered endemic in Italy with an estimated prevalence of carriers of ~6% and an expected incidence of 220 births/year, in the absence of any preventive program. The prevalence of β-TM carriers in Italy differs markedly by region, with some regions historically having a much higher prevalence than others, such as Sardinia, where the estimated carrier rate is around 13%. The island has policies in place to reduce the incidence of hemoglobinopathies, including thalassemia, since the 1970s, with free carrier screening and genetic diagnosis **(17).**

In Ferrara (Italy), a voluntary screening programme for school children and young adults initiated in 1976-1977 has brought down the birth rate of those affected with thalassemia to almost zero. The preventative actions also include legislative action, a public awareness campaign, screening and carrier diagnostics, genetic counselling, and prenatal diagnosis **(18).** These preventative measures have led to a reduction in the number of children born with thalassemia, particularly in Sicily, which has seen an 85% decrease in the incidence of β-thalassemia over the last 30 years **(9).**

Screening methods include full blood count and biochemical analysis including quantitative analysis of hemoglobin by HPLC or capillary electrophoresis. Molecular genetic confirmation by detecting

pathogenic variants establishes the diagnosis. For neonatal and antenatal screening, identification of affected newborns or carriers is achieved by hematological tests. DNA analysis supports definitive diagnosis, and additionally facilitates prenatal diagnosis procedures **(20).**

Many of the molecular defects characterized and spread throughout the world can be found with different frequencies in the various Italian regions. These defects are reported in Table 1, where the marked homogeneity of β-TM in Sardinia and the significant heterogeneity in Sicily can be observed **(21).**

Table 1: *Percentage frequency and heterogeneity of the main thalassaemic defects in some geographical areas in Italy (adapted from ref. 21)*

Defect		Sardinia	Sicily	Lazio	Campania	Calabria	Puglia	Delta PO River	North	West
Cod 39	c.118C>T	95,7	33,9	47,3	41,5	29,6	34,8	60,6	42,7	
IVSI-110	c.93-21G>A	<1	23,5	16,3	22,8	22,2	27,7	25,9	22,6	
IVSI-6	c.92+6T>C	<1	12,6	6,8	9,0	14,9	10,8	6,2	8,7	
IVSII-745	c.316-106C>G	<1	5,2	10,2	4,1	6,5	4,8	2,3	7,8	
IVSI-1	c.92+1G>A	<1	9,8	4,8	7,0	7,5	14,2	<1	4,0	
-87	c.-137C>G	<1	1,9	1,8	1,0	6,5	<1	1,0	3,6	
IVSII-1	c.315+1G>A	<1	1,7	3,2	3,0	2,6	2,8	1,0	2,0	
Cod 6	c.20delA	2,2	2,0	2,3	<1	2,4	<1	<1	3,5	
-101	c.-151C>T	<1	<1	1,2	<1	<1	1,6	<1	<1	
IVSI-2	c.92+2T>A	<1	1,0	<1	<1	<1	<1	<1	<1	
-92	c.-142C>T	<1	<1	<1	<1	<1	<1	<1	<1	
IVSII-844	c.316-7C>G	<1	<1	<1	<1	<1	<1	<1	<1	
Hb Lepore Boston		<1	2,6	2,0	7,0	<1	<1	<1	<1	
δβ-Talassemia siciliana		<1	1,3	1,2	1,0	5,3	<1	<1	<1	

For SCD, the most popular methods for detection are the blood cell count, Hb electrophoresis, and high-performance liquid chromatography (HPLC). Genetic tests are recommended to validate borderline cases and detect unusual and novel variants. Recently, different portable and rapid devices have been described to diagnose SCD, including platforms based on immune assay, density-based separation, and sensor-based technologies **(22).**

ITALIAN LEGISLATION AND HEALTHCARE ORGANIZATION FOR ASSISTANCE OF HEMOGLOBINOPATHIES

The Minister of Health, with his own decree (Law n. 205/2017 Art. 1 paragraphs 437 and 438.437), has established the National Network of Thalassemia and Hemoglobinopathies, which includes the already existing treatment centers and regional networks, that adopts specific guidelines for the correct application of therapeutic protocols and assistance pathways **(23).**

The purpose was to improve access to diagnosis and to provide quality health care for all patients; to accompany the patient in diagnosis and therapy through a multidisciplinary treatment modality; to improve training and promote research for these pathologies; to offer information and prevention activities and to share data and initiate evaluation mechanisms **(23).**

The essential goals are to identify uniform and coherent diagnostic-therapeutic and assistance pathways according to criteria of appropriateness, effectiveness and efficiency; to simplify the phases of access to services through the implementation of direct access methodology and the development of diagnosis and therapy pathways; to ensure high standards of treatment; to overcome the territorial differences in health services provided; to bring the

services closer to the context of the person's life; to disseminate research results and facilitate access to innovative therapies; to promote the prevention of clinical risk and the dissemination of good practices and to guarantee the quality and safety of care **(23).**

The Regional Reference Centers are identified by the Regions on the basis of the level of healthcare and scientific excellence, competence and experience. These Centers have the task of providing secondary and tertiary level services, of coordinating the activities of the regional network, developing relationships between the Network principals to diffuse and consolidate diagnostic and therapeutic protocols. It is the exclusive duty of the Center to inform the patient, at the end of the process, of the results of the diagnostic evaluation for the purpose of sector consultancy, assuming responsibility and epidemiological management. Inter-Regional Centers can be established (on a proposal from the National Coordination Committee) and on the initiative of two and/or more Regions through specific agreements to assign supra-regional tasks to one or more Regional Reference Centers with a view to saving resources, providing better quality of services and prevention activities **(23).**

As per the Ministerial Decree establishing the National Network, €100,000 per year is allocated to create informative tools, implement awareness-raising activities and gather data to monitor the effectiveness of the services offered through the National Network **(24).**

NATIONAL REGISTRY FOR THALASSEMIA AND HEMOGLOBINOPATHIES

It is generally recognized that registries are important tools for detecting demographic patterns, allocating resources, monitoring patient outcomes, and guiding decisions. The establishment of the register is a fundamental step in optimizing, also at a regional level, the planning of transfusion medicine activities, the management of blood resources and to prevent any deficiencies.

With the Decree of the President of the Council of Ministers of 3 March 2017, published in the Official Gazette no. 109 of the following 12 May 2017, "Identification of surveillance systems and registers of mortality, tumors and other pathologies", the National Register of Thalassemia and other Hemoglobinopathies was established at the National Blood Center (CNS) **(23).** The aim of the registry is to provide an accurate census of the patients affected by haemoglobinopathies, which was previously based on estimated prevalence (given that some regions did not have registers) and to improve the care for these patients.

The registry is coordinated by the Italian National Institute of Health (ISS). In order to fulfil the legal obligations of the registry, the National Blood Centre (CNS) of the ISS set up a Steering Committee and Technical-Scientific Committee, which involved representatives of scientific societies, patient groups, the National Centre for Rare Diseases of the ISS, Dr. Deborah Mascalzoni, a European expert on bioethics, and the Heads of the Coordination Structures of the transfusion activities in Sardinia and Sicily, the regions with the highest number of patients affected by haemoglobinopathies **(25, 26)**.

Three independent regional registries for thalassemias have been implemented in Italy: the Italian Multiregional Thalassaemia Registry (HTA-Thal), the Sicilian Registry for Thalassaemia and Haemoglobinopathies (ReSTE), and WebThal on Italian thalassaemia patients.

a. HTA-Thal Registry

The HTA-Thal Registry project aimed to identify the services available in Italy and to collect epidemiological and clinical data on the thalassemic population. The Registry included 22.6% of all the survey's centers located in the North, 41.4% in the Center, 34.3% in the South, and 44.2% in the Islands with a patients/center ratio equal to 12.7, 14.4, 47.6, and 48.8, respectively, with centers having a considerably higher number of patients in the South and Islands **(27)**.

Of a total of 1,899 patients in the Registry, 1,873 had a confirmed diagnosis of β-TM and were included in the analysis. The remaining 26 were excluded from the analysis because they were affected by NTDT **(27)**.

The gender distribution was balanced between males (47.5%) and females (52.5%). The overall mean age was 30.21 ± 11.04 years. The youngest patient was 50 days-old and the oldest patient was 65 years old. Data showed that 68.5% of patients

aged more than 45 years were women (74 out of 108). Overall, 259 patients were of pediatric age (13.8% out of the total) of whom 8.1% were under 12 years of age **(27)**.

In terms of chelation treatment, 448 β-TM patients (23.9%) received deferoxamine (DFO), 383 (20.5%) deferiprone (DFP), 616 (32.8%) deferasirox (DFX), and 399 patients (21.3%) a combination of DFO and DFP. Twenty-one children, all aged less than 4 years, were not treated with any chelator. When treatment was stratified by age groups, DFO was shown to be the commonest chelator in the age group > 45 years, while DFX was the most used chelator in children and young adults (aged less than 25 years) The mean age for combined iron chelation therapy was 30.9 ± 8.6 years for associated DFO/DFP therapy and 33.1 ± 8.2 years for sequential DFO/ DFP **(27)**.

The analysis stratified by Center showed a great variability of medical prescriptions. The prescription rate for DFO varied from 2.8 to 67.8% of patients and the prescription rate for DFP monotherapy ranged from 2 to 50%, depending on the referring Center. DFX was consistently used as monotherapy with a frequency of 75% in some Centers. The DFO/DFP combined therapy was prescribed in almost every Center. No correlation with geographic area, centers' size or other center characteristics was identified by the analysis **(27)**.

b. Sicilian Registry for Thalassaemia and Haemoglobinopathies (ReSTE).

Since 1990, Sicily has maintained a regional register for research on thalassemia and haemoglobinopathies (RESTE) and is the only region that has reliable epidemiological data, registering patients into a mandatory regional registry.

c. WebThal on Italian thalassaemia

This is a cross-sectional evaluation of epidemiological, transfusional and clinical data entered into WebThal on Italian thalassaemia patients who were alive on 31st December 2017. This project, promoted by the Turin Centre, has been expanded in recent years and, at present, includes 36 Italian centres **(28)**.

An analysis of patients who were alive on 31st December 2017 revealed a total number of 3,986 with thalassaemias. These 3,986 patients represent a significant proportion (>50%) of the total population of approximately 7,000 thalassaemia patients living in Italy at the time.

The primary diagnosis was TDT in 3,149 patients (79%), NTDT in 696 patients (17%), and 141 patients (4%) had unspecified thalassaemia. The mean age of patients with β-TM was 37.0 years (range 0.41–78.9 years). Only 8% of patients with β-TM were aged over 50 years **(28)**.

The mean pre-transfusion Hb levels were in line with international guidelines. Data reported for 1,162 patients showed that only 9% had pre-transfusion haemoglobin levels < 9.0 g/dL, 63% had levels between 9 and 10 g/dL, and 28% had levels ≥10 g/dL **(28)**.

The mean ALT levels (30.3± 26.8 U/L; range 6.0–32 U/L; normal range 10–30 U/L) during 2017 were available for 1,376 patients. In terms of iron overload severity, many of the 756 patients with reported data had acceptable hepatic iron levels (74% had a liver iron concentration <7.5 mg/g dry weight). Similarly, at the end of 2017, 85% of patients had no sign of cardiac iron load (MRT2* >20 ms), and only 3% of patients showed signs of a high-risk heart condition (T2* measurement < 10 ms). Other major complications included hepatitis (13.7%), cholelithiasis (11.9%), diabetes mellitus (8.3%), and nephrolithiasis (4.9%) **(28)**.

The overall survival probability at 30 years, reported by Forni et al. **(29)** in a cohort of 709 transfusion-dependent β-TM patients (51.1% males) born between 1970 and 1997 and followed through 2020 by 7 Italian Centers, was 83.6% in the oldest birth cohort (1970–1974) compared with 93.3% in the youngest birth cohort (1985–1997) (P: 0.073). Females showed better survival than males (P: 0.022).

There were a total of 93 deaths at a median age of 23.2 years with the most frequent disease-related causes being heart disease (n = 53), bone marrow transplant (BMT) complication (n = 10), infections (n = 8), liver disease (n = 4), cancer (n = 3), thromboembolism (n = 2) and severe anemia (n = 1) **(29)**.

From the year 2000 onwards there was a steady decline in the number of deaths due to heart disease, and no death from BMT was observed after the year

2010. A total of 480 (67.7%) patients developed ≥1 complication. Patients in younger birth cohorts showed a better complication-free survival (P: < 0.001) which was comparable between sexes. Independent risk factors for death in multivariate analysis included heart disease (P=0.002), serum ferritin >1,000 ng/mL (P:< 0.001), and splenectomy (P: < 0.063) **(29)**.

DIRECT MEDICAL COSTS ASSOCIATED WITH TDT

Most previous studies have documented that the direct medical costs associated with TDT are high, and the management of the disease consumes considerable healthcare resources **(30)**.

The costs of TDT treatment varies across countries and ranged from USD 563 to USD 128,062. Different factors could cause this variation, including the age of patients, different treatment regimens, and patients' clinical characteristics **(31)**.

In Italy, in 2017, the total estimated annual direct medical care cost per patient with β-TM was € 31,883. Iron chelation therapy (ICT) was the most expensive cost component (€ 22,519), followed by transfusions (€ 6,115), treatment of clinical complications (€ 1,427), admissions to hospital (€ 1,213), and laboratory tests (€ 392). The medical annual care costs for NTDT were € 31,183 (€ 22,963 were attributable to ICT) **(32)**.

Additionally, the National Institute for Social Welfare (INPS) provides workers suffering from thalassaemia major or from SCD, who have paid contributions for more than 10 years, with an annual pension. Depending on the severity of their disease and complications, children with SCD also have the right to an 'accompanying allowance', which is granted when the child is not able to carry out daily activities independently. Moreover, the region of Sicily also offers an annual pension to all SCD patients at the time of their diagnosis **(33)**.

PREVALENCE, COMPLICATIONS AND THERAPEUTIC APPROACHES TO SCD PATIENTS IN ITALY

A survey was developed in 1998 by researchers at the Department of Hematology and Pediatric Oncology of the University of Catania and distributed to all Italian centres of Pediatrics and Hematology. It found that, of the 673 SCD cases in people with a known place of residence, 60% lived in Sicily, 20% in Southern Italy, 6% in Central Italy and 13% in Northern Italy. In an update of the survey in 2003, the proportion of SCD patients in Northern Italy had increased to 20% and the proportion in Sicily had decreased to 53% **(34)**.

However, the epidemiology of SCD has changed considerably in the country over recent years, in particular in response to changes in migratory patterns and migration from countries with high SCD prevalence. In Italy, 2018 data from the GREATalyS (Generating Real World Evidence across Italy in SCD) retrospective observational study estimated a total number of 7,977 patients with SCD (1,690 young and 6,287 adult patients). 1,279/7,977 patients had a diagnosis of SCD with concomitant acute vaso-occlusive crisis (VOC) and 5,894 had SCD without concomitant VOC. The proportion of those with one VOC/SCD complication was 3.7% and 15.2% in children compared to adults, with two VOC/SCD complications was 2.3% and 6.2%, and with ≥ 3 VOC/SCD complications was 7.7% and 12.8%, respectively **(35)**.

The mean total health care costs per patient were € 7,918 (€ 2,201 for medications, € 3,320 was for hospitalizations, and € 2,397 for outpatient specialist services) **(35)**.

A national survey was carried out recently by the Società Italiana della Talassemia ed Emoglobinopatia (SITE), SIMTI and the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP) to collect information on the different therapeutic approaches used to treat SCD patients in Italy, the preliminary results of which were published in 2018. The results show that out of 1,579 SCD patients (36):

- 23% did not receive any therapy
- 12% received hydroxyurea alone
- 10% received acute transfusion regimens alone
- 14% received chronic transfusion regimens alone
- 40% received a combination of transfusions and hydroxyurea

A sub-analysis of paediatric data from another nationwide study published in 2018 found a tendency to treat children with lower doses of hydroxyurea

than is recommended. In addition, although guidelines recommend commencing hydroxyurea treatment during the first few months of life, in the cohort of children studied, this treatment was not administered before 11 months of age. Otherwise, the results of the survey indicate relatively good adherence to the guidelines **(37)**.

De Franceschi et al.(38) reported the Italian experience on new cases of SCD and other hemoglobinopathies in refugees between 2014 and 2017 at 13 Italian reference centers for hemoglobinopathies. A total of 70 patients with hemoglobin disorders were identified (61 new patients with SCD, 6 with TDT, and 3 with other hemoglobinopathies), the majority of whom were male. Half were adults with the median age of 21 years and the other half were children. Most came from West African countries Senegal and Nigeria, as well as Morocco, Egypt, and Tunisia in North Africa, and Syria in the Middle East. Approximately two-thirds of the 70 patients were diagnosed after an acute complication requiring emergency care and some of these complications were life-threatening and likely preventable with early diagnosis and treatment **(38)**.

To expedite the identification of SCD – and mitigate complications – De Franceschi et al. **(39)** undertook a pilot study in which they performed point-of-care screening of refugees seen in a single refugee center during October 2017. More than 400 individuals were screened for using one of the new rapid point-of-care screening devices (SickleSCAN® BioMedomics, Inc.), the results of which were then validated using the gold standard laboratory test. Front-line health providers in refugee centers and emergency department personnel were trained to recognize the signs and symptoms of SCD and intervene to provide necessary care. Three percent were found to have SCD and 20% were found to have the heterozygous AS genotype. The majority of sickle hemoglobin (HbS) carriers were from West Africa, and the authors noted that "none of the newly identified SCD patients were aware of their condition."

These findings prompted the researchers to propose several initiatives to improve screening of SCD among migrants arriving to Italy. These include **(39)**:

- Routine screening for SCD in refugees from countries endemic for SCD within 10-14 days of their arrival to identify potentially vulnerable patients
- A structured, collaborative national network
- Educating ED physicians to identify and treat acute SCD-related events (such as SCD-related acute vaso-occlusive events)
- Rapid referrals of refugees with SCD or symptomatic HbS-carrier genotype to a comprehensive SCD reference center.
- Earlier initiation of disease-modifying treatment (e.g., hydroxyurea)

NEWBORNING SCREENING

In the past decades, the countries that have been affected by migratory flows from areas with a high incidence of the S gene, such as Great Britain (UK), France, Belgium and the Netherlands, have developed integrated care programs (comprehensive care) on the basis of international and national guidelines. Within these programmes, some diagnostic and therapeutic measures have allowed a considerable drop in mortality in the pediatric age: the inclusion of newborn screening (NS) with consequent early diagnosis, the introduction of prophylaxis with penicillin within two months of life as protection from functional asplenia, intensive vaccination programs, education of the family in the recognition of signs and symptoms of severity (such as enlarged spleen, a possible sign of splenic sequestration, which is an acute life-threatening complication), and screening starting from two years of life using transcranial Doppler for stroke prevention (40). Some NS projects have been developed also on a local initiative in our country.

From 2010 to 2012 in Ferrara, 1,992 newborns were tested and 24 carriers identified (1.2%). The experience was suspended due to lack of funding (41). In 2013, a newborn screening project was launched in Novara aimed at newborns with a parent from areas at risk of hemoglobinopathy. In Modena, a NS program aimed at women at risk due to ethnicity has been active since 2011. The results of the pilot study indicate the presence of hemoglobinopathy in 27% of the 330 women tested (70% program coverage). The subsequent screening of newborns of carrier mothers of the anomaly, performed on the cord and analyzed by HPLC, allowed the identification of 48 carrier newborns and 9 affected **(42, 43)**. The universal antenatal screening program, extended to all

pregnant women and including newborns at risk, for maternal positivity, is currently underway and supported with funding from the Province. Furthermore, a centralized targeted NS program has been active since 2010 in Friuli Venezia Giulia, financed by the Region. The data, not yet published, report 6,018 newborns tested from 2010 to 2015, a percentage of AS between 1.74% and 4.7% depending on the Province (Zanolli F, personal communication). A pilot program of universal NS has been running since May 2, 2016 in Padua and from September 2016 in Monza. In the first 12 months of activity, more than 2,500 newborns were tested with 0.07% affected and 0.58% carriers at the Padua Center and 0.098% carriers at the Monza Center **(44)**.

CONCLUSION

The implementation of prevention and screening programs for hemoglobinopathies has lowered the prevalence and incidence of β-TM in regions with a historically high number of carriers and patients. In addition to reducing incidence, research improving the overall standard of care in Italy has allowed patients with thalassemia to live longer into adulthood. However, despite important improvements in the management of β-TM, there are still many challenges to overcome before global disease control is achievable.

Significant progress has been made in increasing the knowledge regarding subjects with SCD. Nevertheless, early diagnosis is far from universal and its treatment is still unsatisfactory for both acute and chronic clinical complications.

REFERENCES

1. Thein SL. The molecular basis of β-thalassemia. Cold Spring Harb Perspect Med. 2013;3(5):a011700.
2. Kountouris P, Lederer CW, Fanis P, Feleki X, Old J, Kleanthous M. IthaGenes: an interactive database for haemoglobin variations and epidemiology. PLoS One. 2014;9(7):e103020.
3. Modell B, Darlison M, Birgens H, et al. Epidemiology of haemoglobin disorders in Europe: an overview. Scand J Clin Lab Invest. 2007; 67:39-70.
4. McGann PT, Nero AC, Ware RE. Clinical Features of β-Thalassemia and Sickle Cell Disease. Adv Exp Med Biol. 2017; 1013:1-26.
5. Viprakasit V, Ekwattanakit S. Clinical Classification, Screening and Diagnosis for Thalassemia. Hematol Oncol Clin North Am. 2018; 32:193-211.
6. Mekelenkamp H, Zanten Hv, Vries Md, Lankester A and Smiers F. How to Facilitate Decision-Making for Hematopoietic Stem Cell Transplantation in Patients With Hemoglobinopathies. The Perspectives of Healthcare Professionals. Front Pediatr. 2021; 9:690309.
7. Thompson AA, Walters MC, Kwiatkowski J, et al. Gene therapy in patients with transfusion-dependent b-Thalassemia. N Engl J Med. 2018; 378:1479–93.
8. Cooley TB. Anemia in children with splenomegaly and peculiar changes in the bones. Am. J. Dis. Child. 1927; 34:347-63.
9. Rietti F. Sugli itteri emolitici primitivi. Atti Accad. Sci. Med. Nat Ferrara. 1925; Sez. II: 14.
10. Silvestroni E, Bianco I. Prime osservazioni di resistenze globulari aumentate in soggetti sani e rapporto fra questi soggetti e i malati di cosiddetto itterro emolitico con resistenze globulari aumentate. Boll Atti Accad Med (Roma). 1943; 69:29.
11. Silvestroni E, Bianco I, Sulla frequenza della microcitemia nel Ferrarese e in alcune altre regioni d'Italia. Policlinico Sez Prat. 1948; 55:417.
12. Haldane JBS. Disease and evolution. Ric Sci.1949; (Suppl.19):3.
13. Wolman IJ, Ortolani M. Some clinical features of Cooley's anemia patients as related to transfusion schedules. Ann N Y Acad Sci. 1969; 165:407-14.
14. Bannerman RM, Callender ST, Williams DL. Effect of desferrioxamine and D.T.P.A. in iron overload. Br Med J.1962; 2:1573.
15. Brittenham GM, Griffith PM, Nienhuis AW, et al. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. N Engl J Med. 1994; 331(9): 567-73.
16. Lucarelli G, Polchi P, Izzi T, et al. Allogeneic marrow transplantation for thalassemia. Exp Hematol 1984; 12:676.
17. Cao A, Galanello R, Rosatelli MC, et al. Clinical experience of management of thalassemia: the Sardinian experience. Semin Hematol. 1996; 33:66-75.
18. Vullo C , Lucci N, Salsini G, et al. Prevention of thalassemia in the Ferrara area. Haematologica. 1990;75 (Suppl 5):142-52.
19. Cao A. Results of programmes for antenatal detection of thalassemia in reducing the incidence of the disorder. Blood Rev. 1987;1 (3):169-76.
20. Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. Clin Chem. 2000; 46:1284-90.
21. Rosatelli MC, Tuveri T, Scalas MT, et al. Molecular screening and fetal diagnosis of β-thalassemia in the Italian population. Hum Genet. 1992;88:590-2.
22. Arishi WA, Alhadrami HA, Zourob M. Techniques for the Detection of Sickle Cell Disease: A Review. Micromachines (Basel). 2021; 12(5): 519.

23. Italian Republic. Official Journal of the Italian Republic. Rome - Friday, 12 May 2017 (Gazzetta Ufficiale Della Repubblica Italiana Roma - Venerdì, 12 maggio 2017). http://www.gazzettaufficiale.it/eli/gu/2017/05/12/109/sg/pdf. Accessed 1 Nov 2018.
24. Ministry of Health, National Network of Thalassemia and Haemoglobinopathies, Ministerial Decree Speranza, 2020.
25. National Blood Centre, National Register for Thalassemia and Other Haemoglobinopathies, 2019.
26. Higher Institute of Health, National Blood Centre, National Register of Thalassemia and other Haemoglobinopathies, Composition of the Directive Committee and the Scientific Committee, 2017.
27. Conte R, Ruggieri L, Gambino A, et al. The Italian multiregional thalassemia registry: Centers characteristics, services, and patients' population. Hematology 2016;21(7):415-24.
28. Longo F, Corrieri P, Origa R, et al. Changing patterns of thalassaemia in Italy: a WebThal perspective. Blood Transfus. 2021; 19(3): 261–8.
29. Forni GL, Gianesin B, Musallam KM, et al. Overall and complication-free survival in a large cohort of patients with β-thalassemia major followed over 50 years. Am J Hematol. 2023;98(3):381-7.
30. Mantovani LG, Scalone L, Ravera S, Krol M, Uyl-de Groot C, Cappellini MD. Direct and Indirect Cost of Beta Thalassemia Major: Results from the ITHACA Study. Am Soc Hematology; 2006.
31. Alshamsi S, Samer Hamidi S, Narci HO. Healthcare resource utilization and direct costs of transfusion-dependent thalassemia patients in Dubai, United Arab Emirates: a retrospective cost-of-illness study, BMC Health Serv Res. 2022; 22: 304.
32. ANGELUCCI E, ANTMEN A, LOSI S, BURROWS N, BARTIROMO C, HENRY HU XH. DIRECT MEDICAL CARE COSTS ASSOCIATED WITH B-THALASSEMIA CARE IN ITALY. BLOOD 2017;130 (SUPPL. 1):3368.
33. INPS, Evaluation guidelines for haemoglobinopathies, 2018.
34. Russo G, De Franceschi L, Colombatti R, et al. Current Challenges in the Management of Patients with Sickle Cell Disease–A Report of the Italian Experience. Orphanet J. Rare Dis. 2019;14:120.
35. De Franceschi L, Castiglioni C, Condorelli C et al. on behalf of the GREATalyS Study Group. Real-World Evidence on Disease Burden and Economic Impact of Sickle Cell Disease in Italy. J Clin Med. 2023;12:117.
36. Graziadei G, Sainati L, Bonomo P, et al. Transfusion Therapy in a Multi-Ethnic Sickle Cell Population Real-World Practice. A Preliminary Data Analysis of Multicentre Survey. Blood. 2018;132:2389.
37. Colombatti R, Palazzi G, Masera N, et al. Hydroxyurea prescription, availability and use for children with sickle cell disease in Italy: Results of a National Multicenter survey. Pediatr Blood Cancer. 2018;65 (2):e26774.
38. De Franceschi L, Lux C, Piel FB, et al. Access to emergency departments for acute events and identification of sickle cell disease in refugees. Blood. 2019;133:2100-3.
39. De Franceschi L. Routine Sickle Cell Disease Screening Among Migrants May Help Fast-Track Treatment, Save Lives, and Reduce Health Care Costs.www.hematology.org. 9th May, 2019.
40. de Montalembert M, Ferster A, Colombatti R, et al. European Network for Rare and Congenital Anaemias. ENERCA clinical recommendations for disease management and prevention of complications of sickle cell disease in children. Am J Hematol. 2011;86:72-5.
41. Ballardini E, Tarocco A, Marsella M, et al. Universal neonatal screening for sickle cell disease and other haemoglobinopathies in Ferrara. Italy. Blood Transfus. 2013;11:245–249.
42. Venturelli D, Lodi M, Palazzi G, et al. Sickle cell disease in areas of immigration of high-risk populations: a low cost and reproducible method of screening in northern Italy. Blood Transfus. 2014; 12:346–51.
43. Lodi M, Bigi E, Palazzi G et al. Universal Screening Program in Pregnant Women and Newborns At-Risk for Sickle Cell Disease: First Report from Northern Italy. Hemoglobin. 2017;41:230–3.
44. Martella M, Cattaneo L, Viola G, et al. Universal newborn screening for sickle cell disease: preliminary results of the first year of a multicentric Italian pilot project. Abstract E1489 at the 22nd EHA Congress, Madrid 22-25 June 2017.

Conflict of interest statement: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Correspondence Address:
Vincenzo De Sanctis, MD
Coordinator of the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescence Medicine (ICET-A) and Adolescent Outpatient Clinic, Quisisana Hospital
Ferrara, Italy44121 Ferrara, Italy Tel: +39 0532 770243
E-mail: vdesanctis@libero.it

THALASSEMIA AND HEMOGLOBINOPATHIES IN SPAIN

Prof. Dr. Joan-Lluis Vives Corrons MD
Emeritus Prof. University of Barcelona
Red Blood Cell and Hematopoietic Disorders Research
Institute for Leukaemia Research Josep Carreras
Rare Anaemias & Ektacytometry Unit
Clinical Centre for Ambulatory Medicine
Còrsega 209.08036-Barcelona-Spain

ABSTRACT

Thalassemia and hemoglobinopathies are relatively common in Spain, with varying prevalence across different regions. Regions with historical influxes of populations from the Mediterranean, such as Catalonia, Valencia, and the Balearic Islands, have the highest prevalence. Due to migration impact, Sickle cell disease (SCD) is currently the most prevalent hemoglobinopathy Spain and the implementation of neonatal screening programs for the early detection of hemoglobinopathies has improved timely interventions and care for affected individuals. Moreover, neonatal screening has facilitated data collection and the possibility of conducting cohort studies and comparing data with other European registries through the European Network for Rare and Congenial Anaemias (ENERCA). The paper provides insights into the prevalence and management of thalassemia and hemoglobinopathies in Spain, highlighting the importance of early diagnosis and intervention through neonatal screening programs. The establishment of networks like the Catalan Network for Severe Hemoglobinopathies (CATGLOBIN) has been instrumental in improving the care and survival rates of affected individuals with SCD. Moreover, the high survival rates for patients with both thalassemia major and SCD, indicate the effectiveness of early diagnosis and treatment

INTRODUCTION

Thalassemia and hemoglobinopathies are a group of genetic blood disorders that affect the production of hemoglobin, the protein responsible for carrying oxygen in red blood cells (RBCs). Thalassemia is characterized by reduced or absent production of certain types of globin chains, while hemoglobinopathies involve structural abnormalities in the globin chains.

In Spain, thalassemia and hemoglobinopathies are relatively common, particularly among individuals with Mediterranean ancestry. The prevalence of these disorders varies across different regions of the country. The highest prevalence is observed in areas with a historical influx of populations from the Mediterranean, such as Catalonia, Valencia, and the Balearic Islands. The most common types of thalassemia in Spain are beta-thalassemia and alpha-thalassemia. Beta-thalassemia is more prevalent, and it can be further categorized into thalassemia major, thalassemia intermedia, and thalassemia minor. Alpha-thalassemia has different forms as well, including hemoglobin H disease and alpha-thalassemia silent carrier.

Regarding hemoglobinopathies, the most prevalent one in Spain is sickle cell disease (SCD), which is caused by a mutation in the beta-globin gene. SCD primarily affects individuals of African descent but can also be found in individuals from other ethnic backgrounds.

In Spain, various medical centres and healthcare professionals specialize in the diagnosis and management of thalassemia and hemoglobinopathies. Treatment typically involves supportive measures such as blood transfusions, iron chelation therapy, and, in some cases, bone marrow transplantation. Regular monitoring and genetic counselling are also important components of care for individuals with these disorders.

CHARACTERISTICS OF THALASSEMIA IN SPAIN

As in other Mediterranean countries, the main hemoglobin disorder in Spain is beta-thalassemia. Until 2003, however, the prevalence of hemoglobinopathies in Spain was unknown, and only the establishment of universal pilot screening programs of hemoglobinopathies allowed to identification of an overall incidence of 0.33% **(1, 2)**. The first study of hemoglobinopathies in Spain was carried out in 1981, in Barcelona by Baiget et al. **(3)** that reported a global prevalence of hemoglobinopathies in Spain of 0.14%, a value significantly lower value than that reported by the WHO in 1995 **(4)**.

Although after this first publication, many studies have been carried out to establish the prevalence of β-thalassemia in Spain, in only one, the individuals included were representative of almost all Spanish geographical regions **(5)**. This study was undertaken under the auspices of the Spanish Society of Haematology and demonstrated that, in Spain, β-thalassemia distribution is very heterogeneous with a prevalence ranging from 0.1% to 5%, pointing out that most of the HbS, and also HbC and other hemoglobinopathies carriers, were from southern and western geographical regions of Spain. Moreover, control programs based on the screening of couples at risk for thalassemia and the offer of antenatal diagnosis have been demonstrated to be beneficial for reducing the frequency of β-thalassemia major when properly performed **(6)**.

Noteworthy, some of the Spanish regions exhibited a significantly higher prevalence of both β-thalassemia and structural hemoglobinopathies, most probably due to the well-known genetic influence of Arab populations in the past **(7)**. In 1999, Martìn Nùñez et al. **(8)** performed the first detection campaign for hemoglobinopathies and thalassemias among school children in northern Extremadura and from a total of 2,818 screened samples, the global prevalence of hemoglobinopathies was of 0.24% Notably 0.10% of the autochthonous population was found to be a carrier of HbS, a relatively high prevalence of HbS in this geographical area that May be explained by the influence of malaria infection in the past. In 2006, screening for hemoglobinopathies and thalassemia was carried out among 2,436 pregnant women in Lanzarote (Canary Islands) by Calvo-Villas et al. **(9)**, and a hemoglobin variant was found in 23 women (0.94%): HbS trait (13 cases), HbC trait (7 cases), and HbD-Punjab trait (3 cases). In 82.6% of cases, the variant hemoglobins were found in immigrant populations from Africa and Central and South America. This value is three times higher than that recently reported by Modell et al. with an estimated percentage of pregnant woman carriers for hemoglobinopathy of 0.34% **(10).** The results of a very recent hemoglobinopathy study, also performed in the Canary Islands by De las Heras et al. **(11)** reported that a total of 198 hemoglobinopathies were found in an adult population, with 125 of them corresponding to structural variants. In three cases SCD was identified, and in 70 cases HbS trait was identified. It was noteworthy that 60% of the cases identified as HbS trait were born in the Canary Islands **(Table 1)**.

Between 2003 and 2008, a pilot study for neonatal screening of hemoglobinopathies was performed in Catalonia by Mañú et al. with the help of a grant from the Spanish Ministry of Health **(12, 13).** The neonatal blood samples obtained by heel prick and/or umbilical cord sampling were analysed by HPLC and a total of 4696 newborns from at-risk ethnic groups were studied using two different targeted neonatal screening approaches. Newborns were classified into four different categories according to their mother's birthplace: 1, North Africa; 2, sub-Saharan Africa;3, Asia; and 4, Central and South America). The prevalence of hemoglobinopathies and SCD was calculated for each category. The expected number of births in Catalonia for 2006 was 82.300, and there was an estimated prevalence of 0.021% for SCD and 0.37% for AS, with an overall prevalence of hemoglobinopathies of 0.5% **(Figure 1)**. Using the SCD prevalence obtained for the different ethnic categories of high-risk immigrant populations in the targeted screening study of Catalonia, the number of births from non-indigenous populations affected by SCD in each Spanish region has been also calculated **(Table 2)**.

After 2003, the increasing immigration flows, especially from Africa (northern and sub-Saharan regions) led to the emergence of SCD as one of the most common hereditary disorders in Spain, with an impact on the burden of healthcare in several of its geographical regions. In these regions, the prevalence of SCD is directly related to the impact of immigrant populations, mainly from sub-Saharan Africa **(14)**. The national consensus in Spain indicates

that the number of African immigrants has doubled in only 5 years. Furthermore, the distribution of this immigrant African population is very heterogeneous and differs widely from one region to another.

In conclusion, all the studies performed until 2008, demonstrate that in Spain the prevalence of hemoglobinopathies is lower when compared to other Mediterranean countries **(15)** or concerning the European median (0.5 cases for thalassemia and 15 for SCD per 100,000 inhabitants) **(16)**. Moreover, the prevalence of SCD is heterogeneous and strongly influenced by the migratory flows. After 2008, the most updated information on the current situation of Hemoglobinopathies in Spain has been recently published by the Spanish Society of Pediatric Hematology and Oncology (SEHOP) from a multicentric study with the participation of 51 hospitals all over Spain in which 75 thalassemias (62 Major Thalassemia) 826 Sickle Cell Disease (SCD) and 58 other hemoglobinopathies were registered.

The main reason for the diagnosis of beta-thalassemia is anemia and, in some patients, stroke with few post-stroke sequelae. In these patients, magnetic resonance imaging (MRI) of the brain has demonstrated the presence of lacunar infarcts that may explain the neurological alterations, mainly neurocognitive that appear after the stroke. Treatment is based on antibiotic prophylaxis with penicillin, administered for a minimum of 5 years, and chelation therapy with deferasirox (79%), deferoxamine (58%) or deferiprone (17%) for an average duration of 9 years. A small percentage of patients are splenectomised with or without concomitant cholecystectomy. In almost 50% of children with severe thalassemia major (TM) an HLA-identical hematopoietic stem cell transplant (HSCT) can be prescribed with an overall survival of 96.7%, and a mean follow-up of 13.7 years. The main complications of HSCT are chronic graft-versus-recipient disease (GVHD) and graft rejection. Very few patients die due to septicemia or cardiorespiratory failure.

CHARACTERISTICS OF SICKLE-CELL DISEASE IN SPAIN

As mentioned before, the largest registered SCD population in Spain is concentrated in Catalonia and Madrid. The entire sample is diagnosed by universal newborn screening, anemia symptoms, and vaso-occlusive crisis (VOC), with a mean age at diagnosis of 3 years. As in beta-thalassemia, cerebrovascular accidents, with few sequelae can occur in about 3% of patients, which show a somewhat higher percentage of neurocognitive complications and lacunar infarcts. Treatment consists of antibiotic prophylaxis with penicillin at around 2 years, with a mean duration of 5 years and, when necessary chelating treatment with deferasirox in about 90% of cases and deferoxamine in the remaining 10% of cases. Hydroxyurea treatment can be started in, approximately, 40% of the patients and blood transfusions in about 8% with a mean age of onset at 7 years and a mean duration of 3 years. A small percentage of patients had to undergo implantation of a central venous catheter (CVC), usually, Port-a-cath®, and slenectomy, accompanied by cholecystectomy after 10 years of age. In severe cases of TM, the HSCT is an option with an overall survival of 99% at 5 years of age,98% at 15 years, and 96% after 20 years. As in beta-thalassemia, complications of HSCT that may decrease the survival rate are generally unrelated to GVHD and in general associated with prematurity, metabolic disorders, congenital heart disease, and neuroblastoma. The average age of death is around 7 years.

Newborn screening for hemoglobinopathies and SCD in Spain

The first Newborn Screening Program (NSP) in Spain was introduced in Granada in 1968 by the initiative of professors Federico Mayor-Zaragoza, Magdalena Ugarte, and Antonio Martínez Valverde. In 1978, the National Plan for mental retardation was established within the Real Patronato de Educación y Atención a Deficientes (Royal Council of Education and Care for Individuals with Disabilities), and several laboratories were established within its framework. Between 1982 and 1983, the authorities of each autonomous region in Spain took over the management of government-run programs for the early detection of congenital and metabolic disorders (18). Between 2000 and 2015, there were significant differences in the NSP programs of the different Spanish autonomous communities, as many only included 2 or 3 diseases while others included more than 20. To establish the actual benefits of the early diagnosis of diseases susceptible to screening, the Spanish Federation of Phenylketonuria and other metabolic Disorders, along with a group of health professionals, agreed to review the

existing NSP programs in Spain to develop the broadest possible consensus on aspects such as the criteria applied to select diseases for inclusion, the establishment of units for the diagnosis, treatment, and follow-up of the detected diseases, and the creation of a national register of affected patients. They developed the consensus document for Newborn Screening Programs (NSP) for endocrine and metabolic disorders that were approved in 2013 by the general assembly of the "Consejo Interterritorial" that encouraged the establishment of consensus-based protocols within the framework of the National Health Service (NHS) so that screening programs could be uniformly implemented, based on rigorous quality criteria.

As mentioned before, the first neonatal screening of hemoglobinopathies was started in Catalonia by Baiget et al. **(3)** in 1981. Also in Catalonia, in 1998, Cabot et al. **(19)** performed, a targeted screening study limited to newborns of sub-Saharan African mothers, initiated because of the high and increasing delivery rate of black-origin immigrants in this geographical area. However, the first universal pilot screening of hemoglobinopathies was performed by Dulìn et al. **(1)** in the Community of Madrid. A total number of 29,253 specimens, obtained by heel prick and preserved as a dried blood spot on a filter paper (a ''Guthrie spot''), were screened by high-performance liquid chromatography (HPLC), and 98 hemoglobinopathies were identified with an overall incidence of 0.33%. Seventy-one cases were AS with a prevalence of 0.24%, four cases were SCA, and one case was a compound heterozygote for HbS and beta-thalassemia with a prevalence of 0.017%. In 2007, Cela et al. **(1)** reported the results of the first 32 months of running this program, with the study of a total of 190,238 newborns blood samples (Guthrie spots) by HPLC and the identification of 1,060 hemoglobin variants, corresponding to a prevalence of 0.56%. Thirty-one of these cases were SCD, corresponding to a prevalence of 0.016%. In all of these cases, prevention measures consisting of antibiotic administration, vaccination, and comprehensive clinical care were initiated. The results from newborns identified as carriers of HbS, HbC, HbD or HbE were also reported to the family, and family studies were recommended for carriers and SCD patients. In subsequent pregnancies, prenatal diagnosis was performed in three families after a parental investigation.

The Catalan Network for Severe Hemoglobinopathies (CATGLOBIN)

In 2007, The Red Cell Pathology Unit from the Hospital Clinic of the University of Barcelona (HCB) led by Prof. Joan-Lluis Vives Corrons contacted the Catalan Government (Generalitat de Catalunya) to implement a newborn screening program (NSP) for Sickle-Cell Disease (SCD) in Catalonia. This proposal arose as a need created by the large increase in the incidence of SCD in this Country as the result of the high migration impact over at least 30 years. The estimated prevalence of SCD in the neonatal population of Catalonia is 1 in 3,634 babies, and the sickle cell carrier condition is of 1 in 148 babies **(12, 13)**. In 2009, the TV3 Marathon, in its edition dedicated to "Rare Diseases" awarded the HCB in collaboration with the Hospital of Santa Creu and Sant Pau (HSCSP), also in Barcelona, with a Grant to create the Catalan Network for the Diagnosis and Follow-up of Severe Hemoglobinopathies called CATGLOBIN **(Figure 2)**. This allowed to implementation of the diagnostic procedures for hemoglobinopathies and to improve the clinical care of patients with chronic anemia and painful VOC due to SCD. 15 Health Care Providers (HCPs) from all over Catalonia take part in this network, and in 2012, within the framework of the Advisory Committee on Rare Diseases of the Public Health Agency of the Catalan government, presented the proposal to be included in the second phase expansion of the official Catalan NSP preceded by a one-year pilot study carried out in collaboration with CATGLOBIN. At the beginning of 2015, the Public Health Agency of the Catalan government approved the incorporation of the NSP of hemoglobinopathies and SCD within the official Catalan NSP Organisation and the creation of a Diagnostic Confirmation Unit for SCD in Catalonia.

CLOSING REMARKS

Throughout the last 5 years, thanks to the implementation of The Spanish Undiagnosed Rare Diseases Program a great push has been provided to the implementation of neonatal screening for hemoglobinopathies and especially for SCD. After the dissemination of neonatal screening to most of the autonomous communities and the inclusion of patients of all ages in the registry, a high number of cases have been registered to allow the follow-up of each one of them and to increase their survival by more than 95.5% at 20 years

of age in SCD, and 96.7% in thalassemia major. The NSP has become the most widely used diagnostic method, which has made it possible to pave the way for future cohort studies and to be able to compare data with other European registries, as has been done during the first stage of the ENERCA project **(20)**. This has greatly contributed to the awareness of doctors and the general population against this imported disease that affects a large number of European countries. It is also important to mention that with this new dimension adopted to face the health problem, a great advance has been made in the process of the transition of SCD patients from childhood to adulthood through communication between pediatric haematologists and their adult counterparts. Moreover, this has facilitated the possession of evolutionary data from the beginning, which may have an impact on the creation of new protocols and updating of existing guidelines. and recommendations. The consolidation of the registry led by SEHOP will make it possible to propose the realization of new multicentre studies since just by keeping the registry up to date, the epidemiological studies that are deemed necessary can be promoted by the pertinent health authorities.

REFERENCES

1. Dulin Iñiigueza E, Cantalejo López MA, Cela de Julián ME,Garci G. Early detection of sickle cell anemia and other hemoglobinopathies in neonates in the autonomous community of Madrid. A pilot study. An Pediatr (Barc). 2003;58:146-155.
2. Cela de Julian E, Dulin Iñiguez E, Guerrero Soler M, et al.Evaluation of systematic neonatal screening for sickle cell diseases in Madrid three years after its introduction An.Pediatr (Barc). 2007; 66:382-386.

3 Baiget M, del Rìo E, Doménech M, et al. Escrutinio de hemoglobinopatías en sangre de cordon umbilical. Biolog´ıa Cl´ın Hematol. 1981; 3:251-256.

4.Angastiniotis M, Modell B, Englezos P, et al. Prevention and control of hemoglobinopathies. Bull World Health Org.1995; 73:375-386.

5. Study Group for Hemoglobinopathies and Thalassemias. Thalassemia syndromes in Spain.Preliminary epidemiologic data. Sangre (Barc). 1986; 122:609-613.
6. Baiget M. Hemoglobinas estructurales en España. Sangre (Barc). 1985; 30:899-904.
7. Oliva Berini E, Cladera Serra A, Torrent Quetglas M. Campaign for the detection of minor beta-thalassemia and prevention of major beta-thalassemia in the island of Menorca.10-year experience. Med Clin (Barc) 1998; 110:361-4.
8. Martìn Nùñez GA. Incidencia de hemoglobinopatıas estructurales en la alta Extremadura. Estudio neonatal. Madrid:Universidad Complutense, 1989.
9. Calvo-Villas JM, Zapata Ramos MF, Cuesta Tovar J, et al.Prevalence of hemoglobinopathies in pregnant women in the Lanzarote island health sanitary area. An Med Interna. 2006;23:206-212.
10. Modell B, Darlison M, Birgens H, et al. Epidemiology of haemoglobin disorders in Europe: an overview. Scand J Clin Lab Invest. 2007; 67:39-70.
11. De las Heras Flo´rez S, P´erez Hernandez LM. Hemoglobinopatıas diagnosticadas en el area sanitaria del Hospital Universitario Nuestra Señora de Candelaria de Santa Cruz de Tenerife durante un año. An Med Interna (Madrid). 2008; 25:61-66.
12. Mañú-Pereira M, Maya A, Cararach V, et al. Neonatal screening of hemoglobinopathies and glucose-6-phosphate dehydrogenase in Catalonia. The pilot study in an anonymous, not related population. Med Clin (Barc). 2006;126:281-285.
13. Mañú Pereira M, Corrons JL. Neonatal haemoglobinopathy screening in Spain. J Clin Pathol. 2009 62:22-5. doi: 10.1136/jcp.2008.058834. PMID: 19103853.
14. Las Heras Manso G, Juncà Piera J, Feliu Frasnedo E, et al.Haemoglobinopathies and glucose-6-phosphate dehydrognase deficiency in the sub-Saharan immigrant population of the Center and South Maresme region, Catalonia, Spain. Med Clin (Barc). 2008; 131:5-9.
15. Daniel Y, Elion J, Allaf B, et al. Newborn Screening for Sickle Cell Disease in Europe. Int. J. Neonatal Screen. 2019, 5:15.
16. Orphanet. Prevalence of rare diseases. Orphanet report series 2018 .http://www.orpha.net/orphacom/cahiers/ docs/GB/Prevalence of rare diseases by alphabetical list.pdf.
17. Bardón Cancho EJ, García-Morín M, Beléndez C, et al.Update of the Spanish registry of haemoglobinopathies in children and adults. Med Clin.2030; 155: 95-103.
18. Couce ML. Fifty years of neonatal screening for congenital diseases in Spain. An Pediatr (Barc). 2019; 90:205-206.
19. Cabot Dalmau A, Casado Toda M, Barberàn Pèrez J, et al.Screening neonatal de drepanocitosis en el Consorci Sanitari de Mataro. Justificaci´on y primeros resultados. An Esp Pediatr 1998; 49:157-160.
20. Vives Corrons, J.-L.; del Mar Mañú Pereira, M.; Romeo-Casabona, C.; Nicolás, P.; Gulbis, B.; Eleftheriou, A.; Angastiniotis, M.; Aguilar-Martínez, P.; Bianchi, P.; Van Wijk, R.; et al. Recommendations for Centres of Expertise in Rare Anaemias. The ENERCA White Book. *Thalass. Rep.* **2014**, *4*, 4878. https://doi.org/10.4081/thal.2014.487

Correspondence Address:
Prof. Dr. Joan-Lluis Vives Corrons MD
Emeritus Prof. University of Barcelona
Red Blood Cell and Hematopoietic Disorders Research
Institute for Leukaemia Research Josep Carreras
Rare Anaemias & Ektacytometry Unit
Clinical Centre for Ambulatory Medicine
Còrsega 209.08036-Barcelona
Mail: jlvives@clinic.cat

Figure 1 Newborn Screening for Hemoglobinopathies in Catalonia

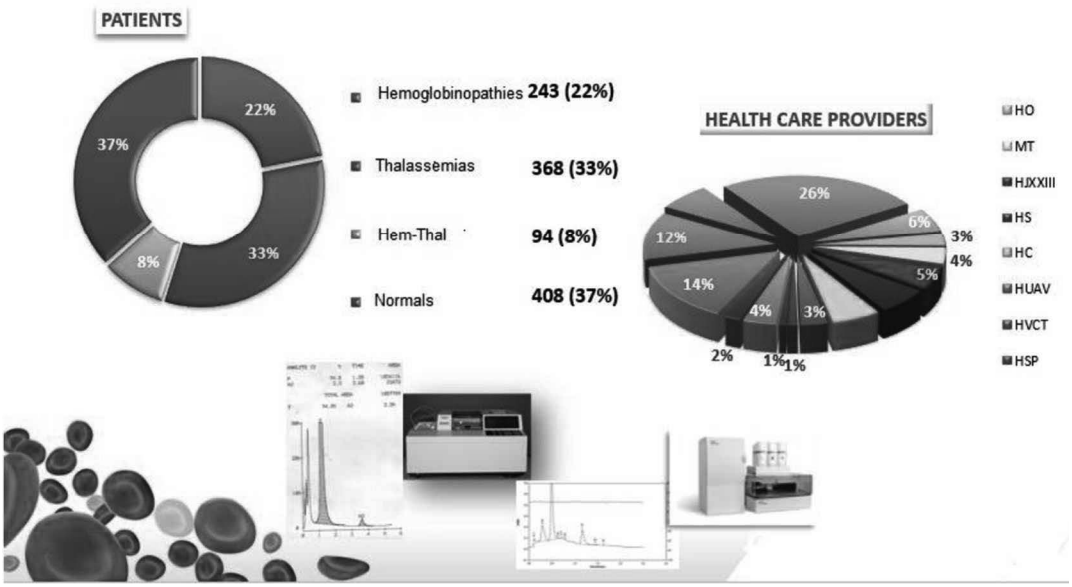


Figure 2. CATGLOBIN

Catalan Network for diagnosis and follow-up of severe hemoglobinopathies. 56 professionals pertaining to 15 Health Care Providers

Table 1. Studies carried out in Spain in order to establish the prevalence of hemoglobinopathies

Reference	Site/population	No. of samples	Prevalence, %	Comments
Baiget *et al* (1981)[13]	Barcelona	3600	Global 0.14	Adult population
De Pablos (1988)[14]	Granada/targeted	16 736	Global 0.13, HbS 0.18	Adult and neonatal population
Martín Nuñez (1989)[15]	Extremadura/universal	4750	Global 0.15	–
Risueño Pacheco (1993)[16]	Cadis/universal	15 789	Global 0.15	Adult or children population is not indicated
Malcorra Aspiazu (1994)[17]	Canary island/targeted	40 192	Global 0.27, HbS 0.14	Autochthonous adult population
Martín Nuñez (1995)[18]	Extremadura/targeted	2818	Global 0.24	School children
Cabot Dalmau (1998)[19]	Mataró/targeted	82	Global 10.98, SCD 1.22	Neonatal screening: sub-Saharan population
Hojas Bernal (2001)[20]	Madrid/universal	1200	Autochthonous global 0.54, migrant global 2.83	Autochthonous and migrant population
Dulín Inigueza (2003)[21]	Madrid/universal	29 253	Global 0.24, SCD 0.017	Neonatal screening
Mañú Pereira (2006)[22]	Catalonia/targeted	1620	Global 2.9, SCD 0.12	Neonatal screening of migrant population
Joyanes (2006)[23]	Madrid/universal	3365	Global 0.77, SCD 0.03	Neonatal screening: specific region of Madrid
Calvo-Villas *et al* (2006)[24]	Lanzarote/targeted	2436	Global 0.94	Pregnant women
Mañú Pereira (2007)[25]	Catalonia/targeted	2439	Global 3.85, SCD 0.21	Neonatal screening of migrant population
Cela de Julián (2007)[26]	Madrid/universal	190 238	Global 0.56, SCD 0.016	Neonatal screening official programme
Las Heras Manso (2008)[26]	Mataró/targeted	204	Global 21.5, HbS 17.6	Sub-Saharan adult population

Hb, haemoglobin; SCD, sickle cell disease.

Mañú M.& Vives-Corrons J Clin Pathol 2009;62:22–25. doi:10.1136/jcp.2008.058834

Table 2. Distribution of African immigrants and indigenous residents of African ethnicity in the different regions of Spain

Region	Total population	African countries (%)	North Africa (%)	Sub-Saharan (%)
Andalusia	8 177 805	120 295 (1.47)	98 298 (1.20)	21 997 (0.27)
Aragón	1 325 272	31 282 (2.36)	19 699 (1.49)	11 583 (0.87)
Asturias	1 079 215	3738 (0.35)	2022 (0.19)	1716 (0.16)
The Balearic Islands	1 071 221	30 027 (2.80)	21 634 (2.02)	8393 (0.78)
The Canary Islands	2 070 465	27 804 (1.34)	16 733 (0.81)	11 071 (0.53)
Cantabria	581 215	2523 (0.43)	1526 (0.26)	997 (0.17)
Castilla-León	2 553 301	20 919 (0.82)	18 068 (0.71)	2851 (0.11)
Castilla-La Mancha	2 038 956	34 209 (1.68)	30 188 (1.48)	4021 (0.20)
Catalonia	7 354 441	275 746 (3.75)	216 180 (2.94)	59 566 (0.81)
Valencia	5 016 348	102 377 (2.04)	82 366 (1.64)	20 011 (0.40)
Extremadura	1 095 894	9847 (0.90)	9378 (0.86)	469 (0.04)
Galicia	2 783 100	8549 (0.31)	5146 (0.18)	3403 (0.12)
Madrid	6 251 876	112 860 (1.81)	78 817 (1.26)	34 043 (0.54)
Murcia	1 424 063	67 863 (4.77)	60 818 (4.27)	7045 (0.49)
Navarra	619 114	12 984 (2.10)	10 069 (1.63)	2915 (0.47)
Basque Country	2 155 546	20 089 (0.93)	13 344 (0.62)	6745 (0.31)
La Rioja	3 17 020	9532 (3.01)	8096 (2.55)	1436 (0.45)
Ceuta*	77 320	2620 (3.39)	2610 (3.38)	10 (0.01)
Melilla*	71 339	5225 (7.32)	5215 (7.31)	10 (0.01)

* Regions in the north of the African continent.

Mañú M.& Vives-Corrons J Clin Pathol 2009;62:22–25. doi:10.1136/jcp.2008.058834

RESULTS FROM THE REPUBLIC OF NORTH CYPRUS THALASSEMIA PREVENTION PROGRAM

Specialist Dr. Gülsen Bozkurt
Pediatric Haematologist
Lefkoşe-CYPRUS

Thalassemia and haemoglobinopathies are global health problems and also are serious health problems in Cyprus. The first scientific studies on thalassemia started in 1976 after a seminar which was organized by the Turkish Hematology Association. Than W.H.O.'s haemoglobinopahy director Dr. Bernadette Modell and local health authorities decided to provide a "thalassemia prevention program" in order to control thalassemia and related disease. The aim was to stop the affected newborns and provide good treatment facilities to the existing thalassemic patients. In 1979, high risk families started to be screened for thalassemia. In 1980, premarital screening was made compulsory by law. In 1984, prenatal diagnosis was started with fetal blood sampling techniques. DNA techniques replaced fetal blood sampling in 1991. After prenatal diagnosis started in 1984, affected birth rates showed a sharp decrease in contrast to an average of 18–20 cases per year. No thalassemic babies have been born in since 2001. But in the last few years some thalaessemic and sickle cell babies born from the uncontrolled population due to latest demographic change in the island.

Keywords: Thalassaemia, thalassaemia prevention program, genetic counseling, prenatal diagnosis

BACKGROUND

Thalassaemia was a great health problem in Cyprus. The first information about the disease was found in Dr. Alan Fawdry's reports, written while he was on duty in Cyprus during 1944–1946 **(1)**. For centuries, malaria was endemic in Cyprus and the incidence of the disease was very high. Malaria caused serious socio-economic damage and a lot of people, especially children, died because of the disease. The survivors of malaria had a microcytic hypochromic anemia and enlarged spleens. Subsequently, babies were born with severe hemolytic anemia with the characteristics of thalassemia. Malaria was completely controlled with successful eradication programs during the years 1946 to 1950 **(2, 3)**. After malaria, thalassemia seemed to be the second most serious public health problem on the island, as one in230 children under 10 years old were found to be thalassemic **(1)**.

THALASSEMIA PREVENTION AND CONTROL PROGRAM

After the Alan Fawdry's reports on thalassemia problem (1944-46), there was no any serious studies were done to stop thalassaemia, because of the political problems in Cyprus during 1950-1974. In 1976, the Turkish Hematology Association organized a seminar in North Cyprus, where Prof. Muzaffer Aksoy, Ayhan Çavdar and Ayten Arcasoy, Çigdem Altay and many more hematologists were present to discuss thalassaemia. In 1978 some doctors and the families of thalassaemic children, formed the "Thalassaemia Association", to fight against the disease. Dr. Bernadette Modell from University College London, UK and the counselor of hemoglobinopathies from the World Health Organization (WHO) was invited to Cyprus to examine thalassemic patients and study ways of controlling and treating thalassaemia. Dr. Modell's report in 1979 also concluded that thalassemia was a major public health problem in Cyprus and it was projected that, if thalassemia were not prevented, the number of thalassemic patients in Cyprus would have increased to 2,000 in 50 years. This would have caused a serious social, economic and psycho-

logical problem. Therefore, it was necessary to establish an effective thalassemia control program in Cyprus. Dr. Ayten Berkalp, the undersecretary of the North Cyprus Health Services and Dr. Modell and local doctors, studied the "Prevention and Control Program". Professor Aksoy and his colleagues visited Cyprus several times and gave their support to set up a thalassemia center. As thalassemia was a genetic disease and possible to control, protective measures had to be undertaken and implemented to stop thalassemic newborns. It was also important to treat the existing thalassemic patients with appropriate treatment modalities to provide them with a better and longer life expectancy.

PREVENTION MEASURES

1- Education and Social Awareness Programs for Thalassemia

At the beginning of the program the public was given information about thalassemia via the media, newspapers and brochures. Conferences on thalassemia took place at schools, universities and military quarters to inform the youth about the risk of thalassemia. Gynecologists were strongly advised to carry out diagnostic tests on pregnant women who had not taken the test before.

2- Laboratory Screening for Thalassemia Carriers and Genetic Counseling

As to control and stop new thalassemic births, since from 1980, all couples have to do, thalassemia carrier screening test before marriage. After identifying the thalassemia carriers, genetic counseling is given in a basic and clear language that can be easily understood. Genetic counseling is given by specialist doctors or by clinical geneticists. Genetic counseling is the provision of advice for couples with a history of inherited anemia (both subjects being thalassemia carriers) and who wish to have children, including the likelihood of having an affected child, the course and management of the disorder. There are situations when couples accept to go through with their marriage and others who change their mind on getting married. Nevertheless, those who decide to go ahead with marriage are strongly advised to contact the Thalassemia Centre, during pregnancy to have prenatal diagnosis.

3- Prenatal Diagnosis

Where couples are both thalassemia carriers, every pregnancy have 25% risk of giving birth to a child affected with thalassemia major. This is a high risk and should not be ignored. Thus, it was necessary to establish a prenatal diagnosis center for thalassemia. For this purpose, gynecologists were sent to England for training. At the same time, qualified staff were trained to carry out tests in the thalassemia laboratory and appropriate equipment was purchased. The prenatal diagnosis program was started in 1984.

METHODS USED IN PRENATAL DIAGNOSIS

At the beginning of 1984, the fetal blood sampling method was used, a practice done throughout the world. At 18–20 weeks' gestation, a fetal blood sample was taken transabdominally from the cord blood of the fetus with a fiberoptic instrument called a fetoscope. This procedure was quite stressful and difficult because of the advanced gestational age of 22–24 weeks.

Since 1991, the procedure with DNA through chorionic villus sampling (CVS) at 10–12 gestational age, has replaced fetal blood sampling. We have been using this technique since 1991. In this method, termination of an affected baby is easier, as the procedure is carried out earlier in the pregnancy, at 10-12 weeks of gestation. Some families are using preimlantation genetic diagnosis (PGD) techniques to have healthy babies, which is available in Cyprus.

TREATMENT OF THALASSEMIA

We had about 200 hundred thalassemic patiens who need, better treatment facilities to live longer and have a better life. Sufficient and safe blood transfusions, effective chelation, prevention from infectious diseases, management of endocrinal, cardiologic, immunological and psychological problems are important for treatment of a thalassemic patient.

Thalassemia major patient depend on life-long red blood cell transfusions. We tried to obtain enough and safe blood to maintain the pre-transfusion hemoglobin (Hb) levels, around 9.0 and 10.5 g/dl

according to the moderate transfusion program that has been universally adopted and recommended by the Thalassemia International Federation (TIF), Nicosia, Cyprus **(4)**. Leukocyte-depleted packed red cells were transfused through bedside leukocyte removal filters, in 2 or 4 week periods to the thalassemic patients.

Effective chelation was essential. Desferal has been available since the early 1980s. Patients were encouraged to use desferal via subcutaneous infu sion pumps. Some heavily iron overloaded patients used desferal with intravenous devices (Port-a-Cath set). Oral chelators were also available. Ferriprox has been used since 2003, Exjade since 2006, especially by patients who have hepatic and/or cardiac iron overload and who have difficulty in using desferal with subcutaneous infusors.

Magnetic resonance imaging (MRI R2 and T2*) are the only reliable, noninvasive assessment for the presence of excess iron in the liver and heart **(5)**. We have been able to perform these techniques since 2002. These techniques have helped us to adjust the chelator doses. The use of desferal alone, or in combination with ferriprox or Exjade, saved patients from the dangers of iron-induced free radicals.

To safeguard patients from viral infections through blood transfusions and immunological reactions was important. Donor screening for Aids (HIV), Hepatitis B (HBV) and Hepatitis C (HCV) viruses and syphillis is routinely done in our Blood Bank at Dr. Burhan Nalbantoglu State Hospital, with improved, fully automated instruments.

The endocrinal complications due to iron overload become apparent in the second decade of life **(6)**. If this complication, were not appropriately managed, somatic and gonad insufficiency would become apparent. Thus, it is necessary to use replacement therapies to achieve a better quality of life. Necessary hormone therapies are given in cases of insufficiency. The incidence of diabetes mellitus is also higher in these patients than in the normal population.

Good psychological and social support to provide these patients with a normal life is important. As psychological problems are often seen in chronic diseases, psychological support and encouragement to live as a normal person, could be useful. We encourage our patients to live a normal life, continue education, find jobs, get married and have children.

RESULTS

More than forty years after the implementation of the "Thalassaemia Prevention Program," significant progress has been achieved. Since 1980, 4,500–5,000 people have been screened every year. Despite of the social awareness studies, some opinion polls (which were carried out a few times) showed that, only 42% of the population is coming voluntarily to be screened, the remaining 58% came as it is compulsory.

The screening program demonstrated that the Turkish Cypriot community had the high incidence of β-thal %16,4 and also α-Thalassemia is also common considering the prevalence of HbH disease. Through DNA testing, it will be possible to detect α-thal carriers more accurately.

MOLECULAR PATHOLOGY

In 1987, blood samples from thalassemic patients, was sent, to Antonio Cao's laboratory in Sardinia with our thalassemia laboratory senior chef for screening beta thalassemia mutations. Five mutations were detected among the Turkish Cypriots **(7)**. A similar study was carried out at Professor Titus H.J. Huisman's laboratory at the Medical College of Georgia, Augusta, GA, USA. The results were similar to those described in the previous study. The following β-thal mutations were found: IVS-I-110 (G>A) 74.1%, IVS-I-1(G>A) 7.3%, IVS-I-6 (T>C) 7.8%, IVS-II-745 (C>G) 6.5%, codon 39 (C>T) 0.9%, unknown 3.4% **(8)**.

In 1993, blood samples from patients with Hb H disease, screened for alfa thalassemia allels at Professor Huisman's laboratory at Augusta, GA, USA. As α-thalassemias were common in Cyprus, the demonstration of α-globin genotypes would be useful for a better understanding of the clinical phenotype of Hb H disease. The samples were studied and 74% deletional and 26% nondeletional alleles were identified. The deletional alleles were as follows: $\alpha^{3.7}$/(30%), $\alpha^{MED\ I}$/(36%), $\alpha^{MED\ II}$/(4%) and

$\alpha^{20.5}$/(4%). The nondeletional types were α^{PA-1}/(12%), α^{PA-2}/(4%), and α^{5nt}/(10%). Eight different α-globin genotypes were detected with various combinations of deletional and nondeletional alleles **(9)**.

SUCCESS OF THE PRENATAL DIAGNOSIS PROGRAM IN NORTHERN CYPRUS

When we started to screen the community and gave genetic counseling to the families at risk for thalassemia, and after the inauguration of the prenatal diagnosis program in 1984, the number of affected newborns decreased dramatically from 18–20 to 6–7 per year **(Figure 1)**. Thereafter, the numbers dwindled down to one or two per year. Two of the affected babies born after1984, were due to misdiagnosis during the first years of our prenatal diagnosis experience. After DNA methods were established in 1991, only five affected babies were born due to different reasons, in a 10- year period. No affected baby has been born since 2001. But in the recent years some thalassemic and sickle cell babies were born because of the uncontrolled population who came from different countries.

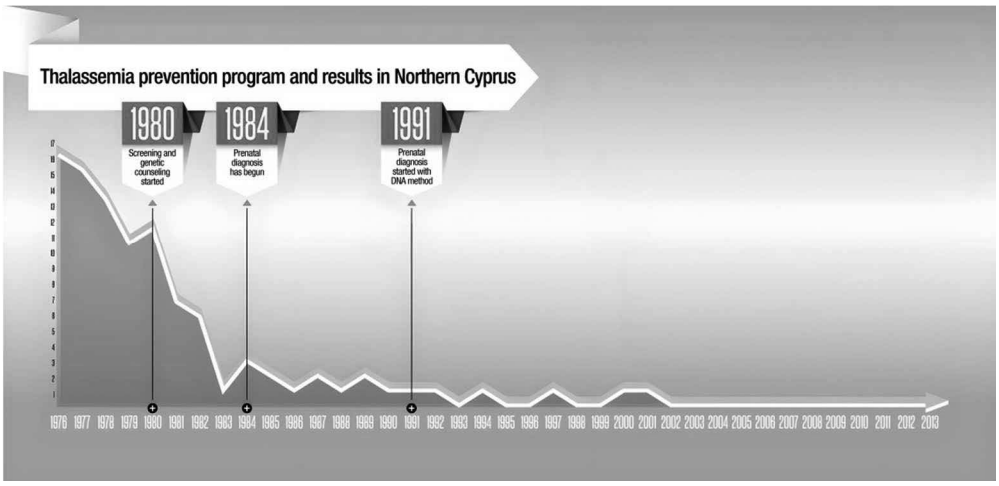


Figure 1: *Graph showing the decrease in the number of babies born with homozygous β-thal in North Cyprus (1976–2006).*

THE SURVIVAL RATE AND QUALITY OF LIFE OF THE THALASSEMIC PATIENTS

Significant progress has been achieved in the quality of life and survival of thalassemic patients. The median survival of the thalasemic patiens is 38,9 years.

We encourage thalassemic patients to have a life as normal persons, work, marry and also have family and children. Most of the adult thalassemic patients have jobs; great majority of them are married and have children. We strongly recommend them to have family with a genetically normal person, so as not to have affected babies. Most of them have normal spouses and healthy children. Interestingly, in recent years there have been marriages between thalassemic subjects, and some have even had babies through *in vitro* fertilization with ovum or sperm from healthy donors.

CONCLUSION

In the past 40 years, the implementation of effective measures have yielded a substantial success in the fight against thalassaemia. Affected new- borns have dramatically decreased and thalassemic patients have a longer and better quality of life.

Thalassaemia and haemoglobinopathies are genetic diseases that are preventable through programs that are implemented on a national level. Considering the fact that genetic diseases will continue for generations, programs to prevent them should be continuous and permanent within the framework of a state policy. Cyprus studies provide a good example of this.

REFERENCES

1. Modell B, Berdoukas V. Thalassaemia in Cyprus. In: Modell B, Ed. The Clinical Approaches to Thalassaemia. London: Grune & Stratton. 1984; 263–277.
2. Aziz M. History of prevention of malaria in Cyprus. Cyprus Med J 1947; 1(2):13–17.
3. Kent G. Total victory over malaria. Reader's Digest February 1951; 77–79.
4. Thalassaemia International Federation: guidelines for the clinical management of thalassaemia. Nicosia, Cyprus TIF, 2000.
5. Wonke B. Clinical management of β-thalassemia major. Semin Haematol 2001; 38(4):350–359.
6. De Sanctis V, Printor C, Gamberini MR. Multicenter study on prevalence of endocrine complications in thalassemia major. Clin Endocrinol 1995; 42:581–586.
7. Sozuöz A, Berkalp A, Figus A, et.al β-Thalassemia mutations in Turkish Cypriots. J Med Genet 1988; 25(11):766–768.
8. Baysal E, Indrak K, Bozkurt G, et.al. The β-thalassaemia mutations in the population of Cyprus. Br J Haematol 1992; 18(4):607–609.
9. Baysal E, Kleanthous M, Bozkurt G, et.al. Alfa - Thalassaemia in the population of Cyprus. Br J Haematol 1995; 89(3):496–499.

Correspondance Address:
Specialist Dr. Gülsen Bozkurt
Pediatric Hematologist
Lefkoşe-CYPRUS
Mail: gulsenbozkurt1@yahoo.com.tr

EPIDEMIOLOGY AND MOLECULAR BASIS OF BETA THALASSEMIA HETEROZYGOTES IN GREECE

Christos Kattamis[1] and Myrto Skafida[2]

1. Emeritus Professor of Pediatrics. Thalassemia Unit, 1st Department of Pediatrics, Medical School, National and Kapodistrian University of Athens, Aghia Sophia Children's Hospital, 11527 Athens, Greece.

2. Pediatric Chief Resident. Department of Pediatrics, SUNY Downstate Health Sciences University, Brooklyn, USA

ABSTRACT

Background. Greece is one of the few European countries with a high prevalence of β-thalassemia trait.

Objectives. The main objectives of this presentation are to review: i) the studies of the prevalence and geographic distribution of β-thalassemia heterozygotes and ii) the type and incidence of β globin gene variants of β-thal. heterozygotes in Greece.

Results. Extensive studies on the prevalence of β-thal heterozygotes demonstrated an incidence of 8.0% for the whole population and a wide heterogeneous geographical distribution with regions (prefectures) of low (<5%), moderate (5-9.9%), high (10-14.9%) and very high (<15% up to 20%) incidence. Sickle cell trait was restricted in areas of few regions with high malaria endemicity in the past.

Molecular studies on 6702 β-thal heterozygotes identified 30 β globin gene variants. The most common with an incidence >5%, were: IVSI-110G>A(β^{+}); CD39C>T (β^{o}); IVSI 1G>A(β^{o}); IVSI-6T>A(β^{++}) and IVSII-745C>G(β^{+}). The five variants covered 87.6% of β-thal-heterozygotes. The mild (β^{++}) and the very mild β^{sil}. variants had very low incidence (<0.50%) except, -87G>A (β^{++}) and -101C>T (β^{sil}.) with an incidence >0.50%. The two variants were mainly identified in parents of patients with β-thalassemia Intermedia.

Identification of β-globin gene variants is crucial in predicting the severity of clinical phenotype on diagnosis, guiding the selection and monitoring of treatment; in assisting genetic counselling and in upgrading prenatal and pre-implantation diagnosis.

The epidemiological studies on β-thal-heterozygotes were used for the evaluation of the impact of β-thalassemia anemia in Greece; the high impact urged the implementation of the national treatment program supplemented later by the prevention program. Prolonged enforcement of both programs leaded to a progressive reduction of the incidence of patients with β-thalassemia, and to an improvement of the burden of β-thalassemia, during the last decades, in Greece

Keywords: β-thalassemia heterozygotes, incidence, molecular basis, Greece

INTRODUCTION

Greece is one of the few European countries with noteworthy incidence of β-Thalassemia that poses a significant challenge on the National Health System (NHS). Originally, on the implementation of universal treatment of patients with frequent transfusions and oral chelation, the burden to NHS was worsening, as treatment was leading to a progressive increase of patients with β-thalassemia due to the considerable improvement of survival. In contrast during the last three decades a progressive decrease in the incidence of β-thalassemia and a reduction of its burden was observed due to the integration of the efficient national prevention program that reduced the annual input of new cases by 95% **(1, 2)**.

In the second half of 20th century screening procedures for the detection of β-thalassemia heterozygotes (β-thal trait, or β-thal carriers), were developed, and epidemiological studies for the incidence of β-thal trait were conducted in European countries with high incidence of β-thalassemia, as Greece,

Italy, and Cyprus. In Greece extensive studies started in the 60's, identified a high incidence of β-thal trait with a wide heterogeneity in geographical distribution **(3, 4)**.

At this period the incidence of β-thal trait was used as an index to assess the expected annual births of neonates with homozygous β-thalassemia. This assessment is based on the incidence of β-thal trait in the population and on the total annual live births. This index was used later by WHO to evaluate the worldwide expected annual rate of births of neonates with β-thalassemia and other hemoglobinopathies, worldwide.

Since the 90's, molecular methods were used for the worldwide characterization of β-globin gene variants. Up to now more than three hundreds β-globin gene variants have been identified. Of interest is that in any population only few variants prevail and these variants are considered as specific for that population **(5)**.

Identification of the type and severity of β-gene variants in a population is of primary importance for predicting the severity of clinical phenotype and precise counselling.

In this presentation we shall review in brief: i) the studies on the epidemiology and geographical distribution of β-thal trait in Greece during 60's to early 70's ii) the type, severity and incidence of β-gene variants prevailing in Greece and iii) the importance of the epidemiology and molecular basis of β-thal trait on the epidemiology of patients with β-thalassemia in Greece.

EPIDEMIOLOGY OF β-THAL. HETEROZYGOUS IN GREECE

Soon after the development of hematological and biochemical methods for screening and diagnosis of β-thal carriers, extensive studies on the epidemiology for β-thal-trait were conducted in Greece, by several groups.

In this chapter we review in brief the findings of these studies that are classified in two groups. One group focused exclusively on the epidemiology of β-thal trait and incidentally on sickle cell trait. This group included studies on populations of recruits from all over Greece (**4, 6)** and on series of micro and macro-population studies in the mainland and the islands **(7, 10)**. The second group of studies was focused on selected areas, of high malaria endemicity in the past, investigating the incidence of three inherited red cells abnormalities: β-thal trait, sickle cell trait and Glucose -6- phosphate dehydrogenase deficiency (G-6-PD). The main goal of these studies was to test the hypothesis that the three red cell abnormalities provided benefits against falciparum infection. The outcomes of the studies aligned with the initial hypothesis (**11, 12)**.

For historical interest, we are describing the algorithm of laboratory process used for detection and diagnosis of β-thal-trait at his period **(Figure 1)**. This algorithm varies in methods, structure, and much more in credibility, from recent algorithms.

The most important and creditable test to detect suspected individuals with β-thal trait, even on the spot, was the osmotic fragility test. For the test, three NaCl buffer solutions were used: 0.32, 0.36 and 0.40%. In our studies we used 0.36% solution which was proved most effective. Of 445 genetically designated and hematological confirmed β-thal carriers 436 (98%)were positive using the osmotic fragility test of 0,36% **(13)**.

In individuals positive to osmotic fragility test the process for final diagnosis included: Complete Blood Count (CBC), namely: Hemoglobin, packed red cell volume, red cell count (done as a rule visually, as automated analysis was not available!), and red cell morphology, studied on stained blood smears **(6)** HbA2 quantification; estimated on paper or starch electrophoresis and later on cellulose acetate while HbF was assessed biochemically by the method of Chernoff and Singer **(6)**.

Thus, the diagnosis of β-thal heterozygotes was based on the hematological criteria. of: decreased osmotic fragility; normal or mildly decreased Hb; changes in red cell morphology (microcytosis, hypochromia, anisocytosis, poikilocytosis and basophilic stippling); low MCV <80 fl and low MCV <27pg and on the biochemical criteria of HbA2 >3;5% and normal or mildly increased <4% HbF.

The hematological and biochemical methods used in this period were not as sensitive and accurate as the recent methods of screening and diagnosis of β-thal carriers. However, they were reliable for the

detection and diagnosis of the classical high HbA2 type of β-thal trait. Rare types of β-thal trait could escape diagnosis as were the two types, (mild and severe), of normal HbA2 and HbF β-thal trait reported later in Greece **(14)**.

The epidemiology and geographical distribution of β-thal trait in Greece is illustrated in Figure 2. The results in brief were: within the whole population the incidence of β-thal-trait was 8% and for sickle - cell trait 1%; for β-thal trait the geographical distribution was extremely heterogenous. There were regions with very low incidence (<5%) mainly in Northern Greece, with moderate (5-9.9%), and with high (10-14.9%), and with very high (>15%) incidence, as were the areas of Karditsa and the islands of Rhodes, Cyprus, Corfu, Lesbos and others **(Figure 2)**.

Of interest were the wide variation in the incidence of β-thal trait, found in micro population studies in the area of Arta between different villages of the area **(8)**.

Although in the past malaria was endemic in many regions of Greece, it is of interest that sickle cell trait was detected in areas of only five regions. As indicated in Figure 2, the prevalence of sickle cell trait in these areas, ranged from 4.5% to 23%.

To evaluate the magnitude of the burden of β-thalassemia disease the total number of the expected infected newborns born during the period 1963-72 was estimated using the index of the expected annual rate of infected newborns based on the incidence of 8% of β thal trait and the total annual number of live births in Greece This index for Greece was and is 160 patients per 100.000 live births During this period the annual rate of births was ~150,000 and that of the expected affected newborns 240-250 ; thus the expected total number of patients for the ten years period was ~2,500 newborns with β-thalassemia. This number was considered as a very high burden for NHS. Similar numbers were found by calculating the expected annual birth rates of neonates affected with thalassemia in the 10-years period, separately for each region of the country.

Based on these findings NHS started a national program for the treatment of affected children, organizing Thalassemia Units, originally in Children's hospitals, starting from our hospital **(15)**; years later, thalassemia Units for adults, joined with hematology Units. were organized. At present more than 30 thalassemia units cover all β-thalassemia in Greece.

As the epidemiology of β-thal-trait remained stable no further extensive epidemiological studies in Greece were organized. One large study in a population of 64.814 recruits, published in 1987 reported similar findings to the studies of the early period **(16)**.

MOLECULAR BASIS OF β- THAL HETEROZYGOTES

Worldwide-studies on the molecular basis of β-thal-trait identified more than 300 β-globin gene variants. Based on the degree of impairment of β chain synthesis these variants are classified in four phenotypes namely: i) β^{o} with a total deficiency of β chains synthesis; ii) β^{+}, with severe impairment; iii) β^{++} with mild impairment; and iv) the very mild, β^{+++} or β silent (β^{sil}) with very mild impairment of β chain synthesis. The very mild variants may escape diagnosis on classical screening.

Worldwide studies demonstrated that in each population only few variants cover more than 80% of the β-thal trait of the country's population; thus, these variants are characterized as the country's specific β-globin gene variants **(5)**.

We will review in brief the results of three studies on the type, severity, and incidence of β-globin gene variants in Greek β-thal heterozygotes published to date.

In these studies, standard molecular methods were used for the identification of β-globin gene variants. They were identified by dot-blot hybridization of nucleotide probes to genomic DNA, amplified by polymerase chain reaction (PCR) procedure, by direct sequencing of amplified DNA, and by gene mapping **(17)**.

The combined data of the three studies cover a cohort of 6,702 β-thal heterozygotes. The type, severity and incidence of the β-globin gene variants are summarized in Table 1.

The number of β-thal- heterozygotes in each, of the three studies were: 348; in Kattamis et al 1990 **(17)**;

3,796 in Bousiou et al. 2008 **(18)**; and 2,558 in Kattamis et al. 2022 **(19)**.

In the whole cohort of 6,702 β-thal heterozygotes, a total of 30 β-globin gene variants were identified. All but two were single point variants. The majority were considered severe phenotypes, β^{o} (16 variants), β^{+} (8 variants); and the remaining mild phenotypes, β^{++}(3 variants) and β^{sil} (3 variants).

The most prevalent variants with an incidence >5%, characterized as specific for the Greek population were the: IVSI-110G>A (β^{+}) with an incidence of 41.49%; CD39C>T (β^{o}), incidence 18.27%; IVSI IVSI-1G>A (β^{o}), incidence 12.54%; IVSI 6T>A (β^{++}), incidence, 8.93%; and IVSII-745G>A (β^{+}), incidence.5.8%. The five variants cover 87.60% of the whole cohort.

In Table 1, there is another interesting subgroup of 7 rather common variants with an incidence varying from 3.07-0.79%. Among them there are two very mild variants, -87C>G and -101C>T and a most interesting novel variant, the IVSI-5G>A plus Corfu delta variant (β+), with an incidence of 0.85%. Up to now this variant have been exclusively reported in β-thal heterozygotes and in homozygous and double heterozygous Greek patients (**19, 20**). This variant was first identified in a homozygous patient from our Unit **(21)**.

In conclusion, the molecular basis of β-thal heterozygotes in Greece is basically related to the severe β^{o} and β^{+} globin gene variants which lead basically to homozygous patients with the severe clinical phenotype of transfusion dependent thalassemia major β^{o}/β^{o}, β^{o}/β^{+}, β^{+}/β^{+}, covering about 80% of β than patients, in Greece **(22)**.

DISCUSSION

The epidemiology of β-thal heterozygotes (trait, or carriers) used to be, and still is, an index for the evaluation of the impact on health of β-thalassemia anemia in countries where it prevails. To this end the incidence of β-thal trait is used to estimate the expected annual rate of births of affected neonates. This index is expressed as the number of affected neonates per 1,000 or 100,000 live births (**1, 2**).

In Greece, prior to the implementation of prevention program, this index was estimated as 1.6 affected neonates per 1,000 or 160 per 100,000 live births. Since the integration of prevention program in mid-90's this index was reduced to only 0.1 per 1,000 or 10 per 100,000 live births **(1, 2)**.

As there was strong evidence that the epidemiology of β-thal trait remains stable no further studies on the epidemiology of β-thal trait were organized in Greece. However, variabilities in the geographical distribution of β-thal trait are expected as there was a considerable influx of people moving from rural to urban areas, during the last decades.

Another factor that may influence the epidemiology, mainly of β-thalassemia anemia, in Greece is the recent influx of immigrant patients from Albania (in northwest) and, from Iran, Pakistan, Philippines, and other countries from the East.

Of major interest are the findings from the molecular characterization of β globin gene variants in 6,702 Greek β-thal heterozygotes. The molecular characterization of β-thal carriers upgraded both prevention and treatment programs.

Regarding the prevention program, identification of β-gene variants allowed: the precise genetic counselling of parents; the transfer of prenatal diagnosis from 15^{th} to 12^{th} week of gestation; the quick and precise antenatal diagnosis; and the. preimplantation diagnosis, based on the β-gene variants of both parents.

Considering treatment, identification of β gene variants in the parents supplemented with molecular characterization of genotype of patient on diagnosis facilitates the prediction of the severity of clinical phenotype guiding the choice of treatment and. its monitoring.

To our experience genotype characterization on diagnosis greatly assists the precise diagnosis of patients with Thalassemia Intermedia (TI). In these patients the genotype contains one of the mild β^{++}, or the very mild β^{sil} variants; as the β^{sil} -101G>T **(23)**,+1480 C>G (**24**), and the β++ -87G>C. The common mild β++ IVSI-6T>A, is not always related to TI, particularly when combined with α βo variant.

The studies on the epidemiology-and molecular basis of β-thal trait give limited information on the burden of β-thalassemia anemia on health services of a country. The precise evaluation of the burden

on health is basically related to the epidemiology of patients with β-thalassemia, which is dynamic, since treatment with frequent transfusions prolonged survival. Since then, the estimation of the prevalence of patients with β thalassemia in a country is a real time event, running as a rule, in parallel to an annual or to a 2-5 or more years intervals national registry of patients with β-thalassemia.

In Greece, two national surveys on β-thalassemia, hemoglobinopathy H disease (HH) and sickle cell disease were carried out in 2010 and 2015 **(25)**. The findings of these surveys, for β-thalassemia and HH are summarized in Table 2.

The total number of patients with β-thalassemia, major and intermedia (TM/TI) were 3,721 in 2010. survey versus 2752 in 2015; a reduction of -14.9%. In contrast the number of patients with HH disease. increased from 178 to 213; an increase of +20%. Furthermore. the estimation of the incidence of β-thalassemia in 2010 was 35.7 patients per 100,000 population versus 26,2 per 100,000 in 2015. In an arbitrary estimation of the incidence of β-thalassemia in 1990, in more than 5,500 patients an incidence of 52.3 patients per 100,000 was found (Unpublished data).

The above data clearly demonstrate the progressive reduction of the incidence of patients with β-thalassemia in Greece This is mainly due to the longstanding effective prevention program, that minimized the annual in-put of new patients, while the out-put remains constant. The progressive reduction of the incidence of β-thalassemia is related to a relative amelioration of the burden of β- thalassemia in Greece. In contrast, hemoglobinopathy H disease for which there is no prevention program, increased by 20% in five years.

In conclusion, from our experience, at present and at the near feature, the most effective schedule to minimize the burden of β-thalassemia in a population where β--thalassemia prevails is to supplement the effective treatment programs with an equally effective prevention program. Otherwise, an efficient treatment program alone, should progressively increase the incidence of β-thalassemia and the burden of the disease. At present this situation seems to exist in most developing and undeveloped countries.

REFERENCES

1. Kattamis C . Efficacy of prolonged implementation of national programs for prevention and treatment of β-thalassemia patients. The Greek experience. *J Hematol Transf* 2016;5(2):1066-71.
2. Ladis V, Karagiorga-Lagana M, Tsatra I, Chouliaras G. Thirty-year experience in preventing haemoglobinopathies in Greece: achievements and potentials for optimisation. *Eur J Haematol*. Apr 2013;90(4):313-22.
3. Kattamis C . *Screening for hemoglobinopathies*. Bickel H, Guthrie R, Hammersen G, Eds Neonatal screening for inborn errors of metabolism Springer Berlin Heidelberg; 137-147.
4. Schizas N, Tegos A, Voutsadakis G, et.al. The incidence and distribution of β-thalassemia and abnormal hemoglobins in Greece. *Hell Armed Forces Med Rev*. 1977;11:197-203.
5. Weatherall DJ,Clegg IB. *The thalassemia syndromes*. 4 ed. Blackwell Science Oxford; 2001.
6. Malamos B, Fessas P, Stamatoyannopoulos G. Types of thalassaemia-trait carriers as revealed by a study of their incidence in Greece. *Br J Haematol*. Jan 1962;8:5-14.
7. Barnicot NA, Allison AC, Blumberg BS, et al. Haemoglobin types in Greek populations. *Ann Hum Genet*. Feb 1963;26:229-36.
8. Fraser GR, Stamatoyannopoulos G, Kattamis C, et al. Thalassemias, Abnormal Hemoglobins and Glucose-6-Phosphate Dehydrogenase Deficiency in the Arta Area of Greece: Diagnostic and Genetic Aspects of Complete Village Studies. *Ann N Y Acad Sci*. Oct 7 1964;119:415-35.
9. Kattamis C, Haidas S, Metaxotou-Mavromati A, et.al Beta-thalassaemia, G-6-PD deficiency, and atypical cholinesterase in Cyprus. *Br Med J*. Aug 19 1972;3(5824):470-1.
10. Kattamis C ,Lagos P, Metaxotou-Mavromati A, et.al. . Iron deficiency, G-6PDd, β- thalassemia and sickle cell trait in Europe. *Hell, Armed Forces Medical Review* 1977;11:191-196.
11. Choremis C, Fessas P, Kattamis C, et al. Three inherited red-cell abnormalities in a district of Greece. Thalassaemia, sickling, and glucose-6-phosphate-dehydrogenase deficiency. *Lancet*. Apr 27 1963;1(7287):907-9.
12. Stamatoyannopoulos G, Fessas P. Thalassaemia, Glucose-6-Phosphate Dehydrogenase Deficiency, Sickling, and Malarial Endemicity in Greece: A Study of Five Areas. *Br Med J*. Apr 4 1964;1(5387):875-9.
13. Kattamis C, Efremov G, Pootrakul S. Effectiveness of one tube osmotic fragility screening in detecting beta-thalassaemia trait. *J Med Genet*. Aug 1981;18(4):

14. Kattamis C, Metaxotou-Mavromati A, Wood WG,et.al. The heterogeneity of normal Hb A2-beta thalassaemia in Greece. *Br J Haematol*. 1979 may;42(1):109-23.
15. Kattamis C, Sofocleous C, Ladis V, Kattamis A. Athens University thalassemia expertise unit: evolution, structure, perspectives and patients' expectations. *Georgian Med News*. Sep 2013;(222):94-8.
16. Tegos C ,Voutsadakis A Paleologou et.al. The incidence and distribution of thalassemia in Greece (a study on 64,814 recruits. *Hell Armed Forces Med Rev*. 1987;(21):27-36.
17. Kattamis C, Hu H, Cheng G, et al. Molecular characterization of beta-thalassaemia in 174 Greek patients with thalassaemia major. *Br J Haematol*. Mar 1990;74(3):342-6.
18. Boussiou M, Karababa P, Sinopoulou K,et.al. The molecular heterogeneity of beta-thalassemia in Greece. *Blood Cells Mol Dis*. May-Jun 2008;40(3):317-9.
19. Kattamis C, Skafida M, Delaporta P, et al. Heterozygosity of the Complex Corfu delta0beta+ Thalassemic Allele (HBD Deletion and HBB:c.92+5G>A) Revisited. *Biology (Basel)*. Mar 11 2022;11(3)doi:10.3390/biology11030432
20. Wainscoat JS, Thein SL, Wood WG, et al. A novel deletion in the beta-globin gene complex. *Ann N Y Acad Sci*. 1985;445:20-7.
21. Traeger-Synodinos J, Tzetis M, Kanavakis E, et. al. The Corfu delta beta thalassaemia mutation in Greece: haematological phenotype and prevalence. *Br J Haematol*. Oct 1991;79(2):302-5.
22. Kattamis C, Metaxotou-Mavromati A, Ladis V,et.al.. The clinical phenotype of beta and delta beta thalassemias in Greece. *Eur J Pediatr*. Oct 1982;139(2):135-8.
23. Maragoudaki E, Kanavakis E, Traeger-Synodinos J, et al. Molecular, haematological and clinical studies of the -101 C --> T substitution of the beta-globin gene promoter in 25 beta-thalassaemia intermedia patients and 45 heterozygotes. *Br J Haematol*. Dec 1999;107(4):699-706.
24. Maragoudaki E, Vrettou C, Kanavakis E, et.al. Molecular, haematological and clinical studies of a silent beta-gene C-->G mutation at 6 bp 3' to the termination codon (+1480 C-->G) in twelve Greek families. *Br J Haematol*. Oct 1998;103(1):45-51.
25. Voskaridou E, Kattamis,A. Frangodimitri E, et. al. National Registry of haemoglobinopathies in Greece: update demographic current trends in affected births and causes of mortality. *Annals Hematology*. 2019;98:55-66.

A. Legend to figures.

Figure 1. Algorithm of laboratory process for screening and diagnosis of β- thalassemia heterozygotes in 60's and early 70's in Greece.

Figure 2. Epidemiology and geographical distribution of β-thalassemia trait in Greece.
(represents areas with coexisting incidence of sickle cell trait in regions with high malaria endemicity in the past)

B. Legends to tables.

Table 1. Type, phenotype and incidence of β- globin gene variants in 6,702 Greek β- thalassemia heterozygotes: data from three publications (17-19.)

Table 2. Prevalence of β-thalassemia Major and Intermedia , and Hemoglobinopathy H Disease reported in the two Greek national Surveys in 2010 and 2015.[25]

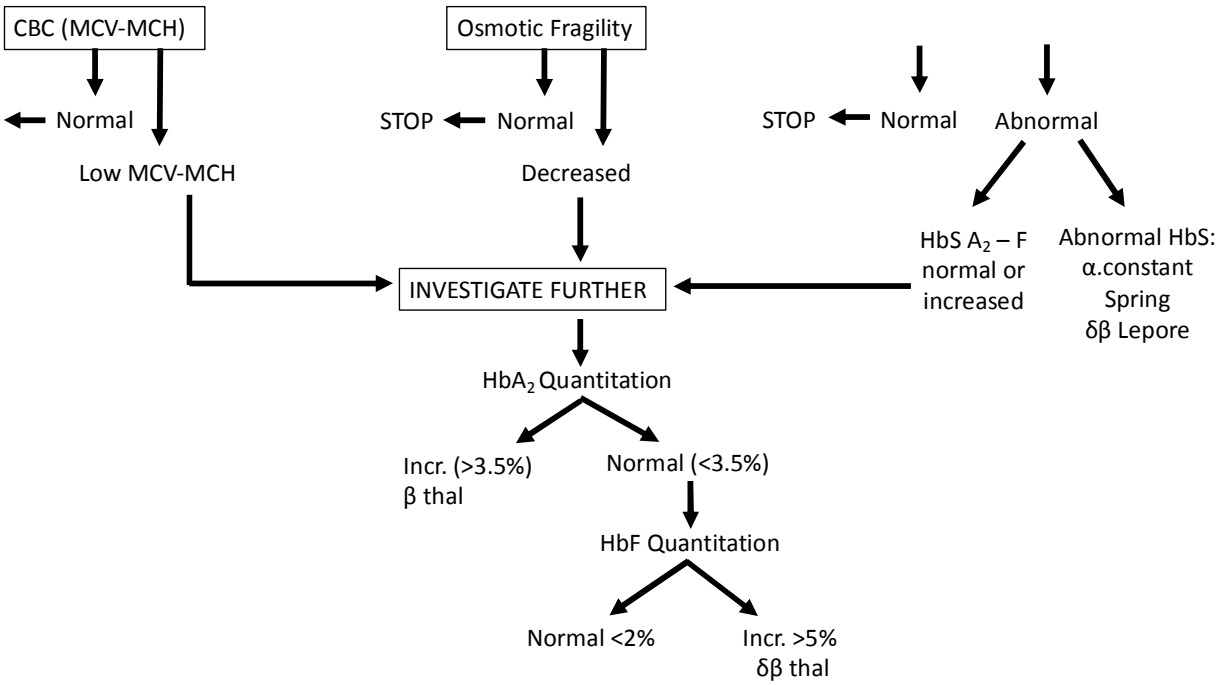


Figure 1: *Algorithm of laboratory process for screening and diagnosis of β-thal heterozygotes in the 60's and early 70's in Greece.*

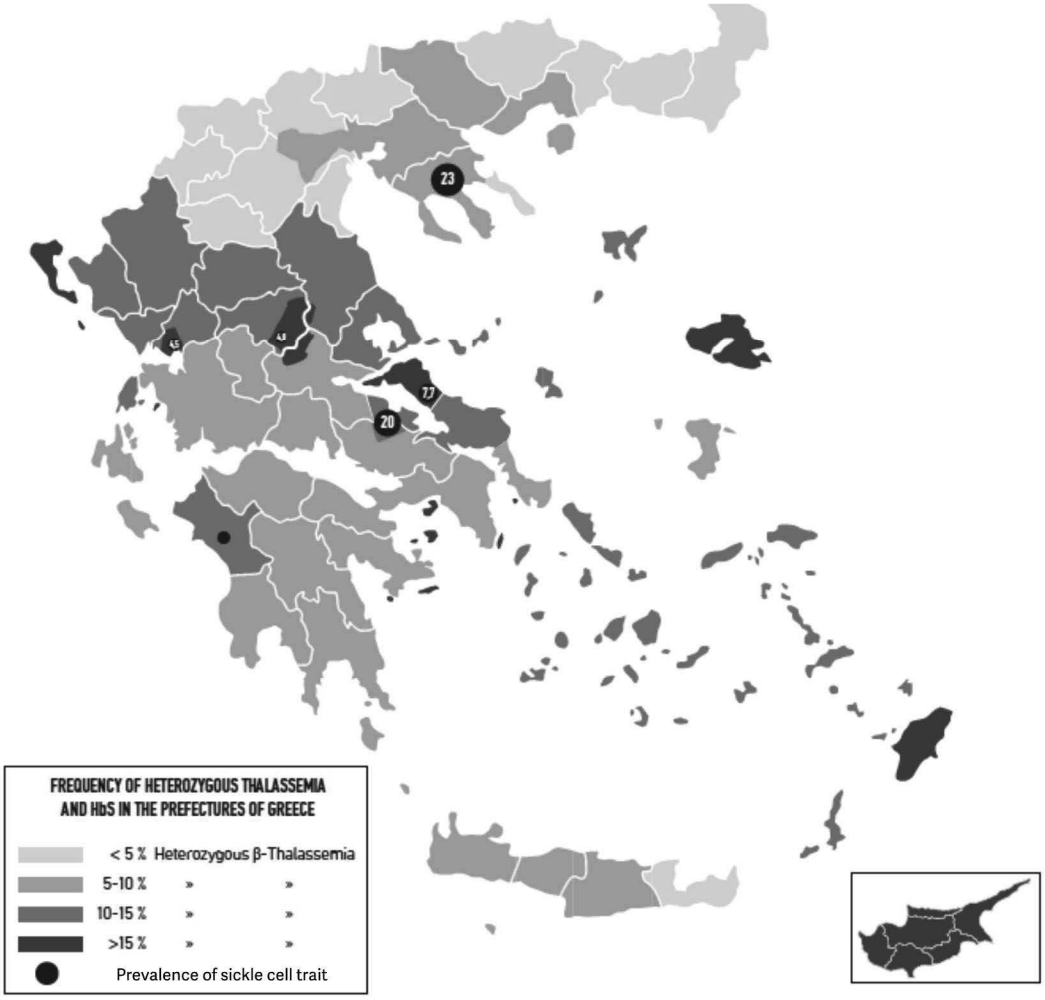


Figure 2: *Epidemiology and geographical distribution of β-thalassemia trait during 1963-72 in Greece. (represents areas with coexisting incidence of sickle cell trait in regions with high malaria endemicity in the past)*

Table 1: *Type, phenotype, and incidence of β- globin gene variants in 6,702 Greek β- thalassemia heterozygotes: data from three publications. (17-19)*

	β-gene variant	**Phenotype**	**No Cases**	**Incidence (%)**
1	IVSI-110 G>A	β^{+}	2781	41.49
2	CD39 C>T	β^{0}	1225	18.77
3	IVSI-1 G>A	β^{0}	839	12.54
4	IVSI-6 T>A	β^{++}	599	8.43
5	IVSII-745 C>G	β^{+}	389	5.8
	Total for variants 1-5		**3.835**	**87.6**
6	IVSII-1 G>A	β^{0}	206	3.07
7	cd6 (- A)	β^{0}	134	1.99
8	-87 C>G	β^{++}	113	1.68
9	-101 C>T	β^{sil}	105	1.56
10	IVSI-5 G>A | δ° Corfu	$\delta^{0}\beta^{+}$	57	0.85
11	CD5 (- CT)	β^{0}	53	0.79
12	CD8 (- AA)	β^{0}	53	0.76
	Total for variants 6-12		**721**	**10.75**
	Total for variants 1-12		**6.554**	**97.8**
	Rare mutations (0.5-0.1%)	$4\beta^{0}$, $1\beta^{+}$, $1\beta^{++}$,$2\beta^{sil}$	128	1.91
	Very rare mutations (<0.1%)	$6\beta^{0}$, $4\beta^{+}$	20	0.29
	Total for variants 1-30	**$16\beta^{0}$,$8\beta^{+}$,$3\beta^{++}$,$3\beta^{sil}$**	**6702**	**100**

Table 2: *Prevalence of β-thalassemia Major and Intermedia , and Hemoglobinopathy H Disease based on the Greek Registries in 2010 and 2015.[25]*

Thalassemia Type	Cases		Difference		Prevalence – patients per 100.000 people	
	2010	2015	Cases	%	2010	2015
TM	2485	2019	-466	-16%		
TI	756	660	-96	-13%		
TM + TI	3241	2759	-472	-14.9%	35.7	26.2
HH	178	213	+35	+20%	1.64	1.96

Legends TM: β- thalassemia major; TI: β-thalassemia intermedia; HH: Hemoglobinopathy H disease

Correspondence Address:

Christos Kattamis
E-mail: ckatamis@med.uoa.gr,
christos.kattamis@gmail.com,
Mail address: Christos Kattamis 41 Themistocleous Street, Alimos 17455, Greece
Tel: +30 210-98-23-639

THALASSEMIA AND HEMOGLOBINOPATHIES IN BULGARIA

Atanas Banchev[1,2], MD, PhD, Simona Simeonova[2], MD Katya Sapunarova[3], MD, PhD
[1.] National Haematology Hospital, Sofia, Bulgaria
[2.] Expert center for Haemophilia and Thalassemia, University Hospital "Tsaritsa Giovanna-ISUL", Medical University-Sofia, Bulgaria
[3.] Haematology department University Hospital "St. George", Plovdiv, Bulgaria, Medical University-Plovdiv, Bulgaria

ABSTRACT

The last two decades led to significant developments in the management of patients with transfusion dependent beta-Thalassemia (TDT) in Bulgaria. The current review discusses the epidemiology, genetics, assess to treatment of the Bulgarian TDT-patients. Special interest is allocated to the recent research and studies executed in the field of thalassemia in regard to the cardiac, pulmonary, liver, renal and endocrinological complications. Finally, the future possibilities for improvement of care for patients with TDT in Bulgaria are discussed.

Keywords: Thalassemia, Bulgaria

INTRODUCTION

In the last two decades significant advances have evolved in the management of patients with transfusion dependent beta-Thalassaemia (TDT). The use of oral iron chelators and the development of non-invasive technologies for the measurement of specific organ iron overload represent the two major advancements that have significantly and positively altered the prognosis of patients with thalassemia major **(1)**. On a regional level in Bulgaria those developments historically overlapped with a political period of transition from socialistic to liberal regimen. The fall of the Berlin wall allowed the TDT patients in Bulgaria to be treated according to the international standards.

The current review discusses the contemporary state of the Thalassaemia management and the advancements achieved over the last 20 years in the Republic of Bulgaria.

EPIDEMIOLOGY OF BETA THALASSEMIA IN BULGARIA

According to the national epidemiology registry for beta-Thalassemia in Bulgaria **(2)** there are 270 patients TDT in the country, of which 52.22% are males, and 47.78% - females aged between 0,5 years and 65 years. The nonstandardised prevalence of TDT confers 3.66/100 000 people. The incidence of heterozygous beta-Talassemia (Thalassemia minor) varies according to different sources, areas and methods of detection between 0.5 and 19% **(3)**. Currently it is postulated, that the frequency of beta-thalassemia trait among the Bulgarian population is about ca. 3% **(4)**, thus, the frequency is comparable to northern Italy and northern Greece **(5)**. Despite being presently located in the periphery of the Mediterranean region, the presence of such a high incidence of beta-Thalassemia trait among the population reflects the complex historical background of the Bulgarian nation, confirming the autochthonous origin of its people **(6)**.

The genetics of the Bulgarian thalassemia patients has been studied extensively over the last two decades and it has been postulated, that the most common mutation is IVS1-110G>A which can be detected in about 31% of the patients with thalassemia trait, followed by bo39C>T in about 20% of them **(Figures 1, 2) (7)**. Apart of the thalassemia mutations very often finding in the genetic analysis of Bulgarian families with congenital anaemias are the hemoglobin variants such as HbO-Arabia, HbG-Coushatta, HbS, Hb Knossos, Delta beta-thalassemia **(7),** Hemoglobin-Lepore **(8)**, as well as the autosomal-dominant Hb Stara Zagora and Hb Jambol. Sickle-cell anemia, as well as alpha Thalassemia are not typical for the Bulgarian genetic pool (9).

THALASSEMIA CENTERS

All of the patients with TDT in Bulgaria are being treated at all together 7 departments for paediatric haematology and clinical haematology located in the university hospitals in Sofia, Plovdiv, Varna and Pleven. In 3 of these departments are in charge expert centres for comprehensive treatment for Thalassemia patients – one in Varna treating both adults and children and two in Sofia – one treating adult and one children **(10)**. In these centres, patients are being managed by multidisciplinary teams of haematologist, paediatric haematologist, genetists, endocrinologists, cardiologists, gastroenterologists, nephrologists on a regular basis. Biannually, all the multidisciplinary teams meet together during a three-days expert meeting to discuss recent needs of the patients, guidelines, changes of therapeutic approaches ect. **(11)**.

ACCESS TO TREATMENT

All TDT-patients are granted free of any charge management concerning the condition and the related complications. All 3 known chelators are marketed and available for the patients. Patients may also be treated by combination therapy consisting of deferiprone and deferoxamine **(12)**.

Chelation therapy is being monitored by MRI T2* in a single radiological centre in Sofia, which is deemed as a reference centre for the method in the country **(13)**.

Bone marrow transplantation as a single curative treatment modality historically has been attempted by a Bulgarian transplantation team in the period 2005–2010 in children, however the results were not favourable, therefore all the consecutive patients considered for bone marrow transplantation have been referred to departments in Italy.

The patients with TDT are granted access to reproductive programs and currently most of the families in which one of the partners is a patient with TDT receive a chance to have their offspring.

SCIENTIFIC WORK OVER THE RECENT YEARS

Over the last 20 years the scientific interest in TDT marked tremendous development and improvement. Researchers based in all university entities, treating patients with TDT have been designing single- and multicentre studies focusing on different aspects of the pathogenesis and treatment of the disease.

In 2008, Marinov et al. have analysed the exercise performance in children with severe beta-thalassemia before and after transfusion **(14)**. The single centre control study included 11 children with TDT and 11 age and sex matched controls who were assessed by comprehensive pulmonary function assessment and incremental exercise test on a treadmill. The researchers concluded that children with TDT have a seriously reduced transfer factor and exercise impairment. The short-term changes in hemoglobin concentration after transfusion are associated with significant improvement in exercise performance.

In 2011, Chakarov et al. has studied the severity of cardiopulmonary damage in children with β-thalassemia in association with the duration and degree of iron overload, so as to determine the hemodynamic risk based on the early changes of the cardiac and pulmonary function. The study aimed at creating a model for prevention, early diagnosis and treatment of the cardiac and pulmonary damage. The analysed cohort consisted of 56 children with TDT, all older than ten years of age and chosen at random, and all receiving monthly hemotransfusions and chelation therapy (Deferoxamin, Deferriprone, Deferasirox). A dynamic assessment of these patients'cardiac status was made by analyzing the main echocardiogram parameters. A structural and functional connection with the levels of iron overload in the myocardium was also determined. The assessment of liver iron overload showed earlier and more severe liver encumbrance, as evaluated through R2* MRI, and T2* MRI. While establishing a correlation between the iron overload in the myocardium and the liver, it became clear that the two are not always connected. Furthermore, in order to predict the genetic risk of myocardial siderosis, GSTM1 and GSTT1 were examined for the first time in Bulgaria. For the first time in the country, a correlation between the levels of ferritin and prohepsidin was determined. The lungs status of patients suffering from β-thalassemia was studied and the types of ventilatory failure identified. The study also demonstrated the role of the pulmonary hypertonia as an additionally aggravating factor leading to heart failure. T2* MRI, were used as basis for the proposed therapeutic regimens offering the possibil-

ity of optimizing the chelation treatment. On the basis of this study, echocardiography for monitoring patients with β-thalassemia is now routinely employed in Bulgaria, and optimal variants for chelation treatment are offered. The results are a basis for determining the therapeutic strategy for other patients with β-TDT. The research concluded that nearly 1/3 of Bulgarian children with BTM show reduced systolic left ventricular function, which is, in fact, a late manifestation of thalassemic cardiopathy **(15)**. The contemporary scientific work by Ganeva et al in 2023, on the contrary, focused on the detection of early myocardial changes, in cardiac function in young asymptomatic patients with TDT and whether they can be diagnosed using modern echocardiographic techniques and parameters and some microRNAs compared to healthy controls which are matched in gender and age to the patient. The case-control study included 26 TDT patients and 56 age matched controls, that some microRNAs are associated with cardiac damage in TDT patients. Significantly lower expression of RQ has-miR-30a-5p, as well as significantly higher expression of has-miR-150-5p, could be associated with increased cardiovascular risk in the future. The next task is to follow patients in the future to test this hypothesis. Since the research of microRNAs is still in the experimental phase, in order to confirm their reliability in clinical practice, it is necessary to conduct larger clinical studies covering a large number of patients **(16)**.

Later on, Georgiev et al. have examined the reduction of liver iron overload by dynamics of serum ferritin concentration and liver MRI T2* in adult patients with β-TDT. This prospective study, which was carried out between 2011–2014, included 46 patients with TDT (male to female ratio =1:1, mean age 33.2±10.9 years) Twenty-one patients (45.7%) were treated with deferasirox, 17 (37%) –with deferiprone, and 8 (17.3%) –with deferiprone in combination with deferoxamine. The patients were allocated into 3 groups based on their initial ferritin level and liver MRI T2*. The analysis demonstated that within 4 years the application of contemporary chelation therapy and ease of the assess to therapy led to significant improvement in the liver iron overload in all studied groups **(12)**.

The renal function in children and adults with TDT has been studied in 2018 by Stoyanova et al. A total of 44 patients-18 children and 26 adults were assessed by nephrological exam, including ultrasound of the kidneys, measurement of protein, microalbumin, β2-microglobulin, NGAL, NAG in first morning urine and the relation of those proteins to the excretory creatinine level of patients and age matched healthy controls. Using modern non-invasive urinary markers for early kidney damage, the study detected very early abnormalities in the tubular function in TDT patients, as well as marked differences in β2-MG and NGAL values in patients and controls and demonstrated statistical significance in the urinary marker NGAL. Urinary NGAL is an early indicator of tubulopathies not only in transfusion-dependent anaemias, but also a marker of renal involvement in the course of acute and chronic diseases. The results confirm that the TDT patients have hyperfiltration and renal insufficiency of I degree. A statistically significant difference in albuminuria and proteinuria in patients and controls was detected. Hyperfiltration and albuminuria are two independent risk factors for the onset and progression of renal insufficiency in these patients **(17)**.

Mineral bone density and bone metabolism was assessed in patients with TDT in a study by Sapunarova et al. **(18)**. In the study, serum levels of sclerostin, osteocalcin, beta-crosslaps, osteoprotegerin, and receptor activator of nuclear factor kappa-B ligand (sRANKL) were assessed in 62 TDT patients and 30 healthy controls. The lumbar spine and femoral neck were used to gauge bone mineral density. The transfusion-dependent beta-thalassaemia patients had a substantially higher sclerostin level (median 565.50 pmol/L) than the healthy controls (median 48.65 pmol/L, p.001) did. The Z-scores at the lumbar spine and femoral neck, osteocalcin, beta-cross laps, osteoprotegerin, sRANKL, pre-transfusion haemoglobin, liver iron content, and female gonadal status all exhibited significant relationships with sclerostin. Sclerostin levels were noticeably greater in patients with fragility fractures and splenectomized TDT patients. Age, sex, BMI, disease severity, serum ferritin, cardiac T2*, and male gonadal status did not exhibit any relationships with sclerostin that were statistically significant. According to the study's findings, sclerostin may contribute to the pathophysiology of the bones in beta-thalassemia patients and may also act as a marker for severe osteoporosis.

FUTURE PERSPEVTIVES

Despite the significant progress observed on a national level in terms of management of the patients with TDT several clinical and social dimensions of the care remain unmet.

First and foremost a national preventive program for Thalassemia is not present, thus granting a steady state of 2 to 3 new-borns with TDT on an annual basis.

Furthermore, a state program for bone marrow transplantation is still missing.

However, the introduction of fully reimbursed disease modifying agents in the very near future as well as the hopes for gene therapy on the horizon promises a better and longer life for the Bulgarian patients with TDT and their relatives.

REFERENCES

1. Cao A, Moi P, Galanello R. Recent advances in β-thalassemias. Pediatr Rep. 2011 Jun 16;3(2):e17.
2. http://raredis.org/pub/Thal_BG_4.pdf
3. Petkov GH, Efremov GD, Efremov DG, et al. Beta-thalassemia in Bulgaria. Hemoglobin. 1990;14(1):25-33.
4. Kalaydjieva L, Eigel A, Horst J. The molecular basis of beta thalassaemia in Bulgaria. J Med Genet. 1989 Oct;26(10):614-8.
5. Efremov, D. G. (1994). Correlation of genotype and phenotype in Beta-thalassemia. [Doctoral Thesis, Maastricht University]. Rijksuniversiteit Limburg. https://doi.org/10.26481/dis.19940422de.
6. De Sanctis V, Kattamis C, Canatan D, et al. β-Thalassemia Distribution in the Old World: an Ancient Disease Seen from a Historical Standpoint. Mediterr J Hematol Infect Dis. 2017 Feb 20;9(1):e2017018.
7. Андонова С, Брадинова И, Савов А. Генетична диагностика на бета-таласемия в България – какво научихме от нашите пациенти. Редки болести и лекарства сираци. 2023Sep.13;14(1):32-8.
8. Efremov GD. Beta-, delta beta-thalassemia and Hb lepore among Yugoslav, Bulgarian, Turkish and Albanian. Haematologica. 1990 Sep-Oct;75 Suppl 5:31-41
9. Petkov GH, Efremov GD. Molecular basis of beta-thalassemia and other hemoglobinopathies in Bulgaria: an update. Hemoglobin. 2007;31(2):225-32.
10. http://www.rd-action.eu/wp-content/uploads/2017/10/Bulgaria-SoA-Report.docx.pdf.
11. Банчев А, Константинов Д. Редките доброкачествени хематологични заболявания – отново във фокуса. Редки болести и лекарства сираци. 2023 Sep.13;14(1):4.
12. Georgiev PG, Sapunarova KG, Goranova-Marinova VS, et al. (2020) Reduction of Liver Iron Load in Adult Patients with β-Thalassemia Major Treated with Modern Chelation Modalities. Folia Medica 62(2): 265-270.
13. Chochkova-Bukova LA, Funken D, Bukova M, et al. Cardiac MRI with late gadolinium enhancement shows cardiac involvement 3-6 months after severe acute COVID-19 similar to or worse than PIMS. Front Cardiovasc Med. 2023 Jan 25;10:1115389.
14. Marinov BI, Terziyski KV, Sapunarova KG et al. Exercise performance in children with severe beta-thalassemia before and after transfusion. Folia Med (Plovdiv). 2008 Oct-Dec;50(4):48-54.
15. Чакъров Ив., Кардиопулмонално увреждане при болни от β-таласемия деца - диагностично терапевтична стратегия, Дисертационен труд Ст. Загора, 2011
16. Ganeva, Dissertation, Possibilities of some echocardiographic techniques and micrornas for detecting subclinical myocardial damage in children and young adults with beta-thalassemia major, Varna 2023.
17. Стоянова, Л., Анализ на уринни маркери за ранна бъбречна увреда и тяхната предиктивна стойност при болни с таласемия майор, МУ-Варна 2019.
18. Katya Sapunarova, Vesselina Goranova-Marinova, Pencho Georgiev et al. (2020) Associations of serum sclerostin with bone mineral density, markers of bone metabolism and thalassaemia characteristics in adult patients with transfusion-dependent beta-thalassaemia, Annals of Medicine, 52:3-4, 94-108

Correspondance Address:
Professor (associate) Atanas Banchev, MD, PhD
Paediatric Haematology and Oncology Department
University Hospital Tsaritsa Giovanna - ISUL
ul. Byalo More 8
1527 Sofia- Bulgaria
E-mail: bantschev@gmail.com

Figures:

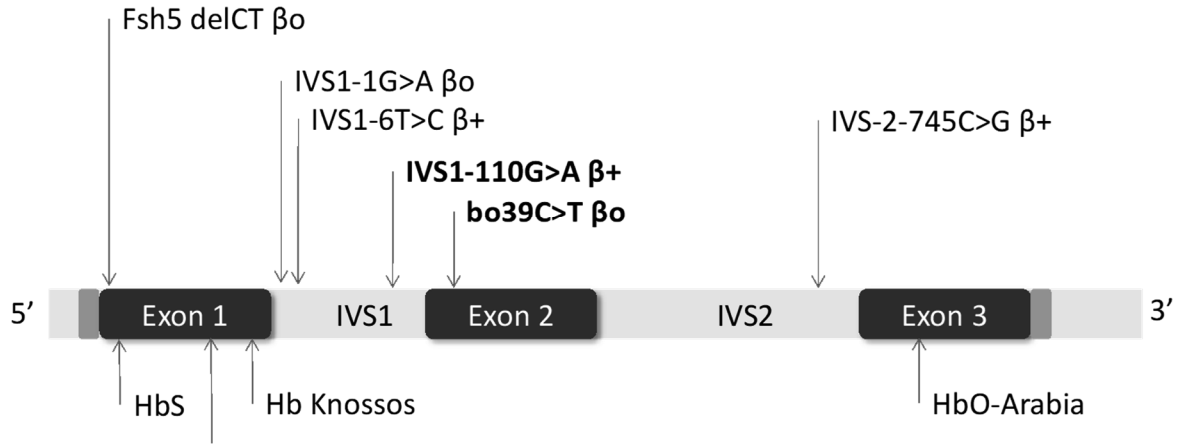


Figure 1: *Most common beta-Hb mutations detected in the Bulgarian population, according to S. Andonova (7).*

Bulgaria:
IVS1-110G>A = 31%
bo39C>T = 20%
IVS1-6T>C = 9%
IVS2-745C>G = 8%
IVS1-1G>A = 6%
Fsh5(-CT) = 6%

Figure 2: *Most common beta-Thalassemia mutations detected in the Bulgarian population, according to S. Andonova (7).*

THALASSEMIA AND HEMOGLOBINOPATHIES IN AZERBAIJAN

Dr. Valeh Huseynov, Dr. Xuraman Jafarova
National Hematology and Transfusiology Center-Baku-Azerbaijan

ABSTRACT

Thalassemia and other hemoglobinopathies are one of the most common genetic diseases in Azerbaijan. Information about thalassemia in Azerbaijan can be found in the literature after the 1950s. Genotyping of β-thalassemia identified through population screening revealed 34 mutations, with codon 8 [-AA]-34.96%, IVS-II-1 [G>A]-16.35%, and IVS-I-110 [G>A]-10.12% leading the spectrum. Genotyping of 45 alleles in **α** thalassemia revealed 9 mutations, including one novel. 20.5 kb and 3.7 kb deletions together accounting for 60 % of the spectrum. The "State Program on Hemophilia and Thalassemia Hereditary Blood Diseases" has been established since 2006. Since 2016 for the prevention of thalassemia, the genetic examination of both carrier couples and fetuses for the purpose of prenatal diagnosis has been carried out. As a result of the current prevention programs, there is a significant decrease in the number of patients registered at the Thalassemia Center. In recent times, due to the regular monitoring and treatment of complications of patients, the quality of life of patients has increased significantly. Stem cell transplantation has been performed in more than 130 patients since 2014. In addition, for the purpose of prevention, numerous educational activities of various nature are carried out of the project "For the sake of a life without Thalassemia". The main purpose of holding these events is to develop voluntary donor service for safe and quality blood supply of people suffering from thalassemia together with the media for educate the public to prevent the spread.

Keywords: Hemoglobinopathies, thalassemia, prevention, diagnostics, Azerbaijan

HISTORY

Hemoglobinopathies are hereditary pathologies, characterized by an abnormality in the synthesis or structure of the hemoglobin molecule, which are passed from parents to their children. Thalassemia is also a type of hemoglobinopathy, a severe hereditary blood disease characterized by impaired hemoglobin synthesis and chronic anemia as a result of a genetic defect. Azerbaijan is among the countries where thalassemia and other hemoglobinopathies are widespread. Information about thalassemia in Azerbaijan can be found in the literature after the 1950s. The first clinical case of β-thalassemia major in our country was reported by Akhundova**.** In addition, the prevalence of thalassemia was first studied in 1981 by Rustamov *et al*, the study covering 26 regions of Azerbaijan was conducted on 6069 people, the carrier frequency was recorded as follows: β-thalassemia in 519 people (8.6%), HbS in 48 people (0.8%), 147 people (2.4%) and glucose-6-phosphate dehydrogenase deficiency was detected. As mentioned in the article that the endemic areas where β-thalassemia carriers are found with a frequency of 10-20% are Agdash, Gabala, Oguz, Sheki, Astara, Sabirabad, Gazakh and Sharur regions **(1-3).** It should also be noted that the frequency and clinical picture of α-thalassemia in Azerbaijan was widely studied between 1983 and 1985 and are published in the articles of Russian scienticsts **(4-7).** The epidemiology of α-thalassemia in Azerbaijan was first studied by Gaziyev. He examined the blood of couples of 1000 newborns and found 0.8-28% Barths hemoglobin in 92 of them. Also, he determined that the prevalence of α-thalassemia is 14% in Gabala region, 9% in Shaki, and 5% in Baku. Besides, in his scientific work, Gaziyev described various clinical forms of α-thalassemia in 233 patients **(8).** Genetic mutations of thalassemia have been investigating in Azerbaijan since the 1980s. IVS-I-110 [G>A] mutation was first discovered in 1987 by Limborskaya and his colleagues in an Azerbaijani patient **(9).** In 1989, Schwartz and colleagues discovered the codon 82/83 [-G] mutation in an Azerbaijani patient for the first

time **(10)**. After that, in 1990, codon 8 [-AA] and codon 39 [C>T] identified by Solovyev and *et al*, and codon 36/37 [-G], codon 44 [-C], IVS-I-6 [T>C] and IVS-II-1 [G>A] mutations were detected by Goltsov **(11, 12)**. The full spectrum of hemoglobin gene mutations which were found in the population of Azerbaijan has been studing in the works conducted by the groups of international researchers in Türkiye, Russia and Azerbaijan. In 1992, a group consisting of employees of Georgia Medical College, Türkiye's Çukurova University and Azerbaijan's Institute of Hematology and Transfusion discovered 20 β-thalassemic mutations in the 99 chromosomes among the Azerbaijani population **(13)**. In 1993, Institute of Hematology and Transfusion of Azerbaijan together with the Institute of Experimental Hematology and Biotechnology of Russia, the Institute of Nuclear Physics and the Institute of Bioorganic Chemistry discovered 15 β-thalassemic mutations in 107 chromosomes. This study adds 3 new mutations to the list of 20 previously discovered mutations. The codon 14 [+T] mutation was found by this group in only one chromosome which was not identified in any subsequent research and is considered characteristic only for the Azerbaijani population **(14)**. In 1994, under the leadership of Kuliev, 15 mutations were found in 135 chromosomes examined in the international research conducted within the HUMAN Thalassemia Program. Two of them are new mutations, and thus the number of β-thalassemia mutations detected in the local population reaches twenty-five **(15)**. A recent study by Aghayev *et al.* at the Thalassemia Center aimed to evaluate the spectrum of common mutations, their impact and co-inheritance and/or the role of polymorphisms in the disease phenotype. The results of study in total, 34 mutations were found in a total of 416 alleles in **β** thalassemia. The top 20 most frequent mutations accounted for 87% of all mutations. The 3 most frequent mutations are as follows: c.25_26delAA (p.Lys9Valfs) 27.9%; c.93-21G>A (IVS1+110G>A) 11.3%; c.315+1G>A (IVS2+1G>A) 8.9%. Genotyping of 45 alleles in **α** thalassemia revealed 9 mutations, with the 3.7 kb deletion being the most common mutation in alpha thalassemia (35.6%), followed by the 20.5 kb deletion (24.4%) and α2 polyA2 (HBA2:c.*92A>G, 13.3%) **(16)**.

Until 2009, the patients suffering from thalassemia and other hemoglobinopathies in Azerbaijan were treated in the hematology departments of various hospitals (The State Clinical Hospital, The Institute of Hematology and Transfusion and others). On February 8, 2005, the "Round Table" held at the initiative of Mehriban Aliyeva, the President of the Heydar Aliyev Foundation, was the beginning of important steps towards solving the problems of thalassemia in our country. The main issue in solving the problems was the creation of a specialized center and a blood bank. The "State Program on Hemophilia and Thalassemia Hereditary Blood Diseases" has been established since 2006 by the decree of the President of the Republic of Azerbaijan in order to increase their life activities, improve their lifestyle and prolong their lives, reduce disability, limited health opportunities and death. The implementation of the State Program has been going on up. In 2009, by the decree of the President of the Republic of Azerbaijan, the Thalassemia Center was established under the Ministry of Health of the Republic of Azerbaijan. From that time, all patients with thalassemia and hemoglobinopathy across the country began to be registered at the dispensary, and their examination and treatment have been carring up today only at this center.

PREVENTION

In a study conducted by Kuliyev in 1994, it was stated that more than 200 children with thalassemia are expected to be born in Azerbaijan every year. This study notes that such a high prevalence indicates the need for a thalassemia control program in the country and lays the foundation for patient management, population screening, genetic counseling, and prenatal diagnosis **(15)**. The implementation of the State Program for the prevention of thalassemia disease in the country was started by the decision of the Cabinet of Ministers of the Republic of Azerbaijan No. 122 dated 04.28.2015. The prophylactic program includes premarital screening of couples for hemoglobinopathy, prenatal diagnosis of couples who are both carriers and a public awareness social campaign and educational activities. Since 2016, within the framework of the State Program for the prevention of thalassemia, the genetic examination of both carrier couples and fetuses for the purpose of prenatal diagnosis has been carried out with customized strip tests *(based on targeted reverse hybridization)* designed for the population specific mutations of Azerbaijan. From 2021, prenatal diagnosis across the country has been started using DNA sequencing and MLPA *(multiplex ligaton dependent probe amlification)* methods at the Genet-

ics Diagnostics Unit, Thalassemia Center. Since 2021, prenatal diagnosis has been performed along with amniocentesis, as well as on materials obtained by aspiration of chorionic villi. **Table 1** shows the number of prenatal diagnosis by years within the prevention program. As can be seen from the table, an increase in the number of prenatal analysis has been observed over the years. **Table 2** shows the number of newborns with various hemoglobinopathies registered in the center by year since 2011. As can be seen from the table, a significant decrease in the number of newborn hemoglobinopathy patients was observed in the country during the year after the prevention programs. **Table 3** shows the number of newborn hemoglobinopathy patients by diagnosis separately. Currently, the state program for the prevention of thalassemia is being successfully continued in Azerbaijan.

STATISTICS

Currently, 3674 patients with hemoglobinopathy are registered in the center's dispensary. 2019 of them are children. 1450 patients are being treated with beta thalassemia major, 639 of them with beta thalassemia intermedia and 413 with alpha thalassemia, 385 patients with sickle cell disorder, and the others with the diagnosis of various hemoglobinopathy (variant hemoglobins). In recent times, due to the regular monitoring and treatment of complications of patients, the quality of life of patients has increased significantly. According to the results of the monitoring conducted in 2022, it was observed that there was a significant decrease in the concentration of iron in the heart and liver in the repeated examinations. Hematopoietic Stem Cell Transplantation (HSCT), which is a radical treatment of the disease, has been performed at the Thalassemia Center since 2014. To date, more than 130 patients with hemoglobinopathy have undergone HSCT. In the article published in 2019, the overall survival rate of patients with HSCT was 97%, and the disease-free survival rate was 88% **(17)**. **Table 4** shows the total number of HSCT patients with hemoglobinopathy and follow up since 2014.

DIAGNOSTICS

As mentioned above, the first detection of thalassemia patients in Azerbaijan dates back to the 1950s. Hb electrophoresis for the diagnosis of thalassemia has been performed since 1965 by the Marengo-Row method at the Institute of Hematology and Transfusiology. In 1977-1978, work was carried out in the Azerbaijan Academy of Sciences to study "Biochemical polymorphism of hemoglobin and enzymopathies in Azerbaijan" using isoelectric focusing (IEF), polyacrylamide gel electrophoresis (PAGE) and amplification methods **(18)**. Hb electrophoresis has been performed at the Thalassemia Center since 2009. Currently, it is performed in the Thalassemia Center by the capillary electrophoresis method on new generation system (Sebia CE). Since 2019, a new generation sequencing laboratory has been established in the Thalassemia Center for the first time in the country and genetic testing performed via molecular methods. In 2018, T2* MRI examinations of liver iron load concentration, which is important for the monitoring of thalassemia patients, was started in a private clinic and used for our patients. In 2019, densitometry, the main diagnostic tool for osteoporosis, which is one of the serious complications of thalassemia patients, was established and put into the use at the center. Furthermore, for the first time in the country, from 2020, T2* MRI examinations of heart iron load concentration have been started.

TRANSFUSION

Since 2006, the implementation of the state program "On the donation of blood, blood components and blood service" has been started. Since then, the patients' need for hemotransfusion has been met at the expense of blood preparations obtained from voluntary donors. Thalassemia patients received erythrocyte masses transplanted with bedside filters until 2022. Since 2022, filter bags have been used in the preparation of erythrocyte masses in the Central Blood Bank. Currently, 1,401 patients are receiving hemotransfusion at the Center. **Table 5** shows the number of patients receiving regular hemotransfusion by diagnosis.

CHELATOR THERAPY

Currently, 1,341 patients are receiving chelator therapy. Desferoxamine (Desferal 500 mg) has been available to patients for the purpose of chelator therapy in our country since 2007. Since 2010, Deferripron (Ferriprox 500 mg) tablet form has been put into use. Since 2011, Deferrosirox (Exjade 500 mg) water-soluble tablets have been used, and since 2019, Deferrosirox (Jadenu 360 mg) has been used in oral tablet

form. Currently, 1,341 thalassemia patients are receiving chelator therapy. **Table 6** also shows the number of patients receiving monotherapy and combined therapy with chelators. Due to available treatment by blood transfusions and iron chelation therapy, it is now possible to extend the life into adulthood though very cumbersome and costly. The medico-economic problem of thalassemia is considered a major public health problem in the countries of the thalassemia endemic zone, and prevention programs, prenatal diagnosis and public education are given more attention as solutions to the problem. In Azerbaijan, in order not to increase the number of people born with hemoglobinopathies, as well as thalassemia, a number of activities are being carried out in the direction of prevention program, prenatal diagnosis and also population education. In the country, work related to prevention of thalassemia disease has been carried out at the state level since 2005. Under the organization of the Thalassemia Center, within the framework of the project "For a Thalassemia-Free Life", numerous educational events of various types are carried out, especially in schools, “ASAN” services, in the higher educational institutions and polyclinics. In 2019, in order to strengthen the prevention of thalassemia, carry out educational work among the population, increase the knowledge and experience of the medical staff working in the relevant field, with the joint organization of the "Regional Development" Public Union of the Heydar Aliyev Foundation and the Thalassemia Center, under the slogan "For a life without thalassemia" were held 8 Regional Symposiums with the participation of doctors, scientists, representatives of non-Governmental organizations, thalassemia patients and their parents in the regions. The main goal of these events, organized by the Thalassemia Center, is to develop voluntary donor service for the safe and quality blood supply of people suffering from thalassemia together with the social media, to educate the public in order to prevent the spread of this disease, and to expand cooperation with a number of countries and organizations fighting thalassemia and to provide the support with other means of education.

REFERENCES

1. Rustamov RSh, et al. Incidence of hereditary hemoglobinopathies in Azerbaijan. Probl Gematol Pereliv Krovi 1981; 9: 12-16.
2. 2. Shirinova EA, et al. Hemoglobin E in the Azerbaijan population. Probl Gematol Pereliv Krovi 1981; 9: 51-53.
3. 3. Gadzhiev EG et al. Heterozygote carrier state of hemoglobin S in Azerbaijan. Gematol Transfuziol 1991; 7: 16-18
4. Rustamov RSh, et al. Incidence of hereditary hemoglobinopathies in Azerbaijan. Probl Gematol Pereliv Krovi 1981; 9: 12-16.
5. 2. Shirinova EA, et al. Hemoglobin E in the Azerbaijan population. Probl Gematol Pereliv Krovi 1981; 9: 51-53.
6. 3. Gadzhiev EG et al. Heterozygote carrier state of hemoglobin S in Azerbaijan. Gematol Transfuziol 1991; 7: 16-18

1. Achundova,A. Thalassemia in Azerbaijan USSR (prevalence, clinic, treatment) Problemı Gematologii 1965;7:10–8.
2. Rustamov R Sh, Gaibov N T, Ahmedova A Lu, et al. Incidence of hereditary hemoglobinopathies in Azerbaijan. Probl Gematol Pereliv Krovi 1981; 9: 12-16
3. Achundova A. Thalassemia, Baku: Azerneshr; 1972.
4. Gaziev, J G. Alpha-thalassemic syndromes (in Russian). Azerbaijan Med Jf 1983;8:43-8.
5. Gaziev J G., Abdullaev GM, Tokarev YN. Bart's hemoglobin in Azerbaijan population (in Russian). Hematol Transfusiol 1983;11:47-53.
6. Abdullaev G M., Gaziev DjG, Mamedova RG. Clinico-laboratory peculiarities of alpha-thalassemia and sickle-cell anomaly combination (in Russian). Azerbaijan Med 1984;2:53-6. 0
7. Gaziev J G. Alpha-thalassemia in Azerbaijan (in Russian). Hematol Transfusiol 1985;10:8-10.
8. Gaziev J. Thalassaemia in Azerbaijan. J Med Genet 1995;32:245. doi:10.1136/JMG.32.3.245
9. Limborskaia S A. , Bukhman V L., Prosniak MI, et al. [Molecular causes of thalassemia. IV. Cloning of the beta-globin gene in a patient with beta-thalassemia from Azerbaijan and determination of the point mutation in a minor intron]. Genetika 1987;23:228–38.
10. Schwartz EI, Gol‘tsov AA, Kaboev OK, et al. A novel frameshift mutation causing beta-thalassaemia in Azerbaijan. Nucleic Acids Res 1989;17:3997.
11. Solovyev GY, Gol‘tsov AA, Surin VL, et al. Molecular nature of mutations causing beta zero-thalassaemia in Azerbaijan. Biomed Sci 1990;1:300–4.
12. Goltsov A, Surin V, Tagiyev A. The nature of mutational damage to the β-globin gene in patients with β-thalassemia in Azerbaijan. Abstracts of the II Congress of medical. geneticists, 1990, p. 101–2.
13. Curuk M, Yuregir G, Asadov C, et al. Molecular characterization of β-thalassemia in Azerbaijan. Hum Genet 1992;90:417–9. doi:10.1007/ BF00220470.
14. Tagiev AF, Surin VL, Gol‘tsov AA, et al. The spectrum of β-thalassemia mutations in Azerbaijan. Hum Mutat 1993;2:152–4. doi:10.1002/humu. 1380020218.

15. Kuliev AM, Rasulov IM, Dadasheva T, et al. Thalassaemia in Azerbaijan. J Med Genet 1994;31:209–12. doi:10.1136/JMG.31.3.209.
16. Agayev A., Huseynov, V T, Jafarova, Kh Kh.,et al. Gene mutation spectrum of thalassemia patients from Azerbaijan. In: EUROPEAN JOURNAL OF HUMAN GENETICS. CAMPUS, 4 CRINAN ST, LONDON, N1 9XW, ENGLAND: SPRINGER-NATURE, 2020. p. 318-319.
17. Aliyeva N., Safarova P, Huseynov V., Experience of Bone Marrow Transplantation in Beta Thalassemia Patients at Thalassemia Center. 1st Azerbaijan International Hematol. Specialists Congress Mater. of the Congress 2019, p. 52–32.
18. Rasulov EM, Movsum-zade KM, Mekhdiev NH. Application of the isoelectric focusing method in PAGE for identifying genetic heterogeneity of **β**-thalassemia. Manuscript deposited. In VNIIMI MH USSR No. 5343-82. M. 1982.-P.11:11.

Correspondence Address:
Dr.Xuraman Jafarova, National Hematology and Transfusiology Center, Thalassemia Unit -Baku-Azerbaijan,
e-mail address - khuraman.jafarova@talassemiya-merkezi.com

Thalassemia and hemoglobinopathies in Azerbaijan.
Supplemental data:

Table 1: *Number of prenatal examinations performed over the years*

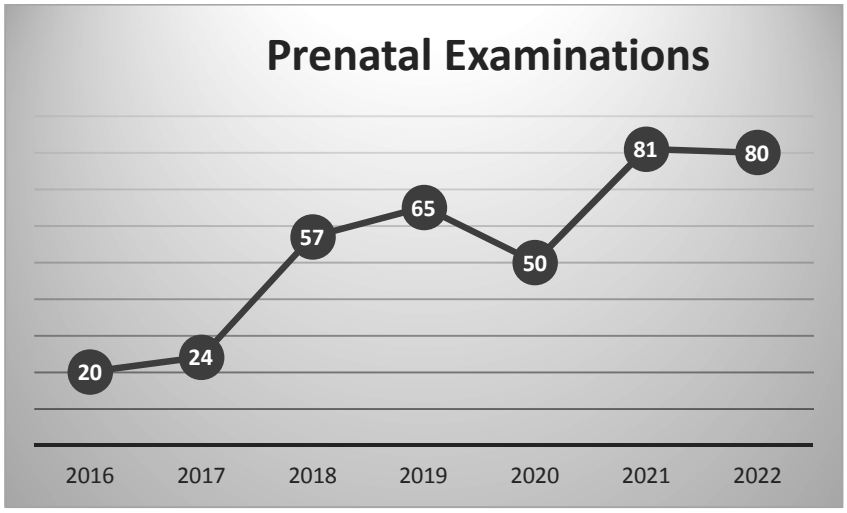


Table 2: *Total Number of newborn hemaglobinopathy patients by year*

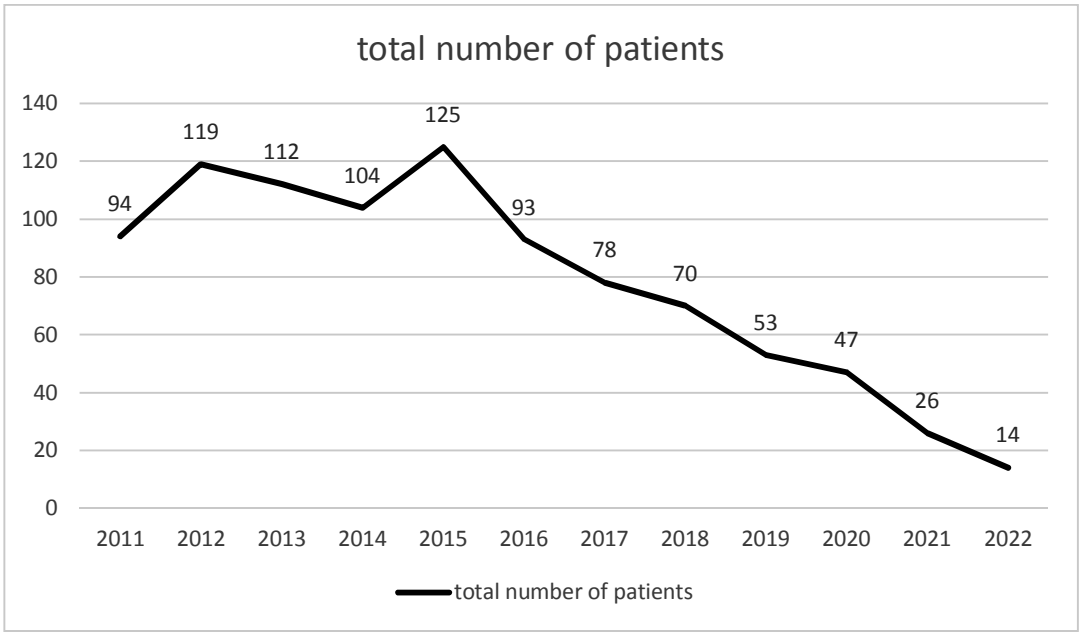


Table 3: *Number of newborn patients with hemaglobinopathy by year*

	β thalassemia major	β thalassemia intermedia	S/β thalassemia	sickle cell disease	other hemoglobinopathies	E/β thalassemia	delta beta thalassemia	HbD/β thalassemia
2011	66	18	8	1	0	0	0	1
2012	68	32	14	2	1	1	0	1
2013	79	19	11	1	2	0	0	0
2014	69	21	12	1	0	1	0	0
2015	79	21	16	2	5	0	1	1
2016	65	20	3	2	0	1	2	0
2017	52	17	4	3	2	0	0	0
2018	44	16	8	1	0	1	0	0
2019	38	6	7	2	0	0	0	0
2020	36	8	1	0	2	0	0	0
2021	23	1	0	1	0	0	0	1
2022	14	0	0	0	0	0	0	0

Table 4: *Total number of patients with hemoglobinopathies and follow up*

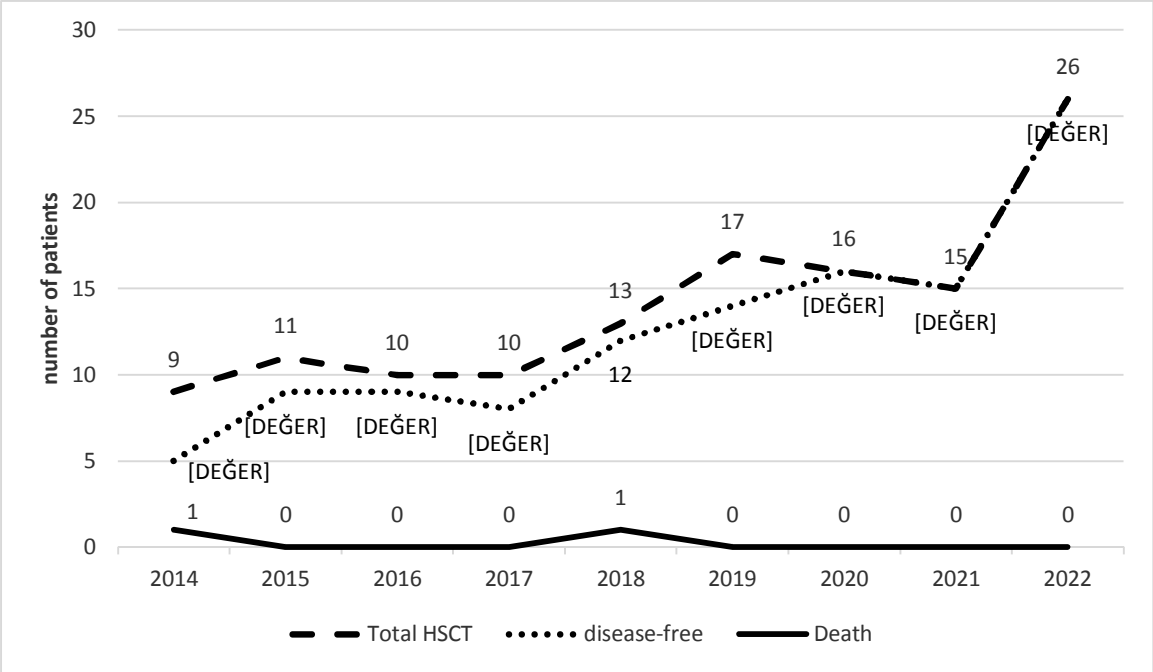


Table 5: *Number of transfusion dependent patients*

Diagnosis	Number of patients
β thalassemia major	1072
β thalassemia intermedia	185
α thalassemia	60
S/β thalassemia	66
Sickle cell disease	9
E/ β thalassemia	8
S/D hemoglobinopathies	1

Table 6: *Number of patients receiving chelator treatment*

Chelator	Number of patients
Desferoxamin (Desferal 500 mg)	93
Deferripron (Ferriprox 500 mg)	53
Deferrosirox (Exjade 500 mg)	63
Deferrosirox (Jadenu 360mg)	415
Desferal 500 mg + Exjade 500 mg	20
Desferal 500 mg + Jadenu 360mg	235
Desferal 500 mg + Ferriprox 500 mg	202
Jadenu 360mg + Ferriprox 500 mg	243
Exjade 500 mg +Ferriprox 500 mg	17

THALASSEMIA AND HEMOGLOBINOPATHIES IN PAKISTAN A REVIEW OF ESCALATING DISEASE BURDEN

Prof. Shabnam Bashir (PhD hematology)
Prof. of Pathology
Allama Iqbal Medical College
Ex-director Punjab thalassemia prevention program
Chairperson task force revamping of blood transfusion service, Punjab-Pakistan

ABSTRACT

Pakistan is a developing country with numerous healthcare related challenges. Among genetic disorders, hemoglobinopathies especially beta thalassemia is posing a matter of great concern with escalating disease burden. For more than three decades focus remained on the disease with much hue and cry but except for depicting our helplessness and concern over worsening situation, little was done by the stakeholders especially the policymakers and government sector. Most of the lukewarm efforts are done by non-government organizations.

Pakistan has estimated carrier frequency of 5-8% and projected birth of additional 5000 affected children every year. Huge disease burden and scarcity of standardized blood transfusion services resulted in substandard management contributing towards high morbidity and mortality. Chelation although available, but increasing cost of treatment resulted in suboptimal use. BMT is again available but in face of huge number of cases, offers little curative solution to few and selected.

Realization of effective preventive strategies is there at all levels. But only govt. of Punjab is providing free of cost diagnostic and preventive services through a network of laboratories and centers throughout Punjab in the form of thalassemia prevention program. Mainstay of prevention is through creating awareness, carrier screening, mostly targeted, voluntary premarital screening and prenatal diagnosis followed by termination of affected pregnancies. Need is to have a National program for thalassemia prevention and also a uniform national blood transfusion policy with strict implementation for both. Total ownership at govt. level both national and provincial, are need of the hour to meet this looming challenge.

Keywords: Pakistan, Hemoglobinopathies, beta thalassemia, blood transfusion services, thalassemia prevention

Review

The inherited hemoglobin disorders are the commonest monogenic diseases. It has been reported that about 7% of the world population are carriers of these disorders and that 300,000-400,000 babies with phenotypically severe diseases necessitating treatment are born each year (De Sanctis et al., 2017). Most affected children born in developed countries have a better chance at survival and are managed as a chronic disorder, while those in low-income countries mostly die before the age of 5 years. Thalassemia is a widespread disease most prevalent in the Mediterranean region, Middle-East, India, and South-East Asia, cutting a swath of high mortality and morbidity in these areas. In Southeast Asia α-thalassemia, β-thalassemia, hemoglobin E and Hb Constant Spring (CS) are prevalent. The abnormal genes in different combinations lead to over 60 different thalassemia syndromes, making Southeast Asia the hub of the most complex thalassemia genotypes (Fucharoen and Winichagoon, 2011).

In a recent report of Thalassemia International Federation (TIF) there is marked shift in prevalence and

incidence parameters especially in countries with effective national thalassemia prevention programs and use of effective treatment strategies have resulted in longevity in this particular disease cohort (Farmakis et al., 2020). In Pakistan disease kinetics are not improving as both available treatment options especially blood transfusion services, coupled with lack of uptake of prevention strategies at state level, are still not prioritized.

AIMS OF REVIEW

This document aims to highlight the current disease burden of thalassemia in Pakistan, availability/ limitations of management including diagnostic facilities, availability of blood transfusion services, chelation and bone marrow transplant. It also reflects upon the current status of prevention strategies and how lack of a unified national policy and strategic plan for thalassemia prevention and management is creating a disastrous situation for thalassemia patients and their families in Pakistan.

THALASSEMIA IN PAKISTAN

Pakistan is among the highest thalassemia burden countries in the world. Pakistan's estimated population is around 225 million. The frequency of β-thalassemia (β-thal) trait ranges from 5- 8%, with more than 10 million carriers. An estimated 5,000 new children with significant disease, are added annually in already existing pool (Sehar K, 2022). The commonly quoted figure for the country is 100,000 transfusion-dependent thalassemia patients. In the absence of a coherent national policy and strategic plan, the number of thalassemics in the country is believed to be increasing, but the exact burden of the disease is unknown owning to lack of national registry. As a result, despite being a preventable blood disorder, thalassemia in Pakistan continues to increase in number and causes misery to the patients and their families. In addition, it also creates a heavy burden on the already resource-constrained and stretched national health-care system, particularly the blood transfusion system. A significant proportion of the blood transfusions carried out in Pakistan are used for thalassemia patients, but the exact figures also remain unknown. There is a need to better address the thalassemia situation and concomitant burden on the national health care and blood transfusion systems in Pakistan. As no baseline survey has ever been conducted in Pakistan, there are no credible statistics about most of aspect of thalassemia in the country. Basic epidemiological and clinical data about the Pakistan thalassemia population do not exist. Information is also not available about treatment facilities and their accessibility to the patients. Without this basic information, it is difficult to convince the policy-makers about allocating resources to control and prevent the thalassemia.

Pakistan is one of those countries in which thalassemia and related hemoglobinopathies are prevalent since long time. In Pakistan very high consanguineous marriages rate is complicating the situation. Consanguineous marriages are norm across Pakistan. It was reported to be as high as 70% in Pakistan and among plethora of acquired and recessively inherited disorders, thalassemia and hemoglobinopathies are significant outcome of interfamily marriages. Inherited disorders among children born in such marriages are twice as likely to have genetic disorders as children of non-related marriage partners (Ullah et al., 2017). Among β-thalassemic patients, 96% had consanguineous marriages with 72% first cousins, 10% distant blood relatives and 14% *Bradari*. Inter-castes marriages were only 4% (Aslam khan et al., 2023). Whatever the notions and dynamics in favour of intermarriages, this particular custom need mass level public awareness and genetic testing followed by counselling to reduce the burden of recessively inherited disorders including thalassemias (Khalid et al., 2019).

SPECTRUM OF HEMOGLOBINOPATHIES IN PAKISTAN

Due to varied geographic distribution and very high consanguinity, various hemoglobinopathies are found in patchy distribution and mutant gene is concentrated among families. However, Beta thalassemia is the most common hemoglobinopathy across Pakistan (Ahmed S. et al., 2002).

COMMON MUTATIONS

IVSI- 5, Fr 8-9, Cd 30, IVSI- 1, Cd-15, Fr 41- 42, Del- 619, Cd 5, Fr 16 and Cap +1 are most common mutations with slight difference in ethnic populations of Pakistan (Ansari et al., 2012).

RARE β-GLOBIN GENE MUTATIONS IN PAKISTAN

HBB: c.92+1G>A (IVS-I-110) (G>A)) and HBB: c.113G>A (codon 37 (G>A)) are the most frequently seen rare mutations in Pakistan (Hussain et al., 2017).

ALPHA THALASSEMIA

Clinically significant alpha thalassemia is much less commonly seen than beta thalassemia. Most common deletion is –α 3.7 and –α 4.2. Thalassemia genotypes in different ethnic groups both within the thalassemia patients and in random control, appeared to be related to clan/ethnicity. The lowest frequency of the –α 3.7 allele is in Pashtoons (1.5%), Punjabis (5.2%), Sindhis (17.1%) while it is the highest in Balochis (20.3%) (Ashraf S., 2008). A similar pattern, with higher frequencies, was seen in the beta thalassemia patients, where Pashtoons had a frequency of 4.1%, while Balochis had 34.0% Khan S et al., 2003). Co inheritance of alpha thalassemia predominantly –α 3.7 deletion with beta thalassemia is also seen quite frequently with potential to change phenotype (Shahid S et al., 2017). Triplication of alpha gene is seen in about 1% of general population (Khan et al, 2003).

OTHER HEMOGLOBIN VARIANTS

In central Punjab generally and in other part of Punjab by and large the most common hemoglobinopathy is beta thalassemia. Other predominant hemoglobin variants are HbD Punjab followed by HbE (Bashir S, 2017).

HBD PUNJAB

It is the second most common Hb variant in Punjab. Though it is considered a benign hemoglobinopathy but in Pakistan due to high frequency of beta thalassemia carriers and HPLC being predominent screening method employed, occasional compound heterozygous cases of Beta/HbD Punjab are missed due to falsely low HbA2, a phenomenon which may pose diagnostic delimma especially in genetic councilling and prenatal diagnosis (Belhoul, 2013). Omission may result in birth of thalassemia major child which will be a failure for a prevention program. Proximity of emerging HbD Punjab peak with HbA2 cause part of A2 to get incorporated in forthcoming HbD peak with falsely low levels of HbA2 on HPLC.

HBD IRAN

This is a clinically silent hemoglobinopathy like hbD Punjab. Both are structural Hb variants with point mutation seen in beta globin gene but at different positions. HbD Punjab also known as Los Angeles, glutamic acid is replaced by glutamine at 121 position on beta chain. whereas HbD-Iran occurs due to replacement of glutamic acid by glutamine at position 22 (Gupta A, 2014). Quite a few numbers of cases of HbD Iran are seen in Southern Punjab. Though the electrophoretic mobility is the same but can be differentiated if both screening methods are employed namely HPLC and CZE as per standard protocols for hemoglobin variant screening workup (Sikander N et al., 2021).

SICKLE CELL DISEASE

Overall, the prevalence of Sickle hemoglobinopathy in Pakistan is unknown but as per data analysis of a reputable lab. with a huge catchment area across Pakistan, 2% of samples coming for hemoglobinopathy work up showed presence of sickle hemoglobinopathy. Its prevalence is highest in Balochistan, followed by Sindh, Khyber Pakhtunkhwa and least in Punjab (Alam H, 2022). Many other studies confirmed its highest prevalence in Balochistan, singly or with other hemoglobin variants (Hashmi N et al., 2008). Certain castes in Pakistan with high consanguinity like Makranis of lyari district and Bohras are known to have sickle gene concentrated among families (Mehboob, A., 2021) likewise in Dera Ismail khan (DIK) Sickle gene was more prevalent in Sherani followed by Bhatni and Ustrana clans (Ullah Z, 2022). Occurrence of other variants especially HbD Punjab and HbE in compound heterozygosity with sickle genes present interesting diagnostic and clinical challenges, rarely seen elsewhere globally (Tauseef, U, 2021).

DELTA BETA THALASSEMIA

Most of time it is a deletional variant, resulting due to loss of both beta and delta genes from chromosome 11. Clinically homozygosity result in presence of 100% HbF and thalassemia intermedia like picture whereas heterozygotes present like thalassemia trait. (Sharma S. et al., 2019). Compound

heterozygous cases with beta thalassemia may result in severe thalassemia major like picture. Though less frequent but regularly seen in various parts of Pakistan (Mansoori H et al., 2016). Delta beta thalassemia is to be distinguish from hereditary persistence of fetal hemoglobin (HPFH), both having high HbF (%) levels. Classically the phenotype of heterozygous individuals is different. Carriers of δβ-thalassemia mutant gene have 5%-20% HbF, which is distributed heterocellularly in red blood cells, whereas heterozygotes of HPFH mutations have 17%-30% HbF, with a pancellular distribution. Additionally, homozygotes of HPFH are asymptomatic as totally benign hemoglobinopathy, whereas δβ-thalassemic homozygotes phenotype is like thalassemia intermedia (Bollekens, JA and Forget, BG., 1991).

Fast moving variants like J meerut and J hope (provisionally diagnosed according to hematological parameters and RT on HPLC) though rare but are occasionally seen during routine hemoglobinopathy workup.

MANAGEMENT OF THALASSEMIA IN PAKISTAN

In Pakistan the mainstay of thalassemia management are blood transfusion as per requirement, iron chelation, management of other complications by medical and surgical means, supplements, social and psychological rehabilitation. Almost all of above mentioned facilities are there, with suboptimal use and varied range of availability. Stem cell /bone marrow transplant are the only permanent curative option for few and selected cases and available in Pakistan. HbF augmentation therapy with hydroxyuria and thalidomide is rampant without any evidence based practice and proving to be both blessing for some and curse for others. Gene therapy and gene editing is not available except in experimental phase in one or two setups.

BLOOD TRANSFUSION SERVICES IN PAKISTAN

Blood transfusion services in Pakistan is very fragmented and a division is between federal and provincial level as well. Though National blood transfusion policy is periodically updated and reviewed but implementation is far from realization. Especially at provincial level the administrative, and regulatory controls is severely lacking. Despite acute realization among all stakeholders, little effort is made at govt. level to address this important health service. Most of the blood transfusion provision to thalassemia patients is in the hands of NGOs, again not totally supervised and monitored by health authorities. On one side there is exceptionally good working by NGOs like Indus hospital health network and Agha Khan hospital providing best quality solutions as far as the safe blood components provision and hemovigilance, and on the other side in some part of Pakistan you may get a unit of blood over the counter from a plethora of mushroomed blood banks, unlicensed and illegal. Unfortunately, most of the patients are forced to avail the services of second type because of financial constraints, illiteracy and accessibility issues. Some slightly better services are provided by Armed forces run medical facilities and tertiary care hospital based blood banks.

PREVALENCE OF TRANSFUSION TRANSMITTED INFECTIONS AMONG THALASSEMIA PATIENTS IN PAKISTAN

In Pakistan exact data about the collection of blood units is not exactly known due to fragmented blood transfusion services and lack of IT based centralized working. Collection of blood units is quite high due to huge disease burden of thalassemia, hemophilia, and other hematological diseases. In addition injudicious bleeding of donors for surgeries and procedures in gyne/obstretrics is very high followed by nonuse and wastage of precious units. A significant portion of all donated blood is discarded prior to transfusion (WHO, 2022).

Transfusion transmitted infections (TTIs) are a huge complicating factor influencing course of disease, morbidity and mortality among thalassemia patients. In a literature review in which the total number of 3786 were tested for TTIs, had hepatitis B virus infection in 3.13 (0.6627.4%) Hepatitis C virus in 26% (5.56268.2%) cases. There were total of 6 cases of HIV. Limited data was available for malaria and syphilis (Ahmad K, 2016).

In another meta-analysis in which a systematic review was done regarding the pooled prevalence of

Hepatitis C virus in beta Thalassemia patients, prevalence of hepatitis C virus was 36.21% (28.98 - 43.75%). In Punjab the prevalence was highest 45.98 (Riaz, 2022). The lack of pre-store red blood cell phenotyping and leukocyte depletion in all thalassemia centers in Pakistan increases alloimmunisation and transfusion requirements, further increasing TTI risk (Zaidi et al., 2015).

This very high infectivity rate is mainly due to directed/replacement donations by donor pool in which the prevalence of these infection is very high in the first place. Most donors are first time donors, young males with age range between 18-30 years. In this single center study of 16,602 male donors (only 45 females), the reactivity in the screening assays was 973 (5.8%), among them. 58 (0.35%) were reactive in more than one assay. The prevalence of HCV, HBV, HIV, syphilis and malaria among studied donor population was 1.8, 1.7, 0.04, 2.1 and 0.07% respectively (Arshad A, 2016).

In a recently published retrospective study in a large cohort of 120 968 donors, 99.0% of the blood donors were young males (age group < 25 years (56 820; 47%), followed by age groups 26–35 years (47 232; 39%). Most of the donors belong to the Islamabad, the capital city of Pakistan and showed 2.0% positivity for at least one viral/parasitic pathogen. Prevalence of HIV was (0.1: 95%CI 0.191–0.283), of HCV (1.5 CI 0.423–0.661) and HBcAg (0.01 95% CI 0.951–1.002). Prevalence of malaria was (0.004%) and that of Syphilis was (0.8%) (Bhatti M, 2022).

IRON CHELATION IN PAKISTAN

Both desferral and oral iron chelators (Deferasirox and Deferiprone) are available in the country but socioeconomic constraints, incomplete knowledge regarding risk of iron overload, increasing frequency of transfusion due to alloimmunisation in multi transfused patients without prior extended antigen panel testing leads to no usage or under usage of iron chelators in two third of cases (Liaquat S et al., 2022).

DIAGNOSIS AND MANAGEMENT OF COMPLICATIONS

Except for very few centers most of thalassemia patients suffer from all sorts of disease complications, like endocrinopathies, bone deformities, massive splenomegaly, hepatic and cardiac issues. This contributes towards very high mortality and morbidity. Except for a very few setups, collective and comprehensive services are not available to most of the patients. In order to improve cardiac morbidity in transfusion dependent thalassaemia (TDT) patients, cardiac T2* MRI (T2*CMR) is available in few centers (Hussain S et al., 2020) but most of the reliance is on serum ferritin testing and echocardiography.

BONE MARROW / STEM CELL TRANSPLANT OR THALASSEMICS IN PAKISTAN

Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for beta thalassemia major. Alternative donor programs are nonexistent and only matched related donor transplants are performed. Haploidentical family donor transplantations was started in 2014.The current requirement is around 10 000 transplantations per year for various disorders and a minimum of 100 bone marrow transplantation (BMT) centers in private and public sectors in the 4 provinces are required to meet this need but only 5 to 6 such centers are offering this facility and for most beta thalassemia is not the priority when more acutely severe diseases like aplastic anemias, acute myeloid leukemia, acute lymphoblastic leukemia, lymphoma, myeloma, primary immune deficiency disorders make it to priority list (Zaidi U et al., 2019).

THALASSEMIA PREVENTION IN PAKISTAN

Beta thalassemia is a preventable disease and many countries in so called “thalassemia belt” have successfully curtailed their disease burden by effective implementation of thalassemia control programs. The only govt. run institution is Punjab thalassemia and other genetic disorder prevention and research institute former Punjab thalassemia prevention program (PTPP). This is a voluntary prevention program based on recommendations of WHO (WHO, 2006).

PTPP was started in 2009 for the 120 million population of Punjab and after initial hiccups of setting up and recruitments, became operational in 2012

onwards. This Program has its Planning, Monitoring & Implementation Unit in Lahore. The services network was extended to all 36 districts of Punjab in November 2015 through 4 divisional centers. Since 2018, the PTTP is working through its 9 divisional centers located in Lahore, Multan, Bahawalpur, Dera Ghazi Khan, Sahiwal, Faisalabad, Gujranwala, Sargodha, and Rawalpindi. These divisional centers have state of the art Hematology labs for carrier screening with facilities of genetic counselling, chorionic villus sampling and termination of affected pregnancy.

SERVICES PROVIDED AWARENESS ABOUT THE DISEASE

The PTPP carried out comprehensive awareness seminars at educational institutes, public places and community gatherings throughout Punjab with free facility of voluntary beta thalassemia carrier screening. The use of electronic and print media contributed in creating mass awareness. This was further enhanced by doing media workshops at press clubs of all regional centers of PTPP. The specially designed awareness material carrying information about disease, inheritance pattern, importance of carrier screening was distributed among the people. Information about prenatal diagnosis and fatwa about termination of affected pregnancy was also provided through pictorial booklets and charts displayed in all hospitals of Punjab. Banners and road steamers also contributed in creating awareness among general public.

CARRIER SCREENING FOR HEMOGLOBINOPATHIES

Extended Family Screening:

Most of the reliance is on extended family or cascade screening as per Thalassemia International Federations (TIF) recommendations mass carrier screening if only advisable if the carrier frequency is high. The carrier frequency of beta thalassemia is 5.4% in Pakistan so the method of choice is extended carrier screening (TIF publication No.21) PTPP has its field staff in all 36 district of Punjab. They visit the blood transfusion centers and identify the beta Thalassemia major children. The parents of affected child are mostly receptive regarding extended family screening due to prior sensitization and actual sufferings. Voluntary carrier screening was offered to all the family members. The blood samples in EDTA vials with pedigree were couriered to the hematology lab at regional centers maintaining cold chain. There is centralized reporting through web based portal with interfacing of lab. equipment. The results prepared are downloaded by field officers who again visit the family and hand over the results to head of each sub family confidentially. Genetic counselling is provided to the sub families regarding marriage of a carrier with a non-carrier individual. The facility of prenatal diagnosis to at risk couples was also offered. The PTPP had registered 9,774 families and this is the largest extended carrier screened pool of thalassemia in Pakistan. The carrier rate of **26.42%** was found in these extended families. Identifying carriers at younger age helps in making better decision.

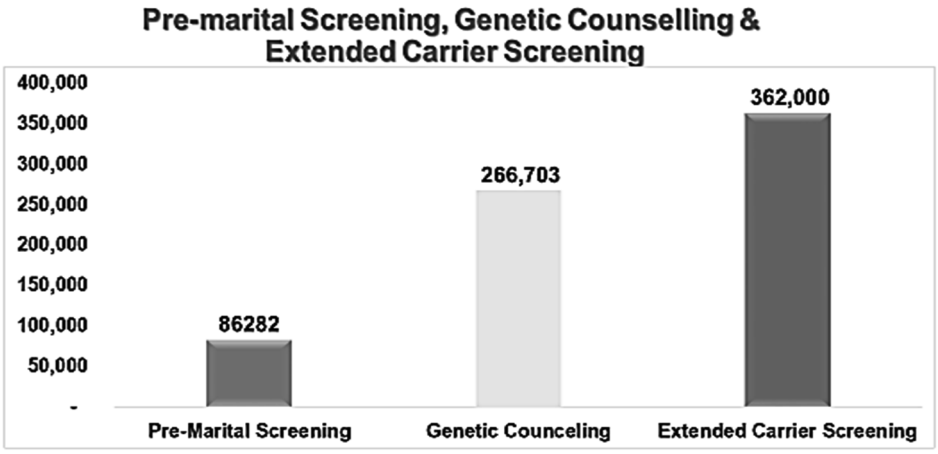


Figure 1: *Number of Pre-Marital Screening, Genetic Counseling & Extended Families Screening till date (PTPP).*

PREMARITAL CARRIER SCREENING

Premarital screening aims to prepare and guide prospective couples towards starting a stable family. PTPP had so far provided pre-marital screening to 86,282 individuals. PTPP had submitted the draft of pre-marital screening law in the Legislation Assembly of the Punjab Province. It is pertinent to mention here that even though the legislation about premarital testing is already made in province of Sindh, Khyber Pakhtoonkhawa (mandatory) and Balochistan (voluntary) but due to lack of comprehensive public sector testing facilities, still not enforced. So, the lesson learned from this experience is, to have a comprehensive National/ provincial thalassemia prevention policy and network available in order to get such laws implemented.

GENETIC COUNSELLING

Genetic Counselling is a communication process involved in human genetic disorders associated with their occurrence in a family. The PTPP had trained genetic counsellors to provide counseling according to the ethical, moral and religious standards and best possible solution is offered to at risk individual or couple. The respect for client's confidentiality is maintained and always non directive counselling is provided. The PTPP staff had provided genetic counselling to 266,703 till date.

PRENATAL DIAGNOSIS

The facility of prenatal diagnosis through Chorionic Villous Sampling (CVS) was provided by PTPP since 2012 at nine divisional centers of the Punjab. Trans-abdominal CVS procedure was performed by trained Gynecologists under real time ultrasound guidance. The calculated miscarriage rate due to procedure was 0.5%. The chorionic villous sample was transported to the DNA lab at Sir Ganga Ram hospital, Lahore on the same day from other regional centers in normal saline maintaining cold chain. About 8,372 families availed the facility of prenatal diagnosis. Medical termination of affected pregnancies were facilitated on couple's request. Availability of a Fatwa or verdict for termination of affected pregnancies by eminent religious scholars facilitated in councilling the family. Another series of CVS included 75 twin gestations. Selective termination of affected fetus was done in case of discordant results in twin gestations by 2-3 cc intra cardiac or intracranial injection of 15% KCl (EhsanY et al.,2020). PTPP established a DNA lab at Sir Ganga Ram Hospital, Lahore and it was made functional in 2015. This is the first, state of the art, public sector DNA laboratory in Pakistan providing free genetic testing facilities for various hemoglobinopathies. The lab has highly qualified staff with national and international trainings. DNA lab is currently using ARMS PCR, High Resolution Melt Curve Analysis (HRM) by Real Time PCR and Direct DNA sequencing for mutation identification.

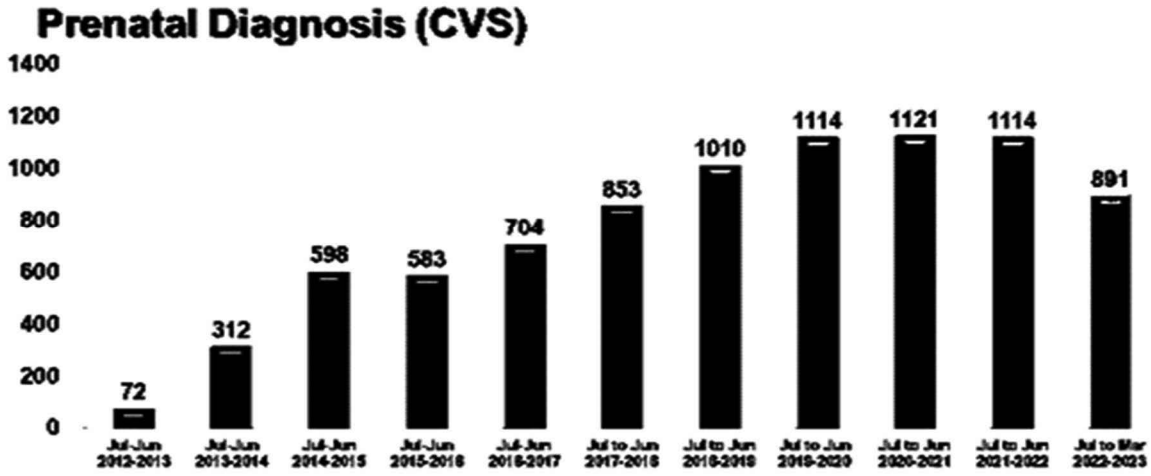


Figure 2: *Prenatal Diagnosis by Chorionic Villus Sampling (CVS)*

PTPP had collaborated with Punjab Information technology Board in effective monitoring and implementation of services. This mutual collaboration had greatly helped in improving quantity and quality of PTPP work.

TOLL FREE HELPLINE

PITB had established a 24 hours, Toll-Free Punjab Thalassemia Prevention Project Helpline (080-099-000).

WEBSITE AND SOCIAL MEDIA

Website (**www.ptpp.punjab.gov.pk**).PTPP is also active on social media through Face book (**www.facebook.com/ptppprogram**).

SOFTWARE FOR ONLINE REPORTING AND PTPP DASHBOARD

PITB had designed a software for on line data entry and online live dash board for PTPP which reflects the daily work performance of the project on the web portal.

THALASSEMIA ELECTRONIC MEDICAL REGISTRY

PTPP is in the process of establishing registry of all thalassemia major/intermedia cases in Punjab. This uphill task of Electronic Medical Registry (EMR) was started with the help of PITB after taking approval from Punjab Health Department. The decrease in incidence and prevalence of Thalassemia can only be documented with initial baseline data. This is the first online thalassemia patient registry in Pakistan featuring real-time data entry and allows enrolled users to observe the aggregated data at any point of time. Secondly, precise micro mapping of thalassemia families in Punjab will help to provide the service of cascade or extended family screening and to identify potential candidates for prenatal diagnosis (PND).

A total of 64 Thalassemia centers were identified out of these 26 are public sector hospitals and 38 were NGOs. A total of 12536 patients were actively registered on EMR. 5042 patients, otherwise enrolled with various NGOs and organizations were lost to follow-up, most likely due to demise. Another 3640 cases are yet to be registered, mainly from PIMS Islamabad and Sundus Lahore.

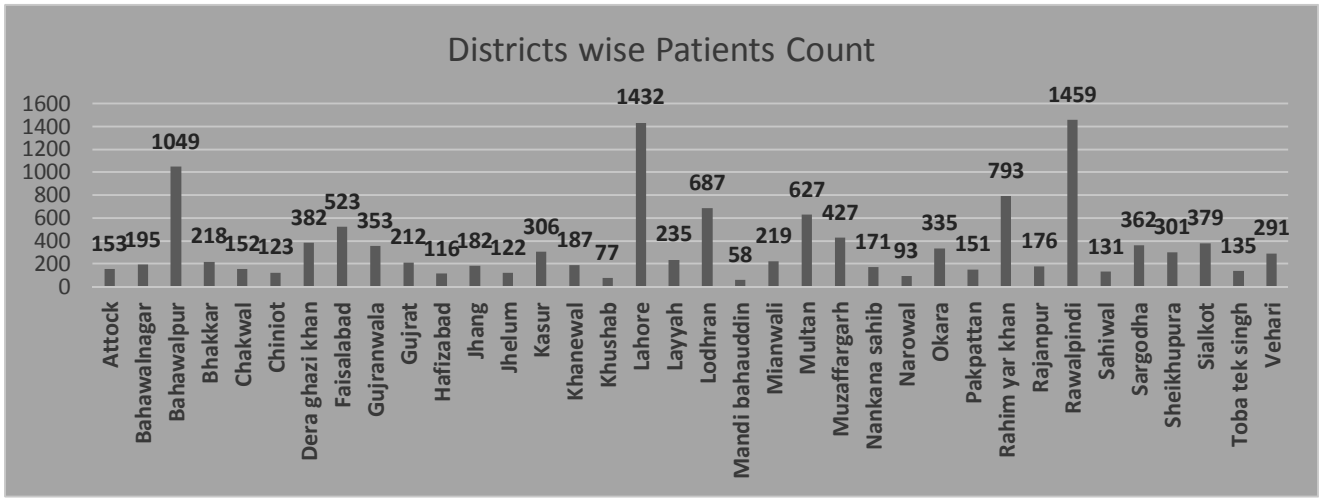


Figure 3: *District wise patient count in all districts of Punjab*

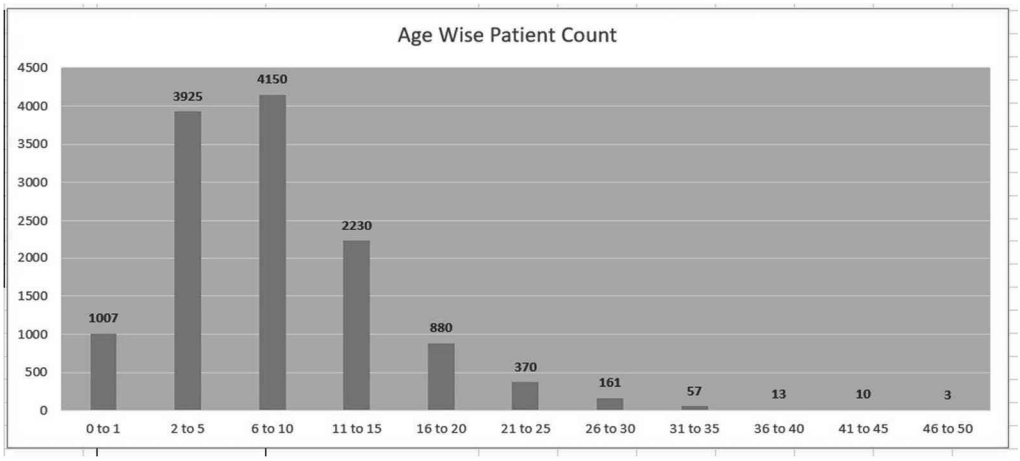


Figure 4: *Age wise distribution*

This data is of utmost importance and as it spans around one year time duration, can roughly tell the incidence of disease (new cases under 1year) and very high mortality as sharp decline in number of cases beyond 10 years. This also reflects our management system failure regarding thalassemia major patients.

Through awareness and provision of diagnostic facilities, a very positive impact is observed in Punjab especially among caregivers of Thalassemia patients. 83.5% of the respondents had adequate knowledge and 98.4% had positive attitudes. Knowledge and attitude were positively correlated (p=0.00). 93% opted for prenatal diagnosis and 91% opted for termination of affected fetus. Among these individuals 12% faced opposition from their family members when they went for diagnostic testing and this opposition rose to 20% when they had to opt for termination. The local religious clerics opposed prenatal diagnosis in 3% of the cases and termination in 7% of the cases (Tariq R, 2021).

In nutshell PTPP is reproducible intervention and should be taken up at National level. Prevention is the only viable option. Furthermore, blood transfusion services in the country needs total revamping as per international guidelines and should be IT based for better management and provision of safe blood components especially to thalassemic patients.

REFERENCES

1. Ahmed, S., Saleem, M., Modell, B. and Petrou, M., 2002. Screening extended families for genetic hemoglobin disorders in Pakistan. New England journal of medicine, 347(15), pp.1162-1168.
2. Ahmed Kiani, R., Anwar, M., Waheed, U., Asad, M.J., Abbasi, S. and Abbas Zaheer, H., 2016. Epidemiology of transfusion transmitted infection among patients with β-thalassaemia major in Pakistan. Journal of blood transfusion, 2016.
3. Alam, H. and Ali, N., 2022. Frequency of sickle cell hemoglobin in high-performance liquid chromatography received in a centralized laboratory. Journal of Applied Hematology, 13(4), p.208.
4. Ansari, S.H., Shamsi, T.S., Ashraf, M.et al 2012. Farzana, T., Bohray, M., Perveen, K., Erum, S., Ansari, I., Ahmed, M.N., Ahmed, M. and Raza, F., 2012. Molecular epidemiology of β-thalassemia in Pakistan: Far reaching implications. Indian journal of human genetics, 18(2), p.193.
5. Arshad, A., Borhany, M., Anwar, N., Naseer, I., Ansari, R., Boota, S., Fatima, N., Zaidi, M. and Shamsi, T., 2016. Prevalence of transfusion transmissible infections in blood donors of Pakistan. BMC hematology, 16, pp.1-6.
6. ASHRAF, S., 2008. Molecular Basis of Thalassemia Intermedia in Pakistani Population (Doctoral dissertation, BAQAI MEDICAL UNIVERSITY KARACHI, PAKISTAN).
7. Aslam khan, M., Qadeer, M.I., Akhtar, M.S., Chudhary, S.A., Maryam, M., Ali, Z., Khalid, A., Irfan, M. and Khan, Y., 2023. CULTURAL CONSANGUINITY AS CAUSE OF β-THALASSEMIA PREVALENCE IN POPULATION. medRxiv, pp.2023-06.
8. Bashir S, Ehsan Y, Jafri, H., 2017. Prevalence of beta thalassemia and other hemoglobinopathies-carriers status of students in educational institutes of central

punjab. Journal of fatima jinnah medical university, 11(4), pp.9-14

9. Bashir S, Ehsan Y, Jafri H, et.al.Prenatal Diagnosis of Thalassemia Major - an effective tool for thalassemia prevention in developing countries like Pakistan. P J M H S. 2017; 11,(3):995-8Bhatti, M.M., Junaid, A. and Sadiq, F., 2022. The prevalence of transfusion transmitted infections among blood donors in Pakistan: A retrospective study. Oman Medical Journal, 37(3), p.e386.
10. Belhoul, K.M., Bakir, M.L. and Abdulrahman, M., 2013. Misdiagnosis of Hb D-Punjab/β-thalassemia is a potential pitfall in hemoglobinopathy screening programs: a case report. Hemoglobin, 37(2), pp.119-123.
11. Bollekens, J.A. and Forget, B.G., 1991. δβ Thalassemia and hereditary persistence of fetal hemoglobin. Hematology/oncology clinics of North America, 5(3), pp.399-422.
12. Ehsan, H., Wahab, A., Anwer, F., Iftikhar, R. and Yousaf, M.N., 2020. Prevalence of transfusion transmissible infections in beta-thalassemia major patients in Pakistan: a systematic review. Cureus, 12(8).
13. Ehsan Y, Bashir S, Sabir F, Ghafoor M, Khaliq S. Six-year experience of prenatal diagnosis for beta thalassemia in twin pregnancies and selective foetal reduction — A case series. J Pak Med Assoc. 2020; 70, (11): 2061-4.
14. Gupta, A., Saraf, A., Dass, J., Mehta, M., Radhakrishnan, N., Saxena, R. and Bhargava, M., 2014. Compound heterozygous hemoglobin D-Punjab/hemoglobin D-Iran: a novel hemoglobinopathy. Indian Journal of Hematology and Blood Transfusion, 30, pp.409-412.
15. Hashmi, N.K., Moiz, B., Nusrat, M. and Hashmi, M.R., 2008. Chromatographic analysis of Hb S for the diagnosis of various sickle cell disorders in Pakistan. Annals of hematology, 87, pp.639-645.
16. Hussain, S., Hoodbhoy, Z., Ali, F., Hasan, E., Alvi, N., Hussain, A., Ishrat, K., Rahman, Z.U., Qamruddin, A., Parvin, A. and Hasan, B.S., 2020. Reduction of cardiac iron overload by optimising iron chelation therapy in transfusion dependent thalassaemia using cardiac T2* MRI: a quality improvement project from Pakistan. Archives of Disease in Childhood, 105(11), pp.1041-1048.
17. John Old. A short Guide for Health Professionals and Laboratory Scientists. Publishers thalassaemia internatıonal federatıon(TIF) publication no. 21.
18. Khan, S.N., Hasan, F., Sollaino, C., Perseu, L. and Riazuddin, S., 2003. Molecular Characterization of α-Thalassemia in Pakistan. *Hemoglobin*, *27*(3), pp.161-166.
19. Khalid, N., Noreen, K., Qureshi, F.M. and Mahesar, M., 2019. Knowledge of thalassemia and consanguinity: A multicenter hospital based retrospective cohort study from metropolitan city of Karachi, Pakistan. The Professional Medical Journal, 26(09), pp.1580-1586.
20. Liaquat, S., Aiman, U.A., Amil, J., Javed, S. and Fatima, N., 2022. Prevalence of Iron Overload and Chelation Therapy in patients of Thalassemia Major and Knowledge and Attitude of Population towards Iron Chelation Therapy in Sargodha, Pakistan. Pakistan Journal of Medical & Health Sciences, 16(12), pp.132-132.
21. Mansoori, H., Asad, S., Rashid, A. and Karim, F., 2016. Delta beta thalassemia: a rare hemoglobin variant. Korean Journal of Hematology, 51(3), pp.213-214.
22. Mansoor N, Meraj F, Shaikh A et al., 2022. Spectrum of hemoglobinopathies with hematological and biochemical profile: a five year experience from a tertiary care hospital. Pakistan journal of medical sciences, 38(8), p.2143.
23. Mehboob, A., 2021. Screening of sickle cell anemia in high prevalence Lyari district of Karachi through different screening tests. *Rawal Medical Journal*, *46*(3).
24. Riaz, M., Abbas, M., Rasool, G., Baig, I.S., Mahmood, Z., Munir, N., Mahmood Tahir, I., Ali Shah, S.M. and Akram, M., 2022. Prevalence of transfusion-transmitted infections in multiple blood transfusion-dependent thalassemic patients in Asia: A systemic review. International Journal of Immunopathology and Pharmacology, 36, p.03946320221096909.
25. Shahid, S., Nadeem, M., Zahid, D., Hassan, J., Ansari, S. and Shamsi, T., 2017. Alpha thalassemia deletions found in suspected cases of beta thalassemia major in Pakistani population. Pakistan journal of medical sciences, 33(2), p.411.
26. Sharma, S., Sehgal, S., Das, R. and Gulati, S., 2019. Phenotypic heterogeneity of delta–beta thalassemia. Indian Journal of Pathology and Microbiology, 62(1), p.185.
27. SIKANDER, N., BASHIR, S., TARIQ, R et al.,2021.Unexpected Discovery of Hemoglobin D Iran Families in Punjab, Pakistan by using two different Screening Methods. P J M H S Vol. 15(11).
28. Tariq, R., Mahmud, T., Bashir, S., Akhtar, S. and Israr, M., 2021. Impact Of Population Screening Programs On The Knowledge, Attitudes And Practices Regarding Prevention Of Thalassemia. Pakistan BioMedical Journal, 4(2), pp.209-214.
29. Tauseef, U., Anjum, M., Ibrahim, M., Baqai, H.S., Tauseef, A., Tauseef, M., Asghar, M.S., Zafar, M., Rasheed, U. and Shaikh, N., 2021. Occurrence of unusual haemoglobinopathies in balochistan: hb sd and hb se-presentation with osteomyelitis. *Revista Paulista de Pediatria*, *39*.

30. Khaliq, S., 2022. Thalassemia in Pakistan. *Hemoglobin*, *46*(1), pp.12-14.
31. Ullah, M.A., Husseni, A.M. and Mahmood, S.U., 2017. Consanguineous marriages and their detrimental outcomes in Pakistan: an urgent need for appropriate measures. Int J Community Med Public Health, 5(1), pp.1-3.
32. Ullah, Z., Rasool, R., Aziz, N., Bano, R., Bashir, S., Ali, S.A. and Khattak, A.A., 2022. Spectrum of Inherited Hemoglobin Disorder in patients presenting for Hb Electrophoresis: A Single Center Study in District Dera Ismail Khan. Pakistan Journal of Medical & Health Sciences, 16(07), pp.151-151.
33. World Health Organization; 2006: Medical genetic services in developing countries The Ethical, Legal and Social Implications of genetic testing and screening Human Genetics Chronic Diseases and Health Promotion.
34. Zaidi, U., Shamsi, T.S., Farzana, T., Ansari, S.H., Borhani, M., Munzir, S., Samad, S., Parveen, K., Anjum, N., Mirza, I.G. and Safdar, D., 2019. Capacity building of stem cell transplantation facilities in Pakistan: joint efforts of NIBD, government, and private-sector institutions. Blood Advances, 3(Supplement 1), pp.41-44.

Correspondence Address:
Prof. Shabnam Bashir (PhD hematology)
Prof. of Pathology
Allama Iqbal Medical College
Ex-director Punjab thalassemia prevention program
Chairperson task force revamping of blood transfusion service, Punjab-Pakistan
E-mail:docshabnam2000@yahoo.com

THALASSEMIA AND HEMOGLOBINOPATHIES IN IRAN

Prof. Dr. Mehran Karimi MD
Professor of pediatric Hematologist-Oncologist, American Hospital Dubai, Dubai, UAE

ABSTRACT

Hemoglobinopathies are the most common single gene disorders in the world population.

Iran has multi-ethnic groups and locates in the thalassemia belt. Therefore, there are many varieties in the molecular genetics and clinical features of hemoglobinopathies in Iran. More than 20000 beta thalassemia major patients have been reported in Iran while alpha thalassemia and sickle cell disease are not rare and so knowing epidemiology, molecular variants, and clinical features of hemoglobinopathies are essential to have a successful national prevention program.

Stablishing a premarital screening program, prenatal diagnosis and legal abortion are the main national successful preventive program in Iran which has reduced thalassemia birth significantly.

Iran is a sample of successful country in controlling hemoglobinopathies including thalassemia and this strategy may be encouraging for other countries in the region.

Keywords: Thalassemia, hemoglobinopathies, Iran

INTRODUCTION

Thalassemia is one of the most common genetic blood disorders globally. It is estimated that 300,000 infants are born with hemoglobinopathies worldwide annually who 60,000 to 70,000 of them are beta thalassemia major (B-TM) patients. B-TM is common in the Mediterranean area, Middle East, Far East, and East Asia **(1-3)**.

Homozygous hemoglobinopathies may be associated with high morbidity and mortality if their diagnosis and management are not timely. Iran is a multi-ethnic, high-population country which beta thalassemia is the most common hemoglobinopathies especially in northern and southern Iran followed by alpha thalassemia and sickle cell disease **(3)**.

Knowing the frequency, clinical characteristics, and demographic of hemoglobinopathies will enable us to draw a proper disease prevention and management strategy in order to minimize the disease burden and make an effective program properly **(4)** especially in counties where financial resources are limited including Iran.

Iran is in thalassemia belt where the rate of hemoglobinopathies is high which could be attributed to the malaria endemicity and high rate of consanguineous marriages. Therefore, the knowledge of genetic epidemiology and clinical features of hemoglobinopathies in the Iran will be valuable in prevention programs and better diagnosis and management of Hb disorders in the country **(5)**.

EPIDEMIOLOGY

Approximately 5 percent of the world's population has a globin variant, but only 1.7 percent has alpha or beta thalassemia trait. Thalassemia affects men and women equally and occurs in approximately 4.4 of every 10,000 live births. Alpha thalassemia occurs most often in persons of African and Southeast Asian descent, and beta thalassemia is most common in persons of Mediterranean, African, and Southeast Asian descent. Thalassemia trait affects 5 to 30 percent of persons in these ethnic groups **(6)**.

Iran is a country with an area of 1,648,19 km2 located in the Middle East. The current population of Iran is 89,301,457 as of September 13, 2023, (equivalent to 1.11% of the total world population) based on Worldometer elaboration of the latest United Nations data **(7)**.

Hemoglobin mutations are as old as the history of evolution. The actual Thalassemia and Sickle Cell

Disease (SCD) mutations are supposed to go back for perhaps 10 to 25.000 years. However, the selection of these mutations (including G6PD) by malaria is supposed to start with the beginning of agriculture or at least when the cohabitation between human and anopheles (which transmits the parasite 3ODVPRGLXP) reached a sufficient population density of both the guests and the parasite.

All major historic events have generated the complex variety of ethnic groups living in various parts of Iran including Persian, Azari (Türkiye), Kurds, Lures, Baluchis and Arabs who are associated with their specific history, culture, customs, and language.

In Iran, according to World Health Organization, about 4 percent of the population, are carriers of the thalassemia gene. In other words, about 2-3 million people are suffering from thalassemia minor. On the other hand, from every 10,000 live births, approximately 4.4% of them have thalassemia **(8)**.

However, thalassemias are more prevalent in Northern and Southern regions of the country, where the carrier rate for β-thalassemia is about 10% **(1)**.

Thalassemia belt is started from Italy followed by Greece, Cyprus, Türkiye, Iran, Pakistan, and India. Iran is one of the countries with high thalassemia population. It is estimated that 25,000 thalassemia major patients in Iran **(9)**. The exact number of Beta thalassemia intermediate is not determined since most of B-TI patients are treated at outpatients' clinics and not registered formally but its frequency is estimated to be 10% of B-TM patients. Ministry of Health is the major organization in Iran which is responsible for registering patients although. Alpha Thalassemia is not as prevalent as β-Thalassemia in Iran **(10)**.

PREVENTION PROGRAM AND PRENATAL DIAGNOSIS (PND)

In view of high rate of thalassemia carrier and birth in Iran, premarital screening test has been started since 1995 and the initial report of prevention program in Iran revealed significant decrease in the birth rate of thalassemia patients **(11)**. On the other hand, prenatal diagnosis (PND) was also started in Iran along with prevention program and abortion is allowed if the fetus is affected by thalassemia major and by law, legal abortion can only be performed before twenty-week gestation in cases with a confirmed diagnosis in Iran. The PND program also showed further decrease in thalassemia birth **(1, 12)**. The success rate of the prevention program was evaluated to be 82.3% in 2009 which demonstrated an effective program in lowering thalassemia births in Iran **(13)**.

PND is available in Iran and more than 10 PND laboratories offer PND services to thalassemic patients. In couples referred to PND, more than 99% of affected fetuses were properly diagnosed and pregnancy was successfully terminated in more than 98% of the affected cases. The most common cause of failure to perform an elective abortion in affected fetuses is late referral to PND centers. Multiple gestation with different results in each fetus and cultural/religious issues are other reasons of not performing an abortion **(1, 14)**.

COMMON GENETIC (STRUCTURAL) VARIANTS

1-The Beta Chain variants

Distribution of different mutations has an extreme divergence in different geographical regions including Iran. In fact, different studies in Iran revealed similar results in B-gene variants which IVS II-1 is the major mutation detected in most regions while in the southern and south-eastern parts of Iran IVS I-5 is the most common mutation. (These 2 mutations were found in 60% of cases.) followed by C 8/9, C 36/37, IVS I-110, C 30, IVS I-1, C 39, C 44, C 22, IVS II-745, IVS I-6, C8, IVS I-25 and C5 (1,15). Figure 1 shows distribution pattern of different mutations of β-Thalassemia in 21 provinces of Iran **(9)**.

Regarding Beta thalassemia intermediate the data is limited, and one study showed the mutations with the highest prevalence are IVS-II-1 followed by IVS-I-110, IVS-I-1 and FSC 8/9 **(16)**.

The sickle cell anemia (SCA) patients are also seen in Iran with high Hb F level like India and Eastern Saudi Arabia which have the benign clinical course.

The prevalence of sickle cell trait and SCA in southern Iran has been estimated to be 1.43 and 0.1%, respectively, while in the center of Iran (Isfahan) the frequency has been reported to be 8.33% **(8)**.

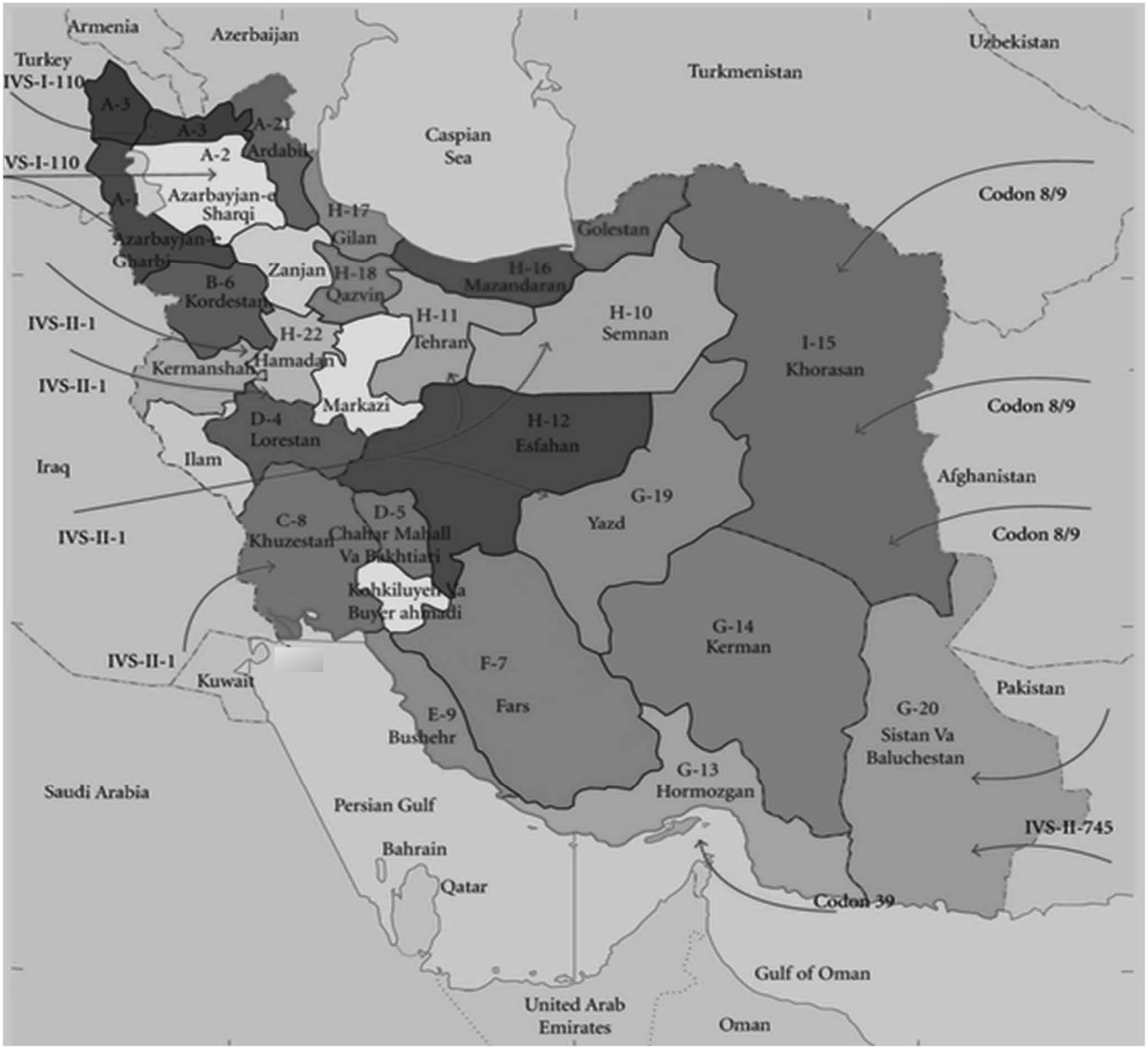


Figure 1: *Distribution pattern of different mutations of β-Thalassemia in 21 provinces of Iran (9)*

2-The Alpha-Chain variants

Alpha-thalassemia as one of the most common monogenetic disorders is widely spread over the Mediterranean, Southeast Asian, and Middle Eastern populations, including Iran. Although beta-thalassemia is much more common than alpha-thalassemia, alpha-thalassemia is still one of the main health problems in Iran.

Allele of α (3.7) mutation was the most prevalent (43.84%) followed by the α(IVS1/-5NT) allele with the prevalence of 4.91%. The less frequent alleles were Hb ICARIA and α (codon16) with the prevalence of 0.04 and 0.01%, respectively. The presence of α-thalassemia 1 and αthalassemia 2 in trans position (- -/-α) is the classic form of HbH disease known as deletional HbH disease. The α-3.7 (single deletion) and - - 20.5 kb and - - MED (double deletions) are reported as the most deletions among Iranian HbH patients, while the α-3.7, α-4.2, - - SEA, - - MED, - - THAI, - - 20.5, - - Tot, - - FIL and -- 5.2 are the most observed mutations of HbH disease in different populations. The most common genotype among Iranians is α 3.7/- - MED.

The data emphasizes the importance of carrier screening, genetic counseling, and prenatal diagnosis to decrease the prevalence of α-thalassemia in Iran which is one of the goals of the national screening program **(8, 17)**.

TREATMENT

All thalassemic patients have access to a general physician in all thalassemia treatment centers under the supervision of a pediatric hematologist. Leuko-reduced Packed cells produced completely by the Iranian Blood Transfusion Organization are available for all thalassemic patients. Transfusions are given to maintain a pretransfusion hemoglobin concentration of 8 to 10 g/ dL. Since 1996 all blood products have been screened for HIV, HBV, and HCV (HBsAg as of 1974, anti-HIV Ab as of 1989).

There are three treatment modalities for Iron overload including deferoxamine, with the average dosage of 30 to 50 mg/kg/d for 5 to 7 d/wk. Deferiprone, an oral iron chelator, at an average dose of 75 mg/kg/day and Deferasirox another oral iron chelator with dose of 14-28 mg/kg/day.

Finally, allogeneic BMT for thalassemic patients has been started since 1993, in Iran **(1)**.

CONCLUSION

Iran is a country with high prevalence of hemoglobinopathies where consanguineous marriage is common. There are many varieties in the molecular genetics and clinical features of hemoglobinopathies in Iran due to presence of various ethnic groups. Among these abnormal variants, β-globin chain variants of beta thalassemia, Hb S, Hb D and α-globin chain variants are more common.

The first step for management of patients with severe form of thalassemia is blood transfusion followed by iron chelation therapy due to iron overload. However, iron overload causes serious complications. So, new therapies have been proposed for disease including bone marrow transplantation and gene therapy/gene editing. Knowing the epidemiology, molecular genetics, and clinical features of hemoglobinopathies lead us to draw a proper prevention program along with diagnose and management of these patients in a timely manner.

National premarital screening program and PND for controlling hemoglobinopathies in countries with a major public health problem could be very successful and Iran is a sample of successful country in controlling hemoglobinopathies including thalassemia and this strategy may be encouraging for other countries in the region.

REFERENCES

1. Abolghasemi H, Amid A, Zeinali S, et al. Thalassemia in Iran Epidemiology, Prevention, and Management. J Pediatr Hematol Oncol 2007; 29:233–238.
2. Galanello R, Eleftheriou A, Traeger-Synodinos J, et al. Prevention of Thalassemias and Other Haemoglobin Disorders. Cyprus: Thalassemia International Federation Publications; 2003.
3. Angastiniotis M, Modell B, Englezos P, et al. Prevention and control of haemoglobinopathies. Bull World Health Organ. 1995; 73:375–386.
4. Modell B, Khan M, Darlison M, et al. A national register for surveillance of inherited disorders: beta thalassaemia in the United Kingdom. Bull World Health Organ. 2001; 79:1006–1013.
5. Rahimi Z. Genetic epidemiology, hematological and clinical features of hemoglobinopathies in Iran. BioMed Res Int. 2013; 2013: 803487.
6. Rund D, Rachmilewitz E. Beta-thalassemia. *N Engl J Med.* 2005;353(11):1135-1146.
7. Updated on July 16, 2023, with the latest July 2023-July 2024 estimates from the United Nations, Department of Economic and Social Affairs, Population Division. World Population Prospects: The 2022 Revision.
8. Nasiri A, Rahimi Z, and Vaisi-Raygani A. Hemoglobinopathies in Iran: An Updated Review. Int J Hematol Oncol Stem Cell Res. 2020 Apr 1; 14(2): 140–150.
9. Rezaee AR, Mehdi Banoei M, Khalili E, and Houshmand M. Beta-Thalassemia in Iran: New Insight into the Role of Genetic Admixture and Migration**.** The Scientific World Journal Volume 2012, Article ID 635183, 7 pages doi:10.1100/2012/635183.
10. M. Garshasbi, C. Oberkanins, H. Y. Law, M. Neishabury, R. Kariminejad, and H. Najmabadi, "α-globin gene deletion and point mutation analysis among Iranian patients with microcytic hypochromic anemia," Haematologica, vol. 88, no. 10, pp. 1196–1197, 2003.
11. Samavat A, Modell B. Iranian national thalassaemia screening programme. BMJ. 2004;329(7475):1134.
12. Hadipour Dehshal M, Ahmadvand A, Darestani SY, Manshadi M, Abolghasemi H. Secular trends in the national and provincial births of new thalassemia cases in Iran from 2001 to 2006. Hemoglobin. 2013;37(2):124-37.
13. Miri M, Tabrizi Namini M , Hadipour Dehshal M, Sadeghian Varnosfaderani F , Ahmadvand A , Yousefi Darestani S ,Manshadi M. Thalassemia in Iran in Last Twenty Years: the Carrier Rates and the Births Trend. IJBC 2013;6(1): 11-18.
14. Moghadam M, Karimi M, Dehghani Sj, Dehbozorgian J, Montazeri S, Javanmardi E, Asadzade R, Amiri A, Saghatoleslam Z, Sotodegan F, Morshedi

N, Imanifard J, Afrasiabi AR. Effectiveness of β-thalassemia prenatal diagnosis in Southern Iran: a cohort study. Prenatal Diagnosis. Volume35, Issue12, December 2015, Pages 1238-1242.

15. Najmabadi H, Karimi-Nejad R, Sahebjam S, et al. The beta-thalassemia mutation spectrum in the Iranian population. Hemoglobin. 2001; 25:285–296.
16. Karimi M, Yarmohammadi H, Farjadian S, Zeinali S, Moghaddam Z, Cappellini MD, Giordano PC. Beta-thalassemia intermedia from southern Iran: IVS-II-1 (G-->A) is the prevalent thalassemia intermedia allele. Hemoglobin. 2002 May;26(2):147-54. doi: 10.1081/hem-120005452.
17. Dehbozorgian J, Moghadam M, Daryanoush S, Haghpanah S, Imani Fard J, Aramesh A, Shahsavani A, Karimi M. Distribution of alpha-thalassemia mutations in Iranian population. Hematology 2015 Jul;20(6):359-62. doi: 10.1179/1607845414Y. 0000000227. Epub 2015 Jan 2.

Correspondence Address:
Prof. Dr. Mehran Karimi MD
Professor of pediatric Hematologist-Oncologist, American Hospital Dubai, Dubai, UAE
E-mail: mkarimi820@gmail.com

THALASSEMIA IN SYRIA

Nada Alaaraj, MD
Consultant Pediatrician and Endocrinologist
Hamad Medical Corporation
Doha, Qatar

ABSTRACT

Thalassemia, a genetic blood disorder affecting hemoglobin genes, is prevalent in the MENA region. Syria reports 5% of its population as carriers of the β-thalassemia gene and 1-5% as carriers of the α-thalassemia gene. The National Thalassemia Program recorded 214 newborns with β-thalassemia major in 2018. In March 2019, 4677 patients were registered across Syria's twelve governorates, receiving lifelong blood transfusion and chelation therapy. Molecular research in Syria has identified numerous β-globin gene mutations, with the eight most common ones, like IVS-I-110 and codon 39, collectively accounting for a significant portion. These mutations show regional variations. For instance, coastal regions have higher frequencies, while specific mutations are more prevalent in different areas.

The Syrian conflict, beginning in 2011, led to extensive internal displacement and an exodus of refugees. This upheaval disrupted healthcare and thalassemia management, impacting accessibility to blood transfusions and iron-chelation therapies. The conflict also damaged healthcare facilities and led to a shortage of healthcare workers. Despite challenges, Syria established a National Thalassemia Program in 1997, offering free blood transfusions and iron chelation therapy. However, gaps in monitoring therapeutic outcomes and ensuring adherence persisted, affecting morbidity and mortality rates.

Among the morbidities observed in thalassemia patients, persistent iron overload, cardiac complications, impaired growth, diabetes mellitus, and hypothyroidism stand out. These complications underscore the need for improved management strategies and continued research to enhance the quality of care for individuals with thalassemia in Syria. Additionally, psychosocial burdens, including educational disruptions and stigmatization, have been identified, emphasizing the need for holistic support for affected individuals.

Keywords: Syria, Thalassemia, prevalence, genetics, morbidities

BACKGROUND

The Syrian Arab Republic (Syria) is located in the Middle East, bordering Lebanon, Türkiye, Iraq, Jordan, and Palestine. It is also bordered by the Mediterranean Sea to the west. Syria is largely a semiarid or arid plateau and encompasses various mountain ranges, desert regions, and the Euphrates River Basin **(1)**.

Thalassemia is a heterogeneous group of genetic blood disorders affecting the hemoglobin genes, causing decreased synthesis of alpha or beta chains of hemoglobin (Hb) and resulting in ineffective erythropoiesis **(2)**. The prevalence of β-thalassemia in the Mediterranean region is the highest in the Middle East and Southeast Asia and lowest in Northern Europe and North America **(3)**. A change in the epidemiological distribution of β-thalassemia has been noticed recently due to multiple factors such as migration, implementation of premarital screening programs, and improved survival rates in the affected population **(3)**. In the Syrian population, it has been reported that 5% of people are carriers of the β-thalassemia gene, and between 1 and 5% are carriers of the α-thalassemia gene **(1, 4)**.

The prevalence of other hemoglobinopathies, such as sickle cell disease, is less than 1%, and G6PD deficiency has been reported in 3% of the Syrian population **(1)**.

The National Thalassemia Program registry reported 214 newborn infants with BTM in 2018. In March 2019, a total of 4677 patients were registered at the Program's satellite centers positioned in the twelve Syrian governorates under government control, to receive chronic lifelong blood transfusion and concurrent chelation therapy **(5)**.

Clinical manifestations of these disorders vary from severe, life-threatening conditions that require frequent blood transfusions and ongoing care (e.g., beta thalassemia major (BTM)) to minor or asymptomatic presentations with mild anemia (e.g., alpha-thalassemia silent carrier) **(6)**.

MOLECULAR TYPES IN SYRIA

More than 300 variants of the β-globin gene have been identified. Most β-thalassemia variants are caused by non-deletions, including single nucleotide substitutions and short insertions/deletions leading to frameshift **(7)**.

A study by Kyriacous, et al, involving 82 patients, eight common beta-thalassemia mutations were found: IVS-I-110 (G-->A), IVS-I-1 (G-->A), codon 5 (-CT), -30 (T-->A), codon 39 (C-->T), IVS-I-6 (T-->C), IVS-II-1 (G-->A), and codon 15 (TGG-->TAG). These mutations accounted for almost 75% of the total beta-thalassemia chromosomes **(8)**.

Murad, et al. studied 636 Syrian affected patients and 94 unrelated carriers, identifying thirty-eight different β-globin gene mutations. The eight predominant mutations were IVS-I.110 [G > A] (22.2%), IVS-I.1 [G > A] (17.8%), Cd 39 [C > T] (8.2%), IVS-II.1 [G > A] (7.6%), IVS-I.6 [T > C] (7.1%), Cd 8 [-AA] (6%), Cd 5 [-CT] (5.6%), and IVS-I.5 [G > C] (4.1%) **(9)**. They observed higher relative frequencies in the coastal regions compared to other regions (37.9% and 22%). There was a clear shift in the distribution of the Cd 39 [C > T] mutation from the northeast region (34.8%) to the northwest region (2.5%), while the IVS-I.5 [G > C] mutation had the highest prevalence in the north regions. The IVS-I.6 [T > C] mutation had a distinct frequency in the middle region. Ten known mutations were found in Syria for the first time: -86 [C > G], -31 [A > G], -29 [A > G], 5'UTR; +22 [G > A], CAP + 1 [A > C], Codon 5/6 [-TG], IVS-I (-3) or codon 29 [C > T], IVS-I.2 [T > A], IVS-I.128 [T > G], and IVS-II.705 [T > G] **(9)**.

Cevirici, et al., detected 16 types of mutations in 35 Syrian refugees with beta-thalassemia major. The most common mutation is IVS-I110 (n = 8). Other mutations, in order of frequency, include IVS-II-745 (n = 3), codon 44 (n = 3), codon 15 (n = 3), IVS-I-110/IVS-I-1 (n = 3), codon 5 (n = 2), IVS-I-1 (n = 2), codon 8/IVS-II-1 (n = 2), codon 44/codon 15 (n = 2), IVS-II-1 (n = 1), codon 39 (n = 1), IVS-I-6/codon 5 (n = 1), codon 9/10 (n = 1), IVS-I-110/codon 39 (n = 1), IVS-I-5/IVS-II-1 (n = 1), codon 39/IVS-II-745 (n = 1) **(10)**.

Gunes, et al. studied 15 Syrian refugees and found that the most common mutations were IVS-I-1 (G>A), IVS-II-1 (G>A), IVS-I-5 (G>C), and codon 5 (-CT), all with a frequency of 15.7%, accounting for 62.8% of all mutations in the Syrian patients. The codon 5 (-CT) mutation was found significantly more frequently in Syrian refugees compared to Turkish citizens (15.7% vs. 0%, p=0.023) **(11)**.

The Syrian conflict in 2011 resulted in an internal displacement of more than 6.1 million individuals and the exodus of 5.6 million refugees **(5)**.

THALASSEMIA MANAGEMENT PROGRAM IN SYRIA

In 1997, the National Thalassemia Program was established in Syria, providing free regular blood transfusions, iron chelation therapy (ICTs), and other related health services to all registered thalassemia patients through a network of national centers. However, due to a lack of efficient monitoring of therapeutic outcomes, evaluation of adherence to chelation therapy, and measures to improve compliance, morbidity and mortality rates remain relatively high **(12)**.

The Syrian conflict had a profound and devastating impact on the lives of Syrians and their healthcare system. Fifty percent of healthcare facilities were damaged, and up to 70% of healthcare workers left the country due to ongoing violence, economic hardships, and social consequences of the war. This had a deleterious effect on thalassemia patients, especially those who were displaced or trapped in conflict areas. Other crucial factors affecting the quality of care for BTM patients during the war included the disruption of blood transfusion accessibility and iron-chelation therapies. War sanctions, along with challenges associated with monitoring

therapeutic adherence and outcomes, exacerbated the problem **(5)**.

The Syrian Ministry of Health (MOH) maintained a well-established system for the management of emergency or strategic blood products before and during the armed conflict. The National Thalassemia Centers, like other governmental hospitals, were mandated to provide blood transfusions to all Syrian BTM patients. However, the primary obstacle during the war was the safety concerns associated with physicians, nurses, paramedical staff, and patients' transportation to and from these medical facilities **(5)**.

MORBIDITIES

A single-center two-phase observational study compared the prevalence of iron overload among BMT patients, before 2009 and during the armed conflict in 2019. The mean serum ferritin level was 3868 and 3757 ng/mL, respectively, indicating persistent insufficient iron chelation. These findings highlight the gap between the expected therapeutic outcomes of iron chelators and real-world patterns and outcomes. (16) Patients on deferoxamine (DFO) showed the highest serum ferritin concentrations compared to deferasirox (DFX) **(5)**.

Cardiac complications represent a significant cause of morbidity in BMT Syrian patients and remain the leading cause of mortality **(16)**. It was observed that cardiac function decreased from 68.64%±6.97% to 60.98%±7.22% in patients on DFO (p=0.0001) and from 67.39%±6.49% to 63.91%±8.51% in patients receiving combination therapy DFO + deferiprone (DFP) (p=0.031). The mean decrease was greater in the DFO regimen (-10.53 ± 11.89) than in patients on combination therapy (-4.74 ± 12.89) (p=0.035) **(12)**.

Hypothyroidism is one of the common endocrine complications described in patients with β-thalassemia major (β-TM) in Syria. Out of the 82 patients included in one study, 24 had subclinical hypothyroidism (29.27%), and one patient had overt hypothyroidism (1.22%). This study demonstrated that noncompliance with DFO therapy increases the risk of thyroid dysfunction by 6.38 times compared to compliance with therapy **(13)**.

In 35 incompliant BTM patients with a mean age of 22.5 years (14 males and 21 females) with a mean Hb level < 8g/dl, the mean BMI was 19.7Kg/m2 for males and 21.7Kg/m2 for females, while the mean height was 161.3cm (Height SDS = -2.17) for males and 150.2cm (HtSDS= -2.04) for females. Most patients were short but within the minimum normal weight. Diabetes mellitus was present in 20% of them, and prediabetes in 40%. There was a significant correlation between chelator usage and impaired glucose tolerance (IGT) **(14)**.

In thalassemic patients, the psychosocial burden affected many aspects of life, including education, time off school, sporting capabilities, differences from friends/siblings, social interactions, family adjustment, anxiety, isolation, and stigmatization. Results indicated a significant association between socio-demographic characteristics such as age, gender, school grade, current schooling, work, family income, and the occurrence of complications with the psychosocial burden variables **(15)**.

CONCLUSION

The Syrian conflict, starting in 2011, disrupted healthcare and thalassemia management, leading to various healthcare challenges. Despite the establishment of a National Thalassemia Program, gaps in monitoring and adherence persist and negatively affect patient outcomes. The morbidities associated with thalassemia include iron overload, cardiac complications, impaired growth, diabetes mellitus, and hypothyroidism. There is a bad need for improved management strategies and comprehensive support for individuals with thalassemia in Syria, considering both medical and psychosocial aspects.

REFERENCES

1. Syrian Refugee Health Profile. https://www.cdc.gov/immigrantrefugeehealth/profiles/syrian/index.html#ref-55 accessed September 12,2023.
2. Bajwa H, Basit H. Thalassemia. [Updated 2023 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545151/
3. Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of β-thalassemia. Eur J Haematol. 2020;105(6):692-703. doi:10.1111/ejh.13512
4. MacVinish, S., van Leeuwen, C., Hoetjes, M. et al. Lessons identified from initiating a thalassaemia programme in a conflict setting: a case study from north-

east Syria. Confl Health 17, 5 (2023). https://doi.org/10.1186/s13031-023-00503-2
5. Touma H, Youssef LA, Al-Salhi L, Ismail Al-Khalil W, AlKeba K. Prevalence and Management of Transfusional Iron Overload in Syrian Beta Thalassemia Major Patients Pre and during the Syrian Conflict. Biomed Res Int. 2023;2023:8911518. Published 2023 Sep 14. doi:10.1155/2023/8911518
6. Bajwa H, Basit H. Thalassemia. [Updated 2023 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545151/
7. Lee JS, Cho SI, Park SS, Seong MW. Molecular basis and diagnosis of thalassemia. Blood Res. 2021;56(S1):S39-S43. doi:10.5045/br.2021.2020332
8. Kyriacou K, Al Quobaili F, Pavlou E, Christopoulos G, Ioannou P, Kleanthous M. Molecular characterization of beta-thalassemia in Syria. Hemoglobin. 2000;24(1):1-13. doi:10.3109/03630260009002268
9. Murad H, Moasses F, Dabboul A, et al. Geographical distribution of β-globin gene mutations in Syria. Hematology. 2018;23(9):697-704. doi:10.1080/10245332.2018.1461291
10. Çevirici, Hatice, Acıpayam, Can, Yenilmez, Ebru Dündar, Belen, Fatma Burcu, Pekpak, Esra, Yaman, Yöntem and Tuli, Abdullah. "Investigation of beta globin gene mutations in Syrian refugee patients with thalassemia major" Turkish Journal of Biochemistry, vol. 44, no. 2, 2019, pp. 126-129. https://doi.org/10.1515/tjb-2018-0492
11. Gunes AK, Gozden HE. The Spectrum of Beta-Thalassemia Mutations in Syrian Refugees and Turkish Citizens. Cureus. 2021;13(6):e15434. Published 2021 Jun 4. doi:10.7759/cureus.15434
12. Youssef A Lama, Hanan Touma, Khawla AlKeba, Osama Maksoud,"Clinical Effectiveness of Iron Chelation Therapies in Syrian Patients with β Thalassemia Major: Suboptimal Clinical Outcomes and High Prevalence of Iron Overload," Blood,Volume 122, Issue 21, 2013, Page 1719, ISSN 0006-4971, https://doi.org/10.1182/blood.V122.21.1719.1719.
13. Yassouf MY, Alquobaili F, Kabalan Y, Mukhalalaty Y. Compliance with Deferoxamine Therapy and Thyroid Dysfunction of Patients with β-Thalassemia Major in Syria. Hemoglobin. 2019;43(3):218-221. doi:10.1080/03630269.2019.1639517
14. Mukhallati L, Farah M, Shoujaa A (2021) Investigation Of Fasting Glucose And Glucose Tolerance In Syrian Beta Thalassemia Major Patients. J Comm Med And Pub Health Rep 2(9): Https://Doi.Org/10.38207/JCMPHR/2021/0209159
15. Gharaibeh H, Amarneh BH, Zamzam SZ. The psychological burden of patients with beta thalassemia major in Syria. Pediatr Int. 2009;51(5):630-636. doi:10.1111/j.1442-200X.2009.02833.x

Correspandence Address:
Nada Alaaraj, MD
Consultant Pediatrician and Endocrinologist
Hamad Medical Corporation
Doha, Qatar
E-mail: nadaalaaraj@gmail.com

HEMOGLOBINOPATHY IN ISRAEL

Carina Levin M.D., Ph.D.
Pediatric Hematology Unit, Emek Medical Center, Rabin Rd. 18101, Afula, Israel

ABSTRACT

The population in Israel is diverse in terms of religion and ethnicity. Consanguineous marriage is common in some of its communities, resulting in an increased risk of genetic diseases. The most frequent hemoglobinopathies are β-thalassemia—with a high frequency in Kurdish Jews and among Arabs, and sickle cell, which is prevalent among descendants of Bedouin tribes and Africans. Great progress has been made in the field of hemoglobinopathies in Israel, including research, diagnosis and prevention of β-thalassemia and sickle cell disease. Patients have access to regular blood transfusions, iron-chelation therapy and novel drugs in clinical trials. The treatment is covered by national health insurance and the patients receive a government allowance. Further optimization of medical care for patients with hemoglobinopathies in Israel requires the creation of specialized centers, a national patient registry and an improved prevention program.

Keywords: Thalassemia, sickle cell, genetics, prevention, Israel

BACKGROUND

The origin of hemoglobinopathies in Israel: Hemoglobinopathies have probably been recognized in Israel since ancient times. Israel is located at an important crossroads between Europe, Asia and Africa. All ancient civilizations and empires—Assyrian, Babylonian and Persian from the Fertile Crescent—have contributed to the history of this country. This fact, together with recent historical and demographic changes, have resulted in diverse ethnic characteristics in Israel and affect the origin and genetics of hemoglobinopathies in this country. Today, the Israeli population consists of Jews, Arabs, Druze, Circassians and other minorities.

β-thalassemia – origin and ethnicity: First evidence for the presence of thalassemia in our region comes from a skeleton with signs of hemolytic anemia, based on bone pathology, found in the submerged Pre-Pottery Neolithic B village of Atlit Yam on the Israeli coast, more than 8000 years ago **(1)**. In modern Israel, β-thalassemia is widespread among a number of different ethnic groups.

Among Jews, it is found mostly in communities of Middle Eastern descent, such as North Africa, Türkiye and Yemen. An unusually high carrier frequency, around 20%, has been found in Jews originated from Kurdistan. Kurdish Jews, who lived as an almost completely isolated community for more than an estimated 27 centuries in three areas of Kurdistan, surprisingly carry 13 different mutations; some are frequent Mediterranean mutations and a few are probably unique to the population of this region **(2, 3)**. Among Jews of Ashkenazi descent—those who lived for centuries in Eastern Europe, the presence of β-thalassemia is extremely rare.

The Arab population that lives in Israel is also diverse in terms of both religion and ethnic origin. Some of the Arab population in this region is made up of people who have lived in the area for centuries, before the spread of Christianity and Islam in the region. Some are believed to have arrived during wars and conquests over the centuries from the East, from Europe and from the Arabian Peninsula. Demographic transition is well-known, occurring in ancient times and in recent periods. More than 40 β-globin mutations have been found among the Arab population, reflecting its diverse origins in Israel **(3)**.

The Druze population in this area is part of the Druze community that spreads from southern Syria, Lebanon, and some villages in the northern part of Israel. This is also an isolated community and just

two β-thalassemia mutations have been found in a few families.

Recently, a new group of immigrants have arrived in Israel, originating from northeast India, on the border with Myanmar. It is believed that they belong to one of the ten lost tribes of the ancient Jews. Hemoglobin E has been found among this group, together with other rare β-globin mutations.

Sickle cell anemia – ethnic origin: The sickle cell mutation originates from central Africa with three known major haplotypes: the Senegal haplotype from West Africa, the Benin or Central African Republic haplotype and the Bantu haplotype found in East Africa. A fourth haplotype has been described—the Arab Indian haplotype, suggested to originate from the Arab Peninsula and the western coast of India.

Slave trade from East Africa became common in the 7th century. Slave centers were located on the island of Zanzibar and in Oman. The slave trade continued until the beginning of the 20th century. The sickle cell trait is found in Israel predominantly in the Bedouin population, principally in the northern part of Israel where malaria was prevalent until the beginning of the 20th century. We can assume that the Bedouin tribes from the southern part of Israel, where sickle cell is not prevalent, have a different origin, probably coming from the Sahara Desert where malaria was also not prevalent. The presence of prevalent African blood group characteristics among the Israeli Bedouin population with sickle cell disease supports the African origin of the sickle cell gene in Israel **(4, 5)**. The sickle cell mutation is very rare in Jews, having been described in only one family, of Kurdish origin (5). Since then, we have found a few more Jewish carriers from various ethnic backgrounds. About 200 years ago, a tribe of African origin settled in a village on the Mediterranean coast of Israel. Sickle cell, β-thalassemia, HgbO-Arab and HgbC are observed in this population.

Interestingly, the sickle cell mutation has not been described in a more recently arrived ethnic group in Israel—the Ethiopian Jews—who have immigrated in the last decades. This probably reflects the fact that this ethnic group lived in closed communities. It is believed that they are descendants of the Queen of Sheba who was related to King Solomon (9th century BC). The last few years have seen the arrival to Israel of migrant workers of African origin and noticeably, some cases of sickle cell as well. HgbC, which also originated in West Africa, is also present in about 3% of the population in the northern part of the country, all of them Arab Bedouins.

β-GLOBIN MUTATIONS IN ISRAEL

Due to the diverse ethnicity of the population in Israel, even a relatively small population, described more than 20 years ago, revealed a wide variety of mutations. In one paper exploring β-globin mutations, their diverse distribution revealed the ethnic differences in different areas **(3)**. Thus, even in the relatively small area of northern Israel, with a population of about 2 million people, a systematic screening conducted for over 35 years (see below) detected more than 60 different mutations in globin genes. About half of these were mutations in β-globin genes **(Table 1, Fig. 1)**, and the others were in α-globin genes **(Table 1, Fig. 2)**.

α-THALASSEMIA IN ISRAEL

Similar to the rest of the world, defects in α-globin genes are common in Israel in all ethnic communities **(Table 1, Fig. 2)**. Nevertheless, clinical α-thalassemia diseases such as HgbH disease (--/-α) or hydrops fetalis—which occurs when all four α genes are deleted or mutated (--/--), are rare in Israel. This is because the incidence of α0-thalassemia (αα/--) in Israel is low.

Several studies have evaluated defects in the α-globin genes in Israel, and a wide diversity of α-globin mutations and clinical presentations was described by Oron-Karni et al. **(6)**. In addition, the molecular diagnosis of α-thalassemia was reported using a diagnostic algorithm that included gap-PCR to detect known deletions, sequencing of the α-globin gene to identify known and novel point mutations, and multiplex ligation-dependent probe amplification to diagnose novel deletions, along with a clinical description. α-Thalassemia was diagnosed in 662 of 975 samples. Deletions, including two novel ones, represented 75.3% of the cases; point mutations comprised 25.4% of the cases, including five novel mutations. The population consisted mostly of Jews (of Ashkenazi and Sephardic origin) and Muslim Arabs, who presented with a

higher rate of point mutations and HgbH disease. Overall, 53 different genotypes were detected, causing a wide spectrum of clinical phenotypes, from asymptomatic to severe anemia **(7)**.

PREVENTION PROGRAM

In 1987, a systematic prevention program for β-thalassemia was initiated, which consists of screening pregnant women, and further detecting couples at risk for having a child with homozygous β-thalassemia. The program covers the entire population living in northeastern Israel. Initially, the program was based on analyzing the blood indices of mean corpuscular volume (MCV) and mean corpuscular Hgb (MCH), and only suspect counts were further analyzed by Hgb electrophoresis. About 4 years later, in 1991, the project strategy was changed to also detect women carrying the HgbS gene, because HgbS disease was also quite frequent in northern Israel. Since then, the strategy has been to conduct a complete blood count, but even with normal MCV and MCH indices, HPLC analysis is performed **(8, 9)**. Obviously, the project was shown to be cost-effective, at least for β-thalassemia patients, taking into consideration the very high cost of treating a thalassemia patient with a minimal life expectancy of 50 years **(10)**.

Today, this program has screened more than 100,000 pregnant women; 8.4% (8557) were detected as being carriers of β-thalassemia and another 1.8% (1816) were carriers of the sickle cell trait. This reflects the high frequency of carriers in the population living in northeastern Israel. The number of couples at risk detected by this program stands at more than 900 **(Table 2)**. Based on the results of this program, since 1993, the Israel Ministry of Health has included detection of β-thalassemia as a routine analysis that is recommended for every pregnant woman who comes to Health and Child Care units all over the country. This recommendation is based on MCV and MCH indexes.

Since the establishment of the screening program, almost all new severe cases (β-thalassemia or sickle cell disease) have been born to couples who decided not to perform prenatal diagnosis or termination of pregnancy after genetic counseling.

PATIENTS WITH HEMOGLOBINOPATHIES IN ISRAEL

For many years, Israeli doctors and scientists have dedicated themselves to the treatment of patients with thalassemia and sickle cell anemia in Israel, as well as to the prevention of new cases and research. Unfortunately, the exact number of patients with hemoglobinopathies in Israel is unknown, because there is no national registry of thalassemia or other hemoglobinopathy patients. Notwithstanding, the level of medical care given to these patients in Israel is considered very high, with access to regular blood transfusions and all types of iron-chelation therapy, and the introduction of new drugs and treatments that are in clinical trials. All of the costs are covered by national health insurance and patients receive an allowance from the government. On the other hand, the number of specialized medical and paramedical personnel is insufficient and there is a need to organize specialized centers for the treatment of hemoglobinopathies in both the center and periphery of the country to ensure equal treatment for all patients, improve the transition of adolescents to adult care and further improve life expectancy, as well as quality of life.

We estimate the number of patients with thalassemia to be around 600, most with β-thalassemia, and the estimated number of sickle cell disease patients at around 200. They are treated all over Israel, principally in the north at Ziv Hospital in Safed, Rambam Medical Center in Haifa, Poria Medical Center in Tiberias, Galilee Medical Center in Nahariya, Emek Medical Center in Afula; in the center at Rabin Medical Center and Schneider Children's Medical Center in Petah Tikva and Hadassa Ein Kerem in Jerusalem; and in the south at Soroka Medical Center in Be'er Sheva.

HEMOGLOBINOPATHY RESEARCH IN ISRAEL

In addition to the genetic characterization and prevention programs, a large number of studies have been carried out in Israel, including both basic and clinical research, which have provided an important focus on understanding the pathophysiology and clinical features of patients with hemoglobinopathies.

(1) Hemosiderosis and iron metabolism in hemoglobinopathies

Iron overload caused by blood transfusions and increased intestinal absorption of iron is a well-known complication of hemoglobinopathies. A highly toxic form of iron—non-transferrin-bound iron (NTBI)—was first recognized in patients with β-thalassemia **(11)**. NTBI promotes the formation of reactive oxygen species which may break down biomolecules such as lipids, sugars, DNA and amino acids. Labile plasma iron is a component of NTBI that is both redox-active and chelatable, and can serve as an indicator of iron overload and iron chelation **(12–14)**.

Cardiac hemosiderosis is a life-threatening complication in transfusion-dependent patients with thalassemia. A study comparing NTBI and cardiac iron overload between patients with transfusion-dependent (TDT) β-thalassemia and those with sickle cell disease found lower NTBI levels and less cardiac hemosiderosis manifestations in the latter, proposing a protective effect of chronic inflammation in iron overload **(12, 13)**.

The principal iron-regulatory mechanism in humans consists of the hormone hepcidin and its receptor ferroportin. These control intestinal iron absorption, storage, recycling and tissue distribution. Hepcidin causes ferroportin internalization and degradation, decreasing iron absorption and mobilization. In β-thalassemia, hepcidin expression is extremely low relative to the degree of iron overload. The determinant factors controlling hepcidin expression in β-thalassemia are those related to anemia and ineffective erythropoiesis, rather than iron status. Exposure of human HepG2 hepatoma cells to sera from patients with β-thalassemia major was studied by Weizer-Stern et al. (15), who found a decrease in hepcidin gene expression. They revealed that hepcidin suppression in β-thalassemic patients results from secretion of a soluble factor that is proportional to the erythroid activity.

(2) Hypercoagulability, thrombotic events and pulmonary hypertension

Hypercoagulability and thrombotic complications are well-known in β-thalassemia. Increased levels of activated platelets and their activation markers, erythrocyte membrane abnormalities and enhanced red blood cell aggregability are known to be part of the hypercoagulability found in thalassemia patients **(16–19)**. Abnormal levels of coagulation factors and their inhibitors, and decreased levels of antithrombin III, protein C and protein S, have been reported in patients with thalassemia **(20, 21)**. Other peripheral blood elements have been reported to contribute to the procoagulant state in thalassemia, such as elevated levels of endothelial adhesion proteins and extracellular vesicles (EVs) **(22, 23)**.

Pulmonary hypertension is a possible complication in β-thalassemia **(24)**. The main risk factors are advancing age and a history of splenectomy; the etiology is multifactorial, involving a complex interaction of platelets, the coagulation system, erythrocytes and endothelial cells. Our group previously found a correlation between vascular endothelial growth factor levels and the severity of β-thalassemia intermedia, which is probably implicated in the endothelial dysfunction found in thalassemia patients **(25)**.

(3) Endocrine and growth abnormalities

The prevalence of endocrine complications in β-thalassemia patients and their relationship with the degree of iron chelation were studied by Shalitin et al. **(26)**. High serum ferritin levels during puberty, with a cut-off mean ferritin level of 2500 ng/mL, were found to be a risk factor for hypogonadism; and high serum ferritin levels during the first decade of life, in the pre-puberty period, with a cut-off mean ferritin level of 3000 ng/mL, predicted final short stature.

(4) Renal dysfunction

Renal complications β-thalassemia have been studied in several cohorts of Israeli patients, showing impairment of renal tubular function in TDT and non-TDT (NTDT) β-thalassemia patients **(27)**; transient proximal renal tubulopathy that was probably related to deferasirox therapy in four pediatric cases with recovery after cessation of the drug **(28)**; and glomerular dysfunction which, contrary to other reports, showed normal or reduced glomerular filtration rate (GFR) in TDT thalassemia patients **(29)**. Further study compared renal function in TDT patients with different chelation regimens. A high prevalence of renal tubular damage and a signifi-

cant decline in GFR over the years was observed, particularly in patients treated with deferasirox **(30)**.

(5) Central nervous system and cognitive dysfunction

Imaging studies confirmed a high prevalence of silent cerebral ischemic lesions in patients with β-thalassemia, especially in splenectomized adults who were NTDT and those with elevated platelet counts **(31)**. In addition, a high prevalence of silent cerebral infarcts was observed in patients with TDT thalassemia **(32)**.

Along with advances in the treatment of β-thalassemia patients and an increase in life expectancy, new foci have emerged to improve their quality of life and incorporate them into society. One is the prevention or improvement of cognitive impairment. Cognitive deficits have been reported in patients with thalassemia. Raz et al. **(33–35)** were the first to examine neurocognitive function using visual event-related brain potential in adults with TDT β-thalassemia. Poorer cognitive performance was found in these patients compared to matched healthy controls, and the difference was more pronounced before blood transfusions. In addition, executive function, attention and response inhibition were also affected, showing longer response times and higher error rates with a negative correlation to Hgb levels.

(6) Hypersplenism, asplenia/splenectomy and infections

Hypersplenism, defined as spleen enlargement and premature destruction of blood cells, is a common feature in patients with hemoglobinopathies. In sickle cell disease, early splenic dysfunction and progressive spleen atrophy are common, but splenomegaly and hypersplenism can also occur. Clinical manifestations of many hematological conditions can be controlled by splenectomy and many thalassemic, and some sickle cell patients, require splenectomy to ameliorate pancytopenia and reduce transfusion burden. However, splenectomy is associated with increased risk of bacterial infections, thrombotic complications and pulmonary diseases **(36, 37)**. Complications of splenectomy in 103 pediatric patients with non-malignant hematological disorders were studied by Yacobovich et al. **(38)**. It was found that patients with thalassemia and central lines were prone to infections and thrombosis. Beyond this subgroup, post-splenectomy infections and thrombotic events were anecdotal. Of note, most of the patients received pre-splenectomy vaccinations.

The association of clinical manifestations and laboratory parameters with spleen status was studied in sickle cell patients. Lower blood cell counts, HgbS levels, C-reactive protein and D-dimer and better metabolic and morphological red blood cell properties were observed in patients with splenomegaly compared to asplenic/hyposplenic sickle cell patients. The protective effect of the spleen and the importance of conserving spleen function were highlighted **(39)**.

The characteristics of EVs have been studied in TDT β-thalassemia patients, revealing the spleen's importance in EV clearance. EV profile reflected spleen status, hypercoagulability state, and the level of HSP70-containing EVs was associated with ineffective erythropoiesis and disease severity **(23)**. In addition, EV microRNA expression, and in particular that of miR-144-3p, was found to be dysregulated. Exposure of cultured cells to β-thalassemia-EVs significantly reduced their viability and increased apoptosis, probably contributing to the mechanism of β-thalassemia organ dysfunction and complications **(40)**.

Infections continue to be among the major causes of mortality among patients with hemoglobinopathies. A large study in 92 homozygous β-thalassemia patients who had been followed longitudinally for decades revealed that infection rate is affected mainly by the duration of the disease, and is increased by splenectomy and, in the long term, by treatment with deferoxamine, the only chelator used today **(41)**. Another 37-year follow-up study investigated the incidence of infections in 54 splenectomized patients with hemoglobinopathies, confirming the long-term high predisposition to serious infections, most caused by Gram-negative microorganisms **(37)**.

(7) Other research

In NTDT β-thalassemia, the clinical severity of the disease is somewhere between the mild symptoms of the β-thalassemia trait and the severe manifestations of TDT β-thalassemia. Patients in Israel with

NTDT β-thalassemia present a wide range of clinical severity, with difficult genotype–phenotype associations challenging genetic counseling **(42)**.

The increased longevity of patients with thalassemia and sickle cell disease introduces new clinical challenges due to the accumulation of disease-related morbidity, psychosocial issues, and adjustments to medical care, highlighting the process of transition to adult **care (43)**. A retrospective study in a single center in Israel described the characteristics of 14 adults with thalassemia who were older than 35 years. Most of the patients were TDT, and three different chelators—alone or in combination—were used at some point during the treatment. Most patients suffered from at least some endocrine dysfunction, 85% of the patients had completed at least a high-school education, 78% were employed, and 64.2% were married **(44)**.

So far great progress has been made in the field of hemoglobinopathies in Israel. With the hope that in the future, patients with hemoglobinopathies will become a national health priority, with the creation of a national registries, optimization of treatment and the prevention of new cases.

I especially want to thank Prof. Ariel Koren for having written the sections on origin and genetics, Dr. Dori Filon for her help with these sections, and Dr. Sari Perez for her help in obtaining data and constructing the graphs. Thanks are also due to the doctors, researchers, nurses and blood bank service for their work and dedication to the detection, prevention and treatment of hemoglobinopathies in Israel.

REFERENCES

1. Hershkovitz I, Ring B, Speirs M, et al. Possible congenital hemolytic anemia in prehistoric coastal inhabitants of Israel. Am J Phys Anthropol 1991; 85(1): 7–13.
2. Rund D, Cohen T, Filon D, et al. Evolution of a genetic disease in an ethnic isolate: β thalassemia in the Jews from Kurdistan. Proc Natl Acad Sci USA 1991; 88: 310–314.
3. Filon D, Oron V, Krichevski S, et al. Diversity of globin mutations in Israeli ethnic groups reflects recent historical events. Am J Hum Genet 1994; 54: 836–843.
4. Levene C, Rachmilewitz EA, Ezekiel E, et al. Blood group phenotypes and hemoglobin S. Acta Haematol 1976; 55(5): 300–305.
5. Rund D, Kornhendler N, Shalev O, et al. The origin of sickle cell alleles in Israel. Hum Genet 1990; 85: 521–524.
6. Oron-Karni V, Filon D, Shifrin Y, et al. Diversity of alpha-globin mutations and clinical presentation of alpha-thalassemia in Israel. Am J Hematol 2000; 65(3): 196–203.
7. Gilad O, Steinberg Shemer O, Dgany O, et al. Molecular diagnosis of α-thalassemia in a multiethnic population. Eur J Haematol 2017; 98: 553–562.
8. Koren A, Levin C, Zalman L, et al. Hb TAYBE: clinical and morphological findings in 43 patients. Eur J Haematol 2016; 97:137–144.
9. Koren A, Zalman L, Palmor H, et al. Sickle cell anemia in Northern Israel: screening and prevention. Isr Med Assoc J 2009; 11(4): 229–234.
10. Koren A, Profeta L, Zalman L, et al. Prevention of beta thalassemia in Northern Israel – a cost-benefit analysis. Mediterr J Hematol Infect Dis 2014; 6: e2014012.
11. Hershko C, Graham G, Bates GW, et al. Non-specific serum iron in thalassaemia: an abnormal serum iron fraction of potential toxicity. Br J Haematol 1978; 40: 255–263.
12. Ghoti H, Goitein O, Koren A, et al. No evidence for myocardial iron overload and free iron species in multitransfused patients with sickle/beta-thalassaemia. Eur J Haematol 2010; 84(1): 59–63.
13. Koren A, Fink D, Admoni O, et al. Non-transferrin-bound labile plasma iron and iron overload in sickle-cell disease: a comparative study between sickle-cell disease and beta-thalassemic patients. Eur J Haematol 2010; 84: 72–78.
14. Breuer W, Ghoti H, Shattat A, et al. Non-transferrin bound iron in thalassemia: differential detection of redox active forms in children and older patients. Am J Hematol 2012; 87: 55–56.
15. Weizer-Stern O, Adamsky K, Amariglio N, et al. Downregulation of hepcidin and haemojuvelin expression in the hepatocyte cell-line HepG2 induced by thalassaemic sera. Br J Haematol 2006; 135: 129–138.
16. Eldor A, Krausz Y, Atlan H, et al. Platelet survival in patients with beta-thalassemia. Am J Hematol 1989; 32: 94–99.
17. Eldor A, Lellouche F, Goldfarb A, et al. In vivo platelet activation in beta-thalassemia major reflected by increased platelet-thromboxane urinary metabolites. Blood 1991; 77: 1749–1753.
18. Chen S, Eldor A, Barshtein G, et al. Enhanced aggregability of red blood cells of beta-thalassemia major patients. Am J Physiol 1996; 270(6 Pt 2): H1951–H1956.

19. Ruf A, Pick M, Deutsch V, et al. In-vivo platelet activation correlates with red cell anionic phospholipid exposure in patients with beta-thalassaemia major. Br J Haematol 1997; 98: 51–56.
20. Levin C, Zalman L, Shalev S, et al. Legg-Calvé-Perthes disease, protein C deficiency, and beta thalassemia major. J Pediatr Orthoped 2000; 20: 129–131.
21. Eldor A, Rachmilewitz EA. The hypercoagulable state in thalassemia. Blood 2002; 99: 36–43.
22. Aharon A, Rebibo-Sabbah A, Tzoran I, et al. Extracellular vesicles in hematological disorders. Rambam Maimonides Med J 2014; 5(4): e0032.
23. Levin C, Koren A, Rebibo-Sabbah A, et al. HSP70-containing extracellular vesicles as biomarkers for ineffective erythropoiesis in beta-thalassemia. Front Physiol 2018; 9: 1214.
24. Du ZD, Rogin N, Milgram E, et al. Pulmonary hypertension in patients with thalassemia major. Am Heart J 1997; 134: 532–537.
25. Shitrit D, Tamary H, Koren A, et al. Correlation of vascular endothelial growth factor with the severity of thalassemia intermedia. Blood Coagul Fibrinolysis 2008; 19: 611–614.
26. Shalitin S, Carmi D, Weintrob N, et al. Serum ferritin level as a predictor of impaired growth and puberty in thalassemia major patients. Eur J Haematol 2005; 74: 93–100.
27. Smolkin V, Halevy R, Levin C, et al. Renal function in children with beta-thalassemia major and thalassemia intermedia. Pediatr Nephrol 2008; 23: 1847–1851.
28. Yacobovich J, Stark P, Barzilai-Birenbaum S, et al. Acquired proximal renal tubular dysfunction in β-thalassemia patients treated with deferasirox. J Pediatr Hematol Oncol 2010; 32(7): 564–567.
29. Milo G, Feige Gross Nevo R, Pazgal I, et al. GFR in patients with β-thalassemia major. Clin J Am Soc Nephrol. 2015; 10(8): 1350–1356.
30. Tanous O, Azulay Y, Halevy R, et al. Renal function in beta-thalassemia major patients treated with two different iron-chelation regimes. BMC Nephrol 2021; 22(1): 418.
31. Musallam KM, Taher AT, Karimi M, et al. Cerebral infarction in β-thalassemia intermedia: breaking the silence. Thromb Res 2012; 130: 695–702.
32. Pazgal I, Inbar E, Cohen M, et al. High incidence of silent cerebral infarcts in adult patients with beta thalassemia major. Thromb Res 2016; 144: 119–122.
33. Raz S, Koren A, Dan O, et al. Cognitive functions in adults with β-thalassemia major: before and after blood transfusion and comparison with healthy controls. Ann NY Acad Sci 2016; 1375(1): 19–27.
34. Raz S, Koren A, Dan O, et al. Executive function and neural activation in adults with β-thalassemia major: an event-related potentials study. Ann NY Acad Sci 2016; 1386(1): 16–29.
35. Raz S, Koren A, Levin C. Attention, response inhibition and brain event-related potentials alterations in adults with beta-thalassaemia major. Br J Haematol 2019; 186(4): 580–591.
36. Musallam KM, Taher AT, Rachmilewitz EA. Beta-thalassemia intermedia: a clinical perspective. Cold Spring Harb Perspect Med 2012; 2: a013482.
37. Sakran W, Levin C, Kenes Y, et al. Clinical spectrum of serious bacterial infections among splenectomized patients with hemoglobinopathies in Israel: a 37-year follow-up study. Infection 2012; 40: 35–39.
38. Yacobovich J, Barzilai-Birenboim S, Steinberg-Shemer O, et al. Splenectomy in childhood for non-malignant haematologic disorders – long-term follow-up shows minimal adverse effects. Br J Haematol 2020; 190(6): 909–915.
39. Peretz S, Livshits L, Pretorius E, et al. The protective effect of the spleen in sickle cell patients. A comparative study between patients with asplenia/hyposplenism and hypersplenism. Front Physiol 2022; 13: 796837.
40. Levin C, Koren A, Rebibo-Sabbah A, et al. Extracellular vesicle microRNA that are involved in beta-thalassemia complications. Int J Mol Sci 2021; 22: 9760.
41. Rahav G, Volach V, Shapiro M, et al. Severe infections in thalassaemic patients: prevalence and predisposing factors. Br J Haematol 2006; 133(6): 667–674.
42. Rund D, Oron-Karni V, Filon D, et al. Genetic analysis of beta-thalassemia intermedia in Israel: diversity of mechanisms and unpredictability of phenotype. Am J Hematol 1997; 54(1): 16–22.
43. Yacobovich J, Tamary H. Thalassemia major and sickle cell disease in adolescents and young adults. Acta Haematol 2014; 132(3–4): 340–347.
44. Shargian-Alon L, Pasvolsky O, Raanani P. Thalassemia major and intermedia in patients older than 35 years: a single center experience. Isr Med Assoc J 2017; 19(12): 767–771.

Correspondence Address:
Carina Levin M.D., Ph.D.
Pediatric Hematology Unit, Emek Medical Center, Rabin Rd. 18101, Afula, Israel
E-mail: Levin_c@clalit.org.il

Table 1: *Most common α- and β-globin defects found in a population in northern Israel*

Gene (old nomenclature)	Number	Percentage
β-globin defects – total (*)	**932**	
HBB:c:20T>A - (HgbS)	259	27.8
HBB:c:93-21G>A (IVS1,110)	185	19.8
HBB:c.118C>T (N39)	93	10.0
HBB:c:315+1G>A (IVS2,1)	84	9.0
HBB:c:316-106C>G (IVS2,745)	56	6.0
HBB:c:113G>A (N37)	40	4.3
HBB:c:92+1G>A (IVS1,1)	40	4.3
HBB:c:25-25delAA (FS8)	32	3.4
HBB:c:92+6T>C (IVS 1,6)	30	3.2
HBB:c.19G>A (HgbC)	30	3.2
HBB:c:-136C>G (-86)	19	2.0
HBB:c.47G>A (N15)	15	1.6
HBB:c:-151C>T (-101)	14	1.5
HBB:c.173A>G (Hgb Cork)	10	1.1
HBB:c:17_18delCT (CD5)	9	1.0
HBB:c.409G>A (Hgb Perpignian)	9	1.0
α-globin genes – total	**847**	
-alpha3.7Kb	514	60.7
HbA2:c95+2_95+6delTGAGG - (IVS1 donor site)	230	27.2
HBA:c.118-120delACC (αCD39)	44	5.2
Triple ααα	28	3.3
HBA2:c:*+92A>G (α poly A)	20	2.4
NG_000006:1g.24664_41064del16401 (-MED)	11	1.3
Total (*)	**1891**	

(*) Including less frequent genes (N = 112) that are not presented in the table. Mostly Arab population.

Table 2: *Number of couples at risk for having an offspring with hemoglobinopathy detected by the prevention program in northeast Israel (*)*

Risk diagnosis for the offspring	**Number**	**Percent**
β-thalassemia homozygous	500	55.0
Sickle cell anemia	210	23.1
Sickle cell β-thalassemia	176	19.4
Sickle cell HgbC disease	16	1.8
HgbC homozygous	5	0.6
Sickle cell HgbD disease	2	0.2
Subtotal for sickle cell disease	44	44
Total	**909**	**100**

(*) Data do not include α-thalassemia results or other rare combinations.

Figure 1:

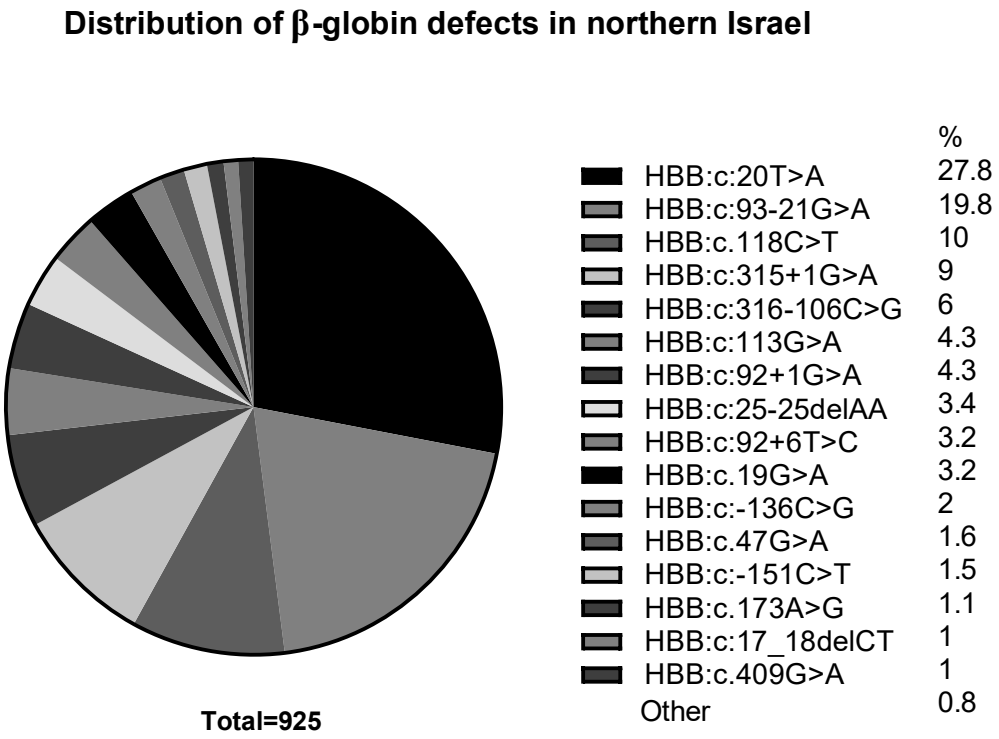
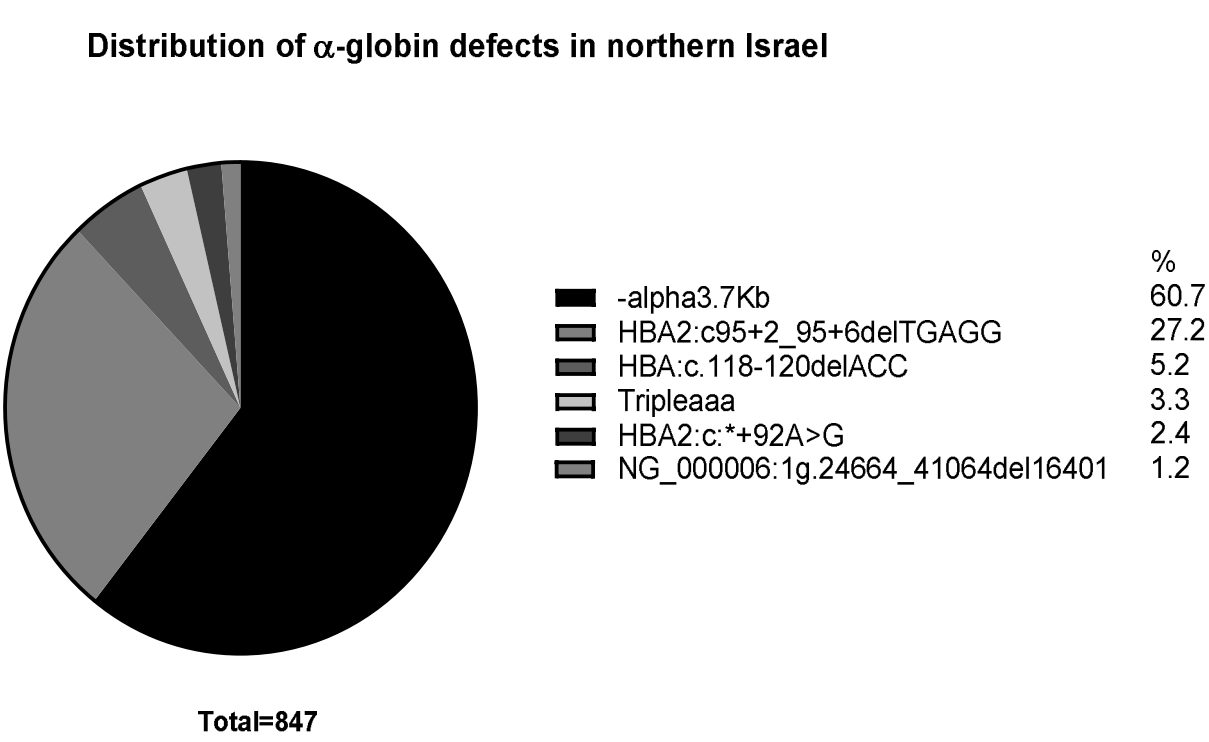


Figure 2:



HEMOGLOBINOPATHIES IN THE UNITED ARAB EMIRATES

Prof. Dr. Erol Baysal, FRCP
Fellow of the Royal College of Physicians, London, UK
Prof. of Genomics
Consultant Molecular Geneticist
Former Head, Molecular Genetics Dept.,
Dubai Genetic and Thalassemia Center
Former Medical Director, UAE Genetic Diseases Association,
Dubai, UAE

ABSTRACT

Hemoglobinopathies are one of the most common monogenic disorders in the UAE. For almost 30 years, the author's laboratory has carried out molecular characterization and mutational analyses of nearly 6000 predominantly UAE national families and over 200,000 PMS tests at the Dubai Genetics Center, a national reference center.

The prevalence of β-globin gene defects in the UAE is quite high at 8.5%. This justifies efforts to control the disease through PMS, PND, and PGD. For couples considering marriage, PMS helps identify the hemoglobinopathy carriers. This was first implemented in the UAE by the author in 2006.

The molecular studies showed that most of the β-thal mutations in the UAE are very severe β^0-thal type. The most common allele was the IVS-I-5 (G>C) *HBB: c.92+5G>C*. Although this allele is β^+-thal, its phenotype is very severe. All other mutations are also severe β^0-thal. High frequency of moderate or severe β-thal mutations have implications in the wide spectrum of clinical manifestations seen in patients whose phenotypes vary from □-thal intermedia (β-TI) to severe transfusion-dependent β-thal major (β-TM).

The PND by CVS was first implemented in the UAE for hemoglobinopathies by the author in 2005. Although termination of pregnancy is illegal in the UAE, many at-risk couples choose PND for its accuracy. This is the most effective way to prevent the birth of affected babies with β-thal major. If the fetus is affected, expatriate couples usually choose their own country for termination. However, most UAE nationals opt for Iran, where termination of pregnancy is allowed by a fatwa (religious decree) for up to 120 days. Some couples decide not to have PND or PGD, even if they are at risk.

Keywords: UAE, β-thalassemia, genotype/ phenotype, premarital screening, prenatal diagnosis

INTROCUCTION

The United Arab Emirates (UAE) is a federation of seven emirates situated on the Eastern Arabian Peninsula bordering Oman, Saudi Arabia, and Qatar. Iran and the Arabian Gulf are in the north and Saudi Arabia is in the west and south. The population of the UAE is diverse. More than 200 nationalities from all over the world live in Dubai. Like other Gulf countries, it comprises immigrants from the Middle East, Africa, India, Pakistan, Iran, Southeast Asia, and Europe. In nearly four decades, the population of the UAE swelled significantly from 600,000 in 1985, to 2.5 million in 1995, 8.5 million in 2010, and 9.3 million in 2020, and now boasts nearly 10 million people (Aug 2023). Life expectancy is one of the highest in the world; 78.21 for males and 81.0 for females.

Dubai is the most populous emirate in the UAE with an estimated 3.55 million inhabitants compared to 1.57 million inhabitants in Abu Dhabi (2023). The oil revenues in the late 60s led to rapid

economic development that transformed Dubai into a modern metropolis. Today, Dubai is a commercial and business hub in the entire Gulf Region.

The distribution of the UAE population is as follows:

The UAE nationals make up 11.48% (1.09 million), of the total population (9.52 mil), while Indians are 27.49%, Pakistanis 12.49%, Bangladeshis 7.40%, Filipinos 5.56%, Iranians 4.76%, Egyptians 4.23%, Nepalese 3.17%, Chinese 2.11%, and others 17.94%.

The expatriates account for 88.52% (8.43 million) of the total UAE population **(Fig. 1)**.

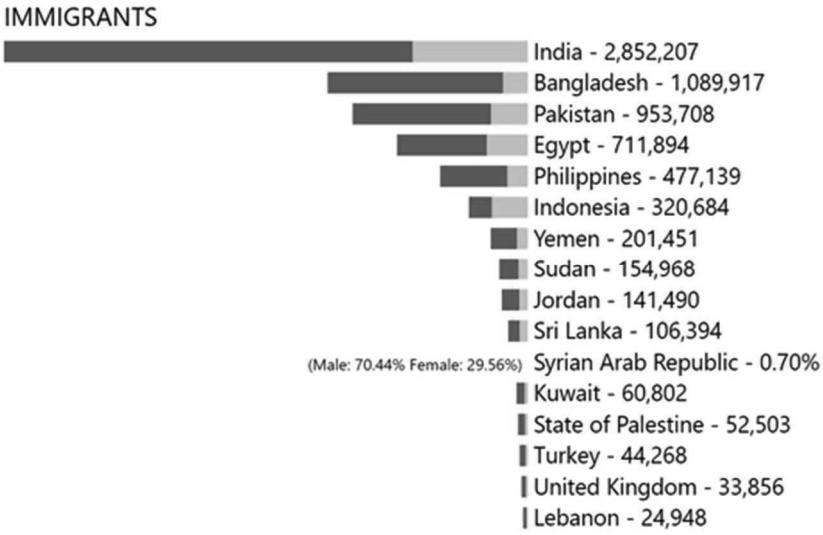
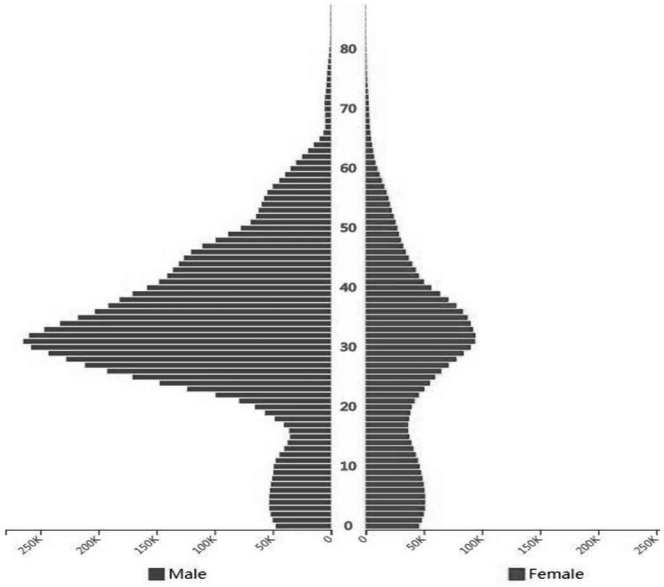


Figure 1: *Distribution of Immigrant Population in Dubai (2019)*
Blue: Males
Pink: Females

The birth rate in the UAE is estimated at 2% and the infant mortality rate is <1%. Live births are estimated at 60,000 per annum. The fertility rate is 1.65 births per national woman.



The age structure in the UAE is a significant consideration as it reflects a bearing on the population growth.

The age groups are represented as follows;
0-14 age group is 14.94% (1.52 mil);
15-24 age group is 12.36% (1.26 mil);
25-54 age group is 64.15% (6.52 mil),
55-64 age group is 6.59% (0.67 mil);
65+ age group is 1.96% (0.20 mil).
Those under the 25-age group comprise 27.3% (2.78 mil) of the entire population.
Those under the age of 55 account for 92% (9.3 mil) of the total population.
In the 25-54 age group, males account for nearly three times the number of females.

Figure 2: *Population age distribution in the UAE (2022)*
Blue: Male Red: Female

β-THALASSEMIA

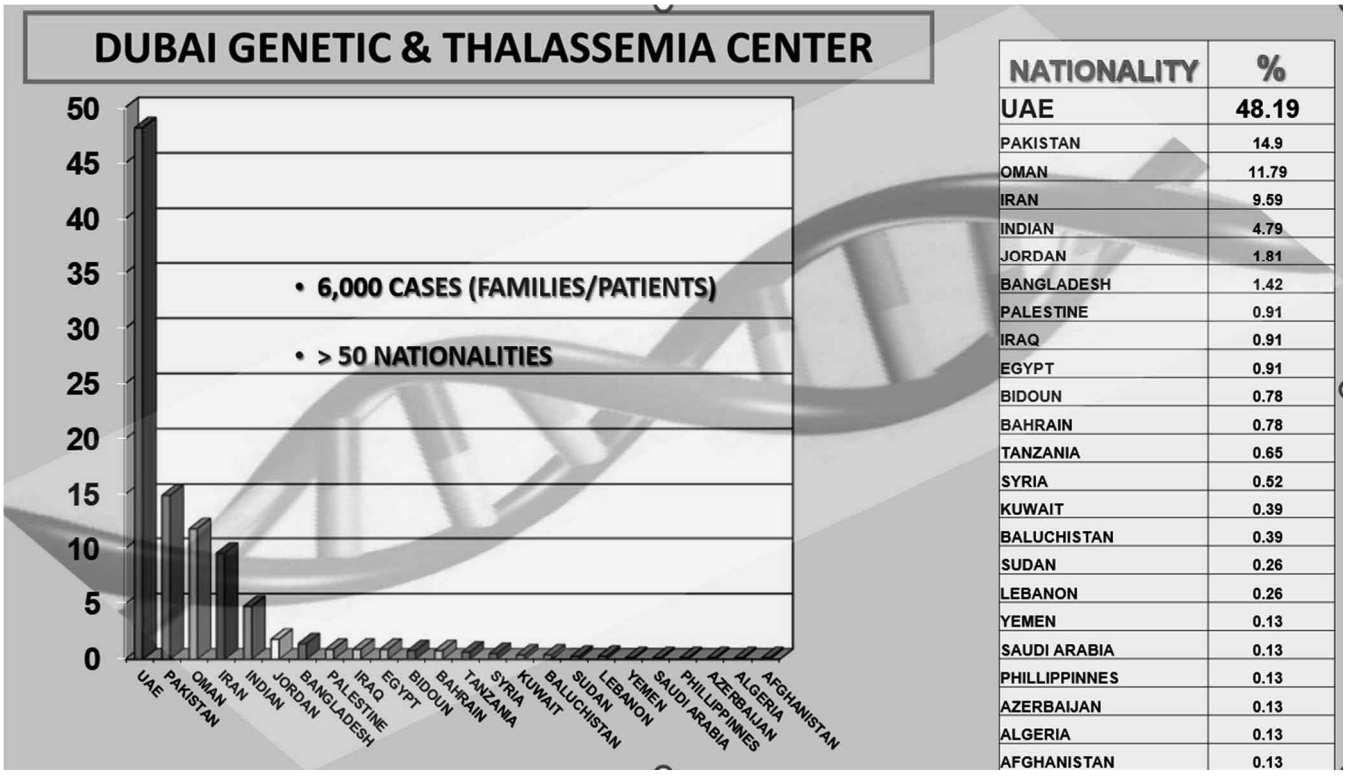
β-thalassemia (β-thal) is one of the most common single-gene disorders affecting almost all the countries in the Mediterranean Basin, the Middle East, South East Asia, the Far East, Australasia, the Americas, and Africa. It is characterized by the deficiency or absence of β-globin chain production. Around 300 different mutations have so far been reported that result in β-thal (Huisman, Carver, and Baysal, 1997; Baysal, 1995). β-thal constitutes a major public health problem in the UAE. Not much was known about the diversity of β-thal mutations in the UAE until the mid-1990s. Preliminary surveys by White et al (1986) showed that β-thal and Hb Sβ-thal and other abnormal Hbs existed in the UAE at high frequencies. Owing to the unavailability of DNA methods at the time, the findings were limited to hematological evaluations such as microcytosis, hypochromia, iron status, and HbA2 values. Due to the large family sizes and the lack of preventive programs previously, most families have more than one affected child.

CONSANGUINITY

The high degree of consanguinity, especially due to first-cousin marriages, resulted in a significant number of homozygotes who are on regular blood transfusion and chelation therapy. The high level of endogamy (intermarriage) originates from centuries-old socio-cultural and religious traditions in Arab societies. Similar observations were made in the expatriate patient population most of whom are Muslims (Bener et al., 1996, Gazali et al., 1997). Our existing data depict that 68% of the UAE nationals (212 out of 313 patients) were characterized as homozygous β-thal thus corroborating consanguinity. This was more than twice the number of compound heterozygotes.

THE DUBAI GENETIC AND THALASSEMIA CENTER

The Dubai Genetic and Thalassemia Center was inaugurated in 1995. The center currently boasts nearly 1500 registered patients of whom approximately 50% are UAE nationals. The other nationalities include India, Pakistan, Bangladesh, and South-East Asian countries **(Fig. 3)**.



NATIONALITY	%
UAE	48.19
PAKISTAN	14.9
OMAN	11.79
IRAN	9.59
INDIAN	4.79
JORDAN	1.81
BANGLADESH	1.42
PALESTINE	0.91
IRAQ	0.91
EGYPT	0.91
BIDOUN	0.78
BAHRAIN	0.78
TANZANIA	0.65
SYRIA	0.52
KUWAIT	0.39
BALUCHISTAN	0.39
SUDAN	0.26
LEBANON	0.26
YEMEN	0.13
SAUDI ARABIA	0.13
PHILLIPPINNES	0.13
AZERBAIJAN	0.13
ALGERIA	0.13
AFGHANISTAN	0.13

Figure 3: *Distribution of thalassemia patients according to their nationalities.*

During 1995-2023, the Molecular Genetics Unit has been actively involved in identifying, characterizing, and elucidating all types of hemoglobinopathies, predominantly in Dubai. Nearly 6000 patients and their families were characterized at the molecular level. Furthermore, the Molecular Genetics Unit has been the reference laboratory for the nationwide mandatory premarital screening (PMS) and is recognized as the 'center of excellence' throughout the Gulf region.

Previous hematology-based surveys showed that the UAE exhibited one of the highest carrier frequencies of β-thal in the Gulf region (White et al, 1986). The first molecular study on the distribution of β-thal in the UAE was reported by Quaife et al (1994) who showed seven β-thal alleles in 50 traits with the most common allele being the IVS-I-5 (G→C) *HBB: c.92+5G>C* substitution. It was suggested that this mutation was introduced to the UAE by population migration from the Baluchistan province of Pakistan, which neighbors Iran and Afghanistan.

The journey to map the genetic make-up of hemoglobinopathies in the UAE started with our initial study which involved 2,000 randomly-selected adult UAE national college students and 800 randomly selected UAE national adults. The results demonstrated that the incidence of β-globin gene defects in the UAE was 8.5% (El Kalla and Baysal, 1998; Baysal 2005; Baysal 2011). Among these anomalies were β-thal mutations, and abnormal Hb variants. The sickle gene (β^S or HbS) contributed significantly to the epidemiology of hemoglobinopathies in the UAE.

The previous studies on the epidemiology of thalassemia included hematological analyses which employed now-defunct methodologies such as column chromatography, cellulose acetate, isoelectric focusing (IEF), and quantitation of Hb types (HbF and HbA2) by column chromatography. The current investigations used highly sophisticated high-performance liquid chromatography (HPLC) and molecular analyses including DNA sequencing.

The molecular characterization and mutational analyses of all β-thal patients were carried out using current molecular techniques including amplification refractory mutation system (ARMS), restriction enzyme analysis (REA), dot-blot hybridization, β-strip hybridization, allele-specific oligonucleotide (ASO), polymerase chain reaction (PCR), gap-PCR and DNA Sequencing. Some of these techniques are now obsolete. Almost all molecular characterizations are currently performed through PCR followed by DNA sequencing using a fully automated ABI PRISM™ 3130 Genetic Analyzer. (Baysal, 2005; Baysal *et al.* 2007; Baysal, 2011).

Molecular studies were carried out on all β-thal patients registered at the Genetic and Thalassemia Center using the latest available techniques. α-Globin genes were routinely investigated according to Baysal and Huisman (1994) as the latter exists at a very high frequency (Baysal, E., 2011a). This topic will be discussed in detail in a separate section. The laboratory analyses included iso-electric focusing (IEF), quantitation of Hb types by column chromatography, PCR, restriction enzyme analysis (REA), β-strip hybridization, allele-specific oligonucleotide (ASO) hybridization as well as manual and automated DNA sequencing.

Molecular studies began with DNA extraction according to the commonly used procedures. The 5'β segment of the β-globin gene was amplified using a forward primer, located in the upstream promoter region 5' to the Cap site, and a reverse primer in the second intervening sequence (IVS-2). A vast majority of the β-thal mutations in the UAE were found in the 5'β segment of the β-globin gene. The 3'β segment was only amplified whenever mutation screening in the 5'β revealed no mutation.

One of the most significant observations derived from our molecular studies is that most of the β-thal mutations in the UAE are severe. Although the most common allele, the IVS-I-5 (G>C) *HBB: c.92+5G>C* is β^+-thal, all other mutations are severe β^0-thal types. High frequency of moderate or severe β-thal mutations have implications in the wide spectrum of clinical manifestations seen in patients whose phenotypes vary from β-thal intermedia (β-TI) to severe, transfusion-dependent β-thal major (β-TM).

In this report, we describe the molecular pathology of a cohort of 838 patients, of which 412 are UAE nationals. Among the UAE nationals, 249 patients were homozygous for the β-thal mutations and abnormal Hb. Table 1 shows the distribution of β-thal mutations in 188 homozygous patients (excluding the abnormal Hb) (Baysal, et al. 2007).

Table 1: *Homozygous β-Thalassemia in the UAE National Patients [modified from Baysal et al, 2007)].*
[a] Excluded from the table are Hb SS (23.7%) and Hb DD (0.8%).

	MUTATION	*n*	Chromosomes	Frequency (%)[a]
1	IVS-I-5(G>C)/IVS-I-5(G>C)	132	264	53.0
2	–25 bp del/–25 bp del	17	34	6.8
3	Codons 8/9(+G)/codons 8/9(+G)	7	14	2.8
4	Codon 39(C>T)/codon 39 (C>T)	6	12	2.4
5	Codon 30(G>C)/codon 30(G>C)	5	10	2.0
6	IVS-II-1(G>A)/IVS-II-1(G>A)	4	8	1.6
7	Codon 5(–CT)/codon 5(–CT)	4	8	1.6
8	–88(C>A)/–88(C>A)	3	6	1.2
9	IVS-I-1(G>A)/IVS-I-1(G>A)	3	6	1.2
10	Codon 15(G>A)/codon 15(G>A)	2	4	0.8
11	Codon 8(–AA)/codon 8(–AA)	2	4	0.8
12	IVS-I-110(G>A)/IVS-I-110(G>A)	2	4	0.8
13	Codons 82/83(–G)/codons 82/83(–G)	1	2	0.4
TOTAL		188	376	

MUTATION ANALYSIS

Table 1 depicts that the most frequent homozygosity was the IVS-I-5/IVS-I-5 (53.0%) *HBB: c.92+5G>C* followed by –25 bp del/–25 bp del *HBB:c.0_92+25del* (6.8%), codons 8/9(+G)/codons 8/9(+G) *HBB:c.27_28insG* (2.8%) and codon 39(C>T)/codon 39(C>T) *HBB: c.118C>T (p.Gln40Ter), HBB: c.118C>T (p.Gln40Ter)* (2.4%). These four mutations account for 65.0% of the homozygous patient population. Also depicted in Table 1 are 13 discrete homozygotes discovered in our UAE national patients in contrast to 23 homozygous mutations in the expatriate population (data not shown). Since the number of homozygous mutations has a direct correlation with the degree of consanguinity, the data shown here corroborate that more than 50% of all marriages in the UAE are between relatives, and more than half of these are between first cousins.

The mutation analysis among the UAE national and expatriate β-thal patients demonstrates that the UAE is arguably the most heterogenous β-thal population in the world with 70 different β-thal mutations reported to date **(Fig. 4)**. It is important to note that most of the β-thal mutations in the UAE are the severe β^0-thal type, except for the most common allele IVS-I-5 (G>C) *HBB: c.92+5G>C*, which is β^+thal.

Fig. 4 depicts our molecular studies which revealed 70 different β-thalassemia mutations in the UAE population to date. From a simple perspective, the total number of mutations in the UAE surpasses the number of mutations reported from China and India, combined! These are the two most populous nations with combined populations of 2.6 billion however the total number of reported mutations is less than 40. Considering Dubai's population of only 3.4 million, the data reported here reflects the considerably diverse molecular heterogeneity in the UAE. However, it must be noted that the genetic diversity of this scale poses major challenges in designing prevention programs and offers alarming prospects to all of us who are engaged in prenatal diagnosis programs for β-thal. However, this problem is generally circumvented by carrying out mutational studies on prospective family members and creating a comprehensive database before performing molecular analysis on the fetus.

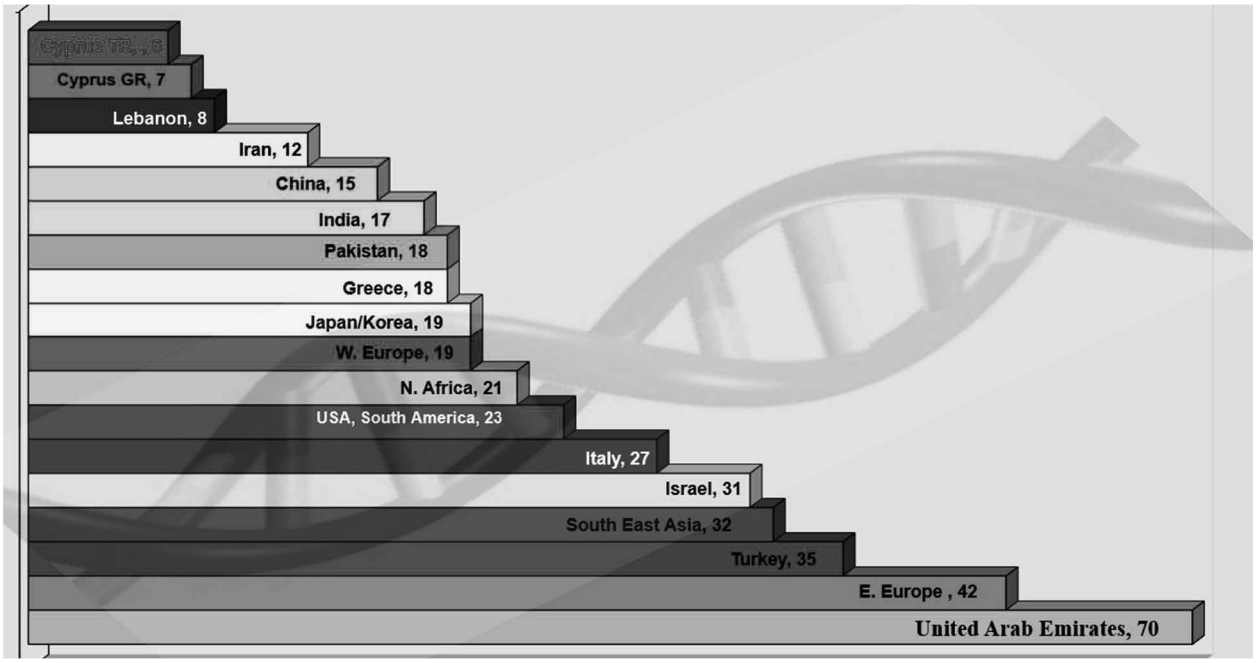


Figure 4: *Heterogeneity of β-thalassemia mutations worldwide as measured by the total number of alleles in each country or region.*

GENE FERQUENCY

Table 2 lists the relative frequencies of the 25 different mutations found in the UAE national patients (*n*:412). The first 10 mutations account for 71.2% of the total β-thal chromosomes (excluding Hb S [β6(A3) Glu→Val, GAG>GTG; *HBB: c.20A>T*), which occurs at 21.1%. Table 2 represents a cohort of homozygous β-thal patients. The most frequent mutation was the IVS-I-5 (G→C) *HBB: c.92+5G>C*, at 44.5%. This is perhaps one of the highest incidences of the IVS-I-5 (G→C) allele reported worldwide. This was followed by the –25 bp del allele at 8.6%.

Table 2: *β-Thalassemia Gene Frequency Among the UAE National Patients (n 412; chr 824)*
[a] Excluded from the table is Hb S (21.1%).

	MUTATION	Chromosomes	Frequency (%)[a]
1	IVS-I-5 (G>C)	367	44.5
2	–25 bp deletion	71	8.6
3	Codons 8/9 (+G)	25	3.0
4	IVS-II-1 (G>A)	23	2.8
5	Codon 39 (C>T)	18	2.2
6	Codon 8 (–AA)	18	2.2
7	Hb D-Punjab (*G*AA>*C*AA)	18	2.2
8	Codon 30 (G>C)	17	2.1
9	Codon 5 (–CT)	17	2.1
10	IVS-I-6 (T>C)	12	1.5
11	–88 (C>A)	9	1.1
12	Codons 82/83 (–G)	8	1.0
13	IVS-I-110 (G>A)	8	1.0
14	IVS-I-5 (G>T)	7	0.9
15	Codon 15 (G>A)	7	0.9

16	Codon 44 (–C)	6	0.7
17	Codon 110 (T>C)	3	0.4
18	IVS-II-848 (C>A)	3	0.4
19	PolyA (AATAA*A*>AATAA*G*)	3	0.4
20	–101 (C>T)	2	0.2
21	Hb Knossos (codon 27, G>T)	1	0.1
22	Codon 37 (G>A)	1	0.1
23	Codons 36/37 (–T)	1	0.1
24	Hb E (codon 26, G>A)	1	0.1
25	δβ deletion	1	0.1

The most common mutation in the UAE is the IVS-I-5 (G→C) *HBB: c.92+5G>C*, a type of β^+ thal. This allele exists in very high frequencies in India and among the neighboring populations. Aside from this, all the subsequent 10 mutations were β^0-thal with a very severe phenotype.

The spectrum of β–thal mutations in the UAE represents an extensive admixture of genes from the Mediterranean, Arabian, and Indian backgrounds. Among the first 11 most common mutations, the following FIVE exist at significantly high levels in the Mediterranean countries:

(1) Cd39 (C→T) *HBB: c.118C>T (p.Gln40Ter)*
(2) IVS-II-1(G→A) *HBB c.315+1G>A*
(3) Cd5 (-CT), *HBB c.17_18delCT*
(4) IVS-I-1(G→A) *HBB):c.92+5G>C*
(5) Cd30(G→C) *HBB):c.92G>C (p.Arg31Thr)*

THREE were prevalent in India;

(1) (IVS-I-5 (G→C) *HBB: c.92+5G>C*
(2) Cd 8/9(+G) *HBB:c.27_28insG*
(3) Hb D-Punjab [β121(GH4) Glu→Gln GAA>CAA *HBB: c.364G>C*)

The other mutations below occur mainly in the Mediterranean, Iran, and the Arabian Peninsula:

(1) -25 bp del, *HBB:c.0_92+25del*
(2) Cd 39 (C→T), *HBB: c.118C>T (p.Gln40Ter)*
(3) IVS-II-I(G→A) *HBB c.315+1G>A.*

Compound Heterozygotes

The β-thal mutations in the UAE nationals showed considerable heterogeneity, similar to that found in the expatriate population. A total of 53 different compound heterozygotes were observed (data not shown). The most prevalent compound heterozygotes were as follows:

1- IVS-I-5 / β^S [β6(A3) Glu→Val, GAG>GTG; *HBB: c.20A>T* (31 patients),
2- IVS-I-5 / –25 bp del *HBB:c.0_92+25del* (17 patients)
3- IVS-I-5 / IVS-I-6 (T>C) *HBB c. 92+6T>C*, (10 patients)
4- IVS-I-5 / Codon 8 (–AA) *HBB c.25_26delAA; p.Lys9ValfsTer14* (10 patients)

Some of the mutations were rare and some were observed only once. It is important to note that for both the homozygous and compound heterozygous patients, the mutations were of the β^0 type except for the IVS-I-5, which is β^+-thal. However, the latter has a very severe phenotype in the homozygous state or when associated with another β^0 allele. This is because only 5% Hb A mRNA is processed through the mutant IVS-I-5 gene, a level insufficient to alleviate severe □-globin chain deficiency.

One cohort consisted of 426 EXPATRIATE □-thal patients. Of these, 256 (60%) were homozygous and 171 (40%) were compound heterozygous. The EXPATRIATE vs UAE NATIONAL patients in this cohort were essentially similar; 426 expatriates and 412 UAE nationals; 50.8 and 49.2%, respec-

tively (Fig.2). Most of the expatriate patients were from Pakistan, Oman, Iran, and India. In the expatriate patients, 78 different combinations of compound heterozygote mutations were defined (data not shown). This was considerably larger than the 53 compound heterozygotes depicted in the UAE nationals. The combined UAE national and expatriate data make the UAE the most heterogeneous population in the world **(Fig 4)**.

Table 3 shows the distribution of β-thal mutations in the UAE vs EXPATS. The data show remarkable similarities in the number of homozygotes, compound heterozygotes, and frequency of IVS-I-5, the most common mutation, thus reflecting a significant overlap of genetic backgrounds. This has a direct influence on religious, cultural, traditional, and historical family planning values.

Table 3: *Comparative Data Showing the Distribution of -Thalassemia Mutations Between UAE National and Expatriate Patients. HbS patients are excluded*

Patients	UAE		EXPATRIATES	
n	412		426	
Homozygotes	249	60.0%	264	62.0%
Compound heterozygotes	163	40.0%	162	38.0%
IVS-I-5(G>C)/IVS-I-5(G>C) (homozygotes	132	54.0%	130	49.2%
IVS-I-5(G>C)/IVS-I-5(G>C) (all patients)	132	32.0%	130	31.0%
IVS-I-5 (G>C) chromosomes	367	44.5%	336	39.4%
Homozygous mutations	15		23	
Compound heterozygous mutation	53		78	

ORIGINS

In contrast to the other countries where only a small spectrum of β-thal alleles occurs; UAE appears to have a significant heterogeneity even among the indigenous population. The IVS-I-5 (G→C) allele was likely introduced to the Arabian Peninsula by gene migration from Baluchistan, a region spanning southern Iran, Afghanistan, and Pakistan. Many of the UAE national families are thought to have their roots in surrounding countries such as Iran and Baluchistan, as evidenced by the common mutations and haplotype studies.

It is well documented that the IVS-I-5 (G→C) mutation was found predominantly in India, Baluchistan, Pakistan, and China but not among the Middle East Arabs (Baysal, 2001; Baysal, 2011). The propensity of this allele in the Arabian Peninsula can be attributed to the population migration from the Indian subcontinent; its low frequency in Kuwait and high frequency in the UAE and Oman favors the speculation that the gene was introduced into the Arabian Peninsula across the Straits of Hormuz. This navigational route still constitutes a major trade link between the Indian subcontinent and the Gulf States.

ABNORMAL HEMOGLOBINS (HBS)

Abnormal Hbs namely HbS, HbD, HbE, HbC, HbO-Arab, and HbKnossos are also important in the epidemiology of hemoglobinopathies in the UAE. Abnormal Hbs, particularly HbS, contribute significantly to the genetic diversity of hemoglobinopathies. The 4.0% prevalence of abnormal Hbs is considered to be similar to that of β-thal. The β^S gene is a major genetic factor in a group of patients with co-inherited Sβ-thal. Molecular studies demonstrated that the β^S gene is indeed the second most common β-globin gene defect in the UAE and exists predominantly in the Arab-Indian haplotype (El Kalla and Baysal, 1998). Meanwhile, HbE (α2β226Glu→Lys) codon 26 (GAG→AAG) *(HBB):c.79G>A (p.Glu27Lys)* is a β-thalassemic hemoglobin, seen particularly in expatriates from South East Asia and Bangladesh.

CONCLUSION

The diversity of hemoglobinopathies in the UAE is most certainly caused by the admixture of genes between different population groups in and around the Gulf region, Indian subcontinent, Middle Eastern countries, and Africa. In recent times this was

exacerbated by the influx of many other nationalities into the UAE. The gene flow and heterogeneity of β-thal mutations represent complex anthropological influences from the East Mediterranean, Asia, India, sub-Sahara, and East Africa corroborating that the diversity of β-thal mutations in the UAE reflects historical events and gene migration in the region. The β-thal distribution, heterogeneity of mutation, and homozygous births due to consanguinity, compounded with cultural traditions and beliefs are some of the important factors that determine the propensity of hemoglobinopathies in the UAE. Public awareness programs, education, and mandatory preventive policies have recently been implemented and seem to be showing exceptional positive effects.

REFERENCES

1. Al Gazali, L.I., Bener, A., Abdulrazzaq, Y.M., et al., Consanguineous *marriages in the United Arab Emirates. J Biosoc Sci. 1997;29(4):491-497.*
2. Baysal, E. and Huisman, T.H.J. *Detection of common α-thalassemia-2 determinants by PCR. American Journal of Hematology 46(3): 208-213, 1994*
3. Baysal, E. *The β- and δβ-thalassemia Repository (eighth edition). Hemoglobin 19 (3&4) 151-164, 1995.*
4. Baysal, E. *Advances in Hemoglobinopathies Research – Hemoglobinopathies in the United Arab Emirates. Hemoglobin. 2001;25(2):247-253.*
5. Baysal, E. *Molecular heterogeneity of □-thalassemia in the United Arab Emirates. Community Genet. 2005;8(1):35-39.*
6. Baysal, E., Yousef, I., Zeinali, M., *et al. Molecular basis of hemoglobinopathies in the UAE: implications for prenatal diagnosis and prevention programs. Emirates Med J. 2007;25(1):7-21.*
7. Baysal, E. *Molecular basis of β-thalassemia in the United Arab Emirates. Hemoglobin 2011;35(5-6):581-8. doi: 10.3109/03630269.2011.634706*
8. Baysal, E., *α-Thalassemia Syndromes in the United Arab Emirates, Hemoglobin; 35 (5-6):574-80, 2011a. PMID: 22074123*
9. El Hazmi, M.A., Al-Swailem, A.R., Warsy, A.S. *Molecular defects in beta-thalassaemias in the population of Saudi Arabia. Hum Hered. 1995*;45(5):278-85.
10. El Kalla, S., Baysal, E. *Genotype-phenotype correlation of sickle cell disease in the United Arab Emirates. Pediatr Hematol Oncol. 1998;15(3):237-242.*
11. Huisman T.H.J., Carver M.A.F. and Baysal E. *A Syllabus of Thalassemia Mutations. The Sickle Cell Anemia Foundation, U.S.A., 1997.*
12. Quaife, R., Al Gazali, L., Abbes, S., *The spectrum of β-Thalassemia mutations in the UAE national population. J Med Genet. J Med Genet. 1994 Jan;31(1):59-61.*
13. White, J.M., Byrne, M., Richards, R., et al Red *cell genetic abnormalities in Peninsular Arabs: sickle hemoglobin, G6PD deficiency, and □ □ thalassemia. J Med Genet.;23(3):245-251, 1986.*

Correspondence Address:
Fellow of the Royal College of Physicians, London, UK
Prof. of Genomics
Consultant Molecular Geneticist
E-mail: erolbaysal@gmail.com

THALASSEMIA AND HEMOGLOBINOPATHIES IN OMAN

Yasser Wali and Esraa Abdelhakem
Pediatric Hematology/Oncology Unit, Child Health Department, Sultan Qaboos University, Muscat, Oman.

ABSTRACT

Oman is a country located at the south-east corner of the Arabian Peninsula. It has close historical ties with Asia (Baluchistan) and East-Africa (Swahili coast, Zanzibar), where part of its population originates. Inherited blood disorders (IBD) have long been described as group-specific diseases. In Oman, the prevalence of sickle cell trait reaches up to 4.8–6% which is the highest between the Arab Gulf States. The most prevalent genotype of SCD is S/S followed by S/β-thalassemia. Three major haplotypes of SCD coexist in Oman: Benin, Arab-India, and Bantu, the distribution of these haplotypes was in excellent agreement with the historical ancient contacts. The incidence of α- thalassemia was 48.5%, based on the presence of Hb Bart's on cord blood testing. Consanguinity must have contributed to such a high prevalence of homozygous α -thalassemia in Omani neonates. The nature of the β- thalassemia mutations that have been determined in the Omani population suggests that the majority have been introduced by gene flow. HbS-Oman is a severe βs gene variant of sickle hemoglobinopathy, it was named because of its high prevalence amongst Omani population. The polymerization and sickling properties of HbS-Oman are considerably greater than that of HbS and it represents one of the "super sickling" forms of Hb. Hemoglobinopathies and thalassemias are one of the main problems faced by public health in Oman. Prevention of such diseases, depends on strict implementation of premarital screening and genetic counseling of high-risk couples.

Keywords: Oman, sickle cell disease, thalassemia, HbS-Oman

BACKGROUND

Oman is a country located at the south-east corner of the Arabian Peninsula. It faces the north-east part of the Arabian Gulf, bordering the United Arab Emirates (UAE) and Saudi Arabia in the west, Yemen in the south and is separated from Iran by a narrow sea strait from the north-east. Oman has close historical ties with Asia (Baluchistan) and East-Africa (Swahili coast, Zanzibar), where trading posts had been implanted. Part of its population originates in these former colonies, but these various origins are mostly passed over in silence by the regime.

Inherited blood disorders (IBD) have long been described as group-specific diseases; sickle-cell anemia has been considered confined to Black populations while thalassemias to Mediterranean and Indian groups. Combined with misinterpretations of population genomics research (which studies distribution and migrations of human genes since prehistoric times), this has led to a global lay representation of IBD as 'diseases of the origins'(**1**). This connection between genotype, territory and ancestry remains in popular discourses, even though genomics has proved it to be not systematic **(2)**.

Zanzibar, Pakistan, and parts of Iran were classified as malaria-endemic areas as well as some parts of the Arabian Peninsula (Malaria Belt). Malaria is one of the main reasons for the occurrence of Sickle cell disease (SCD), thalassemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency and other abnormal hemoglobin as well as erythrocyte defects which are the most common Mendelian diseases. Different populations have evolved different genetic variants to protect against malaria **(3, 4)**. It is known that there has been positive natural selection for hemoglobin S and C in humans despite negative health effects, due to its beneficial role in malaria resistance. However, it is not known if there has been natural selection for hemoglobin E (HbE), which is a common variant in Southeast Asia **(5)**. Moreover, HbC is not present in Oman as per pre-

vious reports which can't be explained by the current literature **(6)**.

Hemoglobinopathies constitute the commonest recessive monogenic disorders worldwide. They fall into two main groups: the thalassemia syndromes and the structural hemoglobin variants (abnormal hemoglobins). α, β, and δβ thalassemias are the main types of thalassemia with clinical importance; the most frequent and clinically important structural hemoglobin variants are HbS, HbE, HbC and HbD. Management of these patients presents a substantial global health burden.

HEMOGLOBINOPATHY STUDIES IN OMMAN

In Oman, the prevalence of sickle cell trait reaches up to 4.8–6% which is the highest between the Arab Gulf States. Omani population represents a variability of HbS genotype combinations with other Hb genotypes that can present with different phenotypic severity. The most prevalent SCD is S/S followed by S/β-thalassemia **(6)**.

The clinical variations in SCD presentation were largely linked to the presence of different β-globin gene haplotypes identified during molecular studies. S/β0 runs almost the same clinical course of S/S. Children with S/ β+thalassemia appears to be more prone to splenic sequestration compared to other genotypes of SCD. Co-inheritance of α-thalassemia, and higher hemoglobin F levels was associated with less hemolysis and milder course **(6)**.

In 1995, a national register of symptomatic hemoglobinopathies in Oman identified 1.757 β-cases of homozygous sickle cell anemia (SCA) and 243 cases of β-thalassemia major in a population of 1.5 million in 1995. 10% had for sickle cell trait and 4% had β-thalassemia trait. They reported the birth prevalence of symptomatic hemoglobinopathies in 23 Omani tribes through screening of a national register, as 3.1 per 1,000 live births during 1989-1992, which included 2.7 per 1.000 live births of homozygous SCD **(7)**.

In 2001, Al-Riyami et al. **(8)**, performed a nationwide survey representative sample of 6.103 Omani and blood samples from 6.342 children aged 0-5 years. Countrywide prevalence rates for the sickle cell and β-thalassemia traits were estimated to be 5.8% and 2.2%, respectively, with no significant gender differences. The prevalence of SCD was 0.2% and homozygous β-thalassemia was 0.07%. The prevalence of sickle cell trait was relatively higher than those reported from Saudi Arabia (1.2%) and United Arab Emirates (1.9%).

In 2010, Alkindi et al.**(9)**, studied consecutive cord blood samples from a total of 7,837 neonates for complete blood counts and for hemoglobin (Hb) profile by high performance liquid chromatography (HPLC). They observed that the overall incidence sickle cell trait was 4.8%, sickle cell disease 0.3%, β-thalassemia trait was 2.6%, Hb E trait was 0.9%, Hb D trait was 0.8%, and homozygous beta- thalassemia was 0.08%. In addition, cases of HbS Oman, a variant of HbS were identified in a few families. Nevertheless, it was striking to note that the incidence of α- thalassemia was 48.5%, based on the presence of Hb Bart's with more than 60% of the samples were homozygous for α+ thalassemia (−α 3.7 deletion), 0.06% had the α T-Saudi mutation in the heterozygous state, and the remaining subjects were α+-thalassemia heterozygotes (mostly α- 3.7 deletions with a few α - 4.2 deletions). Again, consanguinity must have contributed to such a high prevalence of homozygous α -thalassemia in Omani neonates.

The nature of the β- thalassemia mutations that have been determined in the Omani population suggests that the majority have been introduced by gene flow. There was active commerce between Oman and the area around the Indus Valley for centuries. Historically, Oman was a major maritime nation with links to the Far East and India on the one hand and East Africa and Egypt on the other, as well as trade in the Arabian Gulf **(10)**.

In 2010, Hassan et al. **(11)**, studied 87 unrelated individuals of both genders previously diagnosed with β-TM, β- TI or minor or with sickle cell disease. They reported that the IVS-I-5 (G>C) was the most prevalent (73%), while Hb Monroe [IVS-I (–1) or codon 30 (G>C), b30(B12)Arg→Thr], codon 5 (–CT) and IVS-I (–25 bp) 3′ end mutations were the second in frequency (4.5%) together with codon 39 found in immigrants. Codons 8/9 (+G) was third in prevalence (3.3%) and codons 41/42 fourth at 2.2% but in immigrants only. The IVS-I-5 mutation was predominant in Batinah, Muscat and Dhakhiliyah (73.7, 86.6 and 83.3%, respectively), while the

same mutation was absent in Musandam with Hb Monroe being the most prevalent.

In 2015, the same group **(12)** studied 446 unrelated Native Omani individuals either affected with β-TM, or Hb S (HBB: c.20A4T)/ β-thalassemia or carriers of β-thalassemia. Their ages ranged from 1 to 48 years. They reported 32 different β-thalassemia mutations with IVS-I-5 (G4C) was the most frequent mutation (43.6%) in each region except for Dhofar. Codon 44 (–C) and _71 (C4T) both occurred with a frequency of 8.0%, while Hb D-Punjab or codon 121 (G4C) (HBB: c.364G4C) was found at a frequency of 5.0%. The other mutations were IVS-I-128 and Hb La Desirade, occurring at 3.5 and 2.9%, respectively. Hb E [codon 26 (G4A) (HBB: c.79G4A], was found at 2.7%, while the codon 5 (–CT), IVS-I, _25 bp deletion and Hb Sheffield [codon 58 (C4A) (HBB: c.176C4A)] mutations were all observed at 2.5%.

In summary, IVS-1-5 (G>C), a severe β+ allele, is the most prevalent of the mutations thus far described in Oman. A detailed analysis of the distribution of β- thalassemia mutations in Pakistan shows that the IVS I-5 (G>C) mutation is widespread in that country, reaching a peak incidence in the Baluchis (76.2%) and having a high prevalence among the Sindhis (43.9%). It is also found at a high frequency in India **(13, 14)**. Other common β-thalassemia alleles common in Oman are the codon 44 (-C), 37 IVS-1-3' –25bp (epicentre Bahrain), 34 IVS-2-1 (G>A) (Indian subcontinent) **(15)** mutations, and the 619bp deletion at the 3' end of the beta globin gene (Indian subcontinent) **(15)** all of which almost certainly were introduced to Oman through gene flow. The only mutation unique to Oman is Hb Dhofar, which has originated in the southern region of the Sultanate and thus far has only been described in Omanis **(14)**.

The origin of each mutation is described in Table 1 along with frequencies observed in the three countries/regions neighboring Oman: Southern Iran (Hormozgan), UAE and Eastern Saudi Arabia.

Clinical variations in SCA presentation were largely related to the presence of different βs-globin gene haplotypes identified during molecular studies. Daar et al. **(16)**, demonstrated the multicentric origin of the sickle mutation in Northern Oman. They reported the coexistence of three major haplotypes coexist: 52.1% Benin (typical and atypical), 26.7% Arab-India, and 21.4% Bantu. The distribution of haplotypes was in excellent agreement with the historical record, which established clear ancient contacts between Oman and sub-Sahara west Africa which explained the presence of the Benin haplotype. Contacts with Iraq, Iran, present- day Pakistan, and India explained the presence of the Arab-India haplotype. The more recent contacts with East Africa (Zanzibar/ Mombasa) explained the presence of the Bantu haplotype **(16)**.

Daar et al.**(16)**, reported that most of the patients (61.2%) were homozygous sickle cell anemia followed by double heterozygous types mainly sickle cell β-thalassemia. In 52 patients with SS genotypes, the Arab-India haplotype in the homozygote form was associated with higher levels of HbF as compared to the Benin and Bantu haplotypes. The Arab-India haplotype in the heterozygous form had an average HbF level lower than homozygous cases of this haplotype were predominant (68.5%) and reported that 80% of those screened for α-globin gene mutations were heterozygous or homozygous for α-thalassemia. They reported that the presence of alpha thalassemia gene mutation and high Hb F levels were important factors modifying the clinical severity of the disease **(9, 17, 18)**.

In a relatively recent study, Hassan et al. **(19)** found 11 haplotype combinations differently distributed in Oman and stated that the Asian/Asian haplotype was the most prominent while Benin/Benin came the second in rank. The higher percentages of Benin haplotype stated in Daar et al. **(16)** study might be due to their selection of patients who were attending the Sultan Qaboos University Hospital which covers mainly one region (Dhakhiliya region). The Benin haplotype has been observed to be present at a high rate in this region **(7, 16, 19)**.

Hassan et al. **(11, 19)** suggested that the Asian haplotype was associated with the highest HbF levels, fewer hospitalizations and painful episodes and acute chest syndrome. They confirmed that the CAR haplotype whether homozygous or combined heterozygous was associated with the lowest HbF level and the highest incidence of organ damage and renal failure.

Al-Lamki et al.**(20)** and Saraf et al. **(21)**, reported that that the absence of co-inheriting α-thalassemia, and low hemoglobin F levels was associated with more hemolysis, and lower hemoglobin oxygen saturations.

In Oman, the most prevalent SCD is S/S followed by S/β thalassemia. Sickle β thalassemia (S/β-thalassemia) is a condition, which results from the coinheritance of a sickle cell gene and a β-thalassemia gene. The clinical phenotype depends on the type of β -thalassemia gene (β +or β0) inherited. S/β0 runs almost the same clinical course of S/S **(10)**. As previously described patients with the Sβ0 phenotype had a higher degree of hematological involvement in comparison to Sβ+ patients, with lower hemoglobin levels, and signs of more intense chronic hemolysis. Sβ0 patients had decreased body mass index and lower bone mineral density. The degree of bone damage correlated to lower body mass index (BMI) and hemoglobin levels, as well as monocytosis and elevated lactate dehydrogenase, possibly reflecting the effects of hemolysis and inflammation upon bone metabolism and body constitution **(22)**. Children with sickle cell-β+ thalassemia may have preserved some splenic function into adulthood. Their spleen might remain till late adulthood, and they rarely have auto-splenectomy. They are more prone to splenic sequestration than other genotypes of SCD **(23, 24)**.

Trying to find a molecular explanation for the different phenotypes seen within similar basic haplotypes, sub-haplotypes were determined by looking at a total of 42 SNPs in 125 homozygous HbS patients. Out of the 42 SNPs, only 15 SNPs were found modifying the 11 identified haplotypes. However, no sub haplotypes were found to be associated with a specific haplotype except for the CAR/OmanI that showed nucleotide variations at the G-g(SNP1) (SNP position: 5232979-5232984) located in the G-g promoter **(11, 19)**.

However, both Daar et al. **(16)** and Hassan et al. **(11, 19)**, suggested that neither the haplotype or sub-haplotype nor the HbF alone appeared to be fully responsible for the variable clinical phenotypes.

Adding to the array of the disease in Oman is the unique existence of HbS-Oman. Hb S-Oman is a severe βs gene variant of sickle hemoglobinopathy that results from 2 simultaneous mutations in the same β- globin chain. The first is the classic Bs mutation (B6 Glu→Val) and the second is in position 121 (B121 Glu→Lys) **(6)**.

HbS-Oman was first described by Langdown et al. **(25)** and is one of these rarer genotypes. It was named because of its high prevalence amongst individuals of the Omani population. The polymerization and sickling properties of HbS-Oman are considerably greater than that of HbS and it represents one of the "super sickling" forms of Hb. Accordingly, heterozygotes of Hb A/S-Oman genotype with HbS-Oman levels of 20% or less present with some of the clinical complications of SCD. This phenotypic dominance of the sickle mutation is shared only with HbS-Antilles, which has a further valine to isoleucine substitution at the b23 residue, additionally to the standard to sickle b6 glutamic acid-valine. By comparison, sickle cell trait individuals (Hb A/S genotype), with red cell levels of Hb S of about 40%, are usually silent carriers and their red cells seldom sickle in vitro. However, SCD patients of the HbS-C genotype, who share a similar, though milder, clinical course with those of the Hb S/S genotype, have Hb S levels of around 50% **(26-28)**.

Compound heterozygotes of Hb-S-SOman were identified. These compound heterozygote patients run a very severe clinical course like transfusion-dependent thalassemia major with hypersplenism early in life. Hypersplenism was not controlled, solely, by hypertransfusion and needed both splenectomy at the age of one year and bone marrow transplantation in the second year of life **(4, 22)**.

Wali et al. **(4)**, reported six patients with compound heterozygosity for Hb-S-SOman, two patients underwent bone marrow transplantation (BMT) as they had extremely severe course of the disease. Fifty-six patients with HbS-Oman heterozygosity were evaluated, their clinical features ranged from being asymptomatic to severe course of the disease. Patients with Heterozygous HbS-Oman and compound heterozygous HbS-S Oman had lower mean Hb, MCV, MCH and high RDW values compared to SCD patients **(28)**.

Al Balushi et al.**(26)**, analyzed the clinical profile and red cell properties of 29 further cases of HbA/S Oman individuals. Levels of sickling were

considerable and could be elicited with levels of HbS-Oman a low as 4% in fully deoxygenated red cells. Considerable numbers of sickled cells were present at arterial O2 tensions. Currently, we have more than 70 carriers of HbS Oman, and 7 cases (Compound heterozygotes SS Oman). The rare form of sickle cell disease, which is Hb SD, and several variants of HbD such as Hb D Punjab, Hb D Iran, Hb D Ibadan, and Hb D Bushman have been noted to co-inherit with HbS. Except for Hb D Punjab, compound heterozygous states of HbS with HbD variants were clinically innocuous. Studies have shown that the clinical presentations of HbSD-Punjab mimicked severe form of sickle cell anemia **(29)**. In Oman, Hb S-D patients behave exactly like Hb S-S disease with almost similar severity **(26)**.

CONCLUSION

From the public health point of view, hemoglobinopathies and thalassemia are one of the main problems faced by public health in Oman. Options offered to prevent the disease, such as premarital screening and genetic counseling of high-risk couples, are limited to partner choice as the prenatal diagnosis, and medical abortion of the affected fetus is not permitted in Oman **(12)**.

Nowadays, in our hematology outpatient clinics, we encounter considerably high number of infants diagnosed with different types of SCD and thalassemias in Oman. In Sultan Qaboos University Hospital, we have registered more than 3000 with SCD and 500 patients with B thalassemia. Unfortunately, we do not have a national registry for yet.

Table 1: (12) *The β-thalassemia mutation frequency observed in Oman in comparison to its neighboring countries: Iran **(30)**, East Saudi Arabia **(31)**, UAE (**32**). The most common mutation in each country is depicted in bold. Twelve mutations, described in the Omani population but not in other nationalities, are in italics. Mutations observed in other countries but not in Oman were not included.*

Mutation Allele	Origin	Oman ($n=446$)	Hormozgan ($n=155$) (9)	East Saudi Arabia ($n=196$) (3)	UAE ($n=412$) (4)
IVS-I-5 (G>C)	Asian Indian	**43.7**	**69.0**	13.3	**44.5**
codon 44 (–C)	Kurdish	7.9	2.5	0.5	0.7
−71 (C>T) (5′)	Omani	7.9	–	–	–
codon 121 (G>C)	Asian Indian; Pakistani	5.1	–	–	2.2
IVS-I-128 (T>G)	Punjabi	3.5	–	–	–
codon 129 (C>T)	Black	2.9	–	–	–
codon 26 (G>A)	Asian Indian	2.7	–	–	0.1
codon 5 (–CT)	Mediterranean	2.5	2.0	3.1	2.1
IVS-I, −25 bp del (3′)	Asian Indian	2.5	1.0	13.0	8.6
codon 58 (C>A)	British; Omani	2.5	–	–	–
IVS-II-1 (G>A)	East Mediterranean	2.3	9.6	22.2	2.8
codon 39 (C>T)	West Mediterranean	2.3	2.4	**25.0**	2.2
codon 29(C>T)/codon 58(C>G)	Omani	2.3	–	–	–
IVS-I-1 (G>A)	Mediterranean	1.8	–	3.8	–
−101 (C>T) (5′)	Turkish	1.4	–	–	0.2
−88 (C>T) (5′)	Kurdish	1.2	0.34	–	1.1
codon 15 (G>A)	Asian Indian	1.0	0.34	–	0.9
codon 121 (G>A)	Arabian; African	1.0	–	–	–
codon 30 (G>C)	African	0.8	0.7	–	2.1
codons 8/9 (+G)	Asian Indian	0.6	0.34	1.5	3.0
IVS-II-849 (A>G)	Black	0.6	–	–	–
codon 6 (G>A)	Black	0.6	–	–	–
codon 8 (–AA)	Turkish	0.6	3.4	2.1	2.2
+108-+112 5 nt del (3′)	Arabian	0.4	–	–	–
IVS-I-6 (T>C)	West Mediterranean	0.4	0.34	7.1	1.5
codon 10 (C>T)	Iraqi	0.4	–	–	–
codon 22 (G>T)	Reunion Island	0.2	–	–	–
codons 36/37 (–T)	Kurdish; Iranian	0.2	0.7	0.5	0.1
+113 (A>G) (3′)	Kurdish	0.2	2.0	–	0.4
codon 30 (G>A)	Bulgarian	0.2	–	–	–
IVS-I-110 (G>A)	East Mediterranean	0.2	–	2.6	–

REFERENCES

1. Bonnet D. Repenser l'hérédité: Archives contemporaines; 2009.
2. Beaudevin C. Old diseases and contemporary crisis. Inherited blood disorders in the Sultanate of Oman. Anthropology & Medicine. 2013;20(2):175-89.
3. Kwiatkowski DP. How malaria has affected the human genome and what human genetics can teach us about malaria. The American Journal of Human Genetics. 2005;77(2):171-92.
4. Wali Y, AlBalushi H, Nazir H. Sickle cell disease in Oman and HBS Oman: a brief review. Hematol Transfus Int J. 2017;4(6):142-3.
5. Ha J, Martinson R, Iwamoto SK, et.al. Hemoglobin E, malaria and natural selection. Evol Med Public Health. 2019;2019(1):232-41.
6. Al-Mashaikhi N, Al-Rawas A, Wali Y, et.al. The prevalence of sickling abnormality in Oman: A review of relevant publications. Acta Biomed. 2022;93(4):e2022289.
7. Rajab AG, Patton MA, Modell B. Study of hemoglobinopathies in Oman through a national register. Saudi medical journal. 2000;21(12):1168-72.
8. Al-Riyami A, Ebrahim G. Genetic blood disorders survey in the Sultanate of Oman. Journal of tropical pediatrics. 2003;49:i1-20.
9. Alkindi S, Al Zadjali S, Al Madhani A, et.al. Forecasting hemoglobinopathy burden through neonatal screening in Omani neonates. Hemoglobin. 2010;34(2):135-44.
10. De Sanctis V, Kattamis C, Canatan D, et.al. β-thalassemia distribution in the old world: an ancient disease seen from a historical standpoint. Mediterranean journal of hematology and infectious diseases. 2017;9(1).
11. Hassan SM, Hamza N, Jaffer Al-Lawatiya F, et.al. Extended molecular spectrum of β-and α-thalassemia in Oman. Hemoglobin. 2010;34(2):127-34.
12. Hassan SM, Harteveld CL, Bakker E, et.al. Broader spectrum of β-thalassemia mutations in Oman: regional distribution and comparison with neighboring countries. Hemoglobin. 2015;39(2):107-10.
13. Hamamy HA, Al-Allawi NA. Epidemiological profile of common haemoglobinopathies in Arab countries. J Community Genet. 2013;4(2):147-67.
14. Daar S, Gravell D, Hussein HM, et.al. Haematological and clinical features of beta-thalassaemia associated with Hb Dhofar. Eur J Haematol. 2008;80(1):67-70.
15. Varawalla NY, Fitches AC, Old JM. Analysis of beta-globin gene haplotypes in Asian Indians: origin and spread of beta-thalassaemia on the Indian subcontinent. Hum Genet. 1992;90(4):443-9.
16. Daar S, Hussain HM, Gravell D, et.al. Genetic epidemiology of HbS in Oman: multicentric origin for the βS gene. American journal of hematology. 2000;64(1):39-46.
17. Al Zeedi MASA, Al Abri ZG. Attitudes and impact among people with abnormal premarital screening test results in Muscat governorate's primary healthcare centers in 2018. Journal of Community Genetics. 2021;12:163-9.
18. Al-Riyami AA, Suleiman AJ, Afifi M, et.al. A community-based study of common hereditary blood disorders in Oman. East Mediterr Health J. 2001;7(6):1004-11.
19. Hassan SM, Al Muslahi M, Al Riyami M, et.al. Haplotypes, sub-haplotypes and geographical distribution in Omani patients with sickle cell disease. Thalassemia Reports. 2015;5(1):4739.
20. Al-Lamki Z, Wali Y, Shah W, et.al. Natural history of sickle hemoglobinopathies in Omani children. International Journal of Pediatric Hematology/Oncology. 2000;7(2):101-7.
21. Saraf SL, Molokie RE, Nouraie M, et.al. Differences in the clinical and genotypic presentation of sickle cell disease around the world. Paediatr Respir Rev. 2014;15(1):4-12.
22. Benites BD, Bastos SO, Baldanzi G, et.al. Sickle cell/β-thalassemia: comparison of Sβ0 and Sβ+ Brazilian patients followed at a single institution. Hematology. 2016;21(10):623-9.
23. Wali YA, Al-Lamki Z, Hussein SS, et.al. Splenic function in Omani children with sickle cell disease: correlation with severity index, hemoglobin phenotype, iron status, and alpha-thalassemia trait. Pediatr Hematol Oncol. 2002;19(7):491-500.
24. Adekile AD, Tuli M, Haider MZ, et.al. Influence of alpha-thalassemia trait on spleen function in sickle cell anemia patients with high HbF. Am J Hematol. 1996;53(1):1-5.
25. Langdown JV, Williamson D, Knight CB, et.al. A new doubly substituted sickling haemoglobin: HbS-Oman. Br J Haematol. 1989;71(3):443-4.
26. Al Balushi HWM, Wali Y, Al Awadi M, et.al. The super sickling haemoglobin HbS-Oman: a study of red cell sickling, K(+) permeability and associations with disease severity in patients heterozygous for HbA and HbS-Oman (HbA/S-Oman genotype). Br J Haematol. 2017;179(2):256-65.
27. Al Jahdhamy R, Makki H, Farrell G, et.al. A case of compound heterozygosity for Hb S and Hb S Oman. Br J Haematol. 2002;116(3):504.
28. Knox-Macaulay H, Ahmed M, Gravell D, et.al. Sickle cell–haemoglobin E (HbSE) compound heterozygosity: a clinical and haematological study. International Journal of Laboratory Hematology. 2007;29(4):292-301.
29. Thalagahage KH, Jayaweera J, Kumbukgolla W, et.al. HbS/D-Punjab Disease: Report of 3 Cases from

Sri Lanka. Indian J Hematol Blood Transfus. 2018;34(2):350-2.
30. Yavarian M, Harteveld CL, Batelaan D, et.al. Molecular spectrum of beta-thalassemia in the Iranian Province of Hormozgan. Hemoglobin. 2001;25(1):35-43.
31. Al Sultan A PS, Suliman A, et al. Spectrum of b-thalassemia mutations in the eastern province of Saudi Arabia. Hemoglobin. 2011;35(2):125–34.
32. Baysal E. Molecular basis of beta-thalassemia in the United Arab Emirates. Hemoglobin. 2011;35(5-6):581-8.

Correspondence Address:
Prof. Dr. Yasser Wali
Pediatric Hematology/Oncology Unit, Child Health Department, Sultan Qaboos University, Muscat, Oman.
Mail: yasser_wali@hotmail.com

HEMOGLOBINOPATHIES IN THE KINGDOM OF SAUDI ARABIA

Hatoon Ezzat, MD, FRCPC[1,4], Soad Khalil Al Jaouni, MD, FRCPC[2*], Mohammad Alshahrani, MD, ABHS, CFHO[3] , Meshaal Alshehri, MSc[1], Mohammed K. Alabdulaali, MD, MPH(Manchu), FKSUP, IFCAP, MSHA (UAB), CPHHA, CSSBB[1]*

[1]Ministry of Health, Riyadh, Kingdom of Saudi Arabia, hezzat@moh.gov.sa

[2]Department of Hematology, Faculty of Medicine, King Abdulaziz University, Jeddah, 21589 Kingdom of Saudi Arabia, saljaouni@kau.edu.sa

[3]Prince Sultan Military Medical City, Riyadh, Saudi Arabia

[4]University of British Columbia, Vancouver, Canada

[*] Correspondence: Hatoon Ezzat and Soad Khalil Al Jaouni

ABSTRACT

Hemoglobinopathies are one of the most common monogenic disorders in Kingdom of Saudi Arabia (KSA). The Sickle cell disease SCD, α and β-thalassemia genes occur with variable frequency in different regions of KSA. Treatment of hemoglobinopathy disorders is complex, expensive and requires a multidisciplinary approach for wellness. It requires an understanding of disease distribution and mutations.

Screening program for hemoglobinopathies was implemented first by Aramco Hospital in 1980 followed by King Abdulaziz University Hospital (KAUH) and Military Hospital 1996. Premarital screening (PMS) program at Ministry of Health (MOH) of KSA was established in 2004. Total of 5,680,860 partners were screened and counselled for Hemoglobinopathies until 2022. Newborn screening (NBS) in KSA was recently implemented for early disease detection, to prevent complications, facilities access to care and improve QoL. The epidemiology of SCD and Thalassemia varies from Eastern to Western Provinces in KSA. In addition to the unique spectrum of β-thalassemia and SCD mutations.

The potential for implementing precision medicine in hemoglobinopathy through molecular testing have been studied heavily in KSA to improve QoL by early detection through screening programs, treatment adherence and disease prevention which became a mandate of MOH in KSA.

Data review shows the importance of comprehensive and integrated approach in Hemoglobinopathy disorders from proper diagnosis to prevention of disease complications, and treatment expansion including Hematopoietic stem cell transplantation. The newly established Health Sector Transformation Program in KSA through Model of care (MOC) aims to ensure the continued development of healthcare services by restructuring the health sector to be a comprehensive, effective, and integrated health system that is based on the health of the individual and society. The development and implementation of dedicated blood disorder programs at MOH in KSA is becoming a fundamental transformation of the health care ecosystem through MOC in KSA. KSA is one of the several countries that adopted effective steps directed toward prevention by implementing screening programs to decrease the incidence of inherited hemoglobinopathy disorders. However, the implementation of the robust IHBD registry remains a unique achievement in the history in the management of Hemoglobinopathies worldwide.

Keywords: Kingdom of Saudi Arabia, Hemoglobinopathy, Genotype/Phenotype, Prevention, Registry, Screening, Quality of life

INTROCUCTION

The Kingdom of Saudi Arabia (KSA) lies at the furthermost part of southwestern Asia. It is bordered by the Arabian Gulf, United Arab Emirates (UAE) and Qatar from the east; Red Sea from the

west; Kuwait, Iraq, and Jordan from the north; Yemen and Oman from the south.

DEMOGRAPHICS OF SAUDI AARABIA POPULATIONS IN 2023

KSA is the fourth largest state in the Arab world, with a reported population of 32,175,224 as of 2022[1]. 41.6% of inhabitants are immigrants. KSA has experienced a population explosion in the last 40 years and continues to grow at a rate of 1.62% per year.

Fig 1: Saudi Arabia population pyramid in 2020

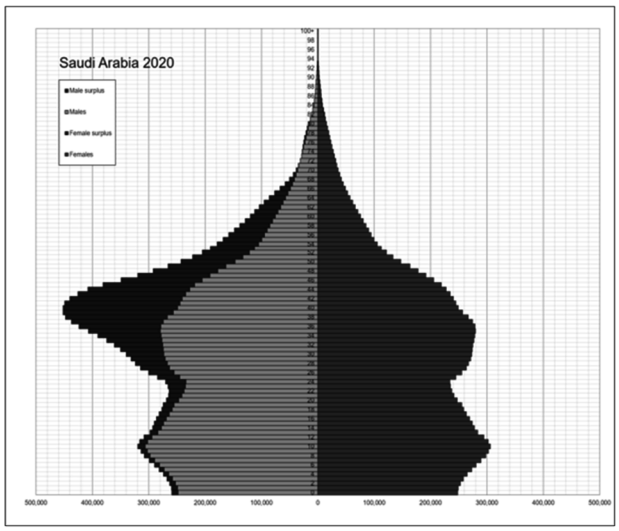


Table 1: Demographics of Saudi Arabia

Population	32,175,224 (Saudi Census 2022)
Growth rate	1.49% (2019)
Birth rate	13.9 births/1,000 population (2023)
Death rate	3.45 deaths/1,000 population
Life expectancy	76.91 years
- **male**	75.33 years
- **female**	78.56 years
Fertility rate	1.89 children born/woman (2023)
Age structure	
0–14 years	24.44%
15–64 years	72.36%
65 and over	3.20%

PREVALANCE OF HEMOGLOBINOPATHIES IN SAUDI ARABIA

Hemoglobinopathies are one of the most common monogenic disorders in KSA. SCD, α and

β-thalassemia genes occur with variable frequency in different regions of the country (**2-9**). SCD is caused by missense mutation of the gene that encodes the beta-globin chain of the hemoglobin molecule. The mutation results in the formation of sickle hemoglobin (HbS), which has the unique feature of polymerizing on deoxygenation. β-thalassemia is one of the most common single-gene disorders affecting countries in the Mediterranean area, Middle East, and Southeast Asia. The disease results from the deficiency or absence of β-globin chain production. α-thalassemia is a disorder caused by the deletion of single or double α-globin genes, and/or point mutations in the α-globin genes. The distribution of α-thal is represented mostly in Southeast Asia. Several local studies shows that the SCD, α and β-thalassemia genes occur with variable frequency in different regions of KSA. The incidence rate (IR) of SCD is believed to be 300,000 to 400,000 live births per year globally. It has been reported that there is a widespread prevalence of SCD in the Arab countries of the Middle Eastern (ME) region with factors such as consanguinity, environmental factors, large sibship size and migration playing a major role in imparting significant inter- and intra- countries differences in the frequencies of the abnormal Hemoglobin S (HbS) genes **(10)**. This is supported by published literature where it was observed that the incidence and prevalence of SCD ranged from 0.04% to 2.1% based on a systemic literature review (SLR) of 23 studies conducted in the ME region (**10**). The prevalence of SCD and Thalassemia varies significantly in different parts of KSA, i.e., 2-27% for sickle cell trait and up to 2.6% had SCD, with the highest rates being observed in the Eastern province, followed by the southwestern provinces (**11**).

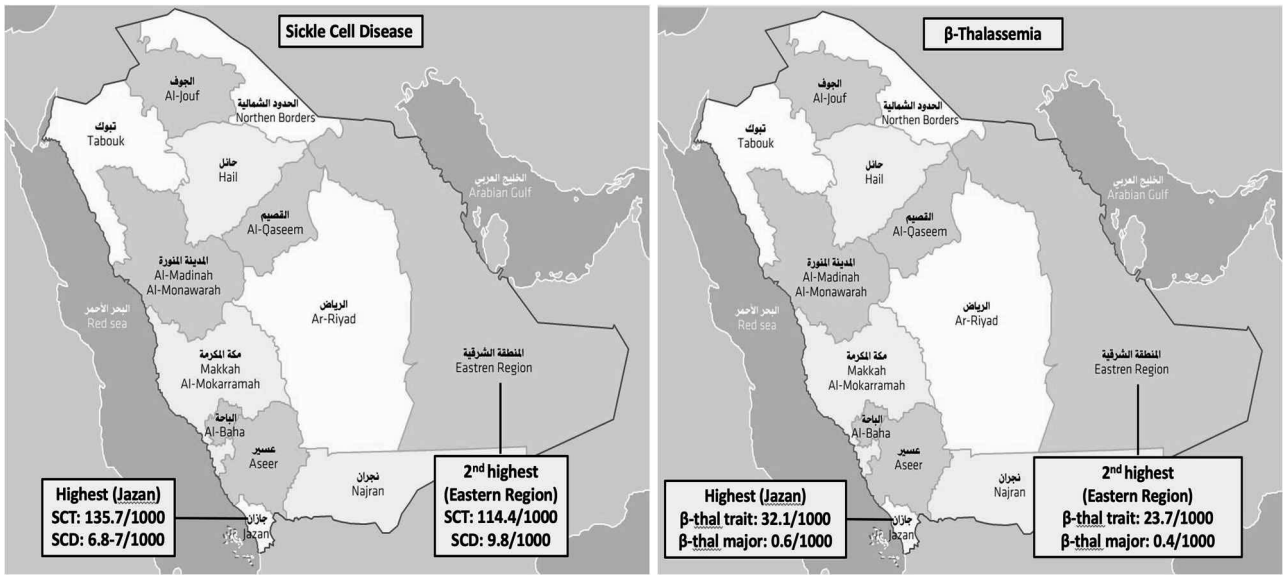


Figure 2: *Prevalence of Hemoglobinopathy in Saudi Arabia*

Clinical and hematological variability exists in SCD in Saudi Arabia with two major phenotypes: a mild phenotype (**12-13)** and a severe phenotype (**14)** as shown in Table 2.

kSCD Phenotype variability in Saudi Arabia

Variable	**Eastern Province**	**Southern West Provinces**
Haplotype	Arab-Indian	Benin
Co-inheritance of α-thalassemia	More	Less
Clinical Severity	Mild/Late	Severe/Early
Vaso-occlusive crisis	Late	Early
Recurrent Acute chest syndrome	Less	More
Avascular necrosis of femoral head	Common	Uncommon
Splenomegaly/splenic sequestration	Common	Uncommon
Stroke	Less	More
Silent Brain Infarct	Late	Early
Priapism	Uncommon	Common
Leg ulcers	Uncommon	Common
Hemoglobin F Level	Higher	Lower

ECONOMIC AND HEALTH BURDEN OF HEMOGLOBINOPATHIES IN SAUDI ARABIA

Treatment of hemoglobinopathies is complex, expensive and requires a multidisciplinary approach. Optimal clinical care is demanding and expensive, but achievable. The increased prevalence of SCD in KSA and complexity of SCD-related vaso-occlusive crisis (VOC) management may lead to higher utilization of medical resources in turn posing a considerable economic burden on the healthcare system, patients, and the society.

Ezzat et al, showed recently in a micro-costing analysis built to estimate the direct and indirect costs associated with SCD management in the KSA over a one-year period both from a payer and societal perspective, that the total SCD burden from societal perspective was 3.6 times higher than the payer's perspective across all age groups. Acute complications represented 91% and 86% of total economic burden of SCD from payer's and societal perspective, respectively. Among acute complications, cost of VOC management was the main driver of SCD burden from both payer's (82%) and societal (76%) perspectives. Among 'VOC management' hospital visit (60%) was the main cost driver from a payer's perspective whereas productivity loss (36%) and out-of-pocket (OOP) expenses (32%) were the main driver from a societal perspective. Patients with ≥5 VOC per year incurred the highest annual cost across all age groups (**15)**. Amerah Bin Zuair et al showed that there were 160 patients (mean age, 31.08 ± 9.06 years; 51.25% female) with SCD included in this study. Most originated from southern Saudi Arabia (45.62%). The average annual number of emergency department (ED) visits was 4, and approximately 19% of patients had ≥3 annual admissions. The mean length of stay was 6 days. The readmission rates at 7, 30, 60, and 90 days were 8%, 24.5%, 13.6%, and 10.8%, respectively. It was demonstrated that SCD generates a significant economic burden on the Saudi society and the effects on the healthcare system and patients' quality of life are evident in the high ED visits, readmission rates and prolonged hospitalization **(16)**.

Study by Al Jaouni et al showed that 115 patients completed the questionnaire. Eighty-seven patients (75.7%) had severe SCD, while 28 (24.3%) had mild disease. Patients with severe disease had a low HRQL (p=0.002). Pain episodes were the main cause of hospitalization (n=59; 51.3%). Thirty-six of patients (31.3%) who had pain episodes were on regular narcotics and had low HRQL scores (p=0.0001). The HRQL scores significantly decreased as pain levels increased. Patients with delayed treatment or those who were not adherent to treatment showed worse HRQL scores (p=0.001). The study concluded that treatment adherence and early intervention in SCD improved HRQL outcomes (**17)**. To assess the prevalence of survival and disease complications among patients with β-thalassemia major treated at King Abdulaziz University Hospital (KAUH); a retrospective chart review was done of all patients followed and treated with a diagnosis of β-thalassemia major from 1990-2004. A total of 360 patients (203 males & 157 females) were transfusion dependent since early childhood and treated with parenteral Deferoxamine. Out of 360 patients, 293 (90.29%) patients were alive, 27 (7.2%) patients had died, 15 (4.2%) patients underwent BMT, and 25 (6.9%) patients follow-up were lost. Twelve (3.3%) patients died from heart disease. 7 (1.9%) patients died from infections; all patients were splenectomized. The serum ferritin levels for patients who died were significantly higher for those patients who survived (7,500 vs. 3, 200; $p < 0.001$). Cardiac constitutes the first important cause of death followed by infection. Complications and deaths among thalassemia patient is iron related organ dysfunction and age related. Most complicated patients were on non-optimal chelation therapy and non-compliance (**18)**.

MOLECULAR AND GENETIC ANALYSIS

Detection of a single base pair mutation in β-globin gene is an important diagnostic tool for SCA. Blood samples from 69 unrelated SCD patients were obtained from the KKUH, Riyadh in Saudi Arabia between 2017-2019. The aim was to study the molecular survey of locus control regions (LCR) in Saudi patients with SCA, and to identify the genetic variables and their clinical manifestations. The results gained from sequencing experiments revealed a wide range of genomic alterations. A total of 69 gene alterations have been recognized in the locus control region; The 1st fragment LCR-HS1 shows 20 alterations; The 2nd fragment LCR-HS2 revealed six changes; The 3rd fragment LCR-HS3 shows many changes; The 5th LCR-HS5 region revealed four changes; The 6th fragment LCR-HS6 revealed eight changes; The 7th LCR-HS7 fragment demonstrates ten changes. This study has successfully identified LCR mutations for random Saudi patients with SCD[19]. Recent studies have indicated that microRNA and VEGF are genetic modifiers and are associated with elevated levels of fetal hemoglobin HbF, and thus they reduce the clinical impact of sickle hemoglobin (HbS) patients. The codominant model, the VEGF-2578-CA genotype was strongly asso-

ciated with increased SCD severity with ($p < 0.003$). The higher expression of HbA1 (65.9%), HbA2 (4.40%), was reported in SCD patients carrying miR-423-AA genotype than miR-423 CA genotype in SCD patients carrying miR-423 CA genotype HbA1 (59.98%), HbA2 (3.74%) whereas SCD patients carrying miR-423 CA genotype has higher expression of HbF (0.98%) and HbS (38.1%) than in the patients carrying AA genotype HbF (0.60%), HbS (36.1%) as shown in Table 3. The study concluded that PCR-amplification refractory mutation system (PCR-ARMS) has been proven to be rapid, inexpensive and is highly applicable to gene mutation screening in laboratories and clinical practices (**20**).

Table 3: *Association of miR-423 rs6505162 C>A gene variation in SCD cases and controls* ***(20)***

Subjects	N	CC	CA	AA	Df Degree of Freedom	χ^2 Chi Square	C	A	*p* Value
Cases	127	18 (14.17%)	61 (48%)	54 (42.51%)	2	6.74	0.34	0.66	0.034
Controls	160	30 (18.75%)	92 (57.5%)	38 (23.75%)			0.47	0.53	

Another study by Abuzenadah aimed at the identification of the spectrum of mutations in patients with β-thal in the western province of KSA. Screening for the mutations was done using the PCR-ARMS technique to test for 12 mutations, and direct automated DNA sequencing for the unknown samples. The study included 172 patients; of these 15 patients had sickle cell anemia and one Hb S [β6(A3)Glu→Val, GAG>GTG]/β-thal. A total of 23 mutations were identified to cause the disease in the western area. Seven common mutations were responsible for the β-thal alleles in 78% of patients and could be detected by the ARMS technique: IVS-II-1 (G>A), IVS-I-110 (G>A), IVS-I-5 (G>C), codon 39 (C>T), codon 26 (G>A) [Hb E or β26(B8)Glu→Lys, GAG>AAG], frameshift codons (FSC) 8/9 (+G), and IVS-I-1 (G>A). DNA sequencing of uncharacterized alleles detected eight less common mutations: FSC 41/42 (-TCTT), IVS-I 25 bp deletion, codon 37 (G>A), FSC 44 (-C), Cap site +1 (A>C), IVS-I-6 (T>C), FSC 5 (-CT) and IVS-I-1 (G>T), and eight rare mutations: -87 (C>G), initiation codon -1 (T>G), codon 15 (G>A), FSC 16 (-C), FSC 20/21 (+G), codon 27 (G>A), IVS-I-130 (G>C) and IVS-II-837 (A>C). Four alleles were normal by DNA sequencing. Genetic heterogeneity was observed in this study, 10 mutations were of Asian or Asian/Indian origin, 2 were Kurdish, 1 Chinese, 1 Turkish, 1 Saudi, and the remainder were of Mediterranean origin. Screening for β-thal mutations using PCR-ARMS for the 7 most frequent mutations in the Saudi population followed by DNA sequencing of the unknown alleles could be useful for the implementation of a strategy for carrier detection and preimplantation genetic diagnosis in high risk families (**20**). At KFSH and RC in Riyadh, Saudi Arabia, samples were collected from preimplantation genetic diagnosis (PGD) and performed gene sequencing for 59 β-thalassemia patients and carriers. Twelve mutations were confirmed in the 5 regions investigated in this study. Cd39 was identified as the most frequent mutation with a frequency of 22.7%, with high prevalence in the central parts of Saudi Arabia. IVS-II-1 G > A was the second frequent mutation observed with a frequency of 21.2%, while IVS-I-1 (G-A) and IVS I-130G>C mutations were observed to be least frequent in the study. Of the 12 gene mutations, 85% were frequently observed in KSA, while 15% were less frequent. The regional distribution of HBB gene mutations varied considerably. The population diversity in KSA contributes to the variability in the prevalence rates of HBB gene mutations. Nevertheless, this study identifies Cd39 and IVS-II-1 G > A as the predominant mutations in HBB gene in Saudi Arabia (**21**).

α -thalassemia is a disorder caused by the deletion of single or double α-globin genes, and/or point mutations in the α-globin genes. There are 2 common types of α-globin genes: HBA2 and HBA1. Recently, it has been discovered that the HBA2 gene is replaced by a unique HBA12 gene convert in 5.7% of the Saudi population. The α-globin genes have been emerging as a molecular target for the treatment of β-thalassemia. Hence, it is essential

to understand the molecular nature of α-globin genes to treat the most prevalent hemoglobin disorders, such as sickle cell disease, α-thalassemia, and β-thalassemia prevalent in Saudi Arabia. Thirty-two different α-globin genotypes have been observed in the Saudi population. This review outlines the classification of the α-globin genes based on their molecular nature and complex combinations of α-globin genes, and their variants predominant in Saudis (**22**).

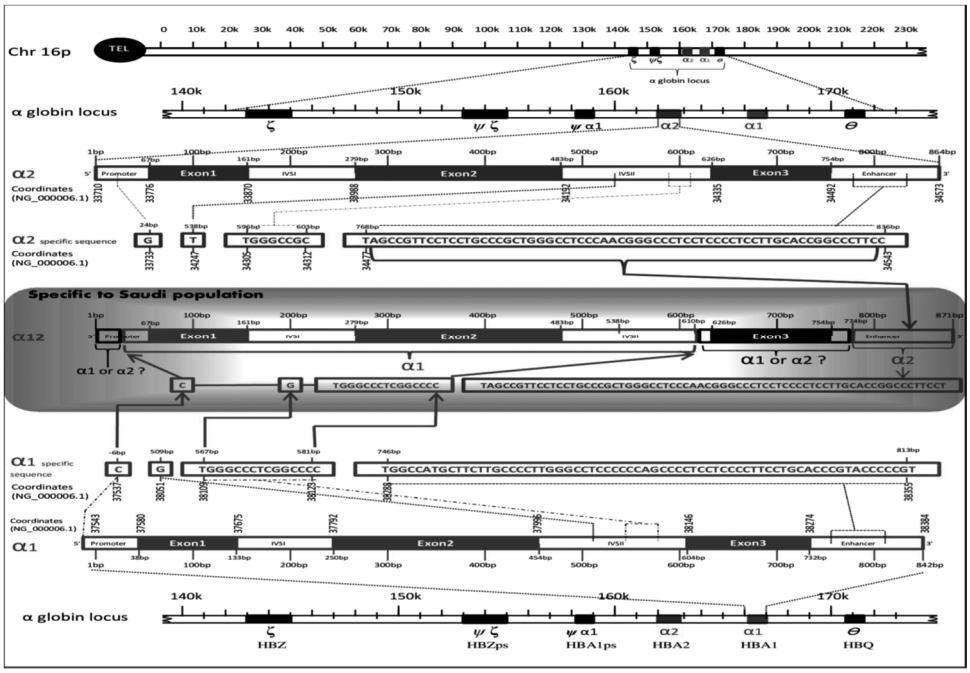


Figure 3: *showing 3 types of globin genes prevalent in the Saudi population: HBA2 (α2), HBA1 (α1), and HBA12 (α12). The α2 gene is colored in nut brown and α1 gene is colored in violet. The undistinguished sequences (α1 or α2?) are colored in black (**23**).*

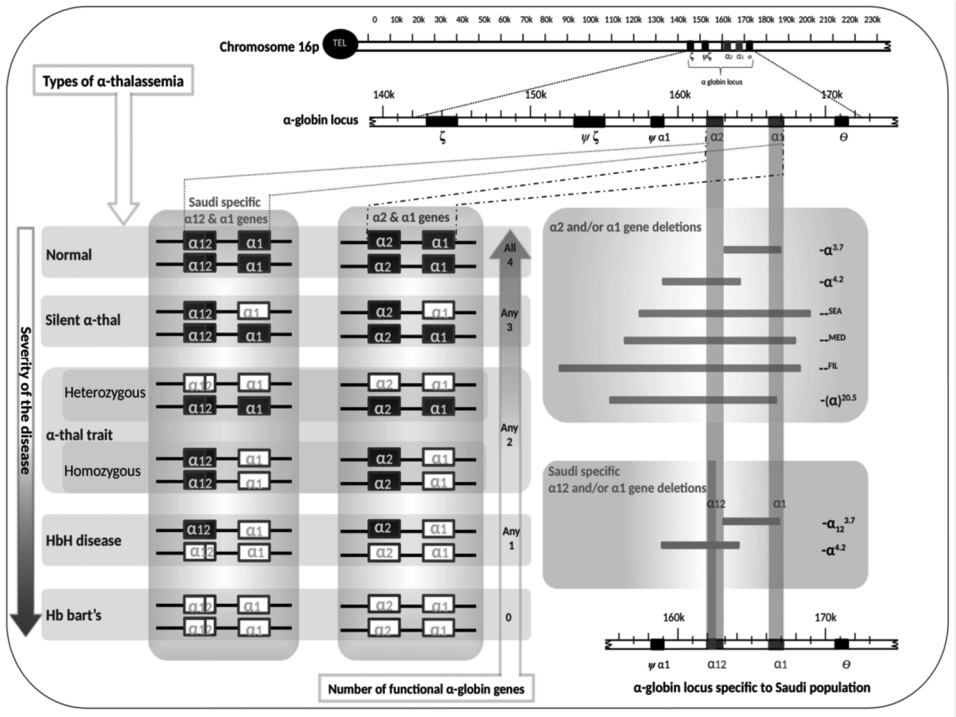


Figure 4: *Molecular types of thalassemia and types of globin gene deletions prevalent in the Saudi population. Filled boxes of genes α1, α2, and α12 indicates normal genes, while empty boxes of genes α1, α2, and α12 indicate the deleted genes*[23]

SCREENING AND PREVENTION PROGRAMS IN SAUDI ARABIA

I-PRE-MARITAL SCREENING PROGRAM (HEALTHY MARRIAGE PROGRAM)

Saudi Arabia is one of the first Middle East countries that started several screening and prevention programs for Hemoglobinopathies. One of the earliest publications concerning the epidemiological situation of hemoglobinopathies in Saudi Arabia was the review by El-Hazmi, published in 2004. The review described how endemicity of malaria aided in increasing prevalence of hemoglobinopathies, such as sickle cell disease and thalassemia in certain regions in the country. With the advancement of transportation systems, movement of Saudi citizens to other regions in the country facilitated spread of these blood disorders all over the kingdom, mandating establishment of a national screening program for hemoglobinopathies, which later included other infectious diseases screened for the premarital screening (**24-25)**. The Premarital screening program (currently called Healthy Marriage Program (HMP)) was initiated in 2004 and involves screening individuals intending to get married, for SCD and thalassemia. In 2011, Memish et al, reported their findings of outcomes of premarital screening covering a longer period between 2004 and 2009, where a marked reduction in overall prevalence of thalassemia traits (**26)**. Additionally, there was a reduction in the number of couples identified as risky and who had decided to proceed with marriage by the end of 2009. Premarital screening program is currently performed at more than 243 healthcare centers across the Kingdom aiming to reduce the numbers of marriage between couples who are at risk of having children affected with SCD and thalassemia (**23)**. Total of 5,680,860 partners were screened and counselled until 2022. An educational program for counselors was developed and published as a national guide to improve counselling, education and Hemoglobinopathy knowledge in the country (**11)**. Until end of 2022, the HMP showed that out of the total screened, 54.2% are sickle cell disease carriers while SCD patients were 0.29%. On the other hand, 1.38% were thalassemia carriers and the diseased were 0.05% (**27-28)**. The Saudi MOH established counseling clinics as an important contributor in this reduction aiding in increased awareness of risky couples and enabling making an informed decision. The proportion of marriages of risky couples' carriers of Hemoglobinopathy disorders had declined significantly after 16 years of establishment of the program due constant efforts to expand on tests availability and HMP clinics, as well as public awareness and knowledge of counselors through training. The effectiveness of premarital screening was evident by reduction of unhealthy marriages from 3% in 2004 to 85% in 2020 in response to HMP results and counseling.

II-NEWBORN SCREENING PROGRAM FOR HEMOGLOBINOPATHY

Performing newborn screening (NBS) can facilitate timely diagnosis, and early access to care to prevent disease complications in hemoglobinopathies. This further permits the establishment of comprehensive care for screen-positive babies. NBS enables the parents to be informed about their child's disorder and the necessary preventive methods for each specific disease. Several institutional efforts were conducted in KSA for NBS Hemoglobinopathy screening for selected newborns. Such screening programs were implemented first by Aramco Hospital in 1980 followed by KAUH and King Fahad Military Hospital in Jeddah 1996. Between July 2001 and January 2002, Al Jaouni and colleagues tested 834 Saudi neonates from the Western region through cord blood sampling using high-performance liquid chromatography (HPLC) technique for hemoglobinopathies. 35.86% were affected with α-thal II; 3.6% had α-thal I; and 0.12% had Hemoglobin H disease. 4.44% neonates had SCT; 0.12% had SCA; 1.92% had HbE trait and 0.12% had HbC trait (**29)**.

In 2022 a pilot study was conducted by Saudi Arabia MOH in Al Ahasa region (East). 5,715 blood samples were collected from newborns on filter paper by HPLC. Dried blood spots were collected by heel prick test between 24-72 hours of age or prior to discharge from the hospital and sent to a laboratory within 24–72 hours after collection. Additional NBS specimen was submitted 90-120 days from the date of transfusion for infants transfused before the screening. Testing was performed within 72 hours, typically on the same day the specimen was received in the laboratory. The results showed

sickle cell trait (SCT) in 811 (14.2%), sickle cell anemia HbSS/Sβ^0 Thal in 25 (0.45%), sickle cell β^+ Thal in 2 (0.04%) **(30)**.

Since the results, NBS for Hemoglobinopathies and G6PD, became a mandatory screening program at MOH and private sectors. Program was launched in March 2023. Until end of August 2023, (108,532) newborns were tested for Hemoglobinopathies (HPLC methodology) and G6PD. 195 newborns tested positive for FS, 5,015 newborns tested positive for Hb variants, and 5,972 newborns tested positive for G6PD. The blood disorders program (BDP) at MOH adopted a clinical pathway for all positive newborns for disease confirmation, parent education, results documentation, and easy access to care for early complication prevention, treatment adherence and improvement of QoL.

INNOVATION IN HEMOGLOBINOPATHY CARE IN SAUDI ARABIA (THE BLOOD DISORDERS PROGRAM AT MOH)

Based on countries' need, MOH in KSA had launched a **blood disorder program** in 2019 to become a globally renowned program, promoting evidence-based science that empowers the community and partners to prevent the incidence of Hemoglobinopathies and improve patients' quality of life. The program's mission is to deliver the highest quality comprehensive healthcare model to affected individuals in KSA in a robust operational context by promoting research, clinical care training and advocacy through the DEEPP strategic planning, to develop, excel, empower, partner, and prevent. The program has implemented many projects such as Virtual Hematology/Hemoglobinopathy department at SEHA virtual hospital, Hemoglobinopathies centers of excellence, Screening programs for Hemoglobinopathy disorders, KPI's, National diseases consensus statements and the Inherited Blood Disorders (IHBD) Registry. Other innovation of care projects is under implementation as HSCT/BMT for SCD and New generation sequencing (NGS) for Hemoglobinopathies.

The IHBD Registry at MOH in Saudi Arabia aimed to estimate the socioeconomic burden associated with SCD and other IHBD management in KSA. A digital platform was deployed to collect data for SCD and other IHBD patients across key MOH sites. The data generated enabled the collection of valuable longitudinal data such as patient medical history, diagnosis, treatment outcomes, morbidity, mortality, and others. The digital platform deployed by the MOH offers multiple useful features as real time data, dashboards, and reports, capturing patients visits and treatments, alerts to care to providers and reminders. Data are collected retrospectively and prospectively. Until date there are comprehensive data on 23,481 patients. The aim is to reach 65,000 patients by end of 2024. The registry data will be leveraged to support multiple MOH systems such as SEHA virtual hospital, National Health Call Center (937) and appointments. The long-term aim of developing value-based healthcare and enhancing medical research at the community and country level **(31)**.

CONCLUSION

Prevention of SCD and thalassemia is a mandate in KSA through newborn screening, premarital screening, and education to treatment adherence to improve QoL and the outcomes. The newly established Health Sector Transformation Program in KSA through Model of care aims to ensure the continued development of healthcare services by restructuring the health sector to be a comprehensive, effective, and integrated health system that is based on the health of the individual and society. Since the launch of the blood disorder program at MOH and the development and implementation of inherited Blood disorders (IHBD) registry it is becoming a fundamental transformation of the health care ecosystem for Hemoglobinopathy in the country.

REFERENCES

1. https://en.wikipedia.org/wiki/Demographics_of_Saudi_Arabia
2. Eman S. Alsaeed, Ghada N. Farhat, Abdullah M. Assiri, et al Distribution of hemoglobinopathy disorders in Saudi Arabia based on data from the premarital screening and genetic counseling program, 2011–2015, Journal of Epidemiology and Global Health 2018, To link to this article: https://doi.org/10.1016/j.jegh.2017.12.001
3. Ziad A. Memish a, Tariq M. Owaidah b, Mohamad Y. Saeedi, Marked regional variations in the prevalence of sickle cell disease and β-thalassemia in Saudi Arabia: Findings from the premarital screening and

genetic counseling program, Journal of Epidemiology and Global Health (2011) 1,61-68.
4. Al-Suliman, Prevalence of β-thalassemia trait in premarital screening in Al-Hassa, Saudi Arabia, Annals of Saudi MedicineVolume 26, 2006
5. Sarah S Alswat1, Raed Alerihi2, Alpha-Thalassemia patterns in Saudi population: a single-center study from Jeddah city, Saudi Arabia , J Hematol Thrombo Dis, ISSN: 2329-8790, January 24-25, 2022
6. Al Jaouni SK. Prevalence of thalassemia disorders and hemoglobinopathies in Jeddah,Western Saudi Arabia. Journal of Applied Hematology.2010;1(1).43-46.
7. Mohammed Makkawi, MSc, PhD, Sultan Alasmari, MSc, PhD, Ali A. Hawan, MD, et al, An update on the prevalence trends in Southern Saudi Arabia, Saudi Med J 2021; Vol. 42 (7)
8. Nisreen Omar Asraf, Saad Abdulrahman Aljibreen, Muath Bader Alotaibi, et al Prevalence of Sickle-Cell Disease in Saudi Arabia: A Systematic Review , Uttar Pradesh Journal Of Zoology, Volume 43, Issue 24, Page 210-223 , Doi: 10.56557/Upjoz/2022/V43i243315 Published: 27 December 2022
9. Wasil Jastaniaha,b Epidemiology of sickle cell disease in Saudi Arabia, October 2010 Ann Saudi Med 2011; 31(3): 289-293 PMID: DOI: 10.4103/0256-4947.81540.
10. El-Hazmi MA, Warsy AS. Sickle cell disease in Middle East Arab countries. *Indian J Med Res*. Nov 2011;134(5):597-610. doi:10.4103/0971-5916.90984
11. Mansour Aljabry, Hatoon Ezzat, Maha Sallam, Decembere 2021, Do it right: The optimal guide to hemoglobinopathy counseling. ISBN-106030383612
12. Amein K. Al-Ali, Ahmed Alsulaiman, Mohammed Alfarhan, et al , Sickle cell disease in the Eastern Province of Saudi Arabia: Clinical and laboratory features, American Journal of Hematology Volume 96, Issue 4Apr 2021
13. Abdulrahman Alsultan, Mohammed K. Alabdulaali, Paula J. Griffin, Ahmed M. AlSuliman, et al, Sickle cell disease in Saudi Arabia: the phenotype in adults with the Arab-Indian haplotype is not benign, British Journal of Haematology, 2014, 164, 597–604
14. Ahmad Ali Hazzazi, Mohssen Hassen Ageeli, Abdulaziz Mohammed Alfaqih, et al, Epidemiology and Characteristics of Sickle Cell Patients Admitted to Hospitals in Jazan Region, Saudi Arabia, Journal of Applied Hematology, 2020.
15. Hatoon Ezzat, Hajer Almudaiheem, Hussain Alsaead, et al, Economic Burden of Sickle Cell Disease in the Kingdom of Saudi Arabia, The journal of Advance Hemaatology, 2023
16. Amerah Bin Zuair,1 Sheikhah Aldossari,1 Rand Alhumaidi et al, The Burden of Sickle Cell Disease in Saudi Arabia: A Single-Institution Large Retrospective Study, Int J Gen Med. 2023; 16: 161–171.
17. Soad K Al Jaouni, Mohammad S Al Muhayawi, Taher F Haalawa, Treatment adherence and quality of life outcomes in patients with sickle cell disease. Saudi Med J. 2013 Mar;34(3):261-5.
18. Al Jaouni SK. Studies on Survival and Disease Complication of Thalassemia Major, Experience of 14 years at King Abdul Aziz University Hospital. JKAU: Med. Sci., Vol. 17 No. 1. pp. 19-28. 2010
19. Faris Q Alenzi, New mutations of locus control region in Saudi sickle patients. Saudi J Biol Sci. 2020 May;27(5):1265-1270. doi: 10.1016/j.sjbs.2020.01.028. Epub 2020 Jan 31.
20. Abdullah Hamadi, Rashid Mir, Ali Mahzari, et al , Molecular Determination of Vascular Endothelial Growth Factor, miRNA-423 Gene Abnormalities by Utilizing

ARMS-PCR and Their Association with Fetal Hemoglobin Expression in the Patients with Sickle Cell Disease, Curr. Issues Mol. Biol. 2022, 44, 2569–2582. https://doi.org/10.3390/cimb44060175.

21. Adel M Abuzenadah, Ibtessam M Ramzi Hussein, Ghazi A Damanhouri, et al, Molecular basis of β-thalassemia in the western province of Saudi Arabia: identification of rare β-thalassemia mutations, Hemoglobin international journal for hemoglobin research Volume 35, 2011.
22. Alotibi RS, Alharbi E, Aljuhani B, Alamri B, Elsayid M, Alhawiti NM, et al. The frequency and spectrum of HBB gene mutation in β-thalassemia patients in Saudi Arabia. J Nat Sc Biol Med 2019;10:97-102.
23. J. Francis Borgio, MSc, PhD. Molecular nature of alpha-globin genes in the Saudi population, Saudi Med J 2015; Vol. 36 (11).
24. M.A.F. El-Hazmi, The natural history and the national pre-marital screening program in Saudi Arabia. Saudi Med J, 25 (November (11)) (2004), pp. 1549-1554
25. Ibrahim M. Gosadi, National screening programs in Saudi Arabia: Overview, outcomes, and effectiveness, Journal of Infection and Public Health Volume 12, Issue 5, September–October 2019, Pages 608-614.
26. Z.A. Memish, M.Y. Saeedi, Six-year outcome of the national premarital screening and genetic counseling program for sickle cell disease and beta-thalassemia in Saudi Arabia. Ann Saudi Med, 31 (3) (2011), pp. 229-235
27. https://www.moh.gov.sa/eServices/Documents/premarital-exam-public-sector.pdf
28. https://www.moh.gov.sa/eServices/Documents/premarital-exam-private-sector.pdf
29. AL JAOUNI SK, GARI MH, DAMANHOURI GA, ET AL , CHROMATOGRAPHY PHENOTYPES DISTRIBUTION OF NEONATAL THALASSEMIA AND SICKLE CELL ANEMIA IN HOSPITALS BIRTHS IN JEDDAH , SAUDI ARABIA,

EGYPTIAN JOURNAL OF PEDIATRICS VOL.20, NO3& 4 , 595-605, 2003.

30. Hatoon Ezzat, Ohoud Kashari, Areej M Alghamdi, et al, Pb2520 Newborn Screening Program in Saudi Arabia for Early Detection of Hemoglobinopathies and G6pd, Hemasphere | 2023;7(S3) EHA2023 Hybrid Congress.
31. Hatoon Ezzat, Areej Alghamdi, Mohammed Al Abdulaali, Revealing the power of disease registries in real-world patient care and research – the Saudi national sickle cell disease registry success story. Hemasphere. 2022 Jun; 6(Suppl): 1358-1359. Published online 2022 Jun 23. doi: 10.1097/01.HS9.0000848760.25119.75

Correspondence Address:
Hatoon Ezzat, MD, FRCPC1,4*, Soad Khalil Al Jaouni, MD, FRCPC2*
1Ministry of Health, Riyadh, Kingdom of Saudi Arabia, hezzat@moh.gov.sa
2Department of Hematology, Faculty of Medicine, King Abdulaziz University, Jeddah, 21589
Kingdom of Saudi Arabia,
E-mail: saljaouni@kau.edu.sa

THALASSEMIA AND HEMOGLOBINOPATHIES IN EGYPT

Ashraf Soliman MD, PhD, FRCP
Professor and Senor consultant of Pediatrics and Endocrinology,
College of Medicine, University of Alexandria, Alexandria, Egypt
Hamad General Hospital, Doha, Qatar

ABSTRACT

In Egypt, Beta Thalassemia is the most common chronic hemolytic anemia (85.1%). It has been estimated that one thousand children out of 1.5 million live births are born each year suffering from the disease. A carrier rate of 9-10.2% has been estimated in 1000 normal random subjects from different geographical areas. More than one-third of relatives of patients with β thalassemia are carriers of the disease. The most common mutations among patients were IVS I-110(G>A) 48%, IVS I-6(T>C) 40%, and IVS I-1(G>A) 24%.

Forty % of BTM patients were HCV Ab positive by ELISA and 19.5% were anti-HCV positive by RIBA; and 29.0% were HBsAg positive. Liver cirrhosis was encountered in 23.5% and variable degrees of liver fibrosis in 35% > 12 years. A direct linear correlation was observed between the fibrosis progression rate and liver iron content (LIC). In 100 patients with BTM (ferritin= 1200 to 8000 ng/ml), 24% had mild to moderate, and 8% had severe cardiac siderosis.

In one study, cardiac dysfunction occurred in (53.2%) of BTM patients but congestive heart failure (CHF) and decreased LVEF in 6.45%. A decrease in left ventricular ejection fraction (LVEF) below the reference range was associated with progression to CHF.

Children and adolescents with BTM had a high prevalence of short stature and delayed puberty. 49% had HtSDS <-2, 83% had HtSDS <-1 and 56% had slow growth velocity (GVSDS <-1). Serum ferritin concentration was correlated significantly with the linear GV. 70% of thalassemic children <12 years had different degrees of underweight and malnutrition. 46% of short children with BTM had low GH peak response to provocation by clonidine and glucagon (< 7 micrograms/l). Almost all children and adolescents with BTM had low circulating insulin-like growth factor 1 (IGF-I) concentrations. Growth and IGF1 response to GH therapy for 1 year were lower than non-thalassemic children with GH deficiency. 73% of boys and 42% patients with BTM (13-21 yrs.) had complete lack of pubescent changes. Males had lower testosterone and basal gonadotrophin level (LH) compared to age-matched adolescents. They had defective nocturnal release of LH and LH pulsatile secretion. Clinical and testosterone response to human chorionic gonadotropin (HCG) were subnormal. Thyroid disorders (subclinical or clinical hypothyroidism) occurred in 9.2%, Dysglycemia in 7.5%, and hypoparathyroidism occurred in 6.66%. High serum ferritin levels were significantly associated with increased endocrine disorders. Combined chelating iron agents significantly decreased the prevalence of endocrine disorders.

Optimization of transfusion, Fe-chelation, and nutrition, as well as early detection and management of complications can markedly improve their health outcome.

Keywords: Egypt, thalassemia, hemoglobinopathies, epidemiology, therapy, complications

REVIEW

In Egypt, Beta Thalassemia is the most common chronic hemolytic anemia (85.1%). A carrier rate of 9-10.2% has been estimated in 1000 normal random subjects from different geographical areas of Egypt. The life expectancy and the quality of life of thalassemic patients remarkably improved over the last 9 years after the development of different centers for their management and care but still they have the

problem of hepatitis C which is still high (76.7%) as diagnosed by HCV-RNA PCR **(1)**.

β thalassemia carrier state constituted the majority among children with microcytic hypochromic anemia (67.59%), with a prevalence rate of 35.84% among the studied relatives of the patients. This is about 3-4 times higher than the estimated carrier rate of 9-10% in the general population. β thalassemia carrier prevalence rate in the studied relatives was 35.84%, with the highest prevalence detected in Al-Sharkia Governorate (51.32%), followed by Kafr-Alsheikh and Al-Dakahilia Governorates (41.78%, 37.13%) respectively, while Al-Menoufia Governorate had the lowest prevalence rate (25.00%). These differences were also highly statistically significant ($p < 0.001$). More than one-third of relatives of patients with β thalassemia are carriers of the disease **(2, 3)**.

Direct DNA sequencing for the frequency of different mutations in patients with BTM was carried on two hundred β-thalassemic Egyptian children covering most Egyptian Governorates (158) (79%) children with Beta thalassemia major (BTM) and 42 (21%) children with intermedia (BTI). The most common mutations among patients were IVS I-110(G>A) 48%, IVS I-6(T>C) 40%, IVS I-1(G>A) 24%, IVS I-5(G>C)10%, IVS II-848 (C>A) 9%, IVS II-745(C>G) 8%, IVS II-1(G>A) 7%, codon "Cd"39(C> T) 4%, −87(C>G) 3%. There was a considerable variation in phenotypic severity among patients resulting from the interaction of different β∘ and β+mutations. Furthermore, no genotype-phenotype association was found among the cases with TM and TI **(4)**.

In another study on 95 Egyptian thalassemic patients from Fayoum in Upper Egypt, Cairo, Alexandria, and Tanta in Lower Egypt and the Nile Delta the results showed that the most common allele encountered was IVS-I-6 (T-->C) (36.3%); the second most common mutation was IVS-I-110 (G-->A) (25.8%). In addition. The study reported three homozygous cases for the promoter region -87 (C-->G) allele with a frequency of 3.2%. DNA sequencing of uncharacterized cases (14 cases, 15 alleles) revealed six cases (six alleles) of codon 27 (G-->T) and three cases (three alleles) of the IVS-II-848 (C-->A) mutation. Codon 37 (G-->A) in the homozygous state was found in one patient with positive consanguinity **(5)**. Another study done on 1000 newborns using (PCR)-based DNA analysis of cord blood samples showed that (9.1%) had at least one of the α genes deleted **(6)**.

Prenatal diagnosis was performed in 71 pregnant mothers at risk for BTM (chorionic villus sampling (n = 57) or amniocentesis (n = 14) between 11 to 14 weeks of gestation. Molecular characterization of fetal DNA by reverse dot blot hybridization and polymerase chain reaction-amplification refractory mutation system techniques was conducted in all cases. Twenty-four women (33.8%) were found to have affected fetuses; 100% of these women opted to terminate the pregnancy. Authors suggested that prenatal diagnosis is feasible and acceptable in Egypt (a Muslim country) **(7)**.

Liver Disorders (Hepatitis, fibrosis, and Cirrhosis) in Egyptian BTM patients were assessed in relation to the hepatitis C virus and compliance with chelation in 111 males and 89 females, with a median age of 13 years. Eighty-one (40.5%) patients were HCV Ab positive by ELISA and 39 (19.5) were anti-HCV positive by RIBA; 58 (29.0%) were HBsAg positive and 13 (6.5%) were anti-HBc positive. Older age, an increased number of transfusion units, and HBsAg seropositivity were significantly associated with a higher prevalence of HCV and HBV **(8)**.

In another study, eighty (80) BTM patients were studied with respect to liver enzymes, ferritin, transferrin saturation, HBsAg, anti-HCV antibody and HCV-PCR for anti-HCV positive patients. Fifty % of the patients were anti-HCV positive and 55% of them were HCV-PCR positive. Patients with elevated ALT and AST levels had significantly higher mean serum ferritin than those with normal levels. Anti-HCV positive patients had higher mean serum ferritin, serum ALT, AST and GGT levels and higher age and duration of blood transfusion than the negative group. Authors reported that iron overload was the main leading cause of elevated liver enzymes, and the presence of HCV infection was significantly related to the increased iron overload **(9)**.

ElAlfy et al, studied fifty-one regularly transfused beta-thalassemia patients above 12 years old who were evaluated for serum alanine transaminase (ALT), serum ferritin (SF), HCV (antibody and RNA), LIC assessed by hepatic R2* and transient elastography (TE) (FibroScan). FibroTest and liver

biopsy were done on 25 patients. Eighty-two% of studied thalassemia patients were HCV antibody positive; 21(49%) of them were viremic (HCV RNA positive); median LIC was 12 mg/gm dry weight. There was a strong positive correlation between the degree of liver stiffness and Ishak fibrosis score assessed in liver biopsy specimens (P = 0.002) and between FibroScan and FibroTest results (P < 0.001). Patients with HCV viremia showed significantly higher ALT, γ-glutamyl transpeptidase (GGT), SF, LIC, and increased liver stiffness compared to patients with no viremia (P = 0.0001, 0.001, 0.012, 0.006 and 0.001) respectively. Liver cirrhosis (TE values > 12.5kPa) was encountered in 23.5% and variable degrees of liver fibrosis (TE values > 6–12.5 kPa) in 35% of studied thalassemic patients **(10)**.

Kamal S et al, evaluated the hepatic fibrosis progression rates were significantly higher in thalassemic patients with chronic HCV and compared to those with chronic HCV alone (1.14±0.48) and (0.35±0.14) (P<0.0001), respectively. A direct linear correlation was observed between the fibrosis progression rate and each of liver iron content (LIC) (R=+0.67; P=0.01) and ferritin (R=0.77; P<0.01). In patients with chronic HCV and thalassemia, the sustained virologic response (SVR) to pegylated interferon-based therapy and direct antiviral agents (DAAS) were 33% and 82% respectively (P<0.0001), while in chronic HCV patients without thalassemia, the SVR rates to PEG-IFN/RBV and DAAs were 51% and 92% respectively. Five patients with concomitant HCV and thalassemia died during the study due to cardiac causes (n=3) and liver cancer (n=2) **(11)**.

A study on 62 BTM patients showed that cardiac complications have occurred in (53.2 %) of them but it caused only congestive heart failure CHF and decreased LVEF less than 45% in (6.45%). Table 2 shows that the cardiac outcome was significantly better in those patients who maintained serum ferritin values lower than 2500 g/L than patients their serum ferritin values higher than 2500 g/L (P = 0.05). The risk of death from iron overload was also significantly increased in this group (p = 0.001). Age and sex showed no effect on the incidence of CHF and mortality in thalassemia patients (P >0.05). A decrease in left ventricular ejection fraction (LVEF) below the reference range was associated with progression to clinical heart failure and death if DFO intensification was not achieved **(12)**.

Another study assessed cardiac functions and arrhythmia in children with BTM and BTI and its relation to cardiac iron overload. Arrhythmia was detected significantly more in BTM patients than in βTI. Nine (30%) BTM and five (16.6%) BTI patients had Sinus tachycardia. Two (6.7%) BTM patients compared to one (3.33%) BTI patient had supraventricular tachycardia runs. Three (10%) BTM and one (3.33%) BTI patients had extreme sinus tachycardia. Two (3.3%) BTI patients had sinus bradycardia, while two (3.3%) BTM patients had incomplete Right bundle branch block. The echocardiographic assessment showed that isovolumic relaxation time (IVRT), Left ventricle myocardial performance index (MPI LV), Right ventricle myocardial performance index (MPI RV), and end-systolic pulmonary artery pressure, were significantly higher in BTM than BTI group (p < 0.05). Fractional shortening, Ejection fractions were significantly lower in BTM than BTI group (p < 0.001). Global myocardial performance is more impaired in BTM than in TI patients. Iron overload has a deleterious effect on cardiac function. A statistically significant negative correlation was found between cardiac T2* and each of (IVRT, MPI LV, MPI RV) (p < 0.05) **(13)**.

These findings were supported by another study on 50 BTM patients which added that serum NT pro-BNP and cardiac troponin (cTnI) were higher in BTM patients compared to controls **(14)**.

Myocardial status was assessed by tissue Doppler and cardiac siderosis was assessed by cardiac magnetic resonance imaging (MRI) T2*.in 100 patients with BTM who had serum ferritin ranging from 1200 to 8000 ng/ml showed that 68 (68%) had no cardiac siderosis, while 24 (24%) had mild to moderate, and 8 (8%) with severe cardiac siderosis. Patients with null genotype Glutathione S-transferase gene polymorphism had significantly higher left ventricular end-diastolic diameter (P = 0.002), and shorter ejection time and was associated with cardiac iron overload independent of serum ferritin in Egyptian patients with BTM **(15)**.

Cardiac function was evaluated in 30 BTM patients aged 15.87 ± 3.19 years before and after 6 months of L-carnitine (50 mg/kg/day) therapy using Dop-

pler and multi-gated equilibrium radionuclide angiography (MUGA). Significant improvement in systolic and diastolic functions was detected after 6 months of L-carnitine therapy **(16)**.

Children and adolescents with thalassemia had a high prevalence of short stature and delayed puberty. Evaluation of growth parameters and sexual maturation in a large cohort of children and adolescents With BTM (n = 72) on regular blood transfusion and iron chelation (subcutaneous desferrioxamine) revealed that the height standard deviation score (HtSDS), growth velocity (GV) (cm/yr), and growth velocity standard deviation score (GVDSD) of children and adolescents with BTM was significantly decreased compared to 200 age-matched normal children ($p < 0.01$). Forty-nine percent of thalassaemic patients had HtSDS less than -2, and 83 percent of BTM patients had HtSDS less than -1. Fifty-six percent of BTM children had GVSDS less than -1. Serum ferritin concentration was correlated significantly with the linear GV in all patients ($r = 0.45$, $p < 0.001$). The mid-arm circumference and skin fold thickness (triceps, subscapular, and biceps) were significantly smaller in children with BTM than in normal children. In thalassaemic patients between the ages of 13 and 21 years, a complete lack of pubescent changes was present in 73 percent of boys and 42 percent of girls. Seventy-four percent of the thalassaemic girls had primary amenorrhea. Males with BTM who had spontaneous testicular development had significantly smaller testicular volume than did normal controls **(17)**.

Thromboembolic events (TEEs) are known complications in BTM patients. Many mechanisms were postulated for thrombosis. In a cross-sectional study on 66 patients Abd El Mabood S et al, found that Protein C and antithrombin III (AT-III) were significantly lower among thalassemic with the main risk factors for their deficiencies being: splenectomy and increasing age **(18)**.

Ragab MAF et al evaluated the pulmonary function for 28 thalassemic children with mean age = 10.9 yrs. Hypoxemia was found in 10 patients and reduced total lung capacity (TLC) was found in 19 of 28 patients (68%). Out of these 19 patients, fourteen (74%) had a moderate (58%) and severe (16%) reduction in TLC. CT scans of the lungs in the 3 patients with a severe reduction in TLC did not show any evidence of pulmonary fibrosis. FEV1 and FEF(25–75%) were less than the predicted values in 28% and 14% of patients respectively. TLC, FVC, FEV1, and FEF (25–75%) were inversely correlated with age, and iron burden. The authors reported that restrictive lung disease becomes more severe with increasing the duration and degree of iron overload. The authors concluded that restrictive lung disease is the more prevalent abnormality of pulmonary functions **(19)**.

Another study on 40 adult patients with BTM (26 men and 14 women); (mean age 23.28 ± 3.595 years). Pulmonary hypertension (PH) was diagnosed in 16 (40%) patients. Significantly higher levels of systolic pulmonary artery pressure sPAP were found in poorly chelated and inadequately transfused patients. Significantly lower levels of protein C, protein S, and antithrombin III were found in patients with PH. There was a significant negative correlation between protein C, protein S, and antithrombin III and (sPAP) **(20)**.

In 1999, Soliman et al studied the growth hormone–insulin–like growth factor 1 (IGF-1) axis in BTM children. BTM children had significantly lower peak GH response to provocation by clonidine and glucagon than age-matched controls. Seven out of 15 children with BTM had a GH peak response of < 7 micrograms/l after provocation. Analysis of their spontaneous nocturnal GH secretion revealed lower mean (2.9 +/- 1.77 micrograms/l) and integrated (2.53 +/- 1.6 micrograms/l) concentrations compared to controls (4.9 +/- 0.29 micrograms/l and 5.6 +/- 0.52 micrograms/l respectively). Both GH deficient and those with normal GH response to provocation had significantly decreased circulating concentrations of insulin-like growth factor 1 (IGF-I) and IGF binding protein 3 (IGFBP-3) compared to normal short children. Serum ferritin concentration correlated significantly with GH peak response to provocation and circulating IGF-I and IGFBP-3 concentrations. Growth and IGF1 response to GH therapy for 1 year were lower than in non-thalassemic children with GH deficiency suggesting partial resistance to GH. MRI studies revealed complete empty sella (n = 2), marked diminution of the pituitary size (n = 4), thinning of the pituitary stalk (n = 3) with its posterior displacement (n = 2), and evidence of iron deposition in the pituitary gland and midbrain (n = 7) in those patients with defective GH secretion (n = 9). These data prove that some children with BTM have a defective GH–

IGF-I–IGFBP-3 axis and suggest the presence of partial resistance to GH **(21, 22)**.

Studying adolescents with BTM showed that their circulating testosterone concentration was significantly lower compared to age-matched adolescents. Their clinical responses and testosterone level, after 3 days, 4 weeks, and 6 months of intramuscular administration of human chorionic gonadotropin (HCG) (2500 U/m2/dose), were markedly decreased. After 6 months of HCG administration, 50 percent (5/10) of the boys did not show significant testicular enlargement or genital changes. Luteinizing hormone (LH) peak responses to GnRH were significantly lower as compared to controls. The mean nocturnal LH and FSH secretion was significantly decreased in all thalassaemic boys as compared to boys with constitutional delay of puberty at the same pubertal stage (testicular volume). MRI studies revealed complete empty sella (n = 5), marked diminution of the pituitary size (n = 5), thinning of the pituitary stalk (n = 3) with its posterior displacement (n = 2), and evidence of iron deposition in the pituitary gland and midbrain (n = 8) in thalassaemic patients, denoting a high incidence of structural abnormalities (atrophy) of the pituitary gland.

The defective testosterone response to long-term (6 months) HCG therapy in some BTM adolescents denoted significant testicular atrophy and/or failure secondary to siderosis. The authors suggested that testosterone replacement might be superior to HCG therapy in these patients **(23)**.

A more recent study on 100 young children (<12 years) with BTM showed that 70% of them had malnutrition. The presence of endocrine disorders was observed in 28/120 (23.33%) patients. The most common endocrine disorders were thyroid disorders, either subclinical or clinical hypothyroidism in 11/120 (9.17%) patients, followed by abnormalities in glucose homeostasis in 9/120 (7.5%) (impaired glucose tolerance occurred in 5 (4.17%) and impaired fasting glucose, occurred in 4 (3.33%)). Hypoparathyroidism occurred in 8/120 (6.66%). High serum ferritin levels were significantly associated with increased endocrine disorders (OR 0.98, 95% CI 0.96–0.99, P = 0.003). Combined chelating iron agents significantly decreased the prevalence of endocrine disorders when compared with monotherapy (OR 0.40, 95% CI 0.16:0.97, P = 0.04) **(24)**.

AlAkhras et al studied 100 children with BTM and found that patients with the β0β0 genotype had a significantly higher prevalence of growth retardation, hypogonadism, hypothyroidism, and hypoparathyroidism compared to those with the β0β+ and β+β+ genotypes (P<0.001, P<0.001, P<0.001 and P=0.037, respectively). Patients with the homozygous IVS-11-745 mutation had a significantly higher prevalence of diabetes (P=0.001). The authors stated that the underlying genetic defect in thalassemia patients is a contributing factor for the development of endocrinal complications, as patients with the more severe defects have a greater rate of iron loading through higher red cell consumption. **(25)**.

ElAlfy et al assessed the Quality of life (QOL) of 127 Egyptian BTM and 65 healthy adolescents using the Pediatric Quality of Life Inventory (PedsQL) 4.0 Generic Core Scale. The BTM patients had lower QOL scores in all domains. Treatment-compliant BTM patients had higher total QOL scores (P = 0.004) compared to non-compliant patients. High pretransfusion hemoglobin levels and low serum ferritin levels were independent predictors of better QOL scores **(26)**.

SUMMARY

These data collectively confirmed the high prevalence of short stature and delayed or absent pubertal development in adolescents with BTM. The etiology of impaired growth includes the contributions of lack of pubertal growth spurt due to delayed/absent puberty, and decreased synthesis of IGF-1 which might be secondary to a disturbed GH-IGF-1 axis and/or undernutrition, probably due to the hypermetabolic status of these children.

RECOMMENDATIONS

It is suggested that newer protocols of treatment, in addition to optimization of transfusion and chelation requirements, and improving the nutritional status of patients with BTM as well as early detection and management of hepatic, cardiac, and growth/pubertal complications can markedly improve their health outcome as well as their quality of life.

REFERENCES

1. El-Beshlawy, A., Kaddah, N., Rageb, L. et al. Thalassemia Prevalence and Status in Egypt. Pediatr Res 45 (Suppl 5), 760 (1999). https://doi.org/10.1203/00006450-199905010-00132
2. El-Shanshory MR, Sherief LM, Hassab HM, Ragab SM, Yahia S, Mansour AK, Ahmed AS, Abdou SH, Helmy AM, Watany MM, Gad ALlah AM, Guindy MA, Mourad ZI, Soliman MA, El-Farahaty RM, El-Dahtory F, Darwish A, Elmabood SA, Kabbash IA, Saied SM. Prevalence of iron deficiency anemia and beta thalassemia carriers among relatives of beta thalassemia patients in Nile Delta region, Egypt: a multicenter study. J Egypt Public Health Assoc. 2021 Oct 11;96(1):27. doi: 10.1186/s42506-021-00088-9. PMID: 34633587; PMCID: PMC8505559.
3. El-Beshlawy A, Kaddah N, Ragab L, Hussein I, Mouktar G, Moustafa A, et al. Thalassemic prevalence and status in Egypt. Abstract 102. In: Proceedings of The Annual Meeting of the American Pediatric Society. San Francisco; 1999 Thalassemia Genotyping in Egypt: (Link between genotype and phenotype)
4. El-Shanshory M, Hagag A, Shebl S, Badria I, Abd Elhameed A, Abd El-Bar E, Al-Tonbary Y, Mansour A, Hassab H, Hamdy M, Alfy M, Sherief L, Sharaf E. Spectrum of Beta Globin Gene Mutations in Egyptian Children with β-Thalassemia. Mediterr J Hematol Infect Dis. 2014 Nov 1;6(1):e2014071. doi: 10.4084/MJHID.2014.071. PMID: 25408857; PMCID: PMC4235483
5. El-Gawhary S, El-Shafie S, Niazi M, Aziz M, El-Beshlawy A. Study of beta-Thalassemia mutations using the polymerase chain reaction-amplification refractory mutation system and direct DNA sequencing techniques in a group of Egyptian Thalassemia patients. Hemoglobin. 2007;31(1):63-9.
6. Youssry I, El Badawy A, Samy RM, Salama N, Abd Elaziz D, Rizk S. Prevalence of α-Thalassemia in the Egyptian Population. Hemoglobin. 2018 Jul;42(4):243-246. doi: 10.1080/03630269.2018.1527231
7. El-Beshlawy A, El-Shekha A, Momtaz M, Said F, Hamdy M, Osman O, Meshaal S, Gafaar T, Petrou M. Prenatal diagnosis for thalassaemia in Egypt: what changed parents' attitude? Prenat Diagn. 2012 Aug;32(8):777-82. doi: 10.1002/pd.3901. Epub 2012 Jun 13. PMID: 22693141.
8. Mansour AK, Aly RM, Abdelrazek SY, Elghannam DM, Abdelaziz SM, Shahine DA, Elmenshawy NM, Darwish AM. Prevalence of HBV and HCV infection among multi-transfused Egyptian thalassemic patients. Hematol Oncol Stem Cell Ther. 2012;5(1):54-9. doi: 10.5144/1658-3876.2012.54. PMID: 22446611.
9. Salama KM, Ibrahim OM, Kaddah AM, Boseila S, Ismail LA, Hamid MM. Liver Enzymes in Children with beta-Thalassemia Major: Correlation with Iron Overload and Viral Hepatitis. Open Access Maced J Med Sci. 2015 Jun 15;3(2):287-92. doi: 10.3889/oamjms.2015.059. Epub 2015 May 28. PMID: 27275237; PMCID: PMC4877869.
10. Elalfy MS, Esmat G, Matter RM, Abdel Aziz HE, Massoud WA. Liver fibrosis in young Egyptian beta-thalassemia major patients: relation to hepatitis C virus and compliance with chelation. Ann Hepatol. 2013 Jan-Feb;12(1):54-61. PMID: 23293194.
11. Kamal S, Abdelhakam S, Ghoraba D, Mohsen MA, Salam AA, Hassan H, Nabeigh L. The Course of Hepatitis C Infection and Response to Anti-viral Therapy in Patients with Thalassemia major and Hepatitis C Infection: A Longitudinal, Prospective Study. Mediterr J Hematol Infect Dis. 2019 Nov 1;11(1):e2019060. doi: 10.4084/MJHID.2019.060. PMID: 31700585; PMCID: PMC6827603.
12. Alama MN, Hindawi S, El Sayes F, Marouf S, Damanhouri GA, et al. (2016) Potential effect of β thalassemia major on cardiac function among patients in Jeddah, Saudi Arabia. Med Res Innov. 1: DOI: 10.15761/MRI.1000115.
13. Salama K, Khaled HZ, Seif El Dien HM. Et al. Assessment of Cardiac Functions and Arrhythmia in Children with Beta-Thalassemia Major and Beta-Thalassemia Intermedia. Macedonian Journal of Medical Sciences 2022. 10. DOI: https://doi.org/10.3889/oamjms.2022.9026
14. Deraz SE, Abd El Naby SA, Mahmoud AA. Assessment of ventricular dysfunction in Egyptian children with Beta-thalassemia major. Hematol Oncol Stem Cell Ther. 2021 Sep;14(3):206-213. doi: 10.1016/j.hemonc.2020.07.003. Epub 2020 Jul 30. PMID: 32758485.
15. Mokhtar GM, Sherif EM, Habeeb NM, Abdelmaksoud AA, El-Ghoroury EA, Ibrahim AS, Hamed EM. Glutathione S-transferase gene polymorphism: Relation to cardiac iron overload in Egyptian patients with Beta Thalassemia Major. Hematology. 2016 Jan;21(1):46-53. doi: 10.1179/1607845415Y.0000000046. Epub 2015 Aug 19. PMID: 26288192
16. El-Beshlawy A, Ragab L, Fattah AA, Ibrahim IY, Hamdy M, Makhlouf A, Aoun E, Hoffbrand V, Taher A. Improvement of cardiac function in thalassemia major treated with L-carnitine. Acta Haematol. 2004;111(3):143-8. doi: 10.1159/000076522. PMID: 15034235.
17. Soliman AT, elZalabany M, Amer M, Ansari BM. Growth and pubertal development in transfusion-dependent children and adolescents with thalassaemia major and sickle cell disease: a comparative study. J Trop Pediatr. 1999 Feb;45(1):23-30. doi: 10.1093/tropej/45.1.23. PMID: 10191589.

18. Abd El Mabood S, Fahmy DM, Akef A, El Sallab S. Protein C and Anti-Thrombin-III Deficiency in Children With Beta-Thalassemia. J Hematol. 2018 May;7(2):62-68. doi: 10.14740/jh392w. Epub 2018 May 10. PMID: 32300414; PMCID: PMC7155864.
19. Ragab MAF. Study of pulmonary function in Egyptian children with homozygous beta thalassemia . Arch Dis Child, 2008;93:pw169.
20. Elbedewy TA, Elshweikh SA, Abd El-Naby AY, Elsheikh EA. Pulmonary hypertension in adult Egyptian patients with b-thalassemia major: correlation with natural anticoagulant levels. Tanta Med J 2015;43:52-9
21. Soliman AT, elZalabany MM, Mazloum Y, Bedair SM, Ragab MS, Rogol AD, Ansari BM. Spontaneous and provoked growth hormone (GH) secretion and insulin-like growth factor I (IGF-I) concentration in patients with beta thalassaemia and delayed growth. J Trop Pediatr. 1999 Dec;45(6):327-37. doi: 10.1093/tropej/45.6.327. PMID: 10667001
22. Soliman AT, El Banna N, Ansari BM. GH response to provocation and circulating IGF-I and IGF-binding protein-3 concentrations, the IGF-I generation test and clinical response to GH therapy in children with beta-thalassaemia. Eur J Endocrinol. 1998 Apr;138(4):394-400. doi: 10.1530/eje.0.1380394. PMID: 9578506
23. Soliman AT, elZalabany MM, Ragab M, Abdel Fattah M, Hassab H, Rogol AD, Ansari BM. Spontaneous and GnRH-provoked gonadotropin secretion and testosterone response to human chorionic gonadotropin in adolescent boys with thalassaemia major and delayed puberty. J Trop Pediatr. 2000 Apr;46(2):79-85. doi: 10.1093/tropej/46.2.79. PMID: 10822933.
24. Mahmoud, R.A., Khodeary, A. & Farhan, M.S. Detection of endocrine disorders in young children with multi-transfused thalassemia major. Ital J Pediatr 47, 165 (2021). https://doi.org/10.1186/s13052-021-01116-2
25. Al-Akhras A, Badr M, El-Safy U, Kohne E, Hassan T, Abdelrahman H, Mourad M, Brintrup J, Zakaria M. Impact of genotype on endocrinal complications in β-thalassemia patients. Biomed Rep. 2016 Jun;4(6):728-736. doi: 10.3892/br.2016.646
26. Elalfy MS, Farid MN, Labib JH, RezkAllah HK. Quality of life of Egyptian b-thalassemia major children and adolescents. Egypt J Haematol 2014;39:222-6

Correspondence Address:
Ashraf Soliman MD, PhD, FRCP
Professor and Senor consultant of Pediatrics and Endocrinology,
College of Medicine, University of Alexandria, Alexandria, Egypt
Hamad General Hospital, Doha, Qatar
E-mail: atsoliman@yahoo.com

HEMOGLOBINOPTHIES IN SUDAN

Shayma Ahmed
Associate Consultant
Pediatrics Endocrine
Hamad Medical Corporation
Doha-Qatar

ABSTRACT

Background: Sickle cell disease (SCD) is one of the most common inherited disorders of hemoglobin in Africa and it is expected that sickle cell trait varies in frequency in different areas in Sudan. Patients and Methods: An extensive PubMed search was carried out and documents published in Sudan that included data on the prevalence of sickle cell anemia and trait. Results: Rates of SCA and trait varied in different areas in Sudan with the highest rates reported from Western and Eastern Sudan where one in every 123 children born in Messeryia tribe in Western Sudan is at risk of having SCD. High consanguinity rates and malaria endemicity are strong related factors with sickle cell gene in Sudan. Conclusion: this review will present epidemiological data, clinical presentations, mortality, treatment protocols and complications of SCD in Sudan.

Keywords: PubMed Sickle cell disease, Sudan sickle cell anemia, sickle cell gene, haplotype, Sudan, Messeryia, tribe, Haemoglobinopathy, Kordofan, Darfur, Khartoum

INTRODUCTION

Sickle cell Disease (SCD) is a global public health concern, with its most significant impact felt in sub-Saharan Africa. The World Health Organization acknowledges SCD as the most common inherited blood disorder in this region, where more than 75% of the worldwide SCD burden is concentrated **(1)**. However, the management of SCD in sub-Saharan Africa faces substantial challenges due to limited healthcare resources, inadequate healthcare staff training, and a lack of public awareness. These factors collectively contribute to the high rates of early mortality associated with SCD in the region **(1)**.

AIM OF THE STUDY

This narrative review aims to comprehensively examine the prevalence, genetic diversity, clinical manifestations, and management of hemoglobinopathies, with a particular focus on SCD , in Sudan. By shedding light on the unique aspects of hemoglobinopathies in Sudan, this review seeks to contribute to our understanding of these disorders within the context of a country characterized by diverse ethnicities, high rates of consanguinity, and distinct migration patterns.

METHODS

This review is based on an extensive search of the available literature spanning the years 2010 to 2023. PubMed served as the primary source for the collection of relevant studies, epidemiological data, genetic analyses, and clinical reports related to hemoglobinopathies in Sudan. Results: 5 papers were reviewed and analyzed.

EPIDIMIOLOGICAL STUDIES

In Sudan, epidemiological studies about SCD are generally scarce. There is no neonatal screening program and a paucity of data regarding children hospitalized by sickle cell anemia. The prevalence of sickle cell anemia in various regions of Sudan varies between 0.8% in central Sudan to 30.4% in Western Sudan. The Messeryia tribe in Kordofan and Darfur represent the highest frequency of sickle cell anemia **(2)**.

High prevalence of sickle cell gene was documented among the population that migrated from Western Africa reported in Al Gadarif state in Eastern Sudan region **(2)**. In Gedarif state, random blood

samples tested for SCA among 100 individuals from different tribes, showed that 20 samples had HbSS, 55 samples had HbAS and 25 samples had HbAA. Sickle cell disease in Darfur and Kordofan showed a prevalence of 30.4% and 18% respectively. It is estimated that one in every 123 children born in Messeryia tribe is at risk of having SCD. Many indigenous tribes that inhabit Darfur region and belong to the Negroid ethnic group and are a part of Nilo-Saharan language family had the highest frequencies of the S gene among them **(3)**.

In 400 children attending Al Fashir hospital the prevalence of sickle cell disease by hemoglobin was 59 (14.8%). Sickle cell trait patients were 11.3% and Sickle cell disease positive patients were 3.5%. Individuals with SCA have consistently low blood Hb concentration, normal MCV and high mean WBC's. Individuals with sickle cell trait had hematological parameters near to those of normal individuals **(2)**.

In Khartoum, Sickle cell disease is the major haemoglobinopathy seen. A study 632 patients attending various clinics at the Khartoum Teaching Hospital, (Capital of Sudan) found that out of this cohort, 5.1% had Hb AS and 0.8% had Hb SS. This was attributed to the migration of tribes from western Sudan because of drought and desertification in the 1970s and 1980s, and the conflicts in Darfur in 2005 **(3)**.

SCA (Hb SS) was highly detected in the Negroid ethnic group; (a Nilo-Saharan language family of North Darfur) living in localities such as Housa 35.7, Fur 28.6%, Zagawa and Bartey 14.3%. While the heterozygous form (HbAS) was detected in 16 tribes of Northern Darfur State **(2)**.

HIGH CONSANGUINITY RATES

A noteworthy contributor to the high prevalence of hemoglobinopathies in Sudan is the exceptionally high rate of consanguineous marriages, which reaches 40-45% of all unions. This practice significantly increases the likelihood of autosomal recessive diseases, including SCD, being inherited **(3)**.

GENETIC DIVERSITY

Genetic analyses in Sudan have revealed significant insights into the origins and spread of SCD. Sudan's ethnically diverse population has resulted in a wide range of genetic variations in hemoglobinopathies. Studies have identified clinically and electrophoretically confirmed SCD cases, that the most frequent haplotype among 143 chromosomes with S gene was the Cameroon (35.0%), followed by the Benin (29.4%), the Senegal (18.2%) and the Bantu (2.8%). The Indian-Arab haplotype was not observed. Three atypical haplotypes were identified in 17 patients, occurring at a combined frequency of 14.6%. One of these, found at the high frequency of 11.8%, possibly represented a new Sudan haplotype **(4)**.

A recent descriptive cross-sectional study conducted between December 2018 and July 2020 reported that among studied patients with SCD 93% and 100% of cases had an absent allele for the e-HindII and Gc-XmnI RFLP sites, respectively. In contrast, the 5 wb-AvaII site was present in most cases (97%). The study detected a new haplotype of the HbS allele in the Sudanese population **(5)**.

Analysis of Y-chromosome haplogroups suggests that the sickle cell gene may have been introduced primarily through males of migrating West African tribes, including Hausa-Fulani and Bagara, during the eighteenth and nineteenth centuries **(4)**.

MIGRATION PATTERN

The migration of various tribes from western Sudan to other regions, including urban areas like Khartoum, has been a notable factor in the spread of SCD. These migrations, driven by drought, desertification, and conflicts in regions like Darfur, have contributed to the dispersion of SCD to different parts of Sudan **(3)**.

CLINICAL PRESENTATIONS AND COMPLICATIONS

Patients with SCD in Sudan commonly experience vaso-occlusive crises, infections, and neurological complications. Treatment approaches include folic acid supplementation, hydroxyurea use, blood transfusions, and prophylactic penicillin. However, there is room for improvement in the utilization of immunization and prophylactic penicillin measures. A recent descriptive, retrospective study conducted in the biggest referral specialized pediatric hospital in Sudan revealed that Vaso-

occlusive crisis (33.3%), infections (13.5%), and neurological complications (10.6%) were the most frequent complications reported during patients visits. After initiation of management, only 3.4% of pediatric patients had hemolytic crises, and 1.4% of the anemic patients had splenomegaly. 100% of patients received folic acid, 73.9% used hydroxyurea, and 69.6% underwent blood transfusion for the management of SCD. Prophylactic penicillin was prescribed for 15% of patients, and 41.1% were immunized with pneumococcal vaccine (PPSV23) **(5)**.

MORTALITY

SCD-related mortality in Sudan predominantly affects children under the age of 5, adolescents, and pregnant women. Accurate mortality rates remain unknown due to the scarcity of data from neonatal screening programs and the absence of prospective natural history studies. Estimates suggest that 50-90% of infants born with SCD in sub-Saharan Africa die before reaching the age of 5 **(1)**.

SCD TREATMENT PROTOCOLS

The SCD treatment protocol in Sudan, involving preventive and symptomatic therapy, aligns with internationally implemented protocols for SCD management. However, there are deficiencies in immunization and prophylactic penicillin approaches. Although Sudanese SCD treatment protocols align with international standards, there is room for improvement in areas such as immunization and prophylactic penicillin approaches. Hydroxyurea and blood transfusions have shown efficacy in reducing fever and vaso-occlusive crises, improving patient outcomes **(5)**.

CONCLUSION

This narrative review underscores the unique challenges and contributions of Sudan to the understanding of hemoglobinopathies, particularly SCD, in a diverse and dynamic context, offering valuable insights into the genetic and clinical aspects of these disorders in the region.

REFERENCES

1. Hassan MM, Ziada MA. Thalassemia major in the Sudan. Br Med J. 1965;1(5430):295. doi:10.1136/bmj.1.5430.295
2. Adam MA, Adam NK, Mohamed BA. Prevalence of sickle cell disease and sickle cell trait among children admitted to Al Fashir Teaching Hospital North Darfur State, Sudan. BMC Res Notes. 2019, 16;12(1):659. doi: 10.1186/s13104-019-4682-5. PMID: 31619285; PMCID: PMC6796395.
3. Sabahelzain MM, Hamamy H. The ethnic distribution of sickle cell disease in Sudan. Pan Afr Med J. 2014, 3;18:13. doi: 10.11604/pamj.2014.18.13.3280. PMID: 25360197; PMCID: PMC4213521.
4. Khalafallah TO, Ajab Eldoor AA, Babker AM, et al. Hematological and molecular analyses of the HbS allele among the Sudanese population. *J Int Med Res.* 2022;50(9):3000605221125050. doi:10.1177/03000605221125050
5. Meysaa Talha, Bashier Osman, Safa Abdalla, Hind Mirghani, Iman Abdoon, "Pediatric Sickle Cell Disease in Sudan: Complications and Management", Anemia, vol. 2022, ID 3058012, https://doi.org/10.1155/2022/3058012

Correspondence Address:
Shayma Ahmed.
Associate Consultant.
Pediatrics Endocrine.
Hamad Medical Corporation.
Doha-Qatar
E-mail: Smohammed5@hamad.qa

HEMOGLOBINOPATHIES IN MOROCCO

Redouane Boulouiz & Mohamed Bellaoui
Genetics Unit, Medical Sciences Research Laboratory, Faculty of Medicine and Pharmacy, University Mohammed Premier, Oujda, Morocco

ABSTRACT

Hemoglobinopathies, hereditary monogenic disorders of hemoglobin, can be divided into three broad categories, depending on whether the pathogenic variant affects the globin protein structure, its synthesis, or globin developmental switching. The two main groups are thalassemias and structural hemoglobin variants. Thalassemias constitute a heterogeneous group in which mutations lead to reduced synthesis or stability of either the α-globin or β-globin chain, causing α-thalassemia or β-thalassemia respectively. While, structural hemoglobin variants arise from the production of structurally defective hemoglobin. Hemoglobinopathies are the most common hereditary diseases worldwide, and Morocco is no exception. Nevertheless, the exact prevalence remains incompletely known, due to a lack of extensive epidemiological studies. Considering the high rate of consanguinity, the geographical location and the historical context, it is obvious to expect a high prevalence as well as a high molecular heterogeneity of hemoglobinopathies in Morocco.

According to the limited literature available for the Moroccan population, the following conclusions can be drawn: First, structural hemoglobin variants exhibit significant heterogeneity, with 14 variants identified, and include prevalent variants such as HbS, HbC, and HbO-Arab. Furthermore, their prevalence varies significantly between different studies. Second, molecular data on β-thalassemia are well documented, and show highly heterogeneous mutational spectrum. However, our knowledge of α-thalassemia is very limited particularly in terms of prevalence and molecular origins. In conclusion, these data imply the urgent necessity to set up screening programs and to implement a national registry. Such measures can play a crucial role in reducing the prevalence of hemoglobinopathies.

Keywords: Hemoglobinopathies, consanguinity, thalassemia, structural hemoglobin variant, mutational spectrum, prevalence, morocco

HISTORY AND CHARACTERISTICS OF MOROCCAN POPULATION

Morocco is a Mediterranean country located in the northwest region of the African continent, covering an area of approximately 710,850 km^2 and a population of 33.85 Million inhabitants (HCP, 2021). Due to both consanguinity and heterogeneous ethnic backgrounds, recessive monogenic disorders are frequently encountered. Indeed, Morocco's genetic heritage has been shaped by a rich historical tapestry, resulting from the influence of various civilizations over time. Although the Berbers were the indigenous people of Morocco, the region has undergone a series of successive civilizations that have left their genetic imprint. These include the Phoenicians (11th century BC), the coastal presence of the Carthaginians (814-146 BC), a brief Roman period (1st to 3rd century AD), a century of influence from the Byzantines of the Justinian Empire in the North and North-West of Morocco, the arrival of the Arabs in the late 7th century AD, and the influence of the Iberian Peninsula population (711-1492 AD). Moreover, the presence of Sub-Saharan populations in Morocco, due to their trade caravans crossing the region, has further enriched the genetic background. Furthermore, in agreement with anthropogenetic studies, it has been demonstrated that the Moroccan population possesses a unique population stratification profile presented by the association or mixture of African, Caucasoid, Asian, Arab, and Berber components (Harich et al., 2002) (Chbel et al., 2003). As a consequence of this genetic diversity various variants have arisen within the gene pool. Therefore, variants in different genes have their origins in various sources (Agouti et al., 2008).

CONSANGUINITY IN THE MOROCCAN POPULATION

Like all other North African and Arab countries, Morocco display a high rate of inbreeding mainly due to cultural, socio-economic, and linguistic factors (Jaouad et al., 2009). In fact, roughly one-third of the population practices consanguineous marriage, with the consanguinity rates and the consanguinity coefficient fluctuating respectively between 25% and 35% and between 0.0094 and 0.0107, depending on the region. Notably, the Oriental and Southern regions report the highest percentages in this regard (Hami et al., 2005) (Talbi et al., 2007). Furthermore, in a population of families carrying autosomal recessive genetic abnormalities, including β-thalassemia, 59.09% of marriages were consanguineous, with 43.18% being marriages between first cousins. Thus, the consanguinity rate among families affected by these diseases is higher than that observed in the general population (Jaouad et al., 2009).

HEMOGLOBINOPATHIES PREVALENCE

Human hemoglobin disorders, known as Hemoglobinopathies, are the most common monogenic disorders, which are caused by pathogenic DNA variants in the genes coding for the globin chains and thus affecting the tetrameric hemoglobin complex. They encompass a group of disorders that can disrupt either the structure, the synthesis of hemoglobin or the globin development switching. Structural disorders lead to the production of abnormal hemoglobin (hemoglobin variant) due to abnormal structure without affecting its level, while the synthesis defects lead to reduced or absence of synthesis of one or both globin chains, giving rise to diseases called thalassemias. There is a third type of hemoglobinopathy called Hereditary Persistence of Fetal Hemoglobin (HPFH) which refers to a cluster of clinically non-pathological conditions that are noteworthy due to their disruption of the perinatal transition from γ-globin to β-globin production.

It should be noted that these hemoglobinopathies, previously known to be characteristic of tropical and subtropical zones, have now spread to all regions of the world, due to migratory flows. Approximately 1.1% of couples around the world run the risk of having a child with hemoglobinopathies. Most affected children born in developed countries can survive with these chronic diseases, whereas in developing countries individual born with these diseases often get affected before reaching the age of 5. It is important to note that hemoglobinopathies contribute to approximately 3.4% of under-5 child mortality worldwide, with a significantly higher impact of 6.4% in Africa. The prevalence of hemoglobinopathies in Morocco, as well as their distribution, vary considerably between studies, depending on the study design or the geographic region under investigation, with a rate ranging from 3.6 % to 72.6% (Agoumi & Sebar, 2003; Alaoui et al., 2020; Dahmani et al., 2017).

HEMOGLOBIN STRUCTURAL VARIANTS

In Morocco, a variety of structural hemoglobin variants have been documented in various state, including homozygous, heterozygous, and composite heterozygous. These variants are found either in association with another variant or in conjunction with β or α thalassemia. The literature reports the presence of nine β-globin variants, three α-globin variants (Hb Philadelphia, Hb Groene Hart and Hb Dunn variants), one γ variant, and one γ triplication (Table 1). Furthermore, four composite heterozygous associations have been identified. Three of these involve β-globin variants and thalassemias (Baklouti et al., 1987; Mears et al., 1981; Delanoe-Garin et al., 1985), while one association combines β-globin variant and α-globin variant (Hb O –Arab /Hb Dunn) (Baklouti et al., 1988). As shown in table 1, it should be noted that although several structural hemoglobin variants have been documented, two important features should be highlighted. Firstly, these variants are predominantly concentrated in the northwestern regions of Morocco, with Kenitra and Larache serving as focal points for hemoglobinopathies, particularly sickle cell anemia. This is in contrast to the higher prevalence of thalassemia in the southern regions. Secondly, two hemoglobin variants dominate: HbS, with frequencies ranging from 1% to 88%, followed by HbC, which varies from 0.5% to 10% (Dahmani et al., 2017; Alaoui et al., 2020; Agoumi & Sebar, 2003; Elmachtani Idrissi et al., 2012).

THALASSEMIAS

Thalassemia, represent a heterogeneous group of hemoglobin diseases caused by reduced expression of one of the two globin chains of the hemoglobin molecule, α-globin gene (MIM *141800 & MIM

141850) and β-globin gene (MIM 141900). This reduction leads to an imbalance in the α:β chains ratio, which underlies the pathophysiological mechanism (Sabath, 2017). Depending on the globin chain affected, we distinguish between α-thalassemia and β-thalassemia.

α-THALASSEMIA

The majority of α-thalassemia cases are caused by gene deletions, while the remaining cases are attributed to point mutation. The presence of 4 α-globin genes complicates the detection of α-thalassemia, particularly when only one or two of these alpha genes are affected. Thus, the frequency of α-thalassemia is probably underestimated because the clinical symptoms may be milder or even asymptomatic, making diagnosis more challenging and require specialized genetic testing (Piel & Weatherall, 2014). Indeed, only a few reports have described cases of α-thalassemia in the Moroccan population. These studies have reported different frequency of α-thalassemia ranging from 0.36% to 44%. This variation is probably attributed to the geographic region or characteristics of the studied populations (Dahmani et al., 2017; Agoumi & Sebar, 2003). Among these studies, only two reports highlighted the molecular aspect of α- thalassemia. The first study, conducted on cord blood sample revealed a 2.2% frequency of Hb-Bart's. While the second report identified the following genotypes in coexistence with β-thalassemia: (_α3.7/_α3.7), (_α3.7/αα) and (_α3.7/ααα) (Lemsaddek et al., 2003; Agoumi & Sebar, 2003). Up to now, no point mutations in *HBA1/ HBA2* genes have been described in the Moroccan population.

β-THALASSEMIA

In contrast to α-thalassemia, about 95% of β-thalassemia cases are the result of point mutations. β-thalassemias are most commonly observed in individuals in the Mediterranean, Middle Eastern, and South Asia, where the prevalence of carriers is relatively higher. In Morocco, the prevalence of β-thalassemia ranges from 0.83% to 3% (Agouti et al., 2007; Agoumi & Sebar, 2003).

The spectrum of β-thalassemia variants is highly heterogeneous and it is important to emphasize that, as expected, variants described in various studies carried out in the Moroccan population are distributed throughout the gene impacting the entire process necessary for the synthesis of an mRNA or a functional protein. The following overview highlights that β-thalassemia results from many different types of molecular abnormalities, predominantly single nucleotide variant (SNV), in the β-globin gene (Table 2). The molecular investigation of β-thalassemia in the Moroccan population indicates a high molecular heterogeneity by describing approximately 28 pathogenic variants which could be categorized as follows: nine (9) variants in the coding region comprising four nonsense substitutions and five (5) frameshift deletions. These variants result in loss of protein production or the degradation of mutated mRNA through the mechanism known as nonsense–mediated mRNA decay (Khajavi et al., 2006). It's worth noting that the majority of β-thalassemia patients (>58%, ranging from 15.5% to 71.15%) display these particular molecular abnormalities. The second group of variants (17 in total) consists of those that impact mRNA splicing and are located in the intron and promoter regions. These variants account for over 34% of β-thalassemia cases. The last described variant is exceptionally rare, occurring in only 1.5% of cases, and it affects the polyadenylation site, leading to the defect in the tailing of β-globin mRNA. Regarding the haplotype analysis, it is noteworthy to mention that the three most common variants, C39 (C>T), FSC8 (-AA), and IVS-I-1 (G>A), suggest these potential origins: Phoenician, Carthaginian, or Roman origin; a Middle Eastern and Turkish origin; and lastly, a Berber origin, respectively.

CONCLUSION

In Morocco, structural hemoglobin variants exhibit significant heterogeneity, with 14 variants identified, and include prevalent variants such as HbS, HbC, and HbO-Arab. Similarly, β-thalassemia is well documented and show a highly heterogeneous mutational spectrum. However, our knowledge of α-thalassemia is very limited, particularly in terms of prevalence and molecular origins. Despite hemoglobinopathies represent a public health problem in Morocco, there are no data regarding the prevalence of hemoglobinopathies and carriers at a national scale. In conclusion, there is an urgent need to carry out extensive epidemiological studies and establish a national registry. Such measures can play a crucial role in reducing the prevalence of hemoglobinopathies and in implementing a carrier screening program as well as appropriate genetic counseling, particularly for prenatal or preimplantation diagnosis.

***Table 1**: Structural hemoglobin variant identified in the Moroccan population.*

Globin genes	Hemoglobin Variant	Ethnic background	Reference
β-Globin	Hb S	African, American Indian,	(Mears et al., 1981; Giordano et al., 1998)
	Hb C	African	(Giordano et al., 1998)
	Hb Tsukumi	Japanese, Moroccan	(North et al., 2001) (Oribe et al., 2000)
	Hb Casablanca	Moroccan	(Wajcman, Drupt, et al., 2000)
	Hb D-Punjab	Australian, Chinese, Dutch, English, Greek, Indian, Pakistani, Turkish	(Chami et al., 1994)
	Hb Hofu	African, Indian, Japanese, Spanish	(Chami et al., 1994)
	Hb Kenitra	Moroccan, Black	(Chami et al., 1994)
	Hb O-Arab	African-American, Arabian, Egyptian	(Chami et al., 1994)
	Hb Pyrgos	African, Greek, Italian, Japanese	(Chami et al., 1994)
α-Globin	Hb G-Philadelphia	African, Chinese, Italian	(Verhoeven & Van Ros, 1968)
	Hb Dunn	African, Indian, Moroccan	(Baklouti et al., 1988)
	Hb Groene Hart	Italian, Moroccan	(Harteveld et al., 2002; Giordano et al., 2007)
γ Globin	triplication ɣ globine	-	(Liu et al., 1988)
	Hb F-Ouled Rabah	Algerian	(Wajcman, Borensztajn, et al., 2000)

***Table 2**: Distribution of β-globin gene pathogenic variants, their location and associated haplotype in Moroccan population*

Region		Molecular basis of mutation	Common Name	Ethnic background	Phenotype	Haplotype	(Lemsaddek et al., 2003)	(Lemsaddek et al., 2004)	(Agouti et al., 2007)	(Agouti et al., 2008)	(Belmokhtar et al., 2022)
Coding region		Nonsens mutation	**C39 (C>T)**	Mediterranean	β^0	I, II, Nd	15,5	26,2	28,05	26,58	71,15
			C37 (G>A)	Saudi Arabian	β^0	VII, I	2	1	-	3,16	-
			C24 (T>A)	African American	β^{++}	IX	-	-	2,44	0,63	-
			C30 (G>C)	North Africa	β^0	-	-	0,5	-	-	-
		Frame shift deletion	**FSC8 (-AA)**	Mediterranean	β^0	IV, VI, VII	15,5	9,6	21,95	13,19	-
			FSC6 (-A)	Mediterranean	β^0	IX, III,	10	13,3	9,76	5,7	3,85
			FSC16 (-C)	Asian	β^0	-	-	2,6	-	-	-
			FSC5 (-CT)	Mediterranean	β^0	III	-	-	-	1,27	5,77
			C39/38 (-C)	Czechoslovak	β^0	-	-	-	-	-	3,85
		Polyadenylation signal defects	**PolyA (T>C)**	African American	β^{++}	-	2	1	-	-	-
Non coding region	Intron I	Splice junction mutations	**IVS-I-1 (G>A)**	Mediterranean	β^0	V, IV, XI	13	8,5	7,32	5,06	1,92
			IVS-I-2 (T>C)	African American	β^0	II, IX	3	2,1	1,22	5,06	-
			IVS-I-2 (T>G)	Tunisian	β^0	IX	-	0,5	-	3,16	-
		Reduces the effectiveness of the normal splice	**IVS-I-5 (G>T)**	Mediterranean	β^+	-	-	0,5	-	-	-
			IVS-I-6 (T>C)	Mediterranean	β^+	VI, VII	14	13,9	2,44	3,16	-
		Splicing site mutation	**IVS-I (25 bp del)**	Middle East	β^0	IX	1	0,5	-	1,27	-
		Cryptic splice site mutations	**IVS-I-110 (G>A)**	Mediterranean	β^+	I, II	2	3,2	-	5,7	3,85
			IVS-I-130 (G>A)	Egyptian	β^0	-	1	0,5	-	-	-

Intron II	**Splice junction mutations**	**IVS-II-1 (G>A)**	Mediterranean	β^0	III	1	3,2	2,44	2,5	1,92
	Cryptic splice site mutations	**IVS-II-726 (A>G)**	Moroccan-Italian	β^{++}	IX	-	-	-	1,27	-
		IVS-II-745 (C>G)	Mediterranean	β^{+}	VII	1	0,5	10,98	7,6	-
Promoter	**Binding transcription factor**	**-28 (A>G)**	African American	β^{+}	I	1	-	-	-	-
		-28 (A>C)	Kurdish, Spanish	β^{+}	-	-	1,6	-	1,9	-
		-29 (A>G)	African American	β^{+}	II, VI, IX, 3black, C	6	4,2	8,54	6,33	3,85
		-56 (G>C)	North Africa	β^{++}	-	-	-	-	-	1,92
		-101 (C>T)	Mediterranean	β^{++}	-	1	0,5	-	-	-
		-190 (G>A)	Moroccan	β^{++}	XI	-	-	-	0,63	-

REFERENCES

1. Agoumi, N. B., & Sebar, A. (2003). Les hémoglobinopathies au Maroc. *Archives de Pédiatrie*, *10*(7), 654-655. https://doi.org/10.1016/S0929-693X(03)00294-X
2. Agouti, I., Badens, C., Abouyoub, A., et al. (2007). Genotypic Correlation Between Six Common β-Thalassemia Mutations and the *Xmn* I Polymorphism in the Moroccan Population. *Hemoglobin*, *31*(2), 141-149. https://doi.org/10.1080/03630260701285050
3. Agouti, I., Badens, C., Abouyoub, A., et al. (2008). Molecular Basis of β-Thalassemia in Morocco : Possible Origins of the Molecular Heterogeneity. *Genetic Testing*, *12*(4), 563-568. https://doi.org/10.1089/gte.2008.0058
4. Alaoui, F. Z., Laghmich, A., Nourouti, N. G., et al. (2020). *Pattern of Hemoglobinopathies : A Cross-sectional Study in North of Morocco*. In Review. https://doi.org/10.21203/rs.3.rs-62915/v1
5. Baklouti, F., Francina, A., Dorléac, E., et al. (1988). Asymptomatic association of hemoglobin Dunn (α6[A4]Asp→Asn) and hemoglobin O-Arab (β121[GH4]Glu→Lys) in a Moroccan man. *American Journal of Hematology*, *27*(4), 253-256. https://doi.org/10.1002/ajh.2830270405
6. Baklouti, F., Francina, A., Dorléac, E., et al. (1987). Association incis of β+-thalassemia and hemoglobin S. *American Journal of Hematology*, *26*(3), 237-245. https://doi.org/10.1002/ajh.2830260305
7. Belmokhtar, I., Lhousni, S., Elidrissi Errahhali, M., et al. (2022). Molecular heterogeneity of β-thalassemia variants in the Eastern region of Morocco. *Molecular Genetics & Genomic Medicine*, *10*(8), e1970. https://doi.org/10.1002/mgg3.1970
8. Chami, B., Blouquit, Y., Bardakdjian-Michau, J., et al. (1994). Hemlobin Variants in North Africa. *Hemoglobin*, *18*(1), 39-51. https://doi.org/10.3109/03630269409014144
9. Chbel, F., de Pancorbo, M. M., Martinez-Bouzas, C., et al. (2003). [Polymorphism of six Alu-insertions in residents of Morocco : Comparative study in Arab and Berber populations and residents of Casablanca]. *Genetika*, *39*(10), 1398-1405.
10. Dahmani, F., Benkirane, S., Kouzih, J., et al. (2017). Profil épidémiologique des hémoglobinopathies : Étude autour du cas index: étude transversale descriptive. *Pan African Medical Journal*, *27*. https://doi.org/10.11604/pamj.2017.27.150.11477
11. Delanoe-Garin, J., Arous, N., Blouquit, Y., et al. (1985). Hemoglobin Kenitra $\alpha_2\beta_2$ 69 (E13) Gly→Arg. A new β Variant of Elevated Expression Associated with α-Thalassemia, Found in a Moroccan Woman. *Hemoglobin*, *9*(1), 1-9. https://doi.org/10.3109/03630268508996977
12. Elmachtani Idrissi, S., El Omri, N., El Jaoudi, R., et al. (2012). Hémoglobinopathie O Arab homozygote dans une famille marocaine. *Immuno-analyse & Biologie Spécialisée*, *27*(5), 268-271. https://doi.org/10.1016/j.immbio.2012.05.002
13. Giordano, P. C., Harteveld, C. L., Heister, A. J. G. M., et al. (1998). The Molecular Spectrum of Beta-Thalassemia and Abnormal Hemoglobins in the Allochthonous and Autochthonous Dutch Population. *Public Health Genomics*, *1*(4), 243-251. https://doi.org/10.1159/000016170
14. Giordano, P. C., Zweegman, S., Akkermans, N., et al. (2007). The First Case of Hb Groene Hart [α119(H2)Pro→Ser, *C* CT→ *T* CT (α1)] Homozygosity Confirms That a Thalassemia Phenotype Is Associated with this Abnormal Hemoglobin Variant. *Hemoglobin*, *31*(2), 179-182. https://doi.org/10.1080/03630260701289490
15. Hami, H., Attazagharti, N., Soulaymani, A., et al. (2005). Homogamie dans la Région du Gharb-Chrarda-Béni Hssen (MAROC). Une enquête prospective (Juin 2003-Février 2004). *Antropo*, *9*, 51-60.
16. Harich, N., Esteban, E., Chafik, A., et al. (2002). Classical polymorphisms in Berbers from Moyen Atlas (Morocco) : Genetics, geography, and historical evidence in the Mediterranean peoples. *Annals of Human Biology*, *29*(5), 473-487. https://doi.org/10.1080/03014460110104393
17. Harteveld, C. L., Delft, P. V., Plug, R., et al. (2002). Hb Groene Hart : a new pro→ser amino acid substitution at position 119 of the **α** 1-globin chain is associated with a mild **α** -thalassemia phenotype. *Hemoglobin*, *26*(3), 255-260. https://doi.org/10.1081/HEM-120015029
18. Jaouad, I. C., Elalaoui, S. C., Sbiti, A., et al. (2009). Consanguineous marriages in morocco and the consequence for the incidence of autosomal recessive disorders. *Journal of Biosocial Science*, *41*(5), 575-581. https://doi.org/10.1017/S0021932009003393
19. Khajavi, M., Inoue, K., & Lupski, J. R. (2006). Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. *European Journal of Human Genetics*, *14*(10), 1074-1081. https://doi.org/10.1038/sj.ejhg.5201649
20. Lemsaddek, W., Picanço, I., Seuanes, F., et al. (2003). Spectrum of β thalassemia mutations and HbF levels in the heterozygous Moroccan population : β Thal Mutations and HbF Levels in Morocco. *American Journal of Hematology*, *73*(3), 161-168. https://doi.org/10.1002/ajh.10358
21. Lemsaddek, W., Picanço, I., Seuanes, F., et al. (2004). The β-Thalassemia Mutation/Haplotype Distribution in the Moroccan Population. *Hemoglobin*, *28*(1), 25-37. https://doi.org/10.1081/HEM-120028884

22. Liu, J. Z., Gilman, J. G., Cao, Q., et al. (1988). Four categories of gamma-globin gene triplications : DNA sequence comparison of low G gamma and high G gamma triplications. *Blood*, *72*(2), 480-484.
23. Mears, J. G., Beldjord, C., Benabadji, M., et al. (1981). The sickle gene polymorphism in North Africa. *Blood*, *58*(3), 599-601.
24. North, M. L., Duwig, I., Riou, J., et al. (2001). Hb Tsukumi [β117(G19)His →Tyr] found in a moroccan woman. *Hemoglobin*, *25*(1), 107-110. https://doi.org/10.1081/HEM-100103076
25. Oribe, Y., Hamaguchi, K., Kusuda, Y., et al. (2000). Hb Tsukumi [β117(G19)His→Tyr] : A New Hemoglobin Variant Found in a Japanese Male. *Hemoglobin*, *24*(2), 117-123. https://doi.org/10.3109/03630260009003430
26. Piel, F. B., & Weatherall, D. J. (2014). The α-Thalassemias. *New England Journal of Medicine*, *371*(20), 1908-1916. https://doi.org/10.1056/NEJMra1404415
27. Sabath, D. E. (2017). Molecular Diagnosis of Thalassemias and Hemoglobinopathies. *American Journal of Clinical Pathology*, *148*(1), 6-15. https://doi.org/10.1093/ajcp/aqx047
28. Talbi, J., Khadmaoui, A. E., Soulaymani, A. E.-M., et al. (2007). Etude de la consanguinité dans la population marocaine. Impact sur le profil de la santé. *Antropo*, *15*, 1-11.
29. Verhoeven, L., & Van Ros, G. (1968). [G Philadelphia and G-2 variants of hemoglobin in a Moroccan immigrant in Belgium]. *Annales Des Societes Belges De Medecine Tropicale, De Parasitologie, Et De Mycologie*, *48*(2), 225-235.
30. Wajcman, H., Borensztajn, K., Riou, J., et al. (2000). Two New $^{G}\gamma$ Chain Variants : Hb F-Clamart [γ17(A14)Lys→Asn] and Hb F-Ouled Rabah [γ19(B1)Asn→Lys]. *Hemoglobin*, *24*(1), 45-52. https://doi.org/10.3109/03630260009002273
31. Wajcman, H., Drupt, F., Henthorn, J. S., et al. (2000). Two new Variants with the Same Substitution at Position β122 : Hb Bushey [β122(GHS)Phe→Leu] and Hb Casablanca [β65(E9)Lys→Met;β122(GHS)Phe →Leu]. *Hemoglobin*, *24*(2), 125-132. https://doi.org/10.3109/03630260009003431

Correspondence Address :
Redouane BOULOUIZ
Genetics Unit, Medical Sciences Research Laboratory, Faculty of Medicine and Pharmacy, University Mohammed Premier, Oujda, Morocco.
E-mail: rboulouiz@gmail.com

HEMOGLOBINOPATHIES (SICKLE CELL DISASES & THALASSEMIA) IN ARAB COUNTRIES

Ahmed Badr[1], Mohamed Yassin[2*]*
[1]College of Medicine, QU Health, Qatar University, Doha 2713, Qatar.
[2]Hematology Section, Medical Oncology, National Center for Cancer Care and Research, Hamad Medical Corporation, Doha 3050, Qatar.
** Correspondence: A.B., ab1906092@qu.edu.qa; M.Y., yassinmoha@gmail.com*

ABSTRACT

Haemoglobinopathies, including sickle cell disease and thalassemias, are widespread autosomal recessive disorders in most Arab nations, characterized by varying prevalence rates and genetic features. Sickle cell disease is a group of inherited disorders that affect the red blood cell causing deficiency in tissues oxygenation. The clinical course of sickle cell disease and its prominent complications are influenced by ethnicity and regional differences. β-thalassemia is present in a diverse range of frequencies in nearly all Arab countries, with carrier rates ranging from 1% to 11%, and with varying mutations. α-thalassemias are also prevalent in the majority of Arab countries, with frequencies ranging from 1% to 58%, with the highest rates reported in Gulf countries. Recent advances in the management of haemoglobinopathies with newer therapies are being introduced to the Western population. However, many of these treatments are yet to be used in the Arabic population. Understanding the genetic variations of the different hemoglobin disorders regionally is essential to anticipate the utilization of new treatments. Despite of the high prevalence of these disorders, many Arab nations face significant difficulties in providing comprehensive and up-to-date care and prevention services. Challenges primarily stem from a lack of accurate data concerning the true scope, health consequences, and economic impact of haemoglobinopathies. To emphasize the importance of prioritizing community-level care and prevention initiatives, this review seeks to illustrate the epidemiological landscape of haemoglobinopathies, including sickle cell disease and thalassemia, in Arab countries. Additionally, it highlights the most common genetic mutations associated with these conditions in the region. Gaining insights into the molecular abnormalities involved enables the development and improvement of diagnostic tests and management protocols for these disorders.

Keywords: Haemoglobinopathies, epidemiology, prevalence, sickle cell disease, β-thalassemia, α-thalassemia, Arab, Arabic countries

INTRODUCTION

Haemoglobinopathies are common autosomal recessive disorders that are characterized by qualitative and quantitative variations in the globin chains. Sickle cell disease (SCD) is one of the most prevalent examples of a qualitative abnormality which is marked by the presence of haemoglobin (Hb)S **(1)**. SCD is marked by autosomal recessive genetic mutations in the hemoglobin beta-subunit, (β6(A3)Glu→Val) **(2)**. These genetic alterations give rise to deformed RBCs that compromise oxygen transport to tissues, leading to sickling, rigidity, and obstruction of small vascular channels **(3, 4)**. Hemolysis and intravascular RBCs clumping associated with SCD cause many complications that contribute to high morbidity and mortality rates, including tissue necrosis, delayed growth, acute chest syndrome, chronic kidney

disease, splenic infarction and multiple organ damage **(5)**. SCD is divided into many subgroups: sickle cell anemia (HbSS), hemoglobin SC disease (HbSC), and hemoglobin sickle-beta-thalassemia (beta-thalassemia positive or beta-thalassemia negative) **(2-4)**.

The highest prevalence of SCD is observed in regions encompassing Sub-Saharan Africa, South Asia, the Middle East, and the Mediterranean, with an estimated 300,000 to 400,000 neonates affected globally each year **(2, 6-8)**. Notably, an analysis revealed variations in SCD prevalence, with significantly higher rates in Africa compared to Europe. The global SCD burden is anticipated to surge by up to 30% by 2050 **(7)**. SCD management is a multifaceted process, involving universal newborn screening for early diagnosis **(9, 10)**. Current medical interventions, such as hydroxyurea, L-glutamine, and emerging therapies like crizanlizumab and voxelotor, aim to reduce complications **(5)**. Blood transfusion and pain management are additional supportive management for symptomatic SCD patients **(11, 12)**. However, there is no current cure for SCD in adults. Bone marrow transplantation has shown promise in pediatric patients, while gene therapy, though in clinical trials, offers potential for the future **(5)**. It is crucial to comprehensively understand the genotypic and phenotypic aspects of SCD in different regions of the world in order to have better treatment options and patient care.

On the other hand, the most common quantitative abnormalities involve a decrease or absence in the synthesis of α- or β-globin chains, resulting in α- and β-thalassemias, respectively. Beta-thalassemia, an autosomal recessive chronic hemolytic anemia, is characterized by reduced hemoglobin levels and impaired red blood cells (RBCs) production **(13, 14)**. The condition results from decreased or absent β-globin chain synthesis, critical for hemoglobin synthesis along with α-globin chains. Mutations in the HBB gene on chromosome 11, comprising more than 200 known mutations, underlie β-thalassemia **(15, 16)**. Variants are classified as β0 (completely abolishing β-globin production due to inactivation of the β gene) and other mutations, categorized based on the reduction degree of β-chain production, such as β+ or β++ (silent) thalassemia **(16)**. B0-thal relies on different mutations including initiation codon, missense, nonsense, splice-site junction, and frameshift mutations **(15)**. B+- and B++- thalassemia are mainly caused by mutation in both the promoter and 5'/3'-UTRs **(15)**. The prolonged reduction in β-globin production leads to the varied clinical presentations among β-thal different spectrums **(13)**. Worldwide, β-thal is a substantial health problem that is most prevalent in low-income countries **(17, 18)**. Annually, a total of 80 million individuals are estimated to be carriers of the disease **(17)**. Among Arab countries, β-thal has a high prevalence with carrier frequencies ranging from 1% to 11% **(1)**.

β-thalassemia can be categorized into four distinct groups: β-thal major, β-thal intermedia, β-thal minor, and silent β-thal **(15)**. Firstly, β-thal major, the most severe manifestation of thalassemia, is manifested by severe anemia and skeletal deformities, demanding regular blood transfusions for survival. Most β-thal major cases result from being homozygous for the β0 allele (β0/β0), with fewer cases arising from compound heterozygosity (β+/β0) **(19)**. Secondly, β-thal intermedia falls between the major and minor presentations. Patients may be either homozygous for the β+ allele (β+/β+) or compound heterozygous for β0 and β+ (β0/β+) **(19, 20)**. The phenotypic spectrum of β-thal intermedia varies from mild to severe anemia, occasionally necessitating blood transfusions. This intermediate form is observed in heterozygous individuals carrying a single dominant β0 allele (β0/BN), homozygous individuals for β0 (β0/β0) with concurrent alpha-thalassemia co-inheritance, or in heterozygous individuals with a dominant β0 (β0/BN) or a recessive β+ allele (β+/BN) alongside a triplication co-inheritance **(19, 20)**. Nevertheless, the complex genotype-phenotype correlations of β-thal intermedia remain incompletely understood. Meanwhile, β-thal minor patients, inheriting either a single β0 or β+ allele, usually remain asymptomatic. Nonetheless, their laboratory results indicate elevated Hb A2 levels and reduced microcytosis and hypochromia. The last and most mild type of the disease is silent β-thal. The carriers of this form possess a heterozygous β++ allele (β++/BN) and typically display normal RBCs and Hb A2 levels **(16)**.

Despite the high prevalence rates of haemoglobinopathies, numerous Arab countries grapple with

significant challenges in delivering comprehensive and current care and prevention services. These challenges primarily arise from insufficient data regarding the true extent, health impact, and economic burden of haemoglobinopathies. To underscore the necessity of setting priorities for implementing community-level care and prevention programs, this review aims to depict the epidemiological landscape of haemoglobinopathies (SCD and thalassemia) in Arab countries and underlining the most common genetic mutations associated with these diseases in the region. Understanding the molecular defects involved allows for the development and enhancement of diagnostic tests and management protocols for these disorders.

METHODS

The search strategy of this review was generated through PubMed's Medical Subject Headings (MeSH) terms, along with other title and abstract keywords. To include all relevant review articles published in English discussing haemoglobinopathies, especially thalassemia and SCD, and their prevalence in the Arab countries, the search terms included the name of the Arab country along with the following search terms: "haemoglobinopathies", "Beta Thalassemia", "Alpha Thalassemia", "Thalassemia", "Sickle Cell Disease", "Sickle Cell Anemia", "SCD", "SCA", "Hemoglobin S Disease", "HbS Disease", "Anemia, Sickle Cell", "Hemoglobin S/O", "sickle/ beta-thalassemia", "SC/SD", "HbSS", "HbSC", "HbSβ+", "HbSβ0", "HbSD", "HbSE", "HbSO Arab", "HBS Oman", "Arab", "Middle East", "Arabian Peninsula", "Gulf countries", "North Africa", "Arab World", "Arab Population", "The Middle East", "Epidemiology", "prevalence" and "incidence". This study had no language or time period restrictions. We employed a polyglot translator to convert our initial search strategy into Embase, Science Direct, Scopus, Web of Science, and Google Scholar, aiming to retrieve a comprehensive set of articles related to our research topic **(21)**. All results identified through this search process were imported into EndNote, which automatically removed duplicate entries. The remaining studies were subsequently transferred to Rayyan, where any residual duplicates were manually removed, initiating the screening process.

This review included studies that directed the epidemiology of haemoglobinopathies (SCD and thalassemia) and associated genetic mutations in the Arabic region only. Studies excluded from this review were: (1) animal studies, (2) non-English articles, and (3) studies that do not focus on the Arabic region in their findings. Across all the five databases, the search strategy generated a total of 1717 articles. All the studies were transferred into EndNote, where 1206 duplicates were identified and automatically removed. The articles were then imported to Rayyan, where 187 additional duplicates were deleted. The process of eligibility assessment was conducted using Rayyan. A total of 271 were assessed to satisfy the eligibility criteria, with only 202 studies succeeded to check the inclusion criteria and were included in this review. Figure 1 depicts a schematic presentation of the identification, selection, and inclusion processes. The included studies were comprehensively assessed and reviewed to identify the epidemiological aspect of the common haemoglobinopathies with the associated genetic mutations in the Arabic region. Due to the high number of included studies, it was impossible to compose a summary table of all the studies.

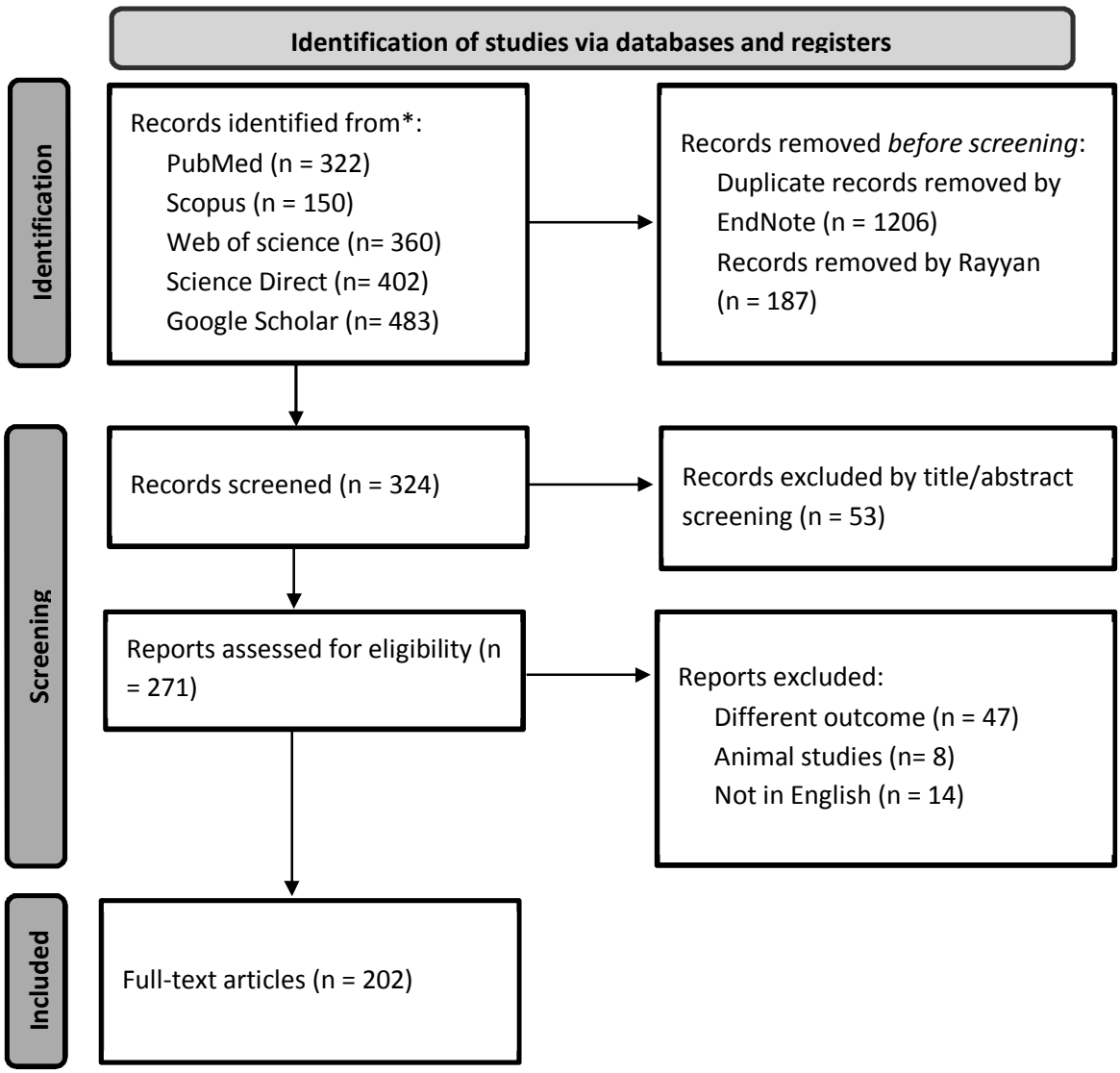


Figure 1: *Schematic Demonstration of the literature review process.*

SONUÇLAR

For the epidemiological profile of SCD in Arab countries, the prevalence of the HbS gene has increased, over the course of generations, in regions with a history of malaria or current malaria endemicity **(1)**. This is because individuals carrying the HbS gene have a selective advantage against falciparum malaria. However, the movement of populations has played a significant role in spreading the HbS gene to regions that do not typically experience malaria outbreaks. SCD is most commonly observed in people from Africa, India, the Caribbean, the Middle East, and the Mediterranean **(1)**. SCD is a condition in which red blood cells tend to take on a sickle shape when deprived of oxygen, primarily due to the presence of HbS **(5)**. This can occur when an individual has two copies of the HbS gene, known as sickle cell anemia-SS, or when they have one copy of the HbS gene and one of the beta-thalassemia gene.

Globally, the βS gene is associated with five major β-globin cluster haplotypes, suggesting a multi-origin pattern **(22-29)**. These haplotypes are named after the regions where they are believed to have originated: Benin (Central West Africa), Senegal (West Africa), the Bantu (Central, East, and Southern Africa), the Cameroon, and the Arab-Indian haplotypes (Arabian Peninsula and India). These haplotypes play a crucial role in determining the severity of the disease and the levels of HbF (fetal hemoglobin) in individuals with sickle cell anemia (SCA). The Arab-Indian and Senegal haplotypes are associated with higher HbF levels and a milder clinical presentation in individuals with two copies of the HbS gene **(5)**. In contrast, the other three haplotypes are linked to lower HbF levels and more

severe clinical symptoms, with the Bantu haplotype being the most severe **(1)**.

Among Arabs, three of the five major haplotypes are most prevalent in association with the β^S mutation: the Benin, Arab-Indian, and Bantu haplotypes **(22-29)**. The other two haplotypes are less common. As expected, this leads to the presence of two major clinical phenotypes among Arabs, with one being milder and associated with the Arab-Indian haplotype, and the other being more severe and linked to the Benin and Bantu haplotypes **(5)**. Table 1 illustrates the distribution of these three major haplotypes in various Arab countries **(1)**. It is evident that in the Eastern Arabian Peninsula, the Arab-Indian haplotype is the most common, while in other Arab countries in the Eastern Mediterranean and North Africa, the Benin haplotype predominates.

This distribution can be explained by the presumed origins of the sickle cell mutation. The Arab-Indian mutation is thought to have originated in the Indus Valley on the Indian subcontinent and subsequently spread to Iran and the Eastern Arabian Peninsula through trade routes and historical interactions **(22-29)**. On the other hand, the Benin haplotype is believed to have originated in Central West Africa and spread vertically through population migration via trans-Saharan routes to North Africa and then across the Mediterranean Sea to Southern Europe and the Eastern Mediterranean region **(22-29)**. Another possible explanation is a direct spread from Africa across the Red Sea to the western part of the Arabian Peninsula and from there to other Arab countries, possibly through slave trade or immigration **(1)**. However, it's important to note that the possibility of any of these haplotypes having independently originated among Arabs cannot be ruled out.

From the studies reviewed, HbSS genotype was the most commonly reported in the Arab population followed by HbSβ0 and HbSβ+. Khamees et al. reported that the HbSE genotype was found in 16 patients in their study which approximately resembles one-quarter of the all HbSE reported cases worldwide **(30)**. In comparison to the US population, the Arab population was found to have higher percentages of HbSS and HbSβthal and lower frequencies of HbSC **(31)**. The HbSO-Arab and HBS Oman are two different genotypic variations that are primarily found in Arab ethnicities. Therefore, genetic therapy may need tailored approaches for these specific genotypic variations when aiming for a curative treatment **(24, 32-35)**. Furthermore, Saudi Arabia accounted for the largest number of SCD cases in the region. El-Hazmi et al. found that this could be mainly due to the high incidence of consanguineous marriages, which constitute as much as 57.7% of all marriages in the country **(24)**.

Table 1: *Distribution of the haplotypes associated with sickle cell gene in Arab countries.(1)*

Countries	*Number of chromosomes*	*Benin (%)*	*Arab-Indian (%)*	*Bantu (%)*	*Other haplotypes (%)*
Algeria	20	100.0	-	-	-
Bahrain	37	2.7	89.2	5.4	2.7
Egypt	28	100.0	-	-	-
Iraq (Northern)	128	69.5	12.5	7.8	10.2
Jordan	20	80.0	20.0	-	-
Kuwait	125	11.2	80.8	5.7	2.4
Lebanon	100	73.0	10.0	15	2.0
Oman	117	52.1	26.7	21.4	-
Palestinian West Bank	118	88.1	-	5.1	6.8
Saudi Arabia (southwestern)	124	98.5	1.5	-	-
Saudi Arabia (eastern)	50	-	94.0	4.0	2.0
Syria	18	66.7	33.3	-	-
Tunisia	66	94.0	-	-	6.0
UAE	94	22.0	52.0	26.0	-

As for the epidemiological profile of β- and α-thalassemias in Arab countries, the reported carrier rates range from 1% to 11% for β-thalassaemia and 1% to 58% for α-thalassaemia **(36)**. Individuals who are heterozygous carriers of β-thalassemia typically do not exhibit any symptoms. However, when someone is homozygous or has compound heterozygosity for β-thalassemia mutations, it typically results in the development of transfusion-dependent β-thalassemia major. This condition poses a significant health challenge in nearly all Arab nations. The highest reports of α-thalassemias were from Gulf countries **(36, 37)**. The majority of reported HBB gene mutations consisted of splice site mutations (27%) and frameshift mutations (26%), as indicated in Figure 2 **(38)**. Other mutation types included promoter mutations (17%), missense mutations (9%), nonsense mutations (6%), deletion mutations (4%), and the remaining (11%) mutations were categorized as 5′-UTR, 3′-UTR, fusion, or a combination of frameshift and nonsense mutations (as depicted in Figure 2) **(38)**. The majority of these mutations were documented in Saudi Arabia (49), followed by Syria (44), Algeria, and the United Arab Emirates (27 each), Palestinian territories (25), Egypt and Iraq (24 each), Tunisia (22), Morocco and Oman (20 each), Jordan and Lebanon (19 each), Kuwait (17), Bahrain (14), Qatar (13), Libya and Sudan (5 each), Comoros (3), Yemen (2), and Mauritania (1) (as shown in Figure 3) **(38)**.

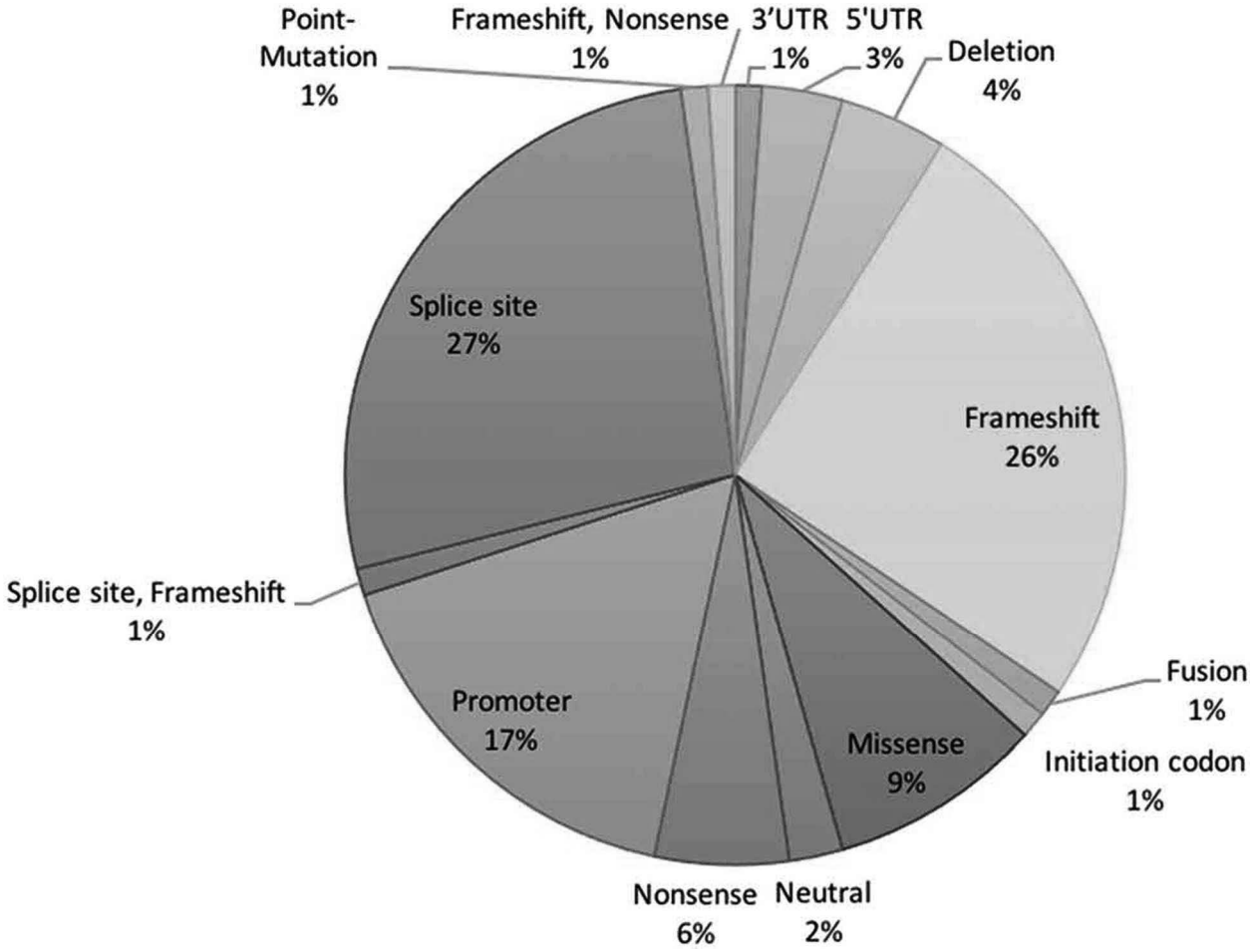


Figure 2: *Different types of β-thal associated HBB gene mutations in the Arab population*

The genetic abnormalities responsible for β-thalassemia primarily involve point mutations, with more than 200 such mutations documented worldwide **(1)**. The prevalence of these mutations in each population varies depending on their geographical origin, interactions with other populations, and the methods used for characterization. Among Arab populations, the diversity of these mutations ranges from 44 distinct mutations in the United Arab Emirates (UAE) to 10 in Eastern Saudi Arabia **(39)**. The most widespread and common mutation among Arabs is IVS-I-110 (G>A) **(40)**. This mutation is most prevalent in Cyprus and Greece, suggesting a possible Greek origin **(41)**. Among Arabs, it is most

frequently observed in Lebanon, Egypt, Syria, Jordan, and certain parts of Saudi Arabia. Its occurrence decreases as we move eastward, where it becomes less common in the Eastern Arabian Peninsula and is replaced by Asian Indian mutations such as IVS-I-5 (G>C), codons 8/9 (+G), and IVS-I (−25 bp del), which are most prevalent in Oman, UAE, and Bahrain **(42)**. IVS-I-110 is still relatively common in Western Arab countries, specifically Tunisia and Algeria, but becomes less frequent as we head west to Morocco **(43)**. In these North African countries, codon 39 (C>T) becomes the most prevalent mutation. Codon 39 is also highly prevalent in the isolated island of Sardinia and mainland Italy, and it is a frequent mutation in the Arabian Peninsula as well **(44)**. Other Mediterranean mutations, including IVS-I-1 (G>A) and IVS-I-6 (T>C), have been reported with varying frequencies in most Arab nations, with the highest rates in the Palestinian territories, Syria, and Egypt **(45)**.

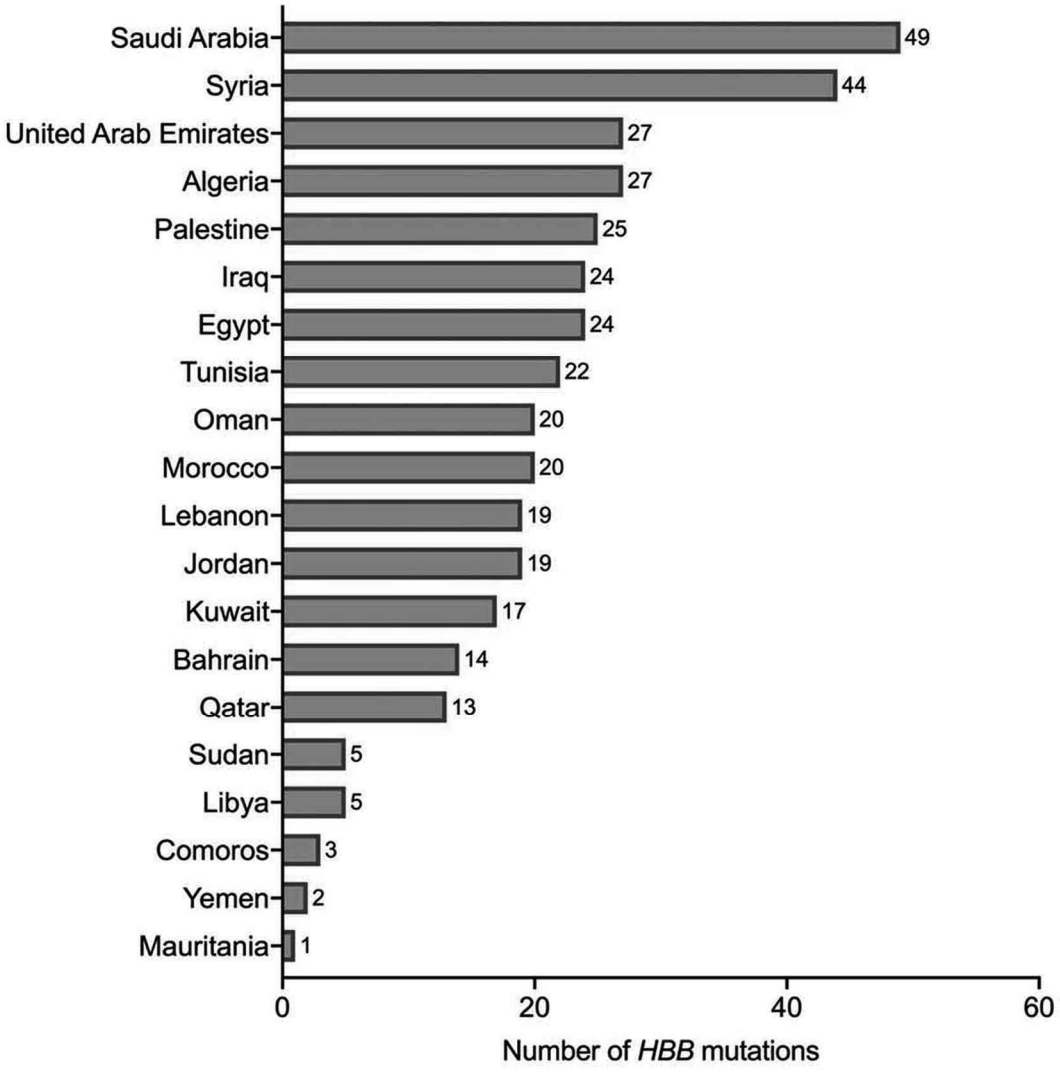


Figure 3: *Numerical distribution of HBB gene mutation among different Arab countries*

On the other hand, the East Mediterranean mutation IVS-II-I (G>A) is more common in the eastern parts of the Arab world, particularly adjacent to Iran, where it reaches its highest worldwide rates (45). Similarly, Kurdish mutations like codons 44 (−C) and 36/37 (−T), observed in the northern Iraqi Kurdish population, are also frequent in nearby Arab countries such as Saudi Arabia, Qatar, and Bahrain. Some mutations, like codon 6 (−A), are believed to have North African origins, with the highest frequencies found in Algeria and Morocco **(46)**. Other mutations, possibly originating in Arab countries, include IVS-II-745 (C>G), which has its highest global frequency in Jordan **(47)**, and IVS-I-2 (T>G), almost exclusive to Tunisia, with rates reaching about 20% **(48)**. Codon 29 (C>T) is ob-

served at rates of approximately 10% in Lebanon, where it may have originated **(49)**. Codon 37 (G>A), on the other hand, may have a Palestinian origin, with rates exceeding 11% **(50)**, and IVS-II-848 (C>A) may have an Egyptian origin, with a reported rate of 11% **(51, 52)**.

In contrast to β-thalassemias, α-thalassemias are primarily caused by deletions involving one or both α-genes, resulting in α+ or α0 defects, respectively. The most commonly reported mutation in the Arab populations, to date, is the ($-\alpha^{3.7}$) deletion, also known as the rightwards deletion **(1, 53)**. Other common mutations include the α2 IVS1 5-bp deletion and the non-deletional α2 polyadenylation signal mutation (AATAAA>AATAAG) **(54)**. The latter mutation which is also called the Saudi polyadenylation signal mutation, because it was first identified among Saudi Arabs, leads to a more severe α+ defect than a single gene deletion. Other α+ mutations, such as ($-\alpha^{4.2}$) (leftwards deletion) and Hb Constant Spring (a mutation involving a termination codon), have been reported, among some Arab countries as well. On the other hand, α0 defects are either rare or only sporadically observed in most Arab populations. Consequently, Hb Bart's hydrops fetalis (resulting from homozygosity to an α0 defect) is almost non-existent, and HbH (caused by compound heterozygosity involving α0 and α+) is not common **(55, 56)**. Therefore, in many Arab countries, particularly in the Arabian Peninsula, the majority of HbH disease cases are actually a result of homozygosity to the common polyadenylation signal mutation ($\alpha^{polyA1}\alpha$/ $\alpha^{polyA1}\alpha$) **(57)**.

DISCUSSION

Consanguinity and haemoglobinopathies

The incidence and prevalence rates of autosomal recessive genetic disorders like haemoglobinopathies may be affected by the demographic and cultural traits of the population under investigation **(5)**. In many Arab nations, specific characteristics are prevalent which affect the genetic diversity significantly, such as early marriage, large family sizes, and older maternal and paternal ages. Consanguineous marriages are customary in most, if not all, Arab communities, constituting 20% to 50% of all marital unions **(58)**. First-cousin marriages, particularly those on the paternal side, are especially common and make up nearly one-quarter of all marriages in various Arab countries. Furthermore, Arab populations demonstrate significant genetic diversity due to their historical migrations and interactions with various populations spanning from East and South Asia to Europe and Africa **(59)**. This genetic intermingling has contributed to the current genetic profile of haemoglobinopathies, which are recognized as common genetic disorders among Arabs.

Different genetic mutations in Arab countries

Haemoglobinopathies in the Arab region have been associated with few significant unique genetic mutations. This was mainly prominent in the Arab reported cases of β-thalassemia **(60)**. The spectrum of β-thalassemia mutations is diverse and often overlaps with those found in other ethnic groups. However, six HBB gene mutations that appear to be specific to Arabs have been discovered in this review. One of these unique mutations is the c.420dupT mutation, found in a young Kuwaiti patient with severe transfusion-dependent β-thalassemia major and chromaturia (reddish/brown discoloration of the urine) **(61)**. This frameshift mutation in exon 3 was caused by a thymidine insertion in codons 139/140. The patient exhibited clinical signs of early-onset β-thalassemia major with ineffective erythropoiesis, necessitating regular blood transfusions. Hematopoietic stem cell transplantation proved to be a successful treatment for this severe form of the disease. Another frameshift mutation was identified in an ethnic Qatari patient with transfusion-dependent β-thalassemia **(62)**. This mutation resulted from a 192 bp deletion within the β-globin gene, spanning exon 1, intron 1, and the first two bases of exon 2, leading to a premature stop codon. Deletions of this kind are quite rare in β-thalassemia, as they account for less than 10% of the reported mutations **(63)**. This particular mutation occurred in compound heterozygosity with the c.92 + 5 G > T mutation, resulting in a β-thalassemia major phenotype.

The third frameshift mutation, c.150delC in exon 2, was found in a patient of Jordanian origin **(47)**. This mutation generated a premature stop codon, leading to a severe form of β-thalassemia major that required frequent blood transfusions. In Sudanese adult males, a deletion of 9.6 kb extending from

exon 1 of the δ-globin gene to the IV-II sequence II of the HBB gene was identified **(64)**. This deletion explained microcytosis and hypochromia in the patient but not the elevated Hb F levels. This mutation is often seen in carriers of δ β-thalassemia or hereditary persistence of fetal hemoglobin (HPFH). In addition to frameshift mutations, a point mutation at the promoter of the HBB gene (c.-240 G > A) was detected in a Moroccan female **(65)**. This mutation resulted in a β-thalassemia intermedia phenotype when in compound heterozygosity with a β0 mutation or a silent phenotype as a heterozygous carrier. The patient initially required transfusions and underwent splenectomy due to an enlarged spleen at the age of 10. However, afterward, she remained asymptomatic without the need for further transfusions. Lastly, the c.92 + 2 T > G mutation, originating from Tunisia and later identified in Morocco, is a splice-site mutation resulting in a severe β0 allele in a homozygous form **(66, 67)**. However, no specific clinical phenotype data were mentioned in the studies that reported this mutation.

Treatment options for haemoglobinopathies in Arab countries

Understanding the epidemiology of the different haemoglobinopathies is crucial for the continuous search process for the best curative treatment and management of the patients. For example, newer therapies are being explored and approved for the management of SCD, with gene therapy showing significant potential **(68)**. Despite various challenges, the rapid advancements and extensive research efforts in gene therapy are expected to benefit not only SCD but also other hemoglobin disorders in the near future **(68)**. Among the recently approved treatments, Voxelotor, an HbS polymerization inhibitor, has received FDA approval for SCD patients aged 12 and older **(69)**. Clinical trials have demonstrated that patients taking Voxelotor experienced a rapid increase in hemoglobin concentration and a potential decrease in morbidity associated with hemolytic anemia in SCD, all without significant side effects compared to a placebo.

Furthermore, L-glutamine has also gained FDA approval for the treatment of SCD. It has been shown to reduce the frequency of pain crises in SCD patients aged 5 and older over a 48-week period **(70, 71)**. The current use of oral anticoagulants in SCD has showed clinical importance in treating serious SCD complications **(72)**. Additionally, the FDA has recently approved Crizanlizumab, a monoclonal antibody targeting P-selectin on platelets and endothelial cells **(73)**. This treatment has shown a significant reduction in the frequency of vaso-occlusive crises in SCD patients aged 16 and older. However, there is a lack of reported cases of patients from Arab countries receiving these newer drugs. Multiple clinical trials are currently underway to assess the efficacy of these treatments in SCD patients in the Arab world, including countries like Egypt, Oman, Lebanon, Saudi Arabia, and Jordan **(5)**. As more data becomes available from these trials, there is hope for a better understanding of how these therapies can benefit different patient populations in the Arab region.

CONCLUSION

Haemoglobinopathies are very common disorders in the Arabic region with insufficient information regarding their epidemiology, associated genetic mutation and guided treatment options. SCD is a prevalent hemoglobinopathy within the Arab population, with genotypic characteristics distinct from other regions and several approved treatments yet to be reported. Our review presents insights into the genetic composition of SCD in Arab countries and its common phenotypic features, aiming to guide further research in the era of genetic therapy. Similarly, while beta-thalassemia (β-thal) is widespread in the Arab world, comprehensive data on prevalent and unique mutations among Arab β-thal patients remains lacking. Such information is vital for enhancing healthcare for individuals with β-thal, encompassing genetic counseling and screening, as well as accurately estimating the frequency of β-thal carriers in the Arab region.

REFERENCES

1. Hamamy, H.A. and N.A. Al-Allawi, *Epidemiological profile of common haemoglobinopathies in Arab countries.* J Community Genet, 2013. **4**(2): p. 147-67.
2. Kato, G.J., et al., *Sickle cell disease.* Nat Rev Dis Primers, 2018. **4**: p. 18010.
3. Lonergan, G.J., D.B. Cline, and S.L. Abbondanzo, *Sickle cell anemia.* Radiographics, 2001. **21**(4): p. 971-94.
4. Mangla, A., et al., *Sickle Cell Anemia*, in *StatPearls.* 2023: Treasure Island (FL).

5. Ata, F., et al., *Genotypic and Phenotypic Composition of Sickle Cell Disease in the Arab Population - A Systematic Review.* Pharmgenomics Pers Med, 2023. **16**: p. 133-144.
6. David, A.N., et al., *Prevalence and impact of sickle cell trait on the clinical and laboratory parameters of HIV infected children in Lagos, Nigeria.* Pan Afr Med J, 2018. **31**: p. 113.
7. Piel, F.B., et al., *Global burden of sickle cell anaemia in children under five, 2010-2050: modelling based on demographics, excess mortality, and interventions.* PLoS Med, 2013. **10**(7): p. e1001484.
8. Wali, Y., V. Kini, and M.A. Yassin, *Distribution of sickle cell disease and assessment of risk factors based on transcranial Doppler values in the Gulf region.* Hematology, 2020. **25**(1): p. 55-62.
9. Ballas, S.K., *Sickle cell disease: clinical management.* Baillieres Clin Haematol, 1998. **11**(1): p. 185-214.
10. Therrell, B.L., Jr., et al., *Newborn screening for sickle cell diseases in the United States: A review of data spanning 2 decades.* Semin Perinatol, 2015. **39**(3): p. 238-51.
11. Tisdale, J.F., S.L. Thein, and W.A. Eaton, *Treating sickle cell anemia.* Science, 2020. **367**(6483): p. 1198-1199.
12. Soliman, A.T., et al., *Does blood transfusion affect pituitary gonadal axis and sperm parameters in young males with sickle cell disease?* Indian J Endocrinol Metab, 2013. **17**(6): p. 962-8.
13. Fibach, E. and E.A. Rachmilewitz, *Pathophysiology and treatment of patients with beta-thalassemia - an update.* F1000Res, 2017. **6**: p. 2156.
14. Galanello, R. and R. Origa, *Beta-thalassemia.* Orphanet J Rare Dis, 2010. **5**: p. 11.
15. Origa, R., *beta-Thalassemia.* Genet Med, 2017. **19**(6): p. 609-619.
16. Thein, S.L., *The molecular basis of beta-thalassemia.* Cold Spring Harb Perspect Med, 2013. **3**(5): p. a011700.
17. Modell, B. and M. Darlison, *Global epidemiology of haemoglobin disorders and derived service indicators.* Bull World Health Organ, 2008. **86**(6): p. 480-7.
18. De Sanctis, V., et al., *beta-Thalassemia Distribution in the Old World: an Ancient Disease Seen from a Historical Standpoint.* Mediterr J Hematol Infect Dis, 2017. **9**(1): p. e2017018.
19. Chen, W., et al., *The molecular basis of beta-thalassemia intermedia in southern China: genotypic heterogeneity and phenotypic diversity.* BMC Med Genet, 2010. **11**: p. 31.
20. Higgs, D.R., J.D. Engel, and G. Stamatoyannopoulos, *Thalassaemia.* Lancet, 2012. **379**(9813): p. 373-83.
21. Clark, J.M., et al., *Improving the translation of search strategies using the Polyglot Search Translator: a randomized controlled trial.* J Med Libr Assoc, 2020. **108**(2): p. 195-207.
22. Adekile, A.D., et al., *Molecular characterization of alpha-thalassemia determinants, beta-thalassemia alleles, and beta S haplotypes among Kuwaiti Arabs.* Acta Haematol, 1994. **92**(4): p. 176-81.
23. Di Rienzo, A., et al., *Molecular basis for HbH disease in Italy: geographical distribution of deletional and nondeletional alpha-thalassemia haplotypes.* Am J Hum Genet, 1986. **39**(5): p. 631-9.
24. el-Hazmi, M.A., et al., *Haplotypes of the beta-globin gene as prognostic factors in sickle-cell disease.* East Mediterr Health J, 1999. **5**(6): p. 1154-8.
25. Inati, A., et al., *Beta-globin gene cluster haplotypes and HbF levels are not the only modulators of sickle cell disease in Lebanon.* Eur J Haematol, 2003. **70**(2): p. 79-83.
26. Kulozik, A.E., et al., *Geographical survey of beta S-globin gene haplotypes: evidence for an independent Asian origin of the sickle-cell mutation.* Am J Hum Genet, 1986. **39**(2): p. 239-44.
27. Perrin, P., et al., *Diversity of sequence haplotypes associated with beta-thalassaemia mutations in Algeria: implications for their origin.* Gene, 1998. **213**(1-2): p. 169-77.
28. Rahimi, Z., et al., *Beta-globin gene cluster haplotypes in sickle cell patients from southwest Iran.* Am J Hematol, 2003. **74**(3): p. 156-60.
29. Samarah, F., et al., *beta(S)-Globin gene cluster haplotypes in the West Bank of Palestine.* Hemoglobin, 2009. **33**(2): p. 143-9.
30. Khamees, I., et al., *Manifestations of HbSE sickle cell disease: a systematic review.* J Transl Med, 2021. **19**(1): p. 262.
31. Hassell, K.L., *Population estimates of sickle cell disease in the U.S.* Am J Prev Med, 2010. **38**(4 Suppl): p. S512-21.
32. Wali, Y.A., et al., *Splenic function in Omani children with sickle cell disease: correlation with severity index, hemoglobin phenotype, iron status, and alpha-thalassemia trait.* Pediatr Hematol Oncol, 2002. **19**(7): p. 491-500.
33. Adekile, A.D., et al., *The Sub-Phenotypes of Sickle Cell Disease in Kuwait.* Hemoglobin, 2019. **43**(2): p. 83-87.
34. Al Balushi, H.W.M., et al., *The super sickling haemoglobin HbS-Oman: a study of red cell sickling, K(+) permeability and associations with disease severity in patients heterozygous for HbA and HbS-Oman (HbA/S-Oman genotype).* Br J Haematol, 2017. **179**(2): p. 256-265.
35. Al Jahdhamy, R., et al., *A case of compound heterozygosity for Hb S and Hb S Oman.* Br J Haematol, 2002. **116**(3): p. 504.

36. Al-Gazali, L., H. Hamamy, and S. Al-Arrayad, *Genetic disorders in the Arab world.* BMJ, 2006. **333**(7573): p. 831-4.
37. Hamamy, H. and A. Alwan, *Hereditary disorders in the Eastern Mediterranean Region.* Bull World Health Organ, 1994. **72**(1): p. 145-54.
38. Khan, A.M., et al., *The spectrum of beta-thalassemia mutations in the 22 Arab countries: a systematic review.* Expert Rev Hematol, 2021. **14**(1): p. 109-122.
39. Al-Ali, A.K., et al., *Molecular bases of beta-thalassemia in the Eastern Province of Saudi Arabia.* J Biomed Biotechnol, 2005. **2005**(4): p. 322-5.
40. Baysal, E., *Hemoglobinopathies in the United Arab Emirates.* Hemoglobin, 2001. **25**(2): p. 247-53.
41. Baysal, E., et al., *The beta-thalassaemia mutations in the population of Cyprus.* Br J Haematol, 1992. **81**(4): p. 607-9.
42. Baysal, E., *Molecular basis of beta-thalassemia in the United Arab Emirates.* Hemoglobin, 2011. **35**(5-6): p. 581-8.
43. Agouti, I., et al., *Genotypic correlation between six common beta-thalassemia mutations and the XmnI polymorphism in the Moroccan population.* Hemoglobin, 2007. **31**(2): p. 141-9.
44. Rosatelli, M.C., et al., *Molecular screening and fetal diagnosis of beta-thalassemia in the Italian population.* Hum Genet, 1992. **89**(6): p. 585-9.
45. Najmabadi, H., et al., *The beta-thalassemia mutation spectrum in the Iranian population.* Hemoglobin, 2001. **25**(3): p. 285-96.
46. Boudrahem-Addour, N., et al., *Molecular heterogeneity of beta-thalassemia in Algeria: how to face up to a major health problem.* Hemoglobin, 2009. **33**(1): p. 24-36.
47. Sadiq, M.F., A. Eigel, and J. Horst, *Spectrum of beta-thalassemia in Jordan: identification of two novel mutations.* Am J Hematol, 2001. **68**(1): p. 16-22.
48. Laradi, S., et al., *[Molecular analysis and prenatal diagnosis of beta-thalassemia: about our experience in central Tunisia].* Ann Biol Clin (Paris), 2000. **58**(4): p. 453-60.
49. Makhoul, N.J., et al., *Genetic heterogeneity of Beta thalassemia in Lebanon reflects historic and recent population migration.* Ann Hum Genet, 2005. **69**(Pt 1): p. 55-66.
50. El-Latif, M.A., et al., *The beta+-IVS-I-6 (T-->C) mutation accounts for half of the thalassemia chromosomes in the Palestinian populations of the mountain regions.* Hemoglobin, 2002. **26**(1): p. 33-40.
51. Hussein, G., et al., *Rapid detection of beta-Thalassemia alleles in Egypt using naturally or amplified created restriction sites and direct sequencing: a step in disease control.* Hemoglobin, 2007. **31**(1): p. 49-62.
52. Hussein, I.R., et al., *Molecular characterization of beta-thalassemia in Egyptians.* Hum Mutat, 1993. **2**(1): p. 48-52.
53. Harteveld, C.L. and D.R. Higgs, *Alpha-thalassaemia.* Orphanet J Rare Dis, 2010. **5**: p. 13.
54. Hadavi, V., et al., *Elucidating the spectrum of alpha-thalassemia mutations in Iran.* Haematologica, 2007. **92**(7): p. 992-3.
55. Al-Allawi, N.A., et al., *Molecular characterization of alpha-thalassemia in the Dohuk region of Iraq.* Hemoglobin, 2009. **33**(1): p. 37-44.
56. Al-Allawi, N.A., M.Y. Shamdeen, and N.S. Rasheed, *Homozygosity for the Mediterranean a-thalassemic deletion (hemoglobin Barts hydrops fetalis).* Ann Saudi Med, 2010. **30**(2): p. 153-5.
57. Hellani, A., et al., *Molecular spectrum of alpha-thalassemia mutations in microcytic hypochromic anemia patients from Saudi Arabia.* Genet Test Mol Biomarkers, 2009. **13**(2): p. 219-21.
58. Hamamy, H. and A.H. Bittles, *Genetic clinics in arab communities: meeting individual, family and community needs.* Public Health Genomics, 2009. **12**(1): p. 30-40.
59. Teebi, A.S. and S.A. Teebi, *Genetic diversity among the Arabs.* Community Genet, 2005. **8**(1): p. 21-6.
60. Tadmouri, G.O., et al., *Consanguinity and reproductive health among Arabs.* Reprod Health, 2009. **6**: p. 17.
61. Croteau, S.E., et al., *Novel dominant beta-thalassemia: Hb Boston-Kuwait [codon 139/140(+T)].* Pediatr Blood Cancer, 2013. **60**(10): p. E131-4.
62. Al-Obaidli, A., et al., *A novel deletional beta-thalassemic variant in an ethnic Qatari patient.* Hemoglobin, 2009. **33**(3): p. 214-9.
63. Patrinos, G.P., et al., *Improvements in the HbVar database of human hemoglobin variants and thalassemia mutations for population and sequence variation studies.* Nucleic Acids Res, 2004. **32**(Database issue): p. D537-41.
64. Waye, J.S., et al., *Sudanese (deltabeta)0-Thalassemia: Identification and Characterization of a Novel 9.6 kb Deletion.* Hemoglobin, 2015. **39**(5): p. 368-70.
65. Agouti, I., et al., *Beta-thalassemia intermedia due to two novel mutations in the promoter region of the beta-globin gene.* Eur J Haematol, 2008. **80**(4): p. 346-50.
66. Chibani, J., et al., *The peculiar spectrum of beta-thalassemia genes in Tunisia.* Hum Genet, 1988. **78**(2): p. 190-2.
67. Lemsaddek, W., et al., *The beta-thalassemia mutation/haplotype distribution in the moroccan population.* Hemoglobin, 2004. **28**(1): p. 25-37.

68. Demirci, S., N. Uchida, and J.F. Tisdale, *Gene therapy for sickle cell disease: An update.* Cytotherapy, 2018. **20**(7): p. 899-910.
69. Howard, J., et al., *Voxelotor in adolescents and adults with sickle cell disease (HOPE): long-term follow-up results of an international, randomised, double-blind, placebo-controlled, phase 3 trial.* Lancet Haematol, 2021. **8**(5): p. e323-e333.
70. Elenga, N., et al., *Real-World data on efficacy of L-glutamine in preventing sickle cell disease-related complications in pediatric and adult patients.* Front Med (Lausanne), 2022. **9**: p. 931925.
71. Niihara, Y., et al., *A Phase 3 Trial of l-Glutamine in Sickle Cell Disease.* N Engl J Med, 2018. **379**(3): p. 226-235.
72. Rozi, W., et al., *Direct oral anticoagulants in sickle cell disease: a systematic review and meta-analysis.* Blood Adv, 2022. **6**(17): p. 5061-5066.
73. Han, J., S.L. Saraf, and V.R. Gordeuk, *Systematic Review of Crizanlizumab: A New Parenteral Option to Reduce Vaso-occlusive Pain Crises in Patients with Sickle Cell Disease.* Pharmacotherapy, 2020. **40**(6): p. 535-543.

Correspondence Address:
Ahmed Badr1*, Mohamed Yassin2*
1 College of Medicine, QU Health, Qatar University, Doha 2713, Qatar.
2 Hematology Section, Medical Oncology, National Center for Cancer Care and Research, Hamad Medical Corporation, Doha 3050, Qatar.
* Correspondence: A.B., ab1906092@qu.edu.qa; M.Y., E-mail: yassinmoha@gmail.com

CHAPTER 2

GENETICS OF THALASSEMIA AND HEMOGLOBINOPATHIES

THE GENETICS OF ALPHA THALASSEMIA

Prof. Dr. Erol Baysal, FRCP
Fellow of the Royal College of Physicians, London, UK
Prof. of Genomics
Consultant Molecular Geneticist
Former Head, Molecular Genetics Dept.,
Dubai Genetic and Thalassemia Center
Former Medical Director, UAE Genetic Diseases Association,
Dubai, UAE

ABSTRACT

α-thalassemia is perhaps the most common monogenic gene disorder worldwide. It is frequent in the Mediterranean, South-East Asia, Africa, the Middle East, and India. The incidence of α-thalassemia in Europe and Northern America has increased appreciably in the last several decades due to migration. It is an inherited autosomal recessive disorder characterized by microcytic hypochromic anemia. Its clinical phenotype varies from almost asymptomatic to fatal hemolytic anemia. In general, α-thalassemia results from the deletion of one or two α-genes from the same chromosome and is classified according to its genotype/phenotype correlation. When the normal complement of four functional α-globin genes is decreased by one or more copies (1-4), the result is a spectrum of clinical phenotypes with increasing severity. All affected individuals have a variable degree of anemia (low Hb), lower MCH and MCV, and normal/low HbA2. Carriers of α^{+}- or α^{0}-thal generally do not need treatment. HbH disease, caused by 3 gene deletions is clinically significant and is characterized by moderate/severe anemia with variable amounts of HbH (1-40%). The type of mutation determines the clinical severity. HbH patients may require occasional blood transfusions. Hb Bart's Hydrops Fetalis syndrome is caused by four α-gene deletions. This is a fatal condition characterized by no functional α-genes with zero α-chain output. In Hb Bart's Hydrops Fetalis, the diagnostic criterion is a significant level of Hb Bart's but no HbF. Genetic counseling must be considered for couples at risk for HbH disease and Hb Bart's Hydrops Fetalis Syndrome. Owing to increased risk to mother and fetus, most pregnancies with potential Hb Bart's Hydrops Fetalis syndrome are terminated.

Keywords: α-thalassemia, genotype/phenotype, deletional, point mutation, HbBart's, Hydrops Fetalis

INTRODUCTION

Thalassemia (thal) can be summarized as insufficient production of hemoglobin polypeptide chains (α-, β-, γ-, δ-) as a result of one or more genetic defects in the globin genes. Thalassemia is an inherited blood disorder that clinically causes mild, moderate, or severe hypochromic (low MCH), microcytosis (low MCV) anemia. Since iron deficiency is the most common cause of hypochromic, microcytotic anemia in children and adults, it should be differentiated from thalassemia. Making this differential diagnosis correctly is important for correct diagnosis and effective treatment.

α-THALASSEMIA

α-Thalassemia (α-Thal) is the most common inherited single-gene disorder in the world, Fig.1 (Weatherall, 2018; Higgs, 1998; Harteveld and Higgs, 2010; Baysal, 2011). α-thal results from underproduction or no production of α-globin chains. A normal human has four α-globin genes and two β-globin genes. These conditions are caused by insufficient production of α-globin chains. They are found in almost every country in the world. The phenotype of α-thal varies, ranging from silent carriers without clinical findings to severe anemia resulting in intra-uterine

death during pregnancy or birth. According to the WHO figures, more than 20% of the world's population is a carrier of some form of α-thal. It is estimated that worldwide approximately 15,000 babies are born every year with the most severe forms of α-thal, namely Hb H disease and Hb Bart's Hydrops Fetalis syndrome.

HEMATOLOGY

5-10% Hb Bart's can be seen in newborn babies, but this disappears within 6 months. Severe α-thal carriers are clinically normal and show similar hematological findings as seen in β-thal carriers; mild hypochromic microcytic anemia; MCV<78fL, MCH<27pg. The CBC depends roughly on the number of α genes present and correlates well with the decrease in α-chain synthesis predicted for each genotype. Persons with homozygous α^{+}-thal have clinical findings like those of α^{0}-thal carriers. These will be discussed in detail in the following sections.

HPLC, combined with CBC, helps to identify α-thal carriers and also quantify abnormal hemoglobin fractions which may be important in HbH cases. Hb Bart's, in newborn carriers, can determine α-thal or any Hb variant associated with an α-thal phenotype. Though Hb Bart's is a very useful diagnostic marker in neonates with α-thal, however, it does not detect all mild α-3.7/αα cases nor does it fully distinguish between the various α thal genotypes (Weatherall, 2018). A reduction in HbA2 levels is sometimes used as a diagnostic marker. HbA2 is indispensable in distinguishing between α and β thal trait but it is not a very useful guide to determine α-thal. Lower HbA2 level is only pertinent in HbH disease patients. The staining of the peripheral RBC with 1% Brilliant Cresyl Blue remains a sensitive method to visualize HbH inclusion bodies in the red cells. The typical golf-ball-like inclusion-body cells are a clear indication of HbH disease **(Figure 8, 9)**.

EPIDEMIOLOGY

Regions with a high incidence of α-thal include the Far East, the Middle East, South East Asia, India, the Mediterranean basin, and Africa. It has been widely reported that α-thal density can reach up to 80-90% in some parts of South East Asia and India (Fodde et al. 1988; Flint et al. 1986). In the Middle East, especially in the Gulf countries, the carrier rate has been reported as 50% in the UAE and 40% in some parts of Saudi Arabia (Fig.2). Thus, α-thal is among the biggest health problems throughout Asia with a significantly high incidence. The number of α-thal cases is also increasing rapidly in Europe, the USA, and Australia due to human migration in recent years. It is well known that hemoglobinopathies are common in places where malaria was endemic in the past, thus exacerbating the protective nature of malaria. HbH disease is predominantly seen in South East Asia (Fischel-Ghodsian, et al, 1988), the Middle East (Baysal, 2011), and the Mediterranean countries (Baysal et al, 1995). Similarly, Hb Bart's Hydrops Fetalis syndrome is mainly seen in South East Asia, especially China (Liao et al, 2007).

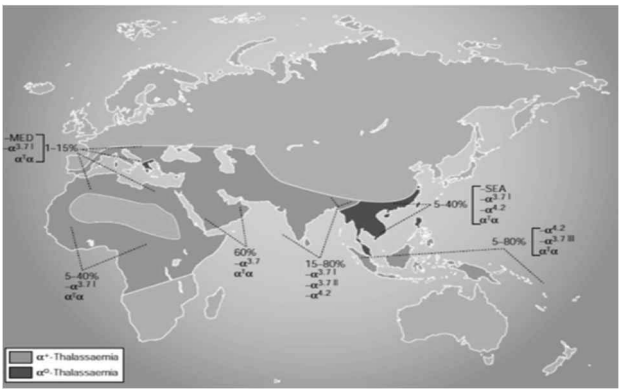


Figure 1: *Distribution of α-thal in the world*

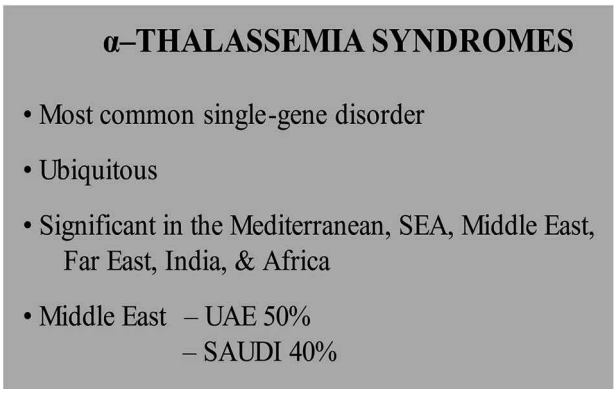


Figure 2: *Distribution of α-thal in the Gulf Region*

The abundance of α-globin gene mutations and their interactions lead to a variety of multiple hematological and clinical phenotypes. The carriers of one or 2 gene deletions have no clinical consequences. HbH patients are clinically well and usually show symptoms of mild to moderate hemolytic anemia. Some of the HbH patients have infrequent transfusions and some may have regular transfusions. Hb

Bart's Hydrops Fetalis is a fatal congenital syndrome that requires intrauterine (in the womb) transfusion. It is seen in the Far East, mainly China. Couples should always be given genetic counseling. The babies generally require regular postnatal transfusion, or bone marrow transplantation after birth.

α-THALASSEMIA IN THE MEDITERRANEAN COUNTRIES AND THE MIDDLE EAST

It is well-documented that malaria is endemic in places with a high incidence of α-thalassemia. HbH disease is also frequently encountered in these regions. In a comprehensive HbH disease study conducted in the Eastern Mediterranean, a significant incidence of α-thal was determined in Cyprus (Baysal et al, 1995; Bozkurt and Baysal, 2019), Türkiye (Öner et al 1997; Çürük 2007), Greece (Traeger-Synodinos et al 1993; Kattamis et al 1996), Italy, Sicily, and Sardinia (Galanello et al 1992). In recent years, it has been reported that α-thal is also common in the Middle East, especially in the Arabian Gulf countries, where point mutations are generally more common, and clinical presentations more pronounced in many HbH patients, some of whom have been reported to receive regular transfusions (Adekile et al, 1994; Kalla and Baysal, 1998; Baysal, 2011). It is now well established that HbH cases in the Gulf countries are genotypically and phenotypically different from those in the Mediterranean countries such as those in Türkiye, Cyprus, and Sardinia (Baysal et al, 1995; Baysal, 2011; Galanello et al, 1992).

While most mutations in the Mediterranean countries are due to large deletions (aka α-thal-1), most of the mutations found in HbH patients in Gulf countries are point mutations (Baysal et al 2007; Baysal 2011). Differences in molecular structure have direct consequences on the phenotype, clinical manifestation, and hematology. The phenotypes among point mutations tend to be significantly more severe. This is particularly evident in patients with the 'Poly-A' mutation whereby patients with homozygous Poly-A mutations have a severe phenotype and may sometimes be dependent on blood transfusion (Bozkurt and Baysal, 2019). This is because the mutant α2 gene reduces the expression of the downstream α1 gene. In other words, the PA-1 (AATAA**A**>AATAA**G**) mutation in the α2 gene downregulates the expression of the downstream α1 gene in the 3' region (Whitelaw and Proudfoot, 1986) and markedly reduces the α-chain production.

EXPRESSION OF α-GLOBIN GENES

Until the first 8 weeks of pregnancy, embryonic hemoglobin Hb Gower-1 (ζ2ε2), Hb Gower-2 (α2ε2), and Hb Portland (ζ2ϒ2) are produced. These embryonic hemoglobins were discovered at University College London (UCL) by Huehns et, al (1961). UCL is situated at the junction of Gower Street and Portland Street, the two large streets that intersect UCL and UCL Hospital (UCLH).

The transition from the embryonic stage to the fetal stage happens around the 9th week when fetal hemoglobin (HbF; α2ϒ2) is synthesized as the predominant hemoglobin **(Fig. 3)**.

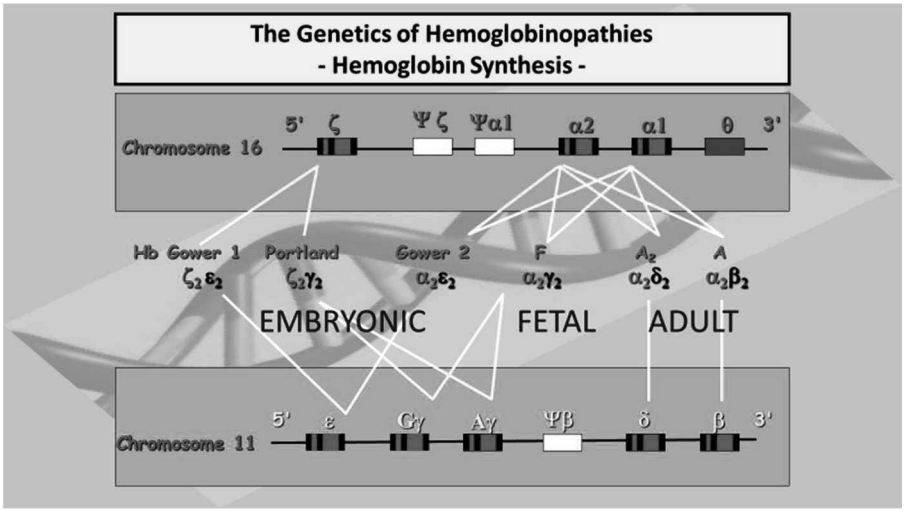


Figure 3: *Expression of globin genes during hemoglobin synthesis*

Immediately after birth until age one, the amount of HbF decreases rapidly to 1%. Adult hemoglobin, HbA (α2β2), appears immediately after birth. HbA constitutes 30% of the total amount of Hb in the blood before birth, 50% at 2 months, and nearly 95% at 6 months of age. HbA2 (α2δ2) appears immediately after birth at levels as low as 1.5-3.5% and is produced throughout life. In normal adults, 95% of Hb is HbA (α2β2), 2-3% HbA2 (α2δ2), and less than 1% Hb F (α2Υ2) is maintained throughout life. The amount of Hb is normally 14-18 g/dL in adult men and 12-16 g/dL in women. In children, it varies with age; in older children (>6 years) Hb less than 11 g/dL is considered anemia.

MOLECULAR BASIS OF α-THALASSEMIA

α-Globin chains are involved in the structure of all major hemoglobin molecules. The α-globin gene cluster responsible for α-globin chain synthesis consists of four functional genes in the subtelomeric region on the short arm of chromosome 16 (16p 13,3): HBZ (OMIM 142310), HBA2 (OMIM 141850), HBA1 (OMIM 141800), and HBQ1 (OMIM 142240). These genes are under the control of the HS-40 region, known as the regulatory critical region (Fig. 4).

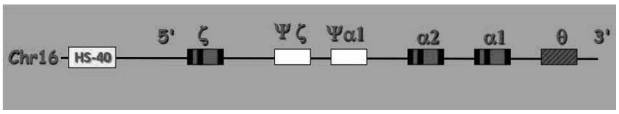


Figure 4: *Chromosome 16 α-globin gene family and the regulatory HS-40 region*

α-Globin genes are located on the short arm of chromosome 16 in the α-globin gene cluster in the following order [telomere- ς2-ψς1-ψα1-α2-α1-ɵ-centromere] (Fig. 3 & 4). α-Globin genes carry the information necessary to encode the 142 amino acids in the α-globin chain in approximately 0.8kb DNA sequence consisting of 3 exons, 2 introns, and 5' and 3' regulatory regions. A normal adult human has four α-genes and hematological findings are generally as follows; Hb 12-15g/dl, MCV 85-100fl, MCH ~30pg, Hb Bart's 0%, α/β chain ratio ~1.0.

The adult HbA molecule consists of 2α and 2β chains making up a tetrameric protein (α2β2). Fetal Hb (HbF) contains (α2γ2) chains. Two separate gene clusters, known as the α gene on chromosome 16 and the β gene on chromosome 11, govern the genetic control of the α, β, γ, δ and other Hb chains.

There are 2 α-globin gene copies (α1 and α2) on each haploid chromosome 16. Diploid chromosomes have 4 alpha genes. The α2 gene is more active than the α1 gene, producing approximately 2.6 times more α-globin chains (Fig. 5). In α-thal syndromes, α-chain production decreases *as per* the mutant α-globin gene and its amount, and the clinical severity changes according to the extent of this decrease.

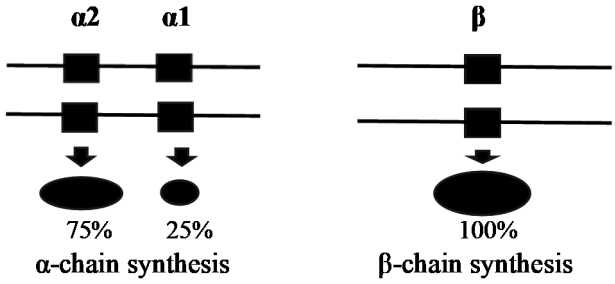


Figure 5: *Hemoglobin Synthesis*

There are a total of four alpha globin genes (αα/αα), two on each of the homologous chromosomes. The α genes on the same chromosome are given the names α2 and α1 in the 5'-3' direction. These genes are structurally copies of each other (duplicated). However, there are slight divergences in their non-coding sequence. These differences may play an important role in the laboratory manipulation of individual α2 and α1 genes by PCR (Baysal and Huisman; 1994). As a result of mutations, α-globin chain synthesis is either reduced or completely abolished (Higgs, 1998; Baysal, 2011). More than 500 mutations of α-globin have been reported to date (Huisman, Carver, Baysal 1997).

α-THALASSEMIA MUTATIONS

There are essentially two groups of α-thal:
1. DELETIONAL 2. NON-DELETIONAL.

It is rather difficult to ascertain an accurate diagnosis due to the overlap of diagnostic criteria between α-thal and iron deficiency anemia.

DELETIONAL α-THAL MUTATIONS are very heterogeneous at the clinical and molecular levels. Clinical forms of α-thal are defined as silent carrier (α^{+}), severe α-thal carrier (α^{0}), HbH disease (--/-α), and Hb Bart's Hydrops Fetalis syndrome (--/--) (Harteveld and Higgs, 2010). Generally, the phenotype of α-thal mutations is directly associated with the number of affected alpha globin genes (in short, 1 gene deletion is considered a silent carrier, 2 gene deletions are carriers, 3 gene deletions are Hb H, 4 gene deletions are Hb Barts Hydrops Fetalis). Currently, there are nearly 150 mutations, whose genotypes and phenotypes are known to be complex and variable. This is not only related to the number of affected α-globin genes but also the type of α-thal mutation; whether it is a small deletion (-α3.7kb and -α4.2kb deletions) or a large deletion (--MED-I, --SEA, -(α)20.5kb) (Fig 6a, 6b).

The α-globin gene cluster encoding the α-globin chains is located in the telomere region at the end of the short arm of chromosome 16. This DNA region is rich in G+C nucleotide sequences. When these genes undergo mutations, they adversely affect the production of α-globin chains. The propensity of deletional α-thal is due to the presence of homologous sequences in the α-genes (α2 and α1). The most common α-thal deletion $-\alpha^{3.7}$ is caused by reciprocal recombination between mispaired Z segments resulting in a chromosome with only one functional α-gene ($-\alpha^{3.7}$ or rightward deletion) and a reciprocal α-triplication (ααα) chromosome with no thal influence. Similarly, reciprocal recombination between mispaired X-boxes results in a 4.2kb deletion, called ($-\alpha^{4.2}$ or leftward deletion) as a result of 98.5% homology between the α2 and α1 genes when the two chromosomes align themselves in the cell nucleus during the meiosis when reciprocal homologous recombination between misaligned chromosomes occurs (Fig.6).

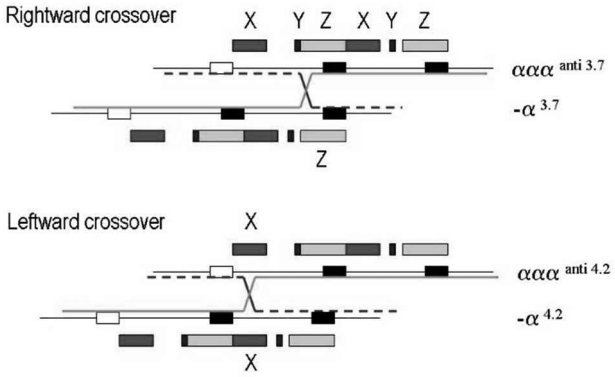


Figure 6: *The formation of rightward $-\alpha^{3.7}$ & leftward $-\alpha^{4.2}$ determinants (Ref: Harteveld & Higgs, 2010).*

α-thal occurs with the disappearance of one or two α-genes or the entire gene cluster. Most of the common deletions range from 2kb-100 kb **(Fig. 6a, b, c**). Sometimes a large deletion may not contain structural genes, but it will almost always abolish the expression of the ς- and α-genes. Deletions involving the telomere of chr 16 cause a new HbH syndrome associated with mental retardation (Fig.6b). These syndromes are outside the scope of this topic and will be discussed briefly later.

The α-globin gene mutations fall into three main groups:

1. SMALL DELETIONS (α-Thal-2): This is the most common cause of thalassemia. The size of the deletions is important and influences the clinical phenotype **(Fig. 6a**).

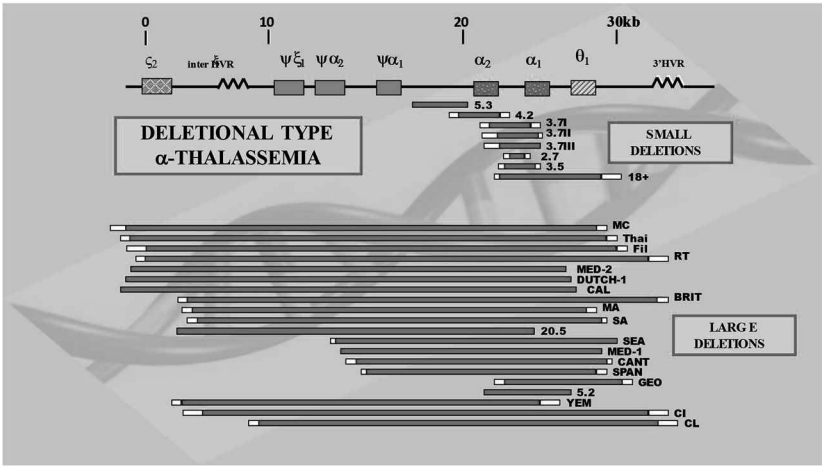


Figure 6a: *Deletional α^{+} and α^{0}-thalassemia mutations.*

2. LARGE DELETIONS (α-Thal-1): If a mutation completely removes gene expression from the chromosome, it is called **α^0 -Thal**, and if it partially reduces expression, it is called **α^+ -Thal**. These are usually in the form of small and large deletions **(Figure 6b)**.

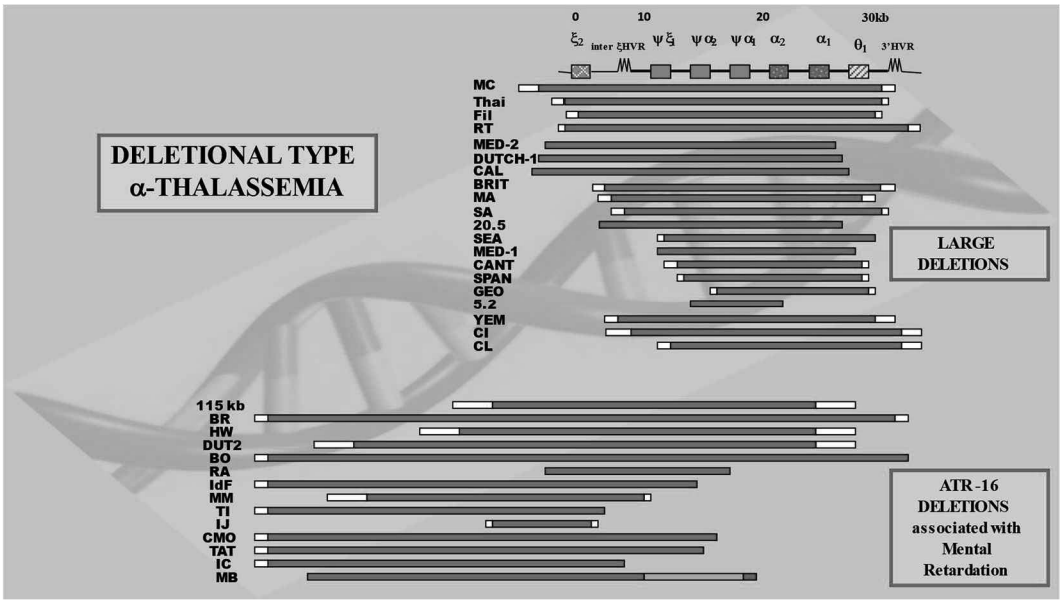


Figure 6b: *Deletional α^0-thalassemia mutations affecting the regulatory regions.*

3. NON-DELETIONAL α-THAL MUTATIONS ($\alpha^T\alpha$ & $\alpha\alpha^T$): Non-deletional α-thal mutations are caused by single or several nucleotide changes or oligonucleotide deletions/insertions in regions critical for α-globin gene expression **(Fig. 6c)**. Generally, the non-deletional α^+-thal determinants tend to give rise to a more severe reduction in α-chain synthesis than the deletional type of α-thal. Many mutations have been described as affecting mRNA processing, mRNA translation, and α-globin stability. The increasing number of α-thal point mutations also causes RNA breakage, RNA fragmentation, and RNA clipping in both the α2 and α1 genes. Such fundamental structural changes in the polypeptide chain ultimately result in unstable hemoglobin.

It has been shown particularly in the Gulf Arab countries that α-thal determinants caused by point mutations are as prevalent as those caused by the more common deletional types (Harteveld and Higgs, 2010; Baysal, 2011). The clinical phenotypes of point mutations have been reported on many occasions to be more severe than the deletional types (Baysal, 2011). In the Middle East and especially in the Gulf Arab countries, non-deletional α-thal type due to point mutations has a significantly high variety and frequency. Fig. 6c shows most of the currently known non-deletion α-thal mutants. Among these the most common non-deletional determinants are the $\alpha^{-5\ nt\ del}\alpha$, polyadenylation site mutations PA-1 α_2^{AATAAG}, PA-2 α_2^{AATGAA}, and PA^{-AA} α_2^{AATA--}. These mutations are often found in the Mediterranean and Middle Eastern populations (Yuregir et al, 1992; Baysal 2011). The others are termination codon mutations leading to elongated Hb variants, such as Hb Constant Spring (HbCS), Hb Icaria, Hb Koya Dora, Hb Seal Rock, and Hb Paksé (Middle East, Mediterranean, and South East Asia). In addition, structural mutations causing highly unstable α-globin variants, Hb Quong Sze, Hb Suan Dok (Chan et al, 1997), Hb Petah Tikvah, Hb Adana (Curuk et al, 1993), Hb Aghia Sophia (Traeger-Synodinos. J., et al 1999). These common mutations are summarized in a comprehensive syllabus (Huisman, Carver, Baysal, 1997) and Fig. 6c.

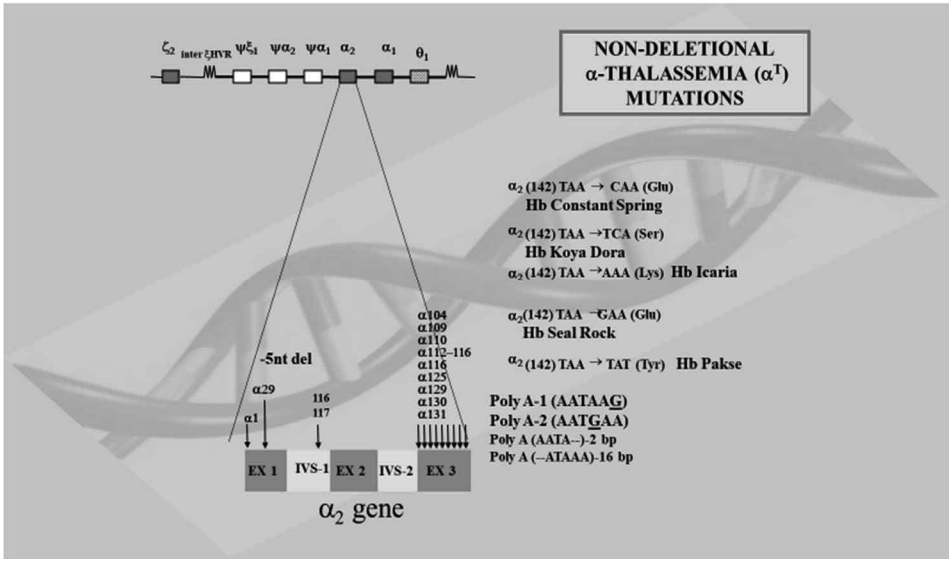


Figure 6c: *Non-Deletional α-thal point mutations*

ATR-16 SYNDROME - Deletional α-Thalassemia Associated with Mental Retardation.

The ATR-16 syndrome is caused by large DNA deletions encompassing the ς- and α-genes. These deletions, which include the telomere at the tip of chromosome 16, can completely abolish the expression of the structural and functional ς2, α2, and α1 genes even though they may not contain them (Gibbons, 2012). In these cases, a new form of α-thal emerges, which is associated with mental retardation. Affected patients may also have classical HbH and dysmorphic deformities, and abnormal facial features. In most family studies, one of the parents was completely normal and that α-thal was passed to the offspring as a *de novo* mutation.

GENOTYPE-PHENOTYPE CORRELATIONS

The number of α-genes is directly related to α-globin chain synthesis, which in turn determines the phenotype. With an ever-increasing number of α-thal mutations, (nearly 150 to date), there is potential for a multitude of possibilities of interactions thus increasing the number of mutant genotypes further. The clinical phenotype severity correlates very well with the degree of α-chain deficiency. It is important to note that interactions involving non-deletional α-thal mutations result in a more severe phenotype than in those with deletional forms of α-thal (Higgs, 2009).

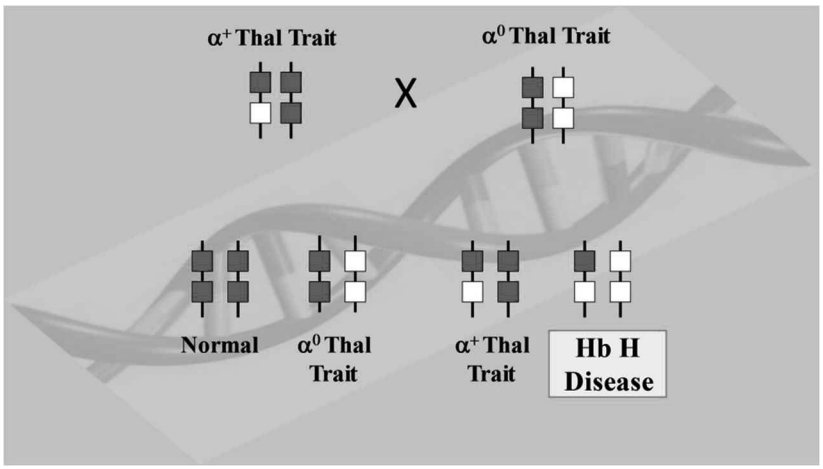


Figure 7: *Possible interactions resulting in various genotypes.*

FOUR DISCRETE CLINICAL PHENOTYPES OF VARYING SEVERITY

1- SILENT CARRIER / α-THAL-2 HETEROZYGOTES ($-\alpha^{+}/\alpha\alpha$): This is caused by a single α-globin gene deletion, resulting in the reduction in α-globin chain synthesis. Silent α^{+}-thal carriers are difficult to detect and occur when only one allele is affected (heterozygote). The α^{+}-thal (-α/αα) phenotype is normal with no clinical significance. There is no abnormal hematological picture or clinical consequences; MCV 75-85, MCH~26, and Hb Bart's can be 0-2%. It is completely asymptomatic or has mild microcytosis and hypochromia, HPLC demonstrating normal HbA_2 and HbF.

2- MODERATE α-THAL CARRIERS ($-\alpha^{0}/\alpha\alpha$): a condition of no clinical significance with below-normal hematological findings (Hb, MCV, MCH). They may be characterized by two forms: **α-thal-2 homozygote (-α/-α)** or **α-thal-1 heterozygote (--/αα).** Both genotypes are hematologically indistinguishable. Distinct hematological anomalies are apparent in these patients; Hb 10-12g/dl, MCV 65-75, MCH ~22, Hb Bart's 2-8%.

3a- HbH DISEASE DUE TO DELETIONS ([--/-α] ; [--/$\alpha^{T}\alpha$]; [--/$\alpha\alpha^{T}$]): These are associated with hemolytic anemia and the characteristic 'golf ball' appearance of adult erythrocytes in peripheral blood (Fig. 8, 9). The most common form of HbH disease is caused by 3 alpha-globin gene deletion (--/-α) (Fig. 7). Other forms are 2 gene deletions and a point mutation in the α2 gene (--/$\alpha^{T}\alpha$) or the α1 gene (--/$\alpha\alpha^{T}$). It is possible to observe 20-40% Hb Bart's during the neonatal period. Hb Bart's turns into adult HbH at a rate of 5-30% after birth. Deletional HbH genotypes are more common in South East Asia and the Mediterranean. Typical values are; MCV 60-70, MCH ~20, α/β chain ratio ~0.4, and HbH 10-20%. Very high Hb Bart's levels in newborns and up to 20% HbH are observed in adult patients.

3b- HbH DISEASE DUE TO 'POINT MUTATION' ([$\alpha^{T}\alpha/\alpha^{T}\alpha$]; [$\alpha\alpha^{T}/\alpha\alpha^{T}$]) HbH disease can result from the combination of two point mutations one on each chromosome i.e. severe point mutations on both α2 ($\alpha^{T}\alpha/\alpha^{T}\alpha$) or severe mutations on both α1 ($\alpha\alpha^{T}/\alpha\alpha^{T}$) genes. Point mutations, which are generally seen in the Middle East and Mediterranean countries, can affect both α2 and α1 genes. In general, these mutations have more severe phenotypes than deletional types (Weatherall, 2018; Harteveld and Higgs, 2010; Huisman, Carver, and Baysal, 1997).

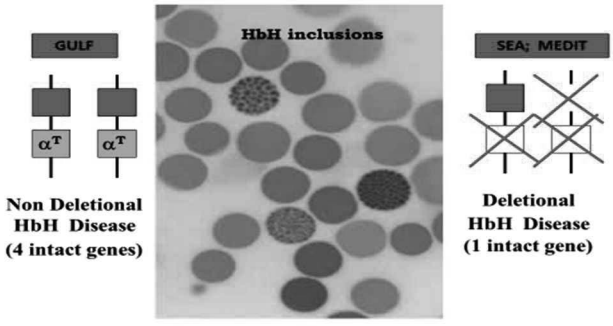


Figure 8: *HbH Disease caused by two discrete genotypes $\alpha^{T}\alpha$ $\alpha^{T}\alpha$ (left) and --/-α (right)*

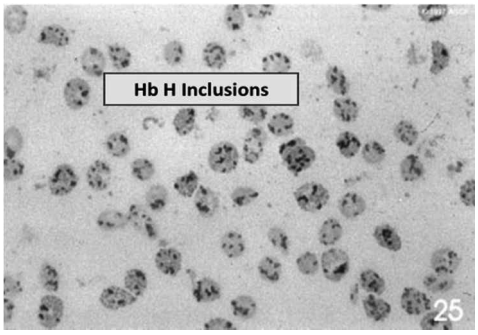


Figure 9: *HbH Inclusion bodies Stained with 1% Brilliant Cresyl Blue)*

4- Hb BART'S HYDROPS FETALIS (--/--): is the most severe form of α-thal, and develops when α-globin cannot be synthesized due to deletion or four inactive α-globin alleles. This syndrome is incompatible with life. Babies with Hb Bart's Hydrops Fetalis are either stillborn or die immediately after birth due to intrauterine anemia, heart, spleen, and liver failure. It is generally caused by a combination of two (--/αα) chromosomes lacking the α genes. This fatal condition is seen in places where α^{0}-thal (--/αα) are most common namely in South-East Asia and the Mediterranean basin. The amount of Hb Bart's (γ4) in the blood can reach up to 80%. The ability of these babies to survive until the last stage of pregnancy is due to the presence of Hb Portland-1 (ς2γ2) and Hb Portland-2 (ς2β2) in the peripheral blood. Since the hematological findings are very low and the α globin chain is not produced

at all, all erythrocytes contain HbH (β4) and Hb Bart's (γ4), both of which cannot bind oxygen. The oxygen transport mechanism is compromised due to a lack of 'heme-heme' interaction. Once the diagnosis is made, the pregnancy is followed by intrauterine transfusions and preterm delivery is possible. The baby is followed up with regular transfusion and iron chelation after delivery.

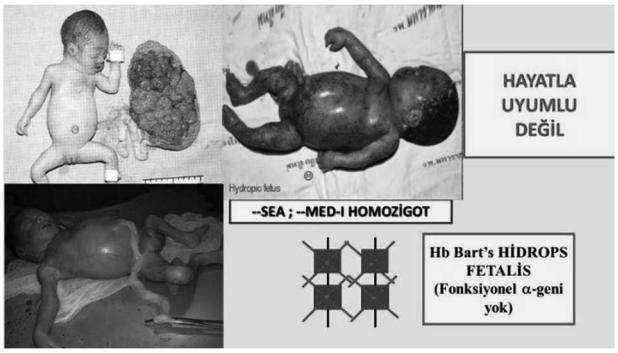


Figure 10: *Hb-Bart's Hydrops Fetalis Syndrome*

PATHOPHYSIOLOGY OF HbH: In HbH disease, the β-chain is relatively more abundant than the α-chain. Excess β chains form a nonfunctional, oxygen-free tetramer, resulting in an unstable HbH (Fig. 8,9). While in α-thal carriers (silent and moderate), unstable HbH, is detected only in cord blood, in HbH disease it is also high in the peripheral blood where hemolytic anemia is observed due to the precipitation of HbH in erythrocytes. These precipitates, called Hb H inclusion bodies, can be demonstrated with reticulocyte staining (1% Brilliant Cresyl Blue) in peripheral blood (Fig 9). In the bone marrow, the maturation process of erythroid mother cells is normal and there is no dyserythropoiesis, so normoblasts are not usually seen in the peripheral smear. The reticulocyte count may be high in proportion to anemia.

Clinically HbH syndromes are generally present at the 'thalassemia intermedia' level. Anemia, jaundice, enlarged spleen and liver, and HbH in peripheral blood are the most common findings. Other complications such as infection, foot ulcers, gallstones, folic acid deficiency, and acute hemolytic crises may also be observed. Iron overload occurs only in transfused adult patients (age >45). There is a direct relationship between the severity of HbH disease and α-globin chain deficiency. Point mutations ($\alpha^T\alpha$), affecting the more active α2 gene, often result in a more severe phenotype than mutations involving the α1 gene. This is because α2-mRNA transcription of α2 genes is 2.6 times higher than α1-mRNA (Liebhaber et al, 1986; Smetanina, et al, 1996). Patients with a point mutation (α^T) are anticipated to be more anemic than patients with deletional type α-thal. They also have higher HbH levels so much so that homozygous patients with ($\alpha^{PA\text{-}1}\alpha/\alpha^{PA\text{-}1}\alpha$) generally require splenectomy and regular blood transfusions (Bozkurt and Baysal, 2019).

REFERENCES

1. Adekile A.D., Gu, L.H., Baysal E., et al, Molecular characterization of alpha-thalassemia determinants, beta-thalassemia alleles, and β S haplotypes among Kuwaiti Arabs; Acta Haematol., 92:176-81, 1994
2. Baysal E. and Huisman T.H.J., Detection of common deletional alpha-thalassemia-2 determinants by PCR. Am J Hematol. 46(3):208-13, 1994.
3. Baysal E., Kleanthous M., Bozkurt G. ve ark., α-thalassemia in the population of Cyprus, British Journal of Haematology, 89, 496-499, 1995.
4. Baysal, E., Yousef, I., Zeinali, M., et al. Molecular basis of hemoglobinopathies in the UAE: implications for prenatal diagnosis and prevention programs. Emirates Med J. 2007; 25(1):7-21.
5. Baysal, E. α-Thalassemia Syndromes in the United Arab Emirates. Hemoglobin; 35 (5-6):574-80, 2011. PMID: 22074123
6. Bozkurt G. and Baysal E., Thalassemia Syndromes, Thalassemia Prevention Programs and their Implications in North Cyprus, 2019
7. Chan V, Chan VW, Tang M, Lau K, Todd D, Chan TK: Molecular defects in Hb H hydrops fetalis. Br J Haematol. 96: 224-228, 1997.
8. Çürük MA. Hb H (β4) disease in Çukurova, southern Türkiye. Hemoglobin 2007;31:265-271.
9. Çürük MA, Dimovski AJ, Baysal E, et al; Hb Adana or alpha 2(59)(E8)Gly-->Asp beta 2, a severely unstable alpha 1-globin variant, observed in combination with the -(alpha)20.5 Kb alpha-thal-1 deletion in two Turkish patients. Am J Hematol. 44: 270-275, 1993.
10. El Kalla S. and Baysal E., Genotype-phenotype correlation of sickle cell disease in the United Arab Emirates. Pediatr Hematol Oncol.;15(3):237-242, 1998.
11. Fei Y.J., Baysal E., Huisman T.H.J. ve ark, 1992, Hb H disease in a Turkish family resulting from the interaction of a deletional α-thalassemia-1and a newly discovered poly A mutation British Journal of Haematology 80, 527-532, 1992.
12. Fischel-Ghodsian N, Vickers MA, Seip M, et al: Characterization of two deletions that remove the en-

tire human zeta-alpha globin gene complex (- -THAI and - -FIL). Br J Haematol. 1988, 70: 233-238.
13. Flint J., Hill A. V. S, Bowden D. K., S. J. High frequencies of α-thalassaemia are the result of natural selection by malaria, Nature 321, 744–750 (1986)
14. Fodde R, Losekoot M, Broek MH, Prevalence and molecular heterogeneity of alfa+ thalassemia in two tribal populations from Andhra Pradesh, India. Hum Genet. 1988, 80: 157-160. 10.1007/BF00702860.
15. Galanello R., Aru B., Dessi C. ve ark HbH Disease in Sardinia: Molecular, Hematological, and Clinical Aspects, Acta Haematol., 88: 1–6, 1992
16. Gibbons, R.J. α-Thalassemia, Mental Retardation, and Myelodysplastic Syndrome; Cold Spring Harbor Perspective Med. (10) 2012
17. Harteveld C.L. ve Higgs D.R. Alpha-thalassaemia, Orphanet J Rare Dis. 28;5:13. doi: 10.1186/1750-1172-5-13, 2010.
18. Higgs D.R., Sharpe J.A., Wood W.G., Understanding alpha-globin gene expression: a step towards effective gene therapy. Seminars in Hematology, 35(2):93-104, 1998;
19. Higgs DR: The molecular basis of alpha-thalassemia. Disorders of Hemoglobin. second edition. Edited by: Steinberg MH, Forget BG, Higgs DR, Nagel RL. Cambridge University Press; 2009.
20. Huisman T.H.J., Carver M.F.H., Baysal E., A Syllabus of Thalassemia Mutations https://globin. bx.psu.edu, 1997.
21. Liao C, Wei J, Li Q, et al: Nonimmune hydrops fetalis diagnosed during the second half of pregnancy in Southern China. Fetal Diagn Ther. 2007, 22: 302-305.
22. Liebhaber S., Cash F. E., Ballas S. K., Human alpha-globin gene expression. The dominant role of the alpha 2-locus in mRNA and protein synthesis J Biol Chem 261(32):15327-33, 1986.
23. Öner C, Gurgey A, Öner R, et al. The molecular basis of Hb H disease in Türkiye. Hemoglobin 1997; 21:41-51.
24. Smetanina N.S., Oner C., Baysal E., et al, 1996 The relative levels of α2 α1 and zeta ς -mRNA in HB H patients with different deletional and non-deletional α-thalassemia determinants, Biochimica et Biophysica Acta 1316 (1996) 176-182, 1996
25. Traeger-Synodinos J., Kanavakis E., Tzetis, M., et al, Characterization of nondeletion α-thalassemia mutations in the Greek population. American Journal of Hematology; 44, 162-167, 1993.
26. Traeger-Synodinos J, Harteveld CL, Kanavakis E, Hb Aghia Sophia [alpha62(E11)Val-->0 (alpha1)], an "in-frame" deletion causing alpha-thalassemia. Hemoglobin., 23: 317-324, 1999.
27. Traeger-Synodinos J., Kanavakis E., Vrettou C., et al., The triplicated α-globin gene locus in β-thalassemia heterozygotes: clinical, hematological, biosynthetic, and molecular studies, British Journal of Haematology, 95, 467-471, 1996.
28. Weatherall D.J., Hematology/Oncology Clinics, The Evolving Spectrum of the Epidemiology of Thalassemia; Review article, 32 (2), 165-175, 2018.
29. Whitelaw E, Proudfoot N: Alpha-thalassaemia caused by a poly(A) site mutation reveals that transcriptional termination is linked to 3' end processing in the human alpha 2 globin gene. EMBO J. 1986, 5: 2915-2922.
30. Yuregir GT, Aksoy K, Curuk MA, et al, Hb H disease in a Turkish family resulting from the interaction of a deletional alpha-thalassemia-1 and a newly discovered poly A mutation. Br J Haematol. 1992, 80: 527-532, 1992.

Correspondence Address:
Fellow of the Royal College of Physicians, London, UK
Prof. of Genomics
Consultant Molecular Geneticist
E-mail: erolbaysal@gmail.com

GENETICS OF BETA THALASSEMIA

Prof. Dr. İbrahim Keser
Akdeniz University, Faculty of Medicine, Department of Medical Biology and Genetics, Antalya, Türkiye

ABSTRACT

Beta-thalassemia which has an important place among hemoglobinopathies, is the only gene disease with well-identified genetic and clinical considerations. Mutational heterogeneity in globin genes, trans or cis particles interacting with these genes cause clinical heterogeneity in the prenatal and postnatal period, and it expands dominantly in thalassemias inherited in a recessive model. In beta-thalassemia, normal beta-globin gene results exceed extremes, resulting in spreading-type clinical phenotypes with non-deletional mutations (transcriptional mutations, variants of RNA genes, translational mutations), deletion-type mutations, modifier genes and mutations, and ends of epigenetic factors. Mutations in the beta-globin gene affect the disease phenotype according to the gene's anatomically location of the mutation. New technologies are used to screen influencing mutation for specific purposes. Different technologies that can diagnose from point mutations at these points to large deletions include genetic diagnostic uses. The study of the basis of the beta-globin gene and its progressive generation requirements has given rise to current approaches for recognizing research and routine diagnose. In this chapter, the genetic sutructure, modifier genes, and phenotypic interactions of beta-thalassemia are actually given. As a result, we think that this study will contribute to the investigation of different aspects of beta-thalassemia, our better understanding of the consequences of beta-thalassemia and to our overall approach to correct diagnosis and treatment.

Keywords: Beta-globin gene, mutation, beta-thalassemia, hemoglobinopathy, Mediterranean Anemia

INTRODUCTION

Beta thalassemia is a hereditary blood disorder characterized by reduced or absent synthesis of the beta globin chains of hemoglobin. This leads to an imbalance in the alpha and beta globin chains, causing a range of clinical manifestations, including anemia, splenomegaly, and bone deformities. The disorder is caused by mutations in the HBB gene, which encodes the beta globin chains **(1, 2)**. Beta-thalassemias, which constitute a group of inherited blood disorders that are heterogeneous at the molecular level, are characterized by abnormality in hemoglobin (Hb) beta (β) chains, resulting in variable phenotypes, ranging from severe anemia to clinically asymptomatic individuals, are the most common autosomal recessive disorders in the world (**1**). It is found in high frequency in Mediterranean, Middle-East, Transcaucasian, Central Asian, Indian Subcontinent and Far East societies. The highest frequency was reported in Cyprus (14%), Sardinia (12%), and Far East Asia **(1-5)**. The high gene frequency of β-thalassemia in these regions most likely contributes to malaria endemic today and in the past, and the distribution of β-thalassemia is very low **(4)**. However, due to social migrations and the slave trade in the past, β-thalassemia is presently common found in Northern Europe, South and North America, and Australia **(5)**. Today, the prevalence of thalassemia has increased almost everywhere due to the increase in immigrant populations in the world, and it is estimated that approximately 7% of the world's population is a carrier of thalassemia **(3)**.

In Türkiye, although it varies between 0.7% and 13% according to the regions, the overall frequency is 2.1%, most common in the Mediterranean (Antalya, Muğla, Mersin, Adana, Hatay Provinces), and less commonly in the Eastern and Southeastern Regions **(4, 6-17)**.

Beta-thalassemia occurs with decreased or absent beta globin chain production, one of the hemoglobin tetramers. According to the severity of the

changing signs and symptoms, three clinical and hematological manifestations are accepted as thalassemia carrier, thalassemia intermedia, and thalassemia major. However, a thalassemia phenotype may also result from constitutive beta chain variants when expressed at a reduced rate, as in the case of Hb E (β26Glu→Lys). Alternatively, variants are very unstable molecules that are rapidly destroyed, leading to functional deficiency, even if they can produce at normal rate. Mutations that completely inactivate the beta globin gene, resulting in a lack of beta-globin production, cause β0-thalassemia. Depending on the degree of quantitative reduction in the production of beta-chains, other mutations that allow some beta-globin productions are classified as β+ or β++ (silent). The occurance of beta-thalassemia phenotype is formed by mutations involving one or a number of nucleotides in beta globin gene and regulator regions localized its around **(18-21)**.

THE LOCALIZATION OF BETA GLOBIN GENE

Beta-globin is encoded as a structural gene, located in a cluster with other β-like genes, extending 70 kilobases (Kb) on the short arm of chromosome 11 (11p15.5) **(Fig. 1)**. The cluster contains five functional genes (5'ε Gγ-Aγ-ψβ, δ-β3') arranged in developmental order of expression. Upstream of the entire beta-globin composition is the locus control region (LCR), which is required for expression of all genes in the composition. This region contains polyandromic sequences spread between 5′ 6 and 20 Kb of the ε gene and transcription factor binding sites such as (AT)x(N)y(AT)z and (CA)x (TA)y) with high affinity. It consists of five DNase 1 hypersensitive (HS) sites containing repeats **(22, 23)**. Another hypersensitive site is at the ~20 Kb 3′ end of the beta-gene (3'HS1). The two extreme HS regions next to the beta compound are suggested to mark the boundaries of the beta-globin gene domain **(23,24)**.

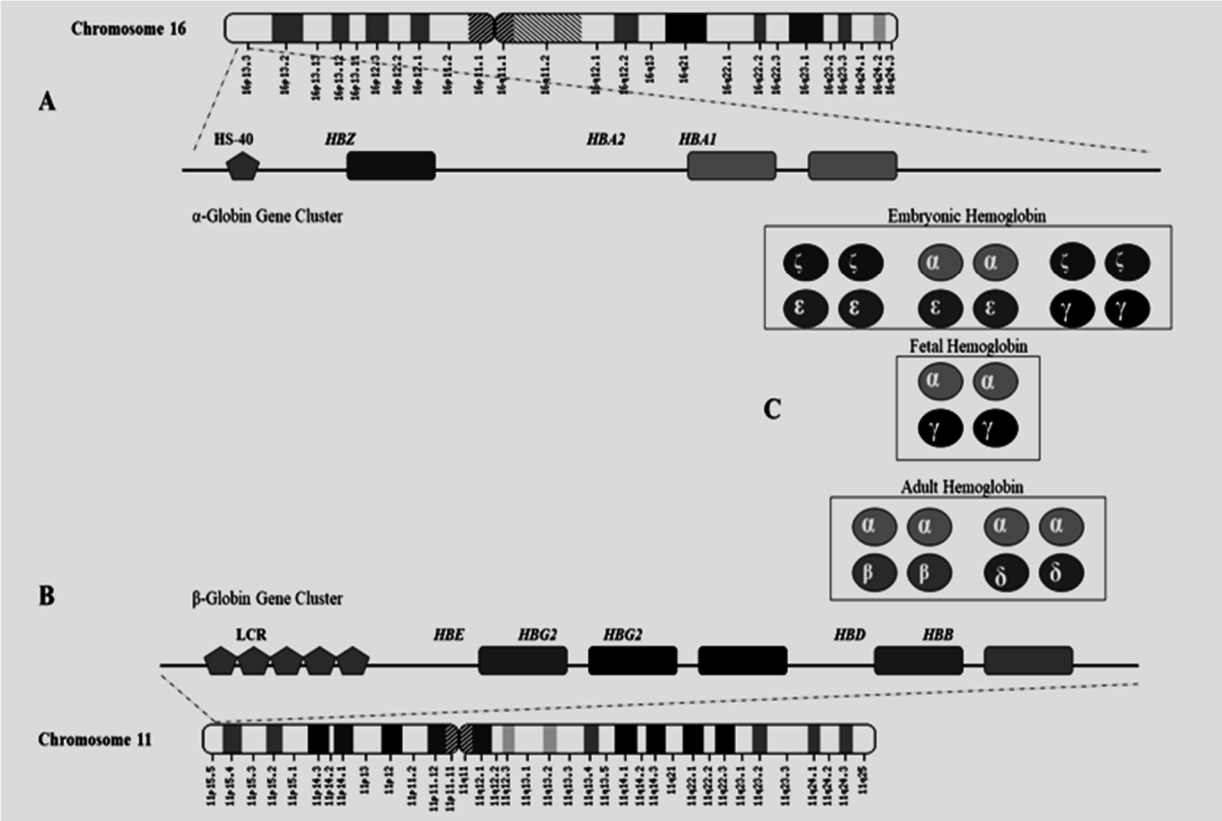


***Figure 1**: **A**) The alpha-globin gene group localized in the 16p13.3 region on the short arm of chromosome 16 and **B)** the β-globin gene group localized in the 11p15.5 region on the short arm of chromosome number 11 in the human genome. **A)** The α-globin gene group contains three functional globin genes, the embryonic ζ gene (HBZ) and two fetal/adult α, α1 and α2, genes (HBA1 and HBA2). **B)** The β-globin gene cluster contains 5 functional genes, the embryonic ε gene (HBE), the two fetal Gy and Aγ genes (HBG2 and HBG1), and the adult δ and β (HBD and HBB) genes. HS-40 α- and locus control region (LCR) β-globin gene*

expression regulatory regions. ***C)*** *Hemoglobin molecules expressed differently in human embryonic, fetal and adult stages.[19] (That figure have been rearranged by the author in the cited reference).*

GENETICS OF BETA THALASSEMIA

Unlike α-thalassemia, which is caused by deletions, beta-thalassemia occurs with point mutations, most of which are single nucleotide changes and short insertions/deletions that lead to frameshifts. More than 300 variants have been described **(16, 25, 26)**. Therefore, beta-thalassemia is also clinically heterogeneous. According to the degree of quantitative reduction in beta-globin production, beta-thalassemia alleles are divided into three categories: 1) absence of beta-globin (β^0); 2) beta-globin is produced, but its amount is reduced (β^+); and 3) beta-globin production is minimally reduced and is also known as "silent" (β^{++}) (Table 1 and Figure 2) **(26, 27)**.

Table 1*: Classification of mutations causing beta thalassemia (The table has been rearranged by the author in the cited reference)****(27)***

A- Deletions
-Large deletions involving the beta LCR with or without beta-gene (cause εγδβ thalassemia).
-Deletions are restricted to beta- gene.
B- Trans-acting mutations
C- Point mutations

I-Transcriptional Mutations	**Type**
Promoter Regulatory Elements	
1) CACCC Box	β^+ to β^{++} (silent)
2) ATA Box β+	
3) 5' UTR (CAP +1 to +45)	β^+ to β^{++} (silent)
II. RNA Processing	
Splice Junctions	
1) IVS1-position 1 and 2	β^0
2) IVS1–3' end (minor deletion of 17 to 44 bp, and insertion of 22 bp)	β^0
3) IVS2-position 1 and 2	β^0
4) IVS2-3' end	β^0
Consensus Splice Sites	
5) IVS1-position 5	β^+ or β^0 depending on nucleotide change
6) IVS1-position 6	β^+
7) IVS1 positions -3, 128, 129	β^+
8) IVS2-5	β^+
9) IVS2–3' end	β^+ to β^{++} (silent)
Cryptic Splice Sites	
10) IVS1-1 positions 110, 116	β^+, β^0
11) IVS2-positions 654, 705, 745, 837	β^+, β^0
12) CD10 (GCC → GCA)	
13) CD19 (AAC AGC) Hb Malay (Asn → Ser)	β^{++}
14) CD24 (GGT → GGA)	β^{++}
15) CD26 (GAG → AAG) (Glu → Lys, Hb E)	β^+
16) CD26 (GAG → GCG)(Glu → Ala, Hb Tripoli)	β^+
17) CD27 (GCC → TCC) (Ala → Ser, Knossos)++	β^{++}

RNA Cleavage - Poly A Signal	
18) AATAAA - Single base changes, small deletions,	β^{+}, β^{++} (silent)
Others- in 3' UTR	
19) Term CD +6, C → G	β^{++} (silent)
20) Term CD +90, del 13 bp	β^{++} (silent)
21) Term CD +47 (C → G	β^{++}
III. RNA Translation	
Start Codon	
1) ATG – Single base substutions, 45 insertions	β^{0}
Nosense Codons	
2) Numerous examples of single base substitions, all leading to premature termination codons	All β^{0}
Frameshift	
3) Numerous examples of minor insertions, deletions, shifting reading frame and leading to premature termination codons	All β^{0}

LCR: Locus control region, UTR: Untranslated region, CD: Codon

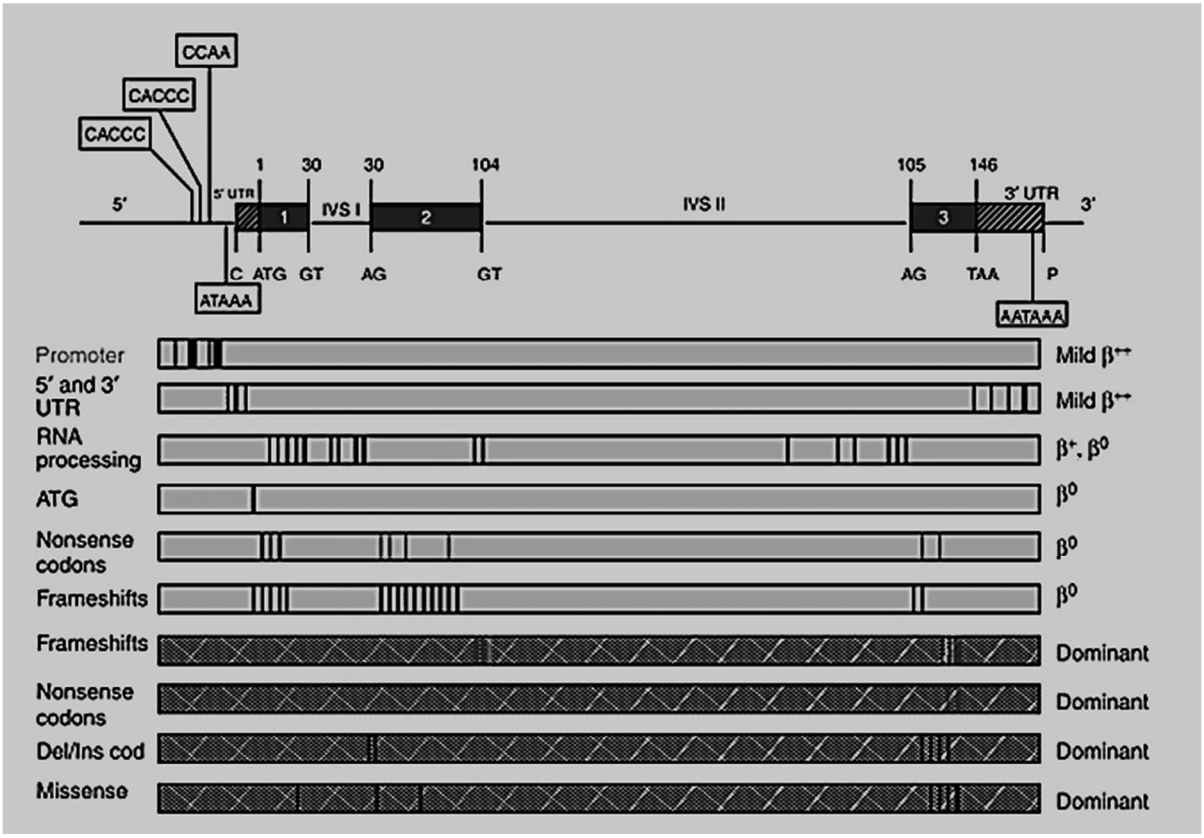


Figure 2: *Map of the human beta-globin (HBB) gene and commonly cited mutations, their location and their effects on gene production. Most of the beta-thalassemia mutations in the world are point mutations that result in aberrant processing of mRNA or premature blockade of the transcription process.*[26]

The general structure of the beta-globin gene is typical of other globin loci **(Fig. 2) (26)**. The genomic sequence covers 1,600 base pairs (bp) and encodes 146 amino acids. The transcribed region contains two introns or three ex-ends separated by intervening sequences (IVS). The first exon encodes amino acids 1 to 29, along with the first two bases of codon 30. Exon 2 encodes the residue portion of codon 30 along with amino acids 31 to 104, and exon 3 encodes amino acids 105 to 146. Exon 2

encodes αβ dimer formation with residues involving heme binding, while exons 1 and 3 encode regions of the beta-globin chain that do not bind to heme. Conserved important sequences for gene function are in the 5′ promoter, exon- It is located at the intron junction and the 3′ untranslated region at the end of the mRNA sequences. The beta-gene promoter contains 3 positive cis-acting elements: the TATA box (positions -28 to -31), the CCAAT box (positions -72 to -76), and the duplicated CACCC motifs (- proximal in position 86 to -90 and distal in position -101 to -105). The CCAAT and TATA elements are found in many eukaryotic promoters, while the CCAAT sequences are generally found in the erythroid cell-specific promoter. Binding of the Erythroid Krüppel-Like Factor (EKLF) to the CACCC motif is crucial for normal adult beta-globin expression. In addition to these motifs, the beta-globin promoter upstream region has two binding motifs for the erythroid transcription factor GATA 1. The importance of these diverse 5′-contiguous sequences for normal gene expression is underlined, particularly through beta-thalassemia resulting from point mutations around and within the TATA box and the CACCC motif in the region -80 to -100. An enhancer is also found in intron 2 and 600 to 900 bp of the downstream globin gene 3′ of the poly(A) region **(26, 27)**.

In beta-globin gene, the 5′ untranslated region (UTR) occupies a 50 nucleotide region between the CAP region, which is the start of transcription, and the initiation code (ATG). Many globin genes (both α and β) have two distinctly conserved sequences in the 5′ UTR. One is the CTTCTG hexanucleotide located 8 to 13 nucleotides downstream of the CAP site. The second conserved sequence is CACCATG, where the last three nucleotides form the start codon (ATG). The importance of these sequences in the regulation of beta-gene expression is through various mutations in the 5′ UTR that cause β-thalassemia. The 3′ UTR forms the region between the termination codon and the poly(A) tail. It consists of 132 nucleotides with the conserved sequence (AATAAA) located 20 nucleotides upstream of the poly(A) tail. This common hexanucleotide serves as the stimulus for the addition of the poly(A) strand, which, by cleaving the 3′ end of the primary copy, stabilizes the processed mRNA and increases translation. β-thalassemia, which is formed as a result of various mutations affecting the AATAAA sequence in the 3′ UTR and other sequences, defines the role these sequences play in gene function. In addition, mutations that affect the beta promoter and eliminate competition for the beta-LCR tend to be associated with variable increases in γ and δ-globin gene expression **(8)**.

Genetics of Beta-Thalassemia Variants

Beta-thalassemias are heterogeneous at the molecular level. More than 300 mutations have been described so far **(19)**. The majority of mutations are single nucleotide substitutions, frameshift deletions or nucleotide insertions. beta-thalassemias rarely result from large gene deletions **(Fig. 2) (26)**. Thus, the vast majority of beta-thalassemias are caused by point mutations within the beta-globin genes or sequences close to it. In thalassemia genetics, the two main groups are β0 and β+-thalassemias. Although the basic classes of mutations constituting these all of groups of thalassemia are summarized in Table 1. In addition, we suggest that it would be more meaningful to examine the genetics of the variants within the framework of the genetics. in order to emphasize the importance of the classifications presented in Table 1.

Dominantly Inherited Beta-Thalassemia

They show a pattern of behavior in contrast to common beta-thalassemia alleles, which are common in malaria regions and inherited in a Mendelian recessive manner. Some forms of β-thalassemia are inherited dominantly. Because, inheritance of a single beta-thalassemia allele results in clinically detectable disease despite normal globin genotype **(8, 28, 29)**. Dominantly inherited beta-thalassemia or beta-thalassemias with inclusion bodies It is highly heterogeneous and is due to mutations at or near the beta-globin gene locus. Most of these are caused by exon 3 mutations in the beta-globin gene. Of the fourthy-four known dominant inherited beta-thalassemia mutations, thirthy-four are located in exon 3 of the beta-globin gene. These mutations are frameshifts, premature chain termination (nonsense) mutations, and complex rearrangements that result in shortened or elongated and highly unstable beta-globin gene products **(26, 28)**.

Dominant beta-thalassemia is generally classified into five different groups: 1) Missens mutations.

Ten different abnormalities have been discovered. A few have been identified by protein analysis alone. All are severely unstable structures and are difficult to define. 2) Deletions or additions of a complete codon. Abnormal hemoglobins formed as a result of the introduction or loss of a new amino acid in beta chain synthesis are also unstable molecules. 3) Early termination of translation. It is caused by a point mutation in the original codon that changes from a frameshift to a nonsense codon, leading to an early terminasyon (stop) codon. The result is a shortened beta chain with 142 residues or less. These shortened beta-chains cannot join with α chains to form an abnormal hemoglobin molecule. 4) Frame shifts resulting in elongated beta chains. Twenty-one of the twenty-three known mutations occurred in exon 3. 5) Complex rearrangements. They are considered to be de novo mutations that cause beta-thalassemia with complex changes in the beta-globin gene sequence, leading to either no beta chain production or the production of abnormal and unstable beta chains **(28)**.

Beta-Thalassemias with Normal Hb A2 Level

One of the diagnostic features of whether beta-thalassemia is β^+ or β^0 is an elevated Hb A2 level in heterozygotes. Many cases with β-thalassemia with normal Hb A2 level are due to co-heredity of δ-thalassemia (δ^+ or δ^0) in the cis or trans position of the β-thalassemia gene, which can be β^+ or β^0 type. It was observed that the $\delta^0$59 (59-A) mutation occurred in the β039 and β+ IVS1-110 mutations in the cis position, and the δ+27 (G>T) mutation occurred in the cis and trans positions in the IVS2-745 mutations **(30-32)**. Normal Hb A2 β -The relatively common form of thalassemia is associated with Hb Knossos (β27 Ala→Ser). Like Hb E, the β27(GCC→TCC) mutation activates the optional junction, which reduces the amount of normal copies containing the variant. Unlike Hb E, Hb A2 level is not elevated in heterozygotes. The molecular basis of normal Hb A2 level is δ^0-thalassemia (Cd59-A) in the cis position to the β27 Ala→Ser mutation **(33)**.

Silent Beta-Thalassemias

Silent beta-thalassemias cause only minimal deficiency of beta-globin production. Heterozygotes have no significant haematological phenotype, with only mild abnormality of globin chain synthesis. Therefore, it is not surprising that these mutations were identified in homozygotes with the typical beta-thalassemia carrier phenotype or in the compound heterozygote with the severe beta-thalassemia allele causing thalassemia intermedia **(34, 38)**. Silent beta-thalassemia alleles, beta-globin gene It is rare except for the C→T or C→G mutation at the -101 position **(36)**. Also, some "promoter" mutations located in the 5′ and 3′ UTRs are silent. Individuals with homozygous CAP +1 A→C allele, thalassemia carrier individuals have hematological values, whether it causes β-thalassemia by reducing beta-globin gene transcription or capping activity and mRNA translation **(34)**. However, the +33 C→G mutation, which is milder than mutations involving promoter elements, results in a reduction of β mRNA, which is 33% of normal β gene production. In beta globin gene, the sequence variation [TA]x[T]y at position -530 may be responsible for silent beta-thalassemia carriage **(38)**.

Beta-Thalassemia Carriers with Extremely High Hb A2

In beta-thalassemia, despite the wide heterogeneity of mutations, elevated Hb A2 levels observed in heterozygosity of different beta-thalassemia alleles in different ethnic groups are extremely uniform, usually between 3,5 and 5,5%, rarely exceeding 6% **(39)**. An unusually high level of Hb A2, over 6,5%, appears to characterize the subgroup of beta-thalassemias, formed by deletions in the beta- promoter that remove regulatory elements. The high level of Hb A2, which often accompanies a moderate increase in Hb F, may be associated with abolition of competition for the up LCR, which allows for increased interaction with the cis δ and γ genes **(40)**.

Beta-Thalassemias Due to The Insertion of Transpossible Elements

In human genome, transposible elements can occasionally disperse human genes and result in inactivation. Insertion of such an element into the LINES (long interspersed elements) repeat sequence family named L1 has been shown to cause the β^+-thalassemia phenomen. Despite 7,7 Kb insertion into IVS2, the affected gene expresses full-length

copies of beta-globin, corresponding to approximately 15% of normal mRNA **(41)**.

Genetic Modifiers of Beta-Thalassemia

Studies have revealed that approximately 1% of beta-thalassemias cannot be identified despite painstaking molecular analysis. Linkage studies show that the beta-thalassemia phenotype separates independently from the β-globin gene cluster, suggesting that genetic determinants are trans-acting **(42)**. In XPD ("Xeroderma Pigmentosum" group D), subunit of transcription factor TFI-III) have been found to be associated with a beta-thalassemia carrier phenotype, often supplemented by reduced beta-globin synthesis levels and reduced beta-globin mRNA **(43)**. It has also been reported that mutations of the transcription factor GATA 1, located on the X chromosome, cause beta-thalassemia **(44)**. The discovery of modifier genes such as Myb and especially BCL11A (B cell lymphoma 11A) in the recent past has contributed to our understanding of many variations in the clinical phenotype of beta-thalassemia **(45)**.

Somatic Deletion of Beta-Globin Gene

Somatic beta-globin gene deletion was recently described in an individual with moderate thalassemia intermedia, a normal genotype, and structurally heterozygous β0 thalassemia **(46)**. Subsequent studies have found that 50% contain the beta-globin gene and 50% contain any beta-globin gene. It was revealed by the identification of an individual with a somatic deletion of the chromosome 11p15 region, consisting of a mixture of beta-globin caused by mosaic cells, without the beta-globin gene. The sum of the beta-globin product is approximately 25% less than that of normally asymptomatic beta-thalassemia carriers. Subsequently, two unrelated Italian patients were shown to have thalassemia intermedia, which is caused by a somatic deletion of chromosome 11p15 in a subpopulation of hematopoietic cells. These unusual cases explain the severity of beta-thalassemia anemia, reflecting the quantitative deficiency of beta-globin chain production **(47)**.

Epigenetic Factors on Beta-Thalassemia

It remains to be determined that methylation patterns in the beta-globin cluster drive fetal to adult hemoglobin change. In a recent study, it was evaluated DNA methylation patterns of the beta-globin cluster from the peripheral blood of 105 β^0/β^0 thalassemia patients and 44 normal controls. In study, it was found that the CpG regions and gamma- and beta-globin promoters in DNase I hypersensitive regions 4 and 3 (HS4-3) in the locus control region (LCR) are particularly hypomethylated in β^0/β^0-thalassemia patientiens. Furthermore, hypomethylations of most of the CpG regions of the HS4-3 core regions were also observed in the bone marrow (BM) of β^0/β^0-patients compared to normal controls. The methylation level of the gamma-globin promoter -50 and +17 CpG regions was negatively correlated with the lower methylation level in patients with high HbF levels compared to those with low HbF levels, and with HbF level in patients with β0-thalassemia. Finally, the gamma-globin promoter +17 and +50 CpG regions also exhibited significant hypomethylation in cord blood (CB) tissues compared with BM tissues from normal controls. These findings revealed that methylation patterns associated with β^0 thalassemia disease and gamma-globin expression in the beta-globin cluster, contributed to the understanding of epigenetic modification in β^0 thalassemia patients, and provided candidate targets for treatments of beta-hemoglobinopathies **(48,49)**.

CONCLUSION

The genetic heterogeneity underlying the phenotypic diversity of beta-thalassemia is the first example of how the broad picture of a monogenic disorder in disease severity can occur at different levels (severity of anemia in the primary level, severity of anemia in the secondary level and severity of treatment-related complications). Although it seems that the elucidation of gene mutations that cause β^0 and β^+-thalassemias in such a genetic diversity seems to have been facilitated by the recently developed diagnostic tools, there are patients with the β-thalassemia phenotype whose molecular diagnosis could not be made. Despite of genetic mutations affecting the beta-globin gene, which cause a severe spectrum of phenotypes, have been tried to be described, studies on the genetics of the β-globin gene, other β-globin genes, βLCR region and trans-acting determinant (modifier) genes should be planned to understand and elucidate variant beta-thalassemias. Despite

the variety of screening methods developed with current DNA technology, identification of beta-thalassemia alleles is still inadequate. Both clinical and laboratory studies show us that there is a long way to go in solving and understanding a dynamic mechanism.

REFERENCES

1. Weatherall, DJ, Clegg, J.B. Inherited haemoglobin disorders: An 1. Increasing Global Health Problem. Bull World Health Organ. 2001; 79: 704-712.
2. Steiner, L.A., Gallagher, P.G. Erythrocyte disorders in the perinatal period. Semin Perinatol. 2007; 31: 254-261.
3. Tolentino, K., Friedman, J.F. An update on anemia in less developed countries. Am J Trop Med Hyg. 2007; 77: 44-51.
4. Keser, İ. Hemoglobinopathies in Türkiye and the World. Turkiye Klinikleri J Med Genet-Special Topics: 2017; 2(1): 1-6
5. Gibbons, R., Higgs, D.R., Olivieri, N.F., et al. The Beta Thalassaemias. In: Weatherall DJ, Clegg JB. Editors. The thalassaemia syndromes. 4th ed. Oxford: Blackwell Scientific Publications: 2001; 287-357.
6. Çürük, A., Kılınç, Y. Alfa talasemi genetiği. Turkiye Klinikleri J Med Genet-Special Topics: 2017; 2(1): 21-26.
7. Keser, I., Mercan, T.K., Bilgen, T., et al. Investigation of Alpha Globin Gene Mutations by Complementary Methods in Antalya. East J Med: 2021; 26(1): 117-122.
8. Tuli, A., Yenilmez, E.D.. Beta-talasemide genetik. Turkiye Klinikleri J Med Genet-Special Topics: 2017; 2(1): 7-14.
9. Keser, I., Sanlioglu, A.D., Manguoglu, E., et al. Molecular analysis of beta-thalassemia and sickle cell anemia in Antalya. Acta Haematol: 2004; 111: 205-210
10. Bilgen, T., Clark, Ö.A., Öztürk, Z., et al. Gap-PCR screening for common large deletional mutations of β-globin gene cluster revealed a higher prevalence of the Turkish Inversion/Deletion (δβ)0 mutation in Antalya. Turk J Hematol. 2016; 33: 107-111.
11. Altay, C. Abnormal hemoglobins in Türkiye. Turk J Haematol. 2002; 19(1): 63-74.
12. Çürük, M.A., Dimovski, A.J., Baysal, E, et al. Hb Adana or alpha 2(59) (E8)Gly->Asp beta 2, a severely unstable alpha 1-globin variant, observed in combination with the -(alpha)20.5 Kb alphathal-1 deletion in two Turkish patients. Am J Hematol: 1993; 44(4): 270-275.
13. Keser, I., Yesilipek, A., Canatan, D., et al. Abnormal hemoglobins aassociated with the beta globin gene in Antalya province, Türkiye. Turk J Med Sci. 2010; 40(1): 127-131.
14. Keser, I., Kayışlı, O.G., Yeşilipek, A., et al. Hb Antalya [codons 3-5 (Leu-Thr- Pro-->Ser-Asp-Ser)]: a new unstable variant leading to chronic microcytic anemia and high Hb A2. Hemoglobin. 2001; 25: 369-373.
15. Keser, I., Öztaş, A., Bilgen, T., et al. First observation of Hemoglobin Kansas [β102 (G4) Asn→ Thr, AAC> ACC] in the Turkish Population. Turk J Haematol. 2015; 32(4): 374-375.
16. Canatan, D., Bilgen, T., Çiftçi, V., et al. First observation of Hemoglobin G Waimanalo and Hemoglobin Fontainebleau cases in the Turkish Population. Turk J Haematol. 2016; 33(1), 71-72.
17. Uysal, A., Genc, A., Taşyurek, N., et al. Prevalence of β-thalassemia trait and abnormal hemoglobin in premarital screening in the Province of Izmir, Türkiye. Pediatr Hematol Oncol. 2013; 30, 46-50.
18. Shafiquea, F., Alic, S., Almansouri, T., et al.Thalassemia, a human blood disorder. Brazilian Journal of Biology. 2023; 83, 1-8.
19. Lee, J-S., Cho, S.I., Park, S.S., et al. Molecular basis and diagnosis of thalassemia. Blood Res. 2021; 56, S39-S43.
20. Sabath, D.E. Molecular diagnosis of thalassemias and hemoglobinopathies: an ACLPS critical review. Am J Clin Pathol: 2017; 148: 6-15.
21. Perumbeti, A. Pathobiology of Human Disease: A Dynamic Encyclopedia of Disease Mechanisms. Elsevier, Hemoglobinopathies and thalassemia syndromes. 2014: 1506–1531.
22. Kukreti S, Kaur H, Kaushik M, et al. Structural polymorphism at LCR and its role in beta-globin gene regulation. Biochimie. 2010;92(9):1199- 206.
23. Bulger M, Bender MA, van Doorninck, JH, et al. Comparative structural and functional analy- sis of the olfactory receptor genes flanking the human and mouse β-globin gene clusters. Proc Natl Acad Sci USA. 2000;97(26):14560-5.
24. Forget BG, Hardison R. The Normal Structure and Regulation of Human Globin Gene Clus- ters. In: Steinberg MH, Forget BG, Higgs DR, Weatherall DJ. eds. Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management. 2nd ed. Cambridge: Cambridge Uni- versity Press; 2009. p.46-61.
25. Giardine, B.M., Joly, P., Pissard, S., et al. Clinically relevant updates of the HbVar database of human hemoglobin variants and thalassemia mutations. Nucleic Acids Research: 49(D1). 2021; 1192-1196.
26. Thein SL. The molecular basis of β-tha- lassemia. Cold Spring Harb Perspect Med 2013;3(5): a011700.
27. Thein, S.L. Molecular basis of β thalassemia and potential therapeutic targets. Blood Cells Mol Dis: 2018; 70: 54–65.

28. Efremov, G.D. Dominantly Inherited Beta-Thalassemia. Hemoglobin. 2007; 31(2): 193-207.
29. Thein SL. Is it dominantly inherited β thalassaemia or just a β-chain variant that is highly unstable? Br J Haematol 1999;107(1):12-21.
30. Traeger-Synodinos J, Tzetis M, Kanavakis E, et al. The Corfu δβ-thalassaemia mutation in Greece: Haematological phenotype and prevalence. Br J Haematol 1991;79(2):302-5.
31. Loudianos G, Cao A, Ristaldi MS, et al. Molecular basis of β-thalassemia with normal fetal hemoglobin level. Blood 1990;75(2):526-8.
32. Trifillis P, Ioannou P, Schwartz E, et al. Identification of four novel delta-globin gene mutations in Greek Cypriots using polymerase chain reaction and automated fluorescence based DNA sequence analysis. Blood 1991;78(12):3298-305.
33. Olds RJ, Sura T, Jackson B, et al. A novel δ0 mutation in cis with Hb Knossos: a study of different genetic interactions in three Egyptian families. Br J Haematol 1991;78(3):430-6.
34. Wong C, Dowling CE, Saiki RK, et al. Characterization of β-thalassaemia mutations using direct genomic sequencing of amplified single copy DNA. Nature 1987;330(6146):384-6.
35. Gonzalez-Redondo JM, Stoming TA, Lanclos KD, et al. Clinical and genetic heterogeneity in black patients with homozygous β-thalassemia from the Southeastern United States. Blood 1988;72 (3):1007-14.
36. Gonzalez-Redondo JM, Stoming TA, Kutlar A, et al. A C→T substitution at nt -101 in a conserved DNA sequence of the promotor region of the beta-globin gene is associated with "silent" beta-thalassemia. Blood 1989;73(6):1705-11.
37. Ho PJ, Rochette J, Fisher CA, et al. Moderate reduction of β-globin gene transcript by a novel mutation in the 5′ untranslated region: A study of its interaction with other genotypes in two families. Blood 1996;87(3):1170-8.
38. Wong SC, Stoning TA, Efremov GD, et al. High frequencies of a rearrangement (+ATA; –T) at –530 to the β-globin gene in different populations indicate the absence of a correlation with a silent β-thalassemia determinant. Hemoglobin 1989;13(1):1-5.
39. Craig JE, Kelly SH, Barnetson R, et al. Molecular characterization of a novel 10.3 kb deletion causing β-thalassaemia with unusu- ally high Hb A2. Br. J. Haematol 1992;82(4): 735-44.
40. Thein SL, Barnetson R, Abdalla S. A β-tha- lassemia variant associated with unusually high Hb A2 in an Iranian family. Blood 1992;79 (10): 2801-3.
41. Lanikova L, Kucerova J, Indrak K, et al. β-Thalassemia due to intronic LINE-1 insertion in the β-globin gene (HBB): molecular mechanisms underlying reduced transcript levels of the β-glo- bin (L1) allele. Hum Mutat 2013;34(10): 1361-5.
42. Wheatherall DJ, Clegg JB. The thalassemia syndromes. 4th ed. Oxford: Blackwell Science; 2001. p.133-91.
43. Viprakasit V, Gibbons RJ, Broughton BC, et al. Mutations in the general transcription factor TFIIH result in b-thalassaemia in individuals with trichothiodystrophy. Hum Mol Genet 2001;10(24): 2797-802.
44. Yu C, Niakan KK, Matsushita M, et al. X- linked thrombocytopenia with thalassemia from a mutation in the amino finger of GATA- 1 affecting DNA binding rather than FOG-1 in- teraction. Blood 2002;100(6): 2040-5.
45. Cao A, Moi P, Galanello R. Recent advances in β-thalassemias. Pediatr Rep 2011;3(2):65- 78.
46. Badens C, Mattei MG, Imbert AM, et al. A novel mechanism for thalassaemia intermedia. Lancet 2002;359(9301):132-3.
47. Galanello R, Perseu L, Perra C, et al. Somatic deletion of the normal β-globin gene leading to thalassaemia intermedia in heterozygous β-thalassaemic patients. Br J Haematol 2004;127(5): 604-6.
48. Bao X, Zuo Y, Chen D, et al. DNA methylation patterns of β-globin cluster in β-thalassemia patients. Clin Epigenetic 2020; 12:187-94.
49. Deng W, Rupon JW, Krivega I, et al. Reactivation of developmentally silenced globin genes by forced chromatin looping. J Cell. 2014;158:849–60.

Correspondence Address:
Prof. Dr. İbrahim Keser
Akdeniz University, Faculty of Medicine, Department of Medical Biology and Genetics, 07079, Konyaaltı, Antalya, Türkiye.
E-mail: ibrahimkeser44@gmail.com, keser@akdeniz.edu.tr

GENETICS OF STRUCTURAL HEMOGLOBINOPATHIES

Esra Isik[1], Ferda Ozkinay[1]
[1]Subdivision of Pediatric Genetics, Department of Pediatrics, Faculty of Medicine, Ege University, Izmir, Türkiye

ABSTRACT

The structure and to functionality of the hemoglobin molecule depend on variations in the genes that encode the globin chains. To date, more than 3,000 variants have been identified in these genes. Some of these genetic alterations can result in quantitative abnormalities in hemoglobin structure, while others result in the formation of structural (qualitative) hemoglobin variants. Mutations leading to structural hemoglobin variants can affect changes in the hemoglobin molecule's structure, affecting the oxygen-carrying capacity of erythrocytes and overall cellular resilience by altering the structure of hemoglobin molecule. These variants, often arising from single-point mutations causing amino acid changes, can result in various abnormalities in the molecule such as instability in the tetramer, deformation of the three-dimensional structure, and other molecular irregularities.

From a clinical perspective, while some abnormal hemoglobin variants remain asymptomatic, others manifest with distinct clinical symptoms. Among these variants, there are diverse classifications, such as sickle cell anemia, hemoglobins with reduced stability, and hemoglobinopathies with altered oxygen affinities. In this section, we will provide a detailed review of the genetic characteristics of structural hemoglobin variants.

Keywords: Genetics, structural, hemoglobinopathy

INTRODUCTION

To date, more than 3000 variants have been reported in globin genes. Some mutations lead to quantitative abnormalities in chain synthesis, while others result in the emergence of different structural forms of hemoglobin (Hb) variants. Mutations that cause structural hemoglobin variants have potential to modify the structure of the Hb molecule, affecting the oxygen-carrying capacity and/or cellular durability of erythrocytes. Structural hemoglobinopathies typically result from single point mutations in globin genes, leading to amino acid changes that can manifest in a range of abnormal physical properties **(1)**. These properties may include (i) tetramer instability; (ii) deformation of the three-dimensional structure; (iii) inhibition of ferrous iron reduction; (iv) alteration of residues interacting with heme, 2, 3-DPG, or the α-β subunit contact region, or (v) other abnormalities in the molecule's characteristics.

Structural hemoglobin variants can exhibit either recessive or dominant inheritance patterns. Some unstable hemoglobin variants typically manifest clinical symptoms in a monoallelic state. While de nova occurrence is predominant, dominant inheritance is also possible. Abnormal hemoglobin variants such as HbS, C, D, and E present clinical symptoms in a biallelic state and are inherited through autosomal recessive transmission **(2)**.

The clinical presentation of hemoglobinopathies varies. Some abnormal hemoglobin variants are clinically silent and are detected during population screenings. Others may be associated with a range of clinical symptoms that can vary from mild to severe.

The qualitative abnormalities caused by structural variants in globin chains are classified as follows **(3)**:

Table 1: *Classification of Hemoglobinopathies*

A. Sickle Cell Anemia
SA, Sickle Cell Trait
SS, Sickle Cell Disease
SC, HbSC Disease
S/β-Thalassemia
S/Other Hb Variants (D, O-Arab., and others; SF, HbS/HPFH)

B. Unstable Hemoglobins
   Congenital hemolitic anemias leading to Heinz body formation
   Acquired ones, e.g., due to drug-induced hemolysis (G6PD deficiency)
C. Structural Hemoglobinopathies with Altered Oxygen Affinity
   Structural Hemoglobinopathies with high oxygen Affinity
   Structural Hemoglobinopathies with low oxygen Affinity
D. Structural Hemoglobinopathies Leading to Methemoglobinemia
E. Posttranslational Modifications

In this section, the genetic characteristics of structural hemoglobin variants will be discussed in detail. Since phenotypic features will be discussed in other sections, a brief mention will be made here about how genetic factors influence the phenotype.

SICKLE CELL DISEASE

Sickle cell disease (SCD) is a disorder resulting from an abnormal Hb molecule, HbS, due to a nucleotide change in the beta globin gene located on chromosome 11. HbS typically leads to a change in the structural shape of normally flexible and biconcave erythrocytes, causing them to assume a sickle shape. These alterations make individuals with sickle cell disease (SCD) prone to microvascular occlusions, circulatory issues, and acute painful crises **(4)**.

Sickle cell disease is one of the most common genetic disorders worldwide, and its prevalence often varies based on ethnic and geographical factors. There are approximately 3 million individuals with sickle cell anemia globally, with the majority of cases being distributed in Africa, the Middle East, and India **(4, 5)**.

The variant c.20T>A in the beta globin (HBB) gene at the 6th codon leads to the substitution of glutamic acid with valine (p.Glu6Val, E6V), resulting in the formation of the HbS variant. HbS is the most common structural hemoglobin variant. HbS polymerizes under conditions of low oxygen concentration or dehydration, causing sickling of erythrocytes. These sickled erythrocytes lose their flexibility and deformability, becoming susceptible to hemolysis.

Sickle cell disease shows an autosomal recessive inheritance pattern. Heterozygous individuals with one allele for HbA and one allele for HbS (HbA/HbS) are healthy carriers. The presence of different variants in biallelic form in the HBB gene, with at least one allele having the Glu6Val variant, may lead to the manifestation of sickle cell anemia symptoms. Among different genotypes, the most common (approximately 70%) is HbS homozygosity (HbSS). Genotypes in which HbS is in compound heterozygosity with other structural hemoglobins or beta thalassemia variants can also occur **(5)**.

In sickle cell disease, the severity of clinical symptoms is influenced by various genetic factors **(6, 7)**. The most important among these is the HbSS genotype, which leads to the sickle cell anemia phenotype. HbS homozygosity is associated with severe clinical symptoms. When combined with other Hb variants, the clinical characteristics may vary; it can result in symptoms similar to HbSS with severe manifestations or present with milder symptoms.

Compound heterozygosity of HbS allele with HbC, HbD-Punjab, or β-thalassemia alleles, the clinical symptoms are generally similar to those seen in homozygous HbS individuals, although they may be milder **(6)**. HbS/β-thalassemia is more commonly observed in India, South Europe, and the Middle East. The type of β-thalassemia variant determines the severity of clinical manifestations. In the presence of β^0 variants, a severe clinical presentation similar to HbSS is expected, while in the presence of β^+ variants, milder clinical symptoms are encountered. However, certain severe β^+ variants such as c.92+5G>C [IVS-I-5 (G>C)] or c.93-21G>A (IVS-I-110 [G>A]) are still associated with a severe clinical profile of sickle cell anemia.

In the β-globin gene cluster, different haplotypes (Bantu, Cameroon, Benin, Senegal, and Arab-Indian) have been identified, which have an impact on the severity of sickle cell anemia clinical symptoms. The frequency of these haplotypes varies by geographical region. Recent genetic studies have demonstrated that these haplotypes share a single ancestral origin **(8)**. These haplotypes represent polymorphic variants in the β-globin gene cluster and influence the phenotypic expression and severity of sickle cell anemia by affecting the levels of HbF (fetal hemoglobin) in the blood. HbF levels are lowest in individuals with the Bantu haplotype and

increase sequentially in those with the Cameroon, Benin, Senegal, and Arab-Indian haplotypes.

HbF levels play a crucial role in the severity of the disease and are directly related to their ability to inhibit the polymerization of deoxygenated HbS. In individuals with SCD, HbF levels range from 3% to 25% (with an average of 7%). Chronic erythropoietic stress induces an increase in HbF **(9)**. The presence of HbF reduces HbS concentration and prevents Hb polymerization when γ-globin chains are incorporated into the Hb tetramer. Molecular factors that increase HbF levels contribute to the alleviation of the clinical symptoms of sickle cell anemia. Polymorphisms associated with increased HbF levels include Xmn1-HBF2 (rs782144) within the β-globin gene cluster on the 11th chromosome (11p), BCL11A (rs1427407 and rs6545816) located on 2p16, and the intergenic HBS1L-MYB (HMIP) region on 6q23 (rs66650371).

The co-occurrence SCD and α-thalassemia is associated with a milder phenotype and has been reported to significantly reduce the risk of acute splenic sequestration, decrease the frequency of painful crises, lower the risk of osteonecrosis, and generally improve survival rates. SCD mutations in combination with α-thalassemia result in lower hemoglobin concentrations. This reduced Hb concentration reduces the likelihood of Hb polymerization. Decreased Hb polymerization and less sickling minimize hemolysis, thereby alleviating clinical symptoms. However, in SCD patients with two α gene deletions, it has been observed that pain does not decrease and may even increase. Additionally, these patients have an increased risk of avascular necrosis and splenic sequestration **(10-12)**. The co-occurrence of α-thalassemia alleles with HbSC, the clinical picture also tends to be milder.

There are several HBB variants, distinct from HbS, that result in a sickle hemoglobin (HbS) phenotype, with approximately 14 different hemoglobins having been described in this manner. These hemoglobins include HbC-Harlem, HbC-Ziguinchor, HbS-Travis, HbS-Antilles, HbS-Providence, HbS-Oman, HbS-Cameroon, HbS-South End, Hb Jamaica Plain, HbC-Ndjamena, HbS-Clichy, HbS-San Martin, HbS-Wake, and HbS-São Paulo **(13)**. These hemoglobinopathies result from different point mutations in the HBB gene, most of which manifest as the sickle hemoglobin phenotype when in a homozygous state or in combination with other hemoglobinopathy alleles, a few of them are inherited dominantly.

Variants in genes other than globin genes can also influence the severity of complications in individuals with sickle cell disease (SCD). Some studies have suggested an increased risk of cerebrovascular complications in individuals carrying *G6PD* gene mutations, while others have failed to confirm this relationship **(14-16)**. Additionally, it has been shown that *APOL1* variants may have an effect on renal complications, and *UGT1A* variants may be influential in cholelithiasis **(6)**.

HbC

Hemoglobin C (HbC) results from a mutation in the beta-globin gene, specifically at the 6^{th} codon, where glutamic acid is replaced by lysine. The HbC mutation leads to a decrease in the solubility of both oxygenated and deoxygenated hemoglobin, resulting in the formation of crystals instead of long polymers **(2)**.

Hemoglobin C variant (HbC) is more commonly reported in West Africa. HbC homozygosity is associated with mild hemolytic anemia and splenomegaly, and vaso-occlusive complications due to sickling are not expected to occur. However, when HbC is in compound heterozygosity with HbS (HbS/C), the clinical presentation of sickle cell anemia is anticipated. Nevertheless, it is associated with a milder clinical picture compared to HbSS, and complications are expected to be less frequent and severe. Vascular retinopathy, avascular necrosis, renal medullary avascular necrosis, and renal medullary microvascular thrombosis have been reported in HbS/C patients **(17)**. In the case of HbC/β-thalassemia compound heterozygosity, the clinical presentation can vary from mild anemia to severe anemia. It has been reported that the clinical picture is more severe in individuals carrying the β^0 allele and in cases where HbA2 levels are low.

HbD-Punjab (Hb Los Angeles)

HbD-Punjab results from a mutation in the β-globin gene at codon 121, causing a change from glutamic acid to glutamine (GAA>CAA). It is the most common mutation in India and Pakistan. Homozygotes typically exhibit very mild symptoms, and

hemoglobin D accounts for over 90% on electrophoresis. When in compound heterozygosity with β-thalassemia alleles, mild anemia is often present. In cases of compound heterozygosity with HbS, a severe clinical presentation similar to HbSS disease can occur. When combined with HBE, normal or slightly reduced hemoglobin levels are observed, while with HbC, a moderate sickle cell disease-like presentation has been observed **(18, 19)**.

Following HbS and HbC, HbD-Punjab is one of the most common hemoglobin variants worldwide. In a haplotype study encompassing 7 polymorphic regions in the β-globin gene, Atalay et al. identified three different haplotypes in HbD-Punjab cases (Mediterranean haplotype, Thai haplotype, and Turkish haplotype) **(20)**.

HbE

HbE is the second most common hemoglobinopathy in the world and is particularly prevalent in East Asia **(2)**. It is caused by a base substitution at codon 26 of the β-globin gene, GAG>AAG, which results in the substitution of lysine for glutamic acid. The mutation activates a cryptic splice site. As a result of competition between normal and cryptic splice site, βE-mRNA production decreases, leading to the emergence of the mild form of β-thalassemia phenotype **(21)**. Homozygotes exhibit a mild phenotype similar to thalassemia carriers, while in compound heterozygosity with other β-thalassemia alleles, a moderate or severe thalassemia phenotype is observed. Compound heterozygosity with HbS (HbS/HbE) is generally asymptomatic, or rarely, mild clinical symptoms may occur. In this case, there is usually no need for treatment, and there is no indication for prenatal diagnosis.

HbE heterozygotes, when coexisting with α+ thalassemia (-α/αα), typically have HbE levels at around 19-21%, and they exhibit mild thalassemic red blood cell changes. However, when HbE is combined with HbH genotype (-/-α), HBE is significantly reduced to approximately 13-15%, resulting in a moderate thalassemic presentation known as "HbAE Bart's disease." In HbE homozygotes and HbE/β thalassemia compound heterozygotes who also carry the HbH genotype (-/-α), moderate anemia (thalassemia intermedia) is observed, along with elevated levels of HbF and HbBart's. This condition is referred to as "EFBart's disease" **(22)**.

Hemoglobin Lepore

Hb-Lepore, a hybrid protein, is formed through unequal crossing over between the homologous chromosomes of the δ-globin and β-globin genes, leading to the creation of a hybrid gene.Three different Hb Lepore variants have been described as a result of crossover at different points: These are Hb Lepore Washington Boston (δ87/β116), Hb Lepore Hollandia (δ22/β50), and Hb Lepore Baltimore (δ50/β86). Hb Lepore Washington Boston (δ87/β116) is the most common **(23, 24)**. In patients with homozygous Hb Lepore, a thalassemia major or intermedia phenotype is observed. When there is compound heterozygosity with β-thalassemia alleles, a thalassemia major phenotype can ocur. In the context of compound heterozygosity with HbS (HbS/ Hb Lepore), the clinical presentation is heterogeneous.

Unstable Hemoglobin Variants

Specific mutations in globin genes can lead to the formation of unstable hemoglobins. These mutations have the potential to modify the primary, tertiary, or tetrameric structure of the globin protein ultimately leading to the formation of an unstable hemoglobin tetramer. Unstable hemoglobin variants undergo rapid denaturation, precipitation, and degradation within erythrocytes. The precipitation within erythrocytes results in the development of dense, globular aggregations recognized as Heinz bodies. These bodies are identifiable through specialized staining techniques. The accumulated structures impede the normal function of erythrocytes, ultimately leading to a shortened erythrocyte lifespan. This condition is associated with symptoms referred to as congenital Heinz body hemolytic anemia syndrome **(2)**.

In the analysis of unstable Hbs, electrophoresis, especially if not performed rapidly after sample collection, may result in a relatively normal hemoglobin electrophoresis pattern due to rapid denaturation. Extremely unstable hemoglobins, on the other hand, cannot be demonstrated using electrophoretic methods because they break down more quickly. Molecular genetic analyses are used in the diagnosis of these extremely unstable hemoglobins **(25)**.

Unstable Hb variants may be associated with mutations in both beta and alpha globin genes, but vari-

ants in the beta globin gene are more commonly observed and are often associated with a more severe clinical picture. Numerous unstable Hb variants have been reported today, with Hb Köln [ß98 (Val->Met) (GTG->ATG)] being the most common among them (2). Examples of unstable hemoglobin resulting from defects in the beta globin chain include Hb Brockton (β138 [H16) Ala > Pro), Hb Philly (β35 [C1) Tyr > Phe), Hb Peterborough (β111 [G13) Val > Phe), Hb Stanmore (β111 [G13) Val > Ala), Hb J-Guantanamo (β128 [H6) Ala > Asp), Hb Khartoum (β124 [H2) Pro > Arg).

Examples of unstable hemoglobins resulting from defects in the alpha globin chain include Prato (α1 or α2 31 [B12) Arg > Ser)), Lombard (α2 103 [G10) His > Tyr), Contaldo (α1 or α2 103 [G10) His > Arg)), Foggia (α2 117 [GH5) Phe > Ser)), Groene Hart (α1 119 [H2) Pro > Ser) **(26-28)**.

Unstable Hb variants are typically identified as heterozygotes and commonly occur de novo. Rarely, autosomal dominant inheritance has also been reported. These variants can lead to a severe hemolytic anemia presentation during childhood and may exhibit a thalassemia phenotype.

Altered Oxygen Affinity Hemoglobinopathies

Around 25 hemoglobinopathies have been described that exhibit high oxygen affinity and lead to erythrocytosis. In this group of hemoglobinopathies, mechanisms that maintain the balance between high oxygen affinity (R state) and low oxygen affinity (T state) have been disrupted due to amino acid changes. The most crucial mechanism maintaining this balance is the α1β2 interaction. Structural changes in these globin chains cause a shift toward the R form, which inhibits the release of oxygen. Examples include Hb Kempsey (β99 [G1) Asp > Asn), Hb Hiroshima (β146 [HC3) His > Asp), and Hb Rahere (β82 [EF6) Lys > Thr).

In Hb variants with low oxygen affinity, the clinical presentation typically involves cyanosis and low oxygen saturation. There is a shift from the R state to the T state in these types of hemoglobins. Individuals carrying these hemoglobins are generally healthy apart from changes in skin color. Examples include Hb Kansas (β102 [G4) Asn > Thr), Hb Beth Israel (β102 [G4) Asn > Ser), and Hb St. Mandé (β102 [G4) Asn > Tyr) **(3, 29)**.

Methemoglobin

Methemoglobins result from variants in methemoglobin reductase enzymes or variants in the globin chain. Iron molecules must be in a reduced state (Fe2+) to effectively bind oxygen. These variants cause the oxidized form Fe3+ to remain stable within the hem molecule. Hemoglobin variants that lead to methemoglobin formation arise from the replacement of two amino acids in the globin chains with tyrosine. This change can take place in α-, β-, or γ-globin subunits (α58 His → Tyr, α87 His → Tyr, β63 His → Tyr, β92 His → Tyr, γ63 His → Tyr, and γ92 His → Tyr). Individuals with these variants are generally normal, except for changes in skin color **(3, 29)**.

Variants causing elongation of globin chains

Mutations causing frame shifts and resulting in the addition of amino acids in the globin chains can lead to the formation of abnormal hemoglobins, leading to damage in red blood cells. The most well-known among these hemoglobins is Hb Constant Spring (α2 142 [HC3) Stop > Gln). This mutation leads to a 31-amino acid elongation of the α2 chain and the formation of an unstable protein. In addition to causing severe HbH disease in the condition of compound heterozygosity with deletional α alleles ((–/αCSα)), this abnormal hemoglobin allele, in isolation as a heterozygote (αα/αCSα) or in homozygosity (αCSα/αCSα), results in a more severe anemia compared to genotypes carrying deletional genes (αα/−α or −α/−α) with the same genes **(30)**.

REFERENCES

1. Williamson D. The unstable haemoglobins. Blood Rev. 1993;7(3):146-63.
2. Old JM, editor Chapter 71 – Hemoglobinopathies and Thalassemias2013.
3. Forget BG, Bunn HF. Classification of the disorders of hemoglobin. Cold Spring Harb Perspect Med. 2013;3(2):a011684.
4. Elendu C, Amaechi DC, Alakwe-Ojimba CE, et al. Understanding Sickle cell disease: Causes, symp-

toms, and treatment options. Medicine (Baltimore). 2023;102(38):e35237.
5. Hardouin G, Magrin E, Corsia A, et al. Sickle Cell Disease: From Genetics to Curative Approaches. Annu Rev Genomics Hum Genet. 2023;24:255-75.
6. Rees DC, Brousse VAM, Brewin JN. Determinants of severity in sickle cell disease. Blood Rev. 2022;56:100983.
7. Menzel S, Thein SL. Genetic Modifiers of Fetal Haemoglobin in Sickle Cell Disease. Mol Diagn Ther. 2019;23(2):235-44.
8. Shriner D, Rotimi CN. Whole-Genome-Sequence-Based Haplotypes Reveal Single Origin of the Sickle Allele during the Holocene Wet Phase. Am J Hum Genet. 2018;102(4):547-56.
9. Stamatoyannopoulos G, Veith R, Galanello R, et al. Hb F production in stressed erythropoiesis: observations and kinetic models. Ann N Y Acad Sci. 1985;445:188-97.
10. Ballas SK, Talacki CA, Rao VM, et al. The prevalence of avascular necrosis in sickle cell anemia: correlation with alpha-thalassemia. Hemoglobin. 1989;13(7-8):649-55.
11. De Ceulaer K, Serjeant GR. Acute splenic sequestration in Jamaican adults with homozygous sickle cell disease: a role of alpha thalassaemia. Br J Haematol. 1991;77(4):563-4.
12. Milner PF, Kraus AP, Sebes JI, et al. Sickle cell disease as a cause of osteonecrosis of the femoral head. N Engl J Med. 1991;325(21):1476-81.
13. Ahmed SG, Ibrahim UA. Non-S Sickling Hemoglobin Variants: Historical, Genetic, Diagnostic, and Clinical Perspectives. Oman Med J. 2021;36(3):e261.
14. Benkerrou M, Alberti C, Couque N, et al. Impact of glucose-6-phosphate dehydrogenase deficiency on sickle cell anaemia expression in infancy and early childhood: a prospective study. Br J Haematol. 2013;163(5):646-54.
15. Joly P, Garnier N, Kebaili K, et al. G6PD deficiency and absence of alpha-thalassemia increase the risk for cerebral vasculopathy in children with sickle cell anemia. Eur J Haematol. 2016;96(4):404-8.
16. Bernaudin F, Verlhac S, Chevret S, et al. G6PD deficiency, absence of alpha-thalassemia, and hemolytic rate at baseline are significant independent risk factors for abnormally high cerebral velocities in patients with sickle cell anemia. Blood. 2008; 112(10):4314-7.
17. Bissonnette ML, Henriksen KJ, Delaney K, et al. Medullary Microvascular Thrombosis and Injury in Sickle Hemoglobin C Disease. J Am Soc Nephrol. 2016;27(5):1300-4.
18. Edison ES, Shaji RV, Chandy M, et al. Interaction of hemoglobin E with other abnormal hemoglobins. Acta Haematol. 2011;126(4):246-8.
19. Hirani S, Gupta S, Veillon D, et al. The curious case of hemoglobin DC disease masquerading as sickle cell anemia. Hematol Rep. 2020;12(1):8519.
20. Atalay EO, Atalay A, Ustel E, et al. Genetic origin of Hb D-Los Angeles [beta121(GH4)Glu-->Gln, GAA-->CAA] according to the beta-globin gene cluster haplotypes. Hemoglobin. 2007;31(3):387-91.
21. Altay C. Abnormal Hemoglobins in Türkiye. Turk J Haematol. 2002;19(1):63-74.
22. Fucharoen S, Weatherall DJ. The hemoglobin E thalassemias. Cold Spring Harb Perspect Med. 2012;2(8).
23. Shaji RV, Edison ES, Krishnamoorthy R, et al. Hb Lepore in the Indian population. Hemoglobin. 2003;27(1):7-14.
24. Ribeiro ML, Cunha E, Goncalves P, et al. Hb Lepore-Baltimore (delta 68Leu-beta 84Thr) and Hb Lepore-Washington-Boston (delta 87Gln-beta IVS-II-8) in central Portugal and Spanish Alta Extremadura. Hum Genet. 1997;99(5):669-73.
25. Rizzuto V, Koopmann TT, Blanco-Alvarez A, et al. Usefulness of NGS for Diagnosis of Dominant Beta-Thalassemia and Unstable Hemoglobinopathies in Five Clinical Cases. Front Physiol. 2021;12:628236.
26. Lacerra G, Scarano C, Musollino G, et al. Hb Foggia or alpha 117(GH5)Phe -> Ser: a new alpha 2 globin allele affecting the alpha Hb-AHSP interaction. Haematologica. 2008;93(1):141-2.
27. Marinucci M, Mavilio F, Massa A, et al. A new abnormal human hemoglobin: Hb Prato (alpha 2 31 (B12) Arg leads to Ser beta 2). Biochim Biophys Acta. 1979;578(2):534-40.
28. Sciarratta GV, Ivaldi G, Molaro GL, et al. The characterization of hemoglobin Manitoba or alpha (2)102(G9)Ser----Arg beta 2 and hemoglobin Contaldo or alpha (2)103(G10)His----Arg beta 2 by high performance liquid chromatography. Hemoglobin. 1984;8(2):169-81.
29. Thom CS, Dickson CF, Gell DA, et al. Hemoglobin variants: biochemical properties and clinical correlates. Cold Spring Harb Perspect Med. 2013; 3(3):a011858.
30. Schrier SL, Bunyaratvej A, Khuhapinant A, et al. The unusual pathobiology of hemoglobin constant spring red blood cells. Blood. 1997;89(5):1762-9.

Correspondence Address:
Prof. Dr. Ferda Ozkinay
Subdivision of Pediatric Genetics, Department of Pediatrics, Faculty of Medicine, Ege University, Izmir, Türkiye
E-mail: f.ferda.ozkinay@ege.edu.tr

GENOTYPE AND PHENOTYPE IN THALASSEMIA

Prof. Dr. Sule Unal Cangul
Hacettepe University, Department of Pediatric Hematology, Ankara, Türkiye

ABSTRACT

Beta thallasemias develop usually due to point mutations in beta globin gene (HBB); whereas alpha thallasemias usually develop related to deletional mutations in alpha globin gene (HBA). However, the clinical severity of the disease in patients with beta thallasemia may vary from mild to severe anemia, indicating genetic modifiers of the disease. Phenotypic severity of beta thalassemia patients is mainly related to the severity of imbalance between α- and non-α globin chain synthesis. Therefore one of the primary modifiers of β-thalassemia severity is the type of β allele inherited (i.e., β^0, β^+, β^{++}). Addionally, accompanying alpha globin gene deletions decrease the severity of the phenotype. Patients with polymorphisms in the HbF modifying genes have been reported to cause a change in disease severity. The disease modifiers are important to know especially for the decision of prenatal diagnosis.

Keywords: genotype, phenotype, thalassemia

BETA THALASSEMIA

Although, generally the homozygous or compound heterozygous point mutations are responsible from the disease state in beta thallasemia; there are various conditions that end up with different phenotypes. Over 350 mutations have been related with beta thalasemias **(1)**. Even within the same family, siblings may have different phenotypes ranging from non-transfusion dependent thallasemi (NTDT) to transfusion dependent thallasemia (TDT). Knowledge about the disease modifying factors in these patients is important to treat the patients appropriately, in addition to accurate prenatal diagnosis of the fetus.

Stop codon or null mutations in β globin gene, result in no β-globin production, causing β^0-thalassemia. Other mutations allowing the production of some amount of β globin production are classified as β^+ or β^{++} thalassemia. When β-globin production is low, this results in accumulation of excess α-globin chains. Phenotypic severity of beta thalassemia patients is mainly related to the severity of imbalance between α- and non-α globin chain synthesis. Therefore, one of the primary modifiers of β-thalassemia severity is the type of β allele inherited (i.e., β^0, β^+, β^{++}). Mutations that involve the promoter regulatory elements such as −101 (C → T), −101 (C → G) or −88 (C → T) are called silent mutations causing β^{++} thalassemia. The carriers of these mutations have normal RBC indices in hemogram and normal HBA2 levels **(2)**. Additionally, the mutations in the 5′ UTR region of the gene also causes silent mutations, such as CAP +1 A→C **(3)**. The homozygous conditions of these silent mutation usually end-up with mild phenotypes and usually with NTDT phenotypes. Ethnic variation exists in phenotypes of patients with homozygotes of the −29 A→G (HBB:c.-79A→G) mutation and patients from Africa have mild disease **(4)** whereas those from Chinese ethnicity develop TDT, possibly related to accompanying *Xmn1* polymorphisms **(5)**. Initiation codon mutations, non-sense mutations or frameshift mutations such as Cd 8/9 +G cause β^0-thalassemia with homozygous states usually ending-up with TDT.

As mentioned earlier, the main determinant of the phenotype is the imbalance between α- and β-globin chain synthesis. Associated α-globin gene deletional mutations with two defective β-globin alleles cause the clinical phenotype to be less severe. This is more prominent in patients who have 2 deletions in α globin genes and biallelic β^+ mutations.

On the contrary, the patients who have a carrier status for β-Thalassemia, and co inheritance of in-

creased α-globin genes due to unequal cross-over during meiosis, such as ααα/αα, ααα/ααα, αααα/αα, αααα/ αααα; will have more severe phenotype despite being a carrier for beta thallasemia, usually ending up with NTDT phenotype **(6-8)**.

Increased HbF due to Xmn1Gγ polymorphisms or trans-acting quantitative trait loci (QTLs) for HbF on Xp22.2-p22.3, 6q23, 8q, and 2p15 may also decline the severity of the phenotype in these patients **(9-11)**. In a recent paper by Jiang et al, 22 patients' homozygous codon 8 frameshift mutations were analyzed and of these 4 had NTDT phenotype; whereas 18 were transfusion dependent and having HMIP on chromosome 6q23 rs66650371 or 3-bp deletion was found to have milder phenotype in the study **(12)**.

A rare form of beta thalassemia is due to dominant inheritance. The family history is usually revealing the diagnosis; however, de novo mutations are not uncommon. The patients with dominant beta thalassemia usually have mild to moderate phenotype.

In a study by Ho et al, 87 patients with NTDT phenotype were molecularly evaluated and the results revealed that 22 had heterozygous mutation in *HBB* gene; whereas 65 had biallelic mutations in *HBB* gene; indicating that by knowing only the *HBB* gene mutation; it is difficult to make an estimation in disease severity **(13)**.

There are several other genetic modifiers of disease severity in patients with beta thalassemia. Associated C282Y mutation in *HFE* gene have been reported to be associated with increased iron accumulation **(14)**. Associated Gilbert's syndrome have also been linked to higher indirect hyperbilirubinemia and cholelithiasis risk in patients with thalassemia **(9, 10)**.

There are other mechanisms which may confuse the prediction of phenotype, such as post-zygomatic mosaicism that have been rarely reported **(15)**.

The clinical severity of E/Beta thallasemias may vary from asymptomatic to TDT depending on the beta globin gene mutation type.

ALPHA THALASSEMIA

Alpha thallasemias usually develop related to deletional mutations and Hb H disease is usually related to deletion of 3 out of 4 alpha globin genes (--/-α). This form of deletional HbH disease may cause mild, moderate to severe phenotype. However, non-deletional mutations may also cause HbH disease (--/$\alpha^T\alpha$) and this form of HbH disease may cause moderate to severe phenotype. Those non-deletional alpha variants include Hb Constant Spring, Hb H Pakse, Hb Quang Sze and Hb Suan Dok **(16)**.

REFERENCES

1. http://globin.cse.psu.edu.
2. Gonzalez-Redondo JM, Stoming TA, Kutlar A, Kutlar F, Lanclos KD, Howard EF, et al. (1989). A C → T substitution at nt −101 in a conserved DNA sequence of the promoter region of the β-globin gene is associated with "silent" β-thalassemia. Blood 73:1705–1711.
3. Wong C, Dowling CE, Saiki RK, Higuchi RG, Erlich HA, Kazazian HH. Characterization of beta-thalassaemia mutations using direct genomic sequencing of ampli- fied single copy DNA. Nature 1987*;*330:384-6.
4. Safaya S, Rieder RF, Dowling CE, Kazazian HHJ, Adams JGI. Homozygous β-thalassemia without anemia. Blood 1989*;*73:324–328.
5. Huang SZ, Wong C, Antonarakis SE, Ro-Lein T, Lo WHY, Kazazian HHJ. The same TATA box β-thalassemia mutation in Chinese and US Blacks: Another example of independent origins of mutation. Hum Genet 1986; 74:162–164.
6. Fucharoen S. Genotypes and Phenotypes of Thalassemia: A Discussion Ann NY Acad Sci. 2005;1054:518-521.
7. Thein SL. Genetic modifiers of β -thalassemia. Hematologica. 2005;90:649-660.
8. Koçak Ü. Hemoglobinopatilerde genotip fenotip ilişkisi. In: Hematolog, Hemoglobinopatiler. Eds: Soysal T, Gurgey A. Türk Hematoloji Derneği, 2014.
9. Rund D, Fucharoen S. Genetic modifiers in hemoglobinopathies. Curr Mol Med. 2008;8:600-608.
10. Thein SL. Pathophysiology of β thalassemia-A guide to molecular therapies. Hematology Am Soc Hematol Educ Program. 2005;31-37.
11. Thein SL. Genetic modifiers of the beta-haemoglobinopathies. Br J Haematol. 2008;141:357-366.
12. Jiang Z, Luo HY, Huang S, Farrell JJ, Davis L, Théberge R, Benson KA, Riolueang S, Viprakasit V, Al-Allawi NA, Ünal S, Gümrük F, Akar N, Başak AN, Osorio L, Badens C, Pissard S, Joly P, Campbell AD, Gallagher PG, Steinberg MH, Forget BG, Chui DH. The genetic basis of asymptomatic codon 8 frameshift (HBB:c25_26delAA) β(0) -thalassaemia homozygotes. Br J Haematol. 2016;172:958-65.

13. Ho PJ, Hall GW, Luo LY, Weatherall DJ, Thein SL. Beta-thalassaemia intermedia: is it possible consistently to predict phenotype from genotype? Br J Haematol. 1998;100:70-8.
14. Piperno A, Mariani R, Arosio C, et al. Haemochromatosis in patients with betathalassemia trait. Br J Haematol. 2000;111:908-914.
15. Badens C, Mattei MG, Imbert AM, Lapouméroulie C, Martini N, Michel G, Lena-Russo D. A novel mechanism for thalassaemia intermedia. Lancet. 2002;359:132-3.
16. Lal A, Vichinsky E. The Clinical Phenotypes of Alpha Thalassemia. Hematol Oncol Clin North Am. 2023;37:327-339.

Correspondence Address:
Prof. Dr. Sule Unal Cangul
Hacettepe University, Department of Pediatric Hematology, Ankara, Türkiye
E-mail: suleunal@hacettepe.edu.tr

CHAPTER 3

CLINIC OF THALASSEMIA AND HEMOGLOBINOPATHIES

CLINIC OF ALPHA THALASSEMIA SYNDROMES

Assoc Prof. Dr. Yesim Oymak
SBU. Dr. Behçet Uz Çocuk Hastalıkları ve Cerrahisi Eğitim ve Araştırma Hastanesi
İzmir-Türkiye

ABSTRACT

Alpha thalassemias are characterised by abnormalities in the production of α globin chains in the haemoglobin molecule. This genetic disorder is prevalent in populations of African, Southeast Asian, and Mediterranean origin. The severity of the disease depends on genetic variants and deletions of α-globin genes. Alpha thalassaemias are divided into two main groups: α+-thalassaemia (deletion or inactivation of one gene pair) and α0-thalassaemia (deletion of both gene pairs).

Alpha-thalassemia carriers (presence of 2 α genes) are usually asymptomatic but can sometimes be mildly anemic. Mutations in a single alpha gene are called silent carriers. Alpha thalassemia can lead to fatal Hb Bart's hydrops foetalis syndrome (β globin tetramers precipitate in red blood cells when no α gene is present) in foetuses in the prenatal period. The clinical picture known as HbH (presence of an α gene) is characterized by anemia, splenomegaly, infections, and other complications. Some patients require regular transfusion and splenectomy, while others can survive with intermittent transfusion. There is a risk of thrombosis after splenectomy. Complications due to iron accumulation can be seen in patients who receive regular transfusions. Diagnosis is based on red blood cell indices, peripheral blood smear, haemoglobin electrophoresis and molecular analysis.

Intrauterine transfusion is performed for severe cases of α thalassaemia. Medical interventions such as in-utero hematopoietic stem cell transplantation are used for these cases. The following discussion includes the clinical features of a broad spectrum of α thalassaemias.

Keywords: Alpha, thalassemia, clinic

BACKGROUND

Hemoglobin disorders are characterized by a reduced or impaired production of hemoglobin's globin chains **(1)**. Alpha-thalassemia is common, especially in populations of African, Southeast Asian, and Mediterranean origin. Variants of α-thalassemia are estimated to be carried by approximately 5% of the global population, with carrier frequencies reaching up to 80-90% in tropical regions **(2-4)**. Owing to migrations, there has been an increase in Alpha-thalassemia major patients in various regions worldwide. For instance, in California, approximately one case of Hb H or Hb H constant spring is observed per 10,000 births **(5)**.

Over 100 genetic variations of α-thalassemia have been identified, which may manifest as either asymptomatic or fatal. The severity of this disorder is closely related to the number of non-functional copies of α-globin genes **(6)**.

PATHOPHYSIOLOGY

The hemoglobin molecule is comprised of globin and heme molecules. The globin structure is made up of two distinct chains, known as alpha and beta, as shown in Figure 1.

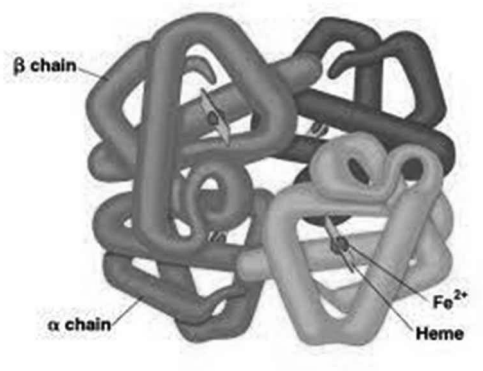


Figure 1: *Structure of hemoglobin molecule*

In adults, hemoglobin consists of pairs of α and β chains (α2β2), whereas fetal hemoglobin is composed of two α chains and two γ chains (α2γ2). The α and γ chain genes exist in duplicate form (αα/αα, γγ/γγ), whereas the β chains are derived from a single gene locus (β/β) **(Figure 2)**. The expression patterns of genes are regulated through complex interactions among transcription factors and regulatory elements, such as promoters and enhancers. These interactions ultimately determine gene activation and deactivation according to tissue type and developmental stage **(7)**.

During primitive erythropoiesis, which originates from the yolk sac, the expression of ζ-globin (from the α-globin locus) and ε-globin (from the β-globin locus) is a distinctive feature. However, these globins are silenced at around 8 weeks' gestation. The transcriptional product from the α-globin locus then becomes α-globin. At the β-globin locus, there is a transition to fetal globin (γ-globin) during fetal development, followed by a secondary switch to adult β-globin.

Fetal oxygen exchange is dependent on embryonic hemoglobin (ξ2γ2) for the initial two months of gestation before being replaced by fetal hemoglobin (α2γ2).

A deficiency in α-globin results in the creation of aggregated γ-globin tetramers (Hb Bart's) before birth and β-globin tetramers (HbH) after birth. These kinds of hemoglobin are more likely to bind with oxygen, causing suboptimal oxygen delivery, hemolysis and damage to erythroid precursors in development, leading to inefficient erythropoiesis **(7)**. Both of these tetramers have remarkably high oxygen affinity, rendering them incapable of efficiently transporting oxygen. Moreover, HbH's instability causes the formation of inclusion bodies within red blood cells and varying degrees of hemolytic anemia.

While the majority of people with a 3 α-globin gene deletion experience only mild symptoms, those with non-deletional mutations such as Hb Constant Spring may require ongoing transfusions and may benefit from stem cell transplantation **(8-10)**.

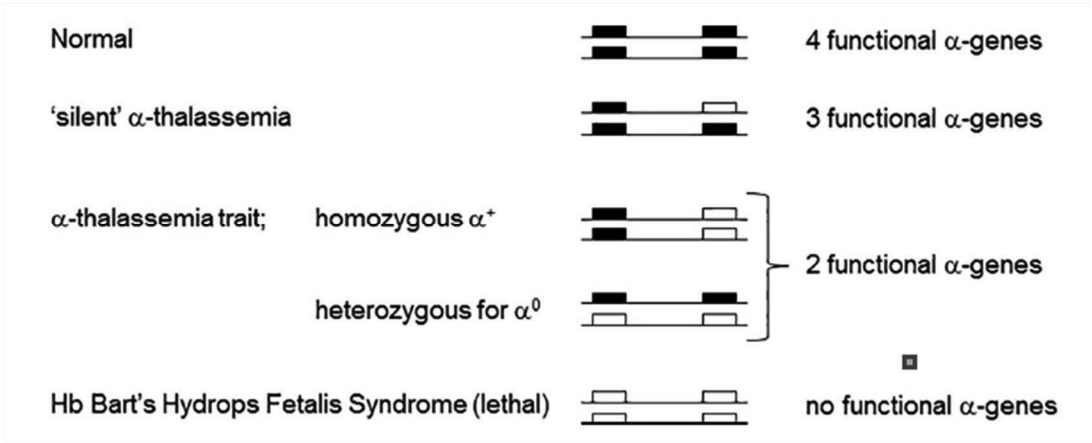


Figure 2: *Location of alpha gene mutations*

The spectrum of α-thalassemia comprises over 100 genetic variants, exhibiting a wide range of clinical manifestations from asymptomatic to life-threatening conditions. The severity of this disorder is generally well-correlated with the number of non-functional copies of the α-globin genes. α-thalassemia can be categorized into two primary subgroups: α+ thalassemia, denoting the deletion or inactivation of one gene pair (−α/αα or ααND/αα, with ND signifying nondeletion), and α^0 thalassemia, signifying the deletion of both gene pairs of α globin genes on the same chromosome (−−/αα) **(11)**.

ALPHA GENE MUTATIONS

Alpha thalassemia most commonly arises from the deletion of one (-α) or both (--) α genes on the chromosome. In rare instances, point mutations occurring in crucial regions of the α2 (αTα) or α1 (ααT) genes can lead to a nondeletional form of

alpha thalassemia. Alpha-thalassemia often results from specific deletions in the α-globin genes. One common deletion is the 3.7 kb rightward deletion, which leads to alpha-thalassemia by leaving only one functional α-gene on the chromosome. Simultaneously, a non-thalassemic α triplication allele is produced. Another type of deletion, the 4.2 kb leftward deletion, also contributes to alpha-thalassemia. Additionally, there are rare and region-specific deletions that result in the loss of a single α gene due to non-homologous recombination events **(12-14)**.

Non-deletion α+ thalassemia mutations often result in a more severe reduction in α-chain synthesis compared to deletion-type mutations. In addition, mRNA processing, translation and α-globin stability are affected by various mutations.

Common non-deletion α+-thalassemia variants include αIVSI(-5 nt)α (found in Mediterraneans), mutations at polyadenylation sites like α2 AATAAG, α2 AATGAA, and α2 AATA-- (found in the Mediterranean and Middle East). Additionally, mutations leading to elongated hemoglobin variants, such as Hb Constant Spring (HbCS), Hb Icaria, Hb Koya Dora, and Hb Seal Rock, have been identified as non-deletional α+-thalassemia mutants **(15-19)**.

Moreover, certain structural mutations give rise to highly unstable α-globin variants, including notable examples like Hb Quong Sze, Hb Suan Dok, Hb Petah Tikvah, Hb Adana, and Hb Aghia Sophia **(20)**.

Deleting both α-genes on a single chromosome leads to a complete absence of α-chain synthesis, resulting in Hb Bart's Hydrops Fetalis Syndrome in homozygotes. Some deletions remove both ζ- and α-genes, posing survival challenges for homozygotes due to their inability to produce essential embryonic and fetal hemoglobins. Rare α0 thalassemia deletions eliminate a regulatory region upstream of the α globin gene cluster, leaving the α genes intact. This region consists of specific sequences, with one known as MCS-R (Multispecies Conserved Sequences or MCS-R1 to R4) crucial for α-globin expression. Alpha thalassemia also rarely arises from deleting the MCS-R regulatory elements, may be marked as (αα) T **(21)**.

CLINIC FEATURES

In clinically significant instances, α-thalassemia primarily involves α^0 thalassemia, which can be inherited either through co-inheritance with α+ thalassemia (−α/−− or ααND/−−), resulting in HbH disease or via inheritance from both parents (−−/−−), leading to the fatal condition known as hemoglobin Bart's hydrops fetalis. Affected fetuses typically succumb to severe oxygen deprivation either early in gestation, as seen in cases such as −−FIL/−−FIL (where FIL denotes a deletion causing α^0-thalassemia, prevalent among Filipinos), or during the third trimester, as observed in cases like −−SEA/−−SEA (where SEA denotes a deletion causing α0-thalassemia, prevalent among individuals from Southeast Asia) **(10)**.

In some rare cases, a small number of children with hemoglobin Bart's hydrops fetalis who received intrauterine transfusions or immediate post-delivery transfusions have managed to survive up to the age of five. However, these survivors require ongoing transfusions and, when appropriate, iron chelation therapy, often experiencing severe clinical complications, congenital anomalies, and developmental delays. The syndrome is typically associated with various congenital malformations and maternal complications, including severe pregnancy anemia, preeclampsia, excess amniotic fluid (polyhydramnios), and significant challenges during fetal delivery and removing the greatly enlarged placenta. Despite these well-documented complications, limited data is available on the frequency of maternal deaths, particularly in developing countries where this condition is prevalent **(14, 22)**.

ALPHA THALASSAEMIA TRAIT

Individuals with α thalassemia trait **(Figure 2)**, regardless of its molecular basis, typically do not exhibit clinical symptoms apart from mild to moderate microcytic hypochromic anemia, which can be detected through routine blood tests. Those carriers (heterozygotes) of α thalassemia are usually clinically asymptomatic. The diagnosis is often made during routine health check-ups or antenatal screening. Complaints related to more severe anemias, such as fatigue, listlessness, and shortness of breath,

are uncommon and are likely attributed to other concurrent medical conditions **(23)**.

HB H DISEASE

HbH disease, typically regarded as a relatively mild disorder, has shown clinically severe manifestations, particularly in cases involving non-deletional variants. This condition exhibits a wide range of phenotypic characteristics. When caused by deletions (−α/−−), it usually presents with moderate anemia and splenomegaly, and blood transfusions are generally unnecessary except during intercurrent infections. However, when it results from the interaction between a non-deletional α-globin gene mutation and α0-thalassemia (ααND/−−), it tends to follow a much more severe clinical course.

Non-deletional forms of HbH disease, mainly caused by the α-globin chain termination mutant hemoglobin Constant Spring, which is prevalent in many Asian countries, tend to be severe. These cases exhibit severe anemia, often early in life, and are linked to increasing splenomegaly, iron buildup, and various clinical issues like infections, leg ulcers, gallstones, and folic acid deficiency. While splenectomy is sometimes necessary, nondeletional HbH disease carries a notably high risk of thrombotic complications, and choosing between splenectomy and lifelong transfusion is challenging **(10)**.

We can observe less severe forms of α-thalassemia due to genetic modifiers that influence the expression of other inherited conditions. Epistatic interactions (one gene exerts an impact on another gene) between α-thalassemia and β-thalassemia or between α-thalassemia and hemoglobin S are examples of this situation **(16)**. Additionally, triplications and quadruplications of the α-globin gene, seen in various populations, can interact with β-thalassemia variants, leading to more severe phenotypes **(24-26)**.

HB BART'S HYDROPS FOETALIS SYNDROME

Hb Bart's Hydrops Fetalis Syndrome is a severe genetic disorder characterized by a lack of α globin expression, primarily caused by the inheritance of no α globin genes from both parents or a severe non-deletion mutation from one parent and no α genes from another parent. Also called alpha thalassemia major (ATM).

In these infants, most of their hemoglobin is made up of non-functional γ4 and β4 homotetramers, with some embryonic Hb Portland (ζ2γ2) being the only functional hemoglobin, crucial for oxygen transport. The clinical symptoms include pale, edematous infants with signs of heart failure, prolonged intra-uterine anemia, hepatosplenomegaly, brain growth retardation, skeletal and cardiovascular malformations, and an enlarged placenta. Sadly, most infants with this syndrome die in utero or shortly after birth, although rare cases require intensive life-support therapy and blood transfusion **(27-34)**.

ATR 16 SYNDROME

Alpha-thalassemia/mental retardation syndrome is a rare condition associated with a large deletion in 16p13.3 resulting in the absence of the alpha-globin gene and other genes around the alpha-globin gene cluster. Aside from severe microcytic hypochromic anemia, individuals with this condition exhibit a variable clinical profile characterized by mild to moderate intellectual disabilities, developmental delays, and a range of less distinct physical features. Patients with ATR 16 syndrome may have a pure monosomy for the short arm of chromosome 16 **(35)**.

ATR X SYNDROME

Another rare syndrome associated with α-thalassemia is X-linked mental retardation syndrome ATR-X. This syndrome is characterized by severe mental retardation and distinctive dysmorphic features shared among patients. Unlike ATR-16, ATR-X presents more severe mental retardation and includes additional clinical features such as significant psychomotor impairment, hypertelorism, and facial abnormalities like a flat nasal bridge, upturned triangular nose, wide mouth, and developmental issues in the urogenital regions. The primary molecular cause of ATR-X is typically attributed to point mutations in the ATRX gene (located on Xq13.3), which encodes a chromatin-associated protein belonging to the SNF2 family of

helicase/adenosine triphosphatases. Although the precise relationship between ATRX mutations and α-thalassemia remains somewhat unclear, it has been determined that the ATRX protein functions as a transcriptional regulator that impacts the expression of α-globin genes. Additionally, the presence of HbH inclusion bodies in red blood cells, which contain insoluble β4 tetramers and become visible as inclusions when stained with 1% Brilliant Cresyl Blue, suggests a down-regulation of α-globin gene expression in some patients with ATRX syndrome. However, it is worth noting that not all patients with ATRX syndrome exhibit HbH inclusions **(36-38)**.

ALFA THALASSEMIA MYELODYSPLASIA (ATMDS)

Patients who previously displayed normal erythropoiesis may develop an acquired form of α-Thalassemia, primarily within hematologic malignancies, especially myelodysplastic syndromes (MDS). This condition is now recognized as α-Thalassemia-myelodysplasia (ATMDS). In its characteristic presentation, ATMDS manifests as a severe HbH disease, marked by pronounced hypochromic microcytic anemia, the presence of numerous HbH inclusions in peripheral blood, and measurable amounts of HbH in the hemolysate (ranging from 1% to 70%). It has become evident that most ATMDS patients have acquired somatic point mutations or mRNA intron-exon splicing abnormalities related to the ATRX gene. Patients with ATMDS are diagnosed at similar ages to those with chronic myeloid disorders without thalassemia (median age: 68 years). They share common marrow characteristics and karyotypic results and have a median survival rate typical of MDS (2–3 years). Their primary causes of death mirror those of MDS, primarily infection-related fatalities and, in around 25% of cases, complications associated with the development of acute myeloid leukemia **(39)**.

DIAGNOSIS

Thalassemia should be considered a potential diagnosis when hypochromic microcytic anemia is present. However, one should not completely discard the possibility of iron deficiency anemia, as it is commonly found in many areas.

The red blood cell characteristics in people with different α-thalassemia genotypes can be understood through laboratory tests. Generally, the severity of microcytic, hypochromic anemia depends on the number of mutated α genes, and this aligns with the reduction in α-chain production predicted for each mutation. To identify specific hemoglobin types like HbH in individuals and Hb Bart's in newborns with α-thalassemia markers or related hemoglobin variants, a combination of techniques like HPLC and Capillary Electrophoresis is essential.

Hb Bart's is present in many newborns with α-thalassemia. However, it may not detect all cases, especially mild α-3.7/αα interactions, and it may not differentiate between various α-thalassemia genotypes. A reduction in HbA2 levels can sometimes indicate α-thalassemia trait, although it does not precisely determine the degree or type of α-thalassemia. It is more distinct in patients with HbH disease.

Initial laboratory testing should encompass a complete blood count with red cell indices and high-performance liquid chromatography (HPLC) or hemoglobin electrophoresis. However, DNA analysis is often preferred as a less complex and straightforward method for diagnosing α-thalassemia.

Nucleated red blood cells and basophilic stippling are frequently encountered in the context of more severe phenotypes, including Hb Bart's hydrops and severe non-deletional Hb H disease. Staining blood cells with Brilliant Cresyl Blue **(Figure 3)** is a sensitive method to visualize inclusion bodies in red cells. Inclusion bodies consist of β4-tetramers that precipitate on the red cell membrane, causing damage and leading to hemolysis. These inclusion bodies, less detectable in older blood samples, typically have a golf-ball-like appearance with stippling evenly distributed over a blue-stained background. They are seen occasionally in carriers of the --/αα genotype and carriers of certain non-deletional defects. Patients with HbH disease often have numerous red cells containing these inclusions in their blood smears **(23)**.

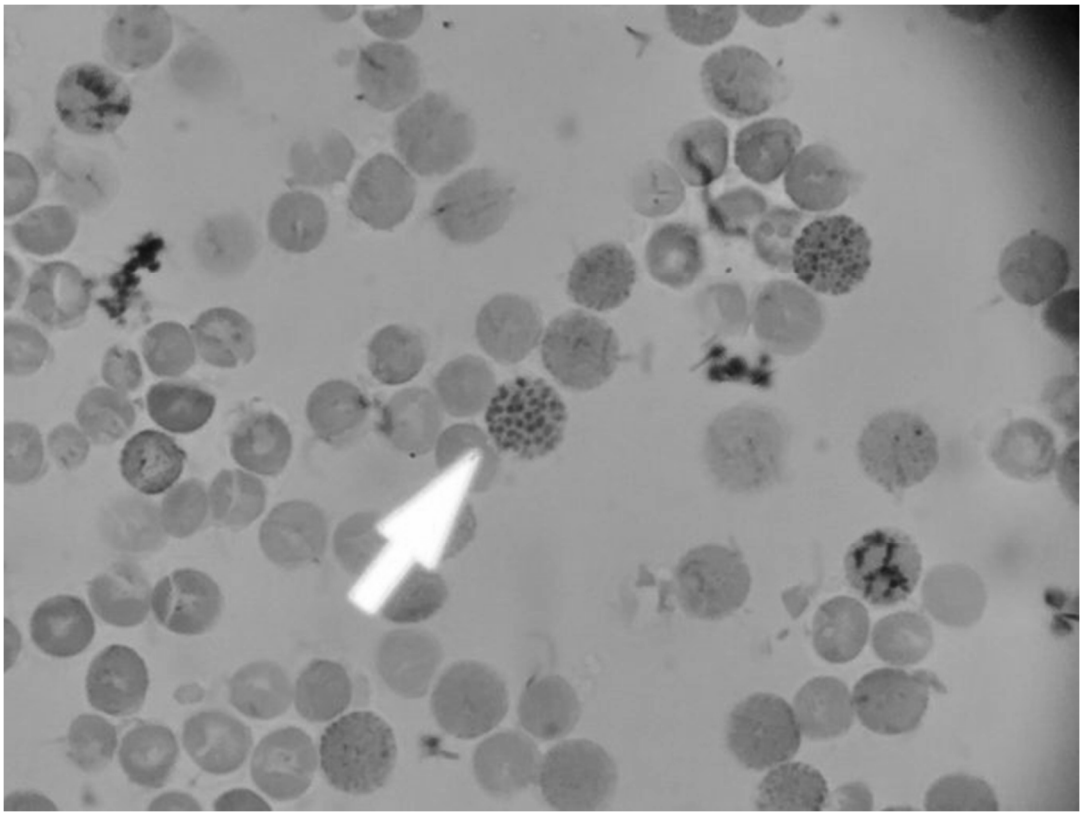


Figure 3: *The presence of inclusion bodies in erythrocytes stained with Brilliant Cresyl Blue.*

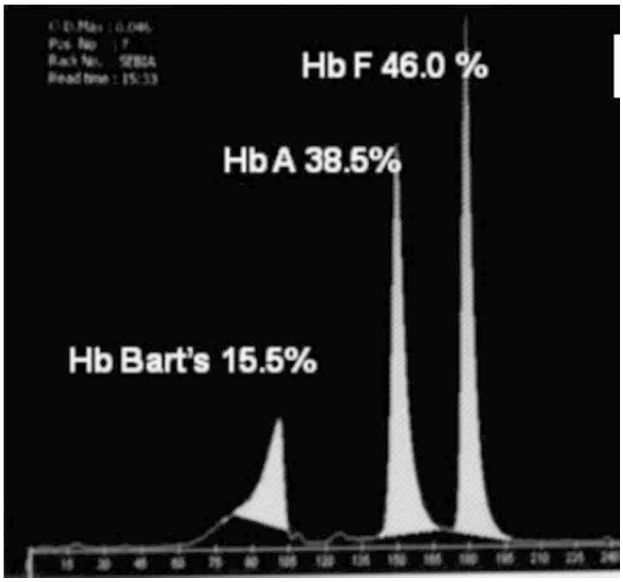


Figure 4: *An illustration of Hb H disorder found through capillary electrophoresis during birth (- -SEA/-α3.7).*

HEMOGLOBIN ELECTROPHORESIS

Hydrops Fetalis without α-thalassemia is a frequently encountered nonspecific symptom in various fetal and maternal conditions. What sets Hb Bart's hydrops fetalis syndrome apart is the presence of Hb Bart's and the absence of HbF, a distinction easily discerned through techniques like HPLC or Hb-electrophoresis **(23)**.

Because of the inherent instability of the Hb H tetramer, delayed processing of blood samples can lead to erroneous adverse outcomes. HPLC is not deemed suitable for measurement due to the inherent instability of Hb H. In contrast, capillary electrophoresis is considered a more suitable approach **(Figure 4) (39)**.

Qualitative and quantitative hemoglobin analysis is crucial in diagnosing α thalassemia syndromes. Electrophoresis can identify fast-moving hemoglobin species, specifically Hb H (β4) and Bart's (γ4), characteristic of α thalassemia syndromes. The measured levels of Hb H can range from less than 1% to as high as 40%, with the typical range being 10-15%. The variation in measurement results can be attributed to test sensitivity, laboratory expertise, instrument type, and the quality of blood samples.

In some cases, Hb H may not be easily identified using specific liquid chromatography platforms, necessitating manual identification based on the presence of hemoglobin species at a specific retention time (RT).

Due to the shortage of available α globin chains, there is a reduction in Hb A2 (α2δ2). In patients with non-deletional Hb H disease, especially Hb H/Hb CS, the Hb CS variant can be detected at a very low level, typically ranging from 1% to 4% **(40)**.

MOLECULAR ANALYSIS

The only definitive and safe way to screen for α-thalassemia is DNA analysis. α- and β-thalassemia mutations can be inherited together. Detecting an α-thalassemia mutation does not simultaneously exclude a β-thalassemia mutation.

Various approaches are used in the molecular diagnosis of thalassemia, including targeted deletion analysis, sequence analysis, and deletions in specific genes and regulatory regions. Initially, GAP-PCR is utilized for targeted deletion analysis to identify common deletions. If common deletions are not identified, non-deletional mutations are detected using reverse dot blot analysis, primer-specific amplification, or PCR with enzymatic digestion. The next step involves analyzing the sequences of α1 and α2 globin genes. Typically, these methods do not detect exon or whole-gene deletions/duplications. For rare or unknown deletions, alternative techniques such as Southern blotting (less commonly used), quantitative PCR, long-range PCR, and multiplex ligation-dependent probe amplification (MLPA) can be applied. These methods are also capable of detecting duplications within the α globin cluster. Furthermore, considering testing for genes associated with conditions resembling α thalassemia, like ATRX and HBB, is a possibility **(40)**.

FETAL DIAGNOSTIC METHODS

Fetal diagnosis is often conducted early in pregnancy through chorionic villus sampling. However, fetal anemia may be diagnosed later during gestation by quantifying the peak systolic velocity in the middle cerebral artery. Various alternative methods for preimplantation and preconception genetic or prenatal diagnosis are still in the early stages of research. These tests involve analyzing maternal blood for fetal DNA or identifying fetal cells in maternal blood using antibodies against globin chains **(41)**.

TREATMENT

Deletion-type patients may require intermittent transfusion therapy during illness and less frequently chronic transfusion therapy. In contrast, patients without deletion type may have moderate splenomegaly requiring regular transfusions and sometimes splenectomy. However, there are significant clinical differences in both categories. Iron overload is rare in HbH disease but may occur in elderly patients and those receiving regular transfusions **(23)**.

Most pregnancies with Hb Bart's hydrops fetalis syndrome are terminated. In very rare cases, early detection of homozygous α0-thalassemia makes it possible to perform intrauterine transfusions and deliver non-hydropic infants. However, most survivors face a difficult perinatal period and a high likelihood of congenital urogenital and limb defects. Those who survive may be candidates for hematopoietic stem cell transplantation. Nevertheless, the severe obstetric complications and the need for long-term transfusion therapy often lead to ATM counseling and selective abortion as recommended options. Couples at risk of having a child with this syndrome should be informed about the increased risk of maternal and fetal morbidity **(11, 23)**.

IN UTERO HEMATOPOİETİC STEM CELL TRANSPLANTATİON (IUHCT)

In utero hematopoietic stem cell transplantation (IUHCT) is being investigated as a potential treatment for ATM. This approach utilizes the fetus's unique immune environment to allow the successful transplantation of maternal stem cells without the need for pre-conditioning. A phase 1 clinical trial currently uses maternal stem cells as donors, capitalizing on the fetus's natural tolerance to maternal antigens and immune regulatory properties. However, IUHCT needs to work on achieving adequate engraftment without conditioning. The two-step protocol involves IUHCT to establish initial engraftment, followed by a postnatal "boost" with maternal stem cells to improve engraftment levels. If successful, this approach could offer a safer alternative to traditional postnatal hematopoietic stem cell transplantation. Promising outcomes have been

observed in animal models for conditions like Sickle Cell Disease and Beta-Thalassemia Major, leading to donor-specific tolerance and peripheral mixed chimerism **(42)**.

In the future, gene therapy and gene editing will hold promise for patients with ATM. Some strategies developed for β-thalassemia could be adapted for the treatment of ATM. For example, lentiviral ex vivo gene therapy could replace the defective α-globin gene or gene editing techniques could introduce functional α-globin genes at a safe genomic site.

Considering the improved survival of ATM patients due to IUT, the number of individuals requiring chronic treatment for ATM is expected to increase. Therefore, developing improved HCT and gene therapy options could make a critical contribution to the ultimate cure of ATM patients. These advanced approaches offer hope for a healthier future for these patients **(42)**.

REFERENCES

1. Weatherall DJ, Clegg JB. Thalassemia — a global public health problem. Nat Med 1996;2:847-9
2. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ 2008;86:480-7
3. Chui DH. Alpha-thalassaemia and population health in Southeast Asia. Ann Hum Biol 2005;32:123-30.
4. Hossain MS. Lack of knowledge and misperceptions about thalassaemia among college students in Bangladesh: a cross-sectional baseline study. Orphanet J Rare Dis. 2020: 21;15(1):54.
5. Feuchtbaum L, Carter J, Dowray S, et al. Birth prevalence of disorders detectable through newborn screening by race/ethnicity. Genet Med. 2012;14(11):937-45.
6. Giardine B, Borg J, Viennas E, et al. Updates of the HbVar database of human hemoglobin variants and thalassemia mutations. Nucleic Acids Res 2014;42: D1063-D10
7. Farashi S, Harteveld CL. Molecular basis of α-thalassemia. Blood Cells Mol Dis. 2018;70:43-53.
8. Songdej D, Babbs C, Higgs DR; Bart's Hydrops Fetalis Syndrome International Consortium. An international registry of survivors with Hb Bart's hydrops fetalis syndrome. Blood. 2017;129(10):1251-1259.
9. Lal A, Goldrich ML, Haines DA, et al. Heterogeneity of hemoglobin H disease in childhood. N Engl J Med. 2011;364(8):710- 718.
10. Weatherall DJ, Clegg JB. The thalassemia syndromes. 4th ed. Oxford, England: Blackwell Science, 2001 D.J.
11. Piel FB, Weatherall DJ. The α-thalassemias. N Engl J Med. 2014;371(20):1908-16.
12. Higgs DR, Pressley L, Clegg JB, et al. Detection of alpha thalassaemia in Negro infants. Br J Haematol 1980, 46:39-46.
13. Higgs DR, Hill AV, Bowden DK, et al. Independent recombination events between the duplicated human alpha globin genes; implications for their concerted evolution. Nucleic Acids Res 1984, 12:6965-6977.
14. Trent RJ, Higgs DR, Clegg JB, et al. A new triplicated alpha-globin gene arrangement in man. Br J Haematol. 1981;49(1):149-52.
15. Bradley TB, Wohl RC, Smith GJ. Elongation of the alpha-globin chain in a black family:interaction with HbG Philadelphia. [abstract]. clinical research 1975, 23:
16. Clegg JB, Weatherall DJ, Milner PF. Haemoglobin Constant Spring--a chain termination mutant? Nature 1971, 234:337-340.
17. Clegg JB, Weatherall DJ, Contopolou-Griva I, et al. Haemoglobin Icaria, a new chain-termination mutant with causes alpha thalassaemia. Nature 1974, 251:245-247.
18. De Jong WW, Meera KP, Bernini LF. Hemoglobin Koya Dora: high frequency of a chain termination mutant. Am J Hum Genet 1975, 27:81-90.
19. Waye JS, Eng B, Patterson M, et al. Identification of a novel termination codon mutation (TAA-->TAT, Term-->Tyr) in the alpha 2 globin gene of a Laotian girl with hemoglobin H disease. Blood 1994, 83:3418-3420
20. Ratrinos GP, Giardine B, Riemer C, et al. Improvements in the HbVar database of human hemoglobin variants and thalassemia mutations for population and sequence variation studies. Nucleic Acids Res 2004, 32:D537-D541
21. Higgs DR: The molecular basis of alpha -thalassemia. In Disorders of Hemoglobin second edition. Edited by: Steinberg MH, Forget BG, Higgs DR, Nagel RL. Cambridge University Press; 2009
22. Higgs DR. Alpha-thalassemia. Baillière's Clin Haematol 1993;6:117-50.
23. Harteveld CL, Higgs DR. Alpha-thalassaemia. Orphanet J Rare Dis. 2010 May 28;5:13.
24. Thein SL. Genetic modifiers of beta thalassemia. Haematologica 2005;90:649- 60
25. Ma SK, Au WY, Chan AY, et al. Clinical phenotype of triplicated alphaglobin genes and heterozygosity for beta0-thalassemia in Chinese subjects. Int J Mol Med 2001;8:171-5. 26.
26. Giordano PC, Bakker-Verwij M, Harteveld CL. Frequency of alpha-globin gene triplications and their in-

teraction with beta-thalassemia mutations. Hemoglobin 2009;33:124-31.
27. Chan V, Chan TK, Liang ST, et al. Hydrops fetalis due to an unusual form of Hb H disease. Blood 1985, 66:224-228.
28. Chan V, Chan VW, Tang M, et al. Molecular defects in Hb H hydrops fetalis. Br J Haematol 1997, 96:224-228
29. McBride KL, Snow K, Kubik KS, et al. Hb Dartmouth [alpha66(E15)Leu-->Pro (alpha2) (CTG-->CCG)]: a novel alpha2-globin gene mutation associated with severe neonatal anemia when inherited in trans with Southeast Asian alpha-thalassemia-1. Hemoglobin 2001, 25:375-382.
30. Beaudry MA, Ferguson DJ, Pearse K, et al. Survival of a hydropic infant with homozygous alpha-thalassemia-1. J Pediatr 1986, 108:713-716.
31. Bianchi DW, Beyer EC, Stark AR, et al. Normal longterm survival with alpha-thalassemia. J Pediatr 1986, 108:716-718.
32. Lee SY, Chow CB, Li CK, et al. Outcome of intensive care of homozygous alpha-thalassaemia without prior intra-uterine therapy. J Paediatr Child Health 2007, 43:546-550.
33. Yi JS, Moertel CL, Baker KSi Homozygous alpha-thalassemia treated with intrauterine transfusions and unrelated donor hematopoietic cell transplantation. J Pediatr 2009, 154:766-768
34. Lal A, Goldrich ML, Haines DA, et al. Heterogeneity of hemoglobin H disease in childhood. N Engl J Med. 2011;364(8):710- 718.
35. Higgs DR, Buckle V, Gibbons R, et al. Unusual Types of Alpha Thalassaemia. In Disorders of Hemoglobin second edition. Cambridge University Press; 2009.
36. Gibbons RJ, Wada T, Fisher CA, et al. Mutations in the chromatin-associated protein ATRX. Hum Mutat. 2008 Jun;29(6):796-802.
37. Gibbons RJ, Pellagatti A, Garrick D, et al. Identification of acquired somatic mutations in the gene encoding chromatin-remodeling factor ATRX in the alpha-thalassemia myelodysplasia syndrome (ATMDS). Nat Genet. 2003, 34(4):446-9.
38. De La Fuente R, Baumann C, Viveiros MM. Role of ATRX in chromatin structure and function: implications for chromosome instability and human disease. Reproduction. 2011, 142(2):221-34.
39. Gibbons RJ. α-Thalassemia, mental retardation, and myelodysplastic syndrome. Cold Spring Harb Perspect Med. 2012, 2(10):a011759.
40. Thalassaemia International Federation. (2021). 2021 Guidelines for the Management of Transfusion-Dependent Thalassaemia (TDT) (4th edition, Version 2.0). In M.D. Cappellini, D. Farmakis, J. Porter, & A. Taher (Eds.). (p. 30, Chapter 1).
41. Vichinsky EP. Alpha thalassemia major — new mutations, intrauterine management, and outcomes. Hematology Am Soc Hematol Educ Program 2009;35-41
42. Horvei P, MacKenzie T, Kharbanda S. Advances in the management of α-thalassemia major: reasons to be optimistic. Hematology Am Soc Hematol Educ Program. 2021, 2021(1):592-599.

Correspondence Address:
Assoc Prof. Dr. Yesim Oymak
SBU. Dr. Behçet Uz Çocuk Hastalıkları ve Cerrahisi Eğitim ve Araştırma Hastanesi, İzmir-Türkiye
Tel : +90 5323554228
E-mail: yesimoymak@hotmail.com

CLINIC IN BETA THALASSEMIA SYNDROMES

Şifa Şahin[1], Zeynep Karakaş[2]
[1]Dr. Istanbul University, Istanbul Medical Faculty, Pediatric Hematology/Oncology,
[2]Prof Dr. Istanbul University, Istanbul Medical Faculty, Pediatric Hematology/Oncology/ İstanbul, Türkiye

ABSTRACT

Beta thalassemia has a very heterogeneous clinical appearance, ranging from asymptomatic cases to severe anemia requiring transfusion. According to clinical findings and genotypes, thalassemias are classified as thalassemia minor, intermedia or major. The classification of patients with β-thalassemia as non-transfusion-dependent thalassemia (NTDT) and transfusion-dependent thalassemia (TDT) is now widely used in international management guidelines for its suitability in clinical practice n of patients with thalassemia plays a key role in making management and follow-up decisions. In recent clinical studies, in the classification of β Thalassemia according to transfusion dependence, receiving at least 6 units of red blood cells in the last 6 months is defined as NTDT. The clinical findings of the disease are mainly caused by chronic anemia, ineffective erythropoiesis findings, and complications resulting from transfusion-related iron overload.

Keywords: β Thalassemia, clinical findings, transfusion

BACKGROUND

Beta Thalassemia (β-Thal) is a blood disease caused by a decrease in the β globin chain synthesis rate in the structure of adult hemoglobin and progresses with anemia. More than 280 β-Thal mutations have been identified to date, including at least 230 point mutations and 18 large deletions, occurring in a wide variety of ethnic groups. Although β-Thal is mostly seen especially around the Mediterranean, the Indian subcontinent, Southeast Asiaand Africa, it has spread all over the world due to migration and has become a public health problem **(1)**. Approximately 150 million people live in the world with Thalassemia carriers. In Türkiye, the carrier incidence for β thalassemia is 2.1%, and in some provinces in the Mediterranean Region, this rate is maintained up to 13% **(2)**.

The severity of the defect of β globin chain is very variable (**3)**. Since normal individuals have two allelic β globin genes, β thalassaemia can exist in a heterozygous or homozygous state. Compound heterozygosity occur with having two different mutant genes. β thalassemia mutations are divided into two broad categories, β0 (beta zero) thalassemia and β+ (beta plus) thalassemia. In the homozygous or compound heterozygous state β0 thalassemia, a total lack of β chain production and a total failure to produce haemoglobin A occur. In homozygous β+ thalassaemia there is reduced but not absent expression of the abnormal β gene with some production of haemoglobin A (**1)**.

In β-thalassemia, a defect in the β-globin gene results in ineffective erythropoiesis, causing reduced rates of differentiation of erythroblasts maturing into the polychromatic and orthochromatic phase. It occurs as a result of various pathogenic mechanisms of anemia. On the one hand, alpha globin recruits cytosolic heat shock protein 70 (HSP70) and prevents its nuclear translocation and protection of the erythroid transcription factor GATA binding factor 1 (GATA1) from cleavage. On the other hand, excess of alpha globin is responsible for the degradation of radical oxygen species (ROS) (the formation of which also increases with iron overload), which activates GDF11, which then activates the SMAD2/3 inhibitory pathway, resulting in the inhibition of the differentiation of erythroids. In this way, individual hematological development occurs (**4)**.

Beta thalassemia has a very heterogeneous clinical appearance, ranging from asymptomatic cases to severe anemia requiring transfusion. According to clinical findings and genotypes, thalassemias are classified as thalassemia major, intermedia or minor. Clinical classification of patients with thalassemia plays a key role in making management and follow-up decisions. However, clinical classification regarding transfusion requirements is used more frequently today (**1**). Phenotype classification based on transfusion requirement, which has begun to replace the traditional phenotypes mentioned above in the past decade, aims to emphasize the need for individual treatment throughout the course of the disease (**5-10**).

Transfusion requirement has importance in terms of associated pathophysiological features and practical management. The classification of patients with β-thalassemia as non-transfusion-dependent thalassemia (NTDT) and transfusion-dependent thalassemia (TDT) is now widely used in international management guidelines for its suitability in clinical practice **(11, 12)**.

The three main β-thalassemia phenotypes are traditionally assigned based on the clinical presentation; Certain genetic profiles are widely, but not exclusively, considered to be associated with certain phenotypes **(Figure 1)**. β-Thalassemia carriage or β-thalassemia minor, resulting from heterozygous inheritance of the β-thalassemia mutation, is characterized by borderline asymptomatic anemia with microcytosis and hypochromia (**7, 8, 13)**.

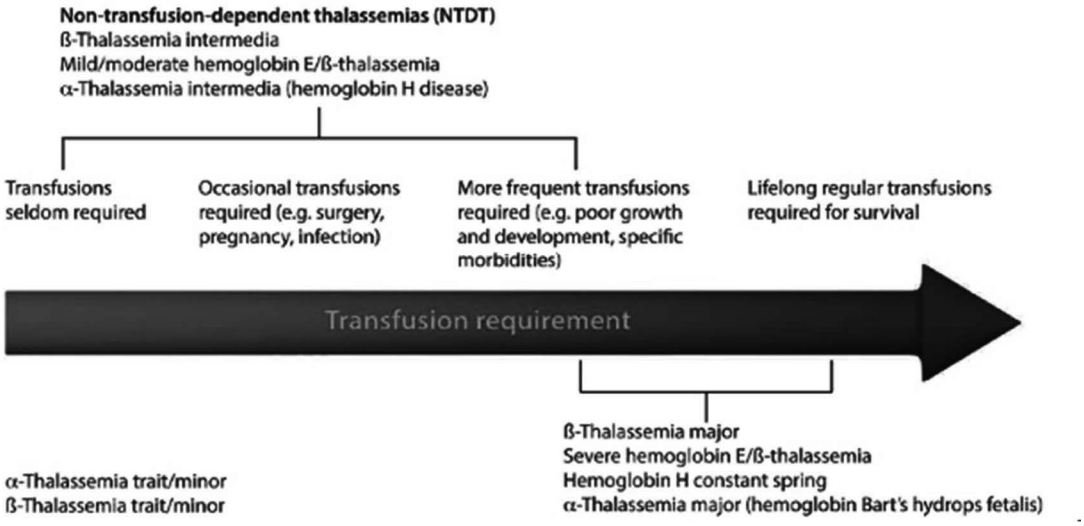


Figure 1: *Thalassemia phenotypes and their relationship to transfusion requirements* [11,12]

THALASSEMIA TRAIT

Beta thalassemia patients may appear as silent carriers with normal hematological parameters and normal Hb A_2 levels. These individuals cannot be distinguished by routine screening. Beta thalassemia carriers usually have hypochromic microcytic anemia associated with high HbA_2 (3.5-8%), and HbF (5-20%) levels (**2**).

Since the hemoglobin A percentage in newborns is low, β thalassemia cannot be diagnosed. However, the average hemoglobin A percentage at 26-12 months is higher than in other infants, and the HbA_2 percentage at six months does not overlap with values seen in infants with normal globin genes. The rate of decline in hemoglobin F is slower than in hematologically normal infants, and adult levels are not reached until childhood **(9)**.

Erythrocytosis (RBC>$5x10^{12}$/L), microcytosis, normal RDW are common in these individuals. In the differential diagnosis, in addition to beta thalassemia carriers, iron deficiency anemia, alpha thalassemia trait and chronic disease anemia should also be taken into consideration (**2**).

Hematological features of thalassemia carriage are microcytosis, hypochromia and usually an increase in the HbA2 percentage. Hemoglobin composition is 92%–95% HbA, >3.8% HbA_2, and variable amounts of HbF (0.5%–4%). Besides microcytosis

and hypochromia, there are marked differences in the size and shape of red blood cells. Red cells with β0-thalassemia have lower mean corpuscular volume than red cells with β+-thalassemia. Historically, the microcytic, hypochromic mild anemia characteristic of thalassemia carriage was thought to have no clinical consequences other than its association with anemia of pregnancy. However, some studies suggest that individuals with thalassemia may experience symptoms of anemia such as headache, drowsiness, fatigue, dizziness and exercise intolerance, even though their hemoglobin levels are within the normal range **(7-10)**.

There was no difference in the frequency of these symptoms between the two groups with mild anemia or those with normal hemoglobin levels. A significant increase in the frequency of infection attacks has also been noted in individuals with β-thalassemia carriers. The incidence of advanced coronary artery disease has decreased in men with thalassemia carriers, and myocardial infarction in men occurs at older ages **(9)**.

THALASSEMIA INTERMEDIA

NTDT clinically includes three distinct forms: β-thalassemia intermedia (β-TI), hemoglobin E/β-thalassemia (mild and moderate forms), and α-thalassemia intermedia (hemoglobin H disease). Although NTDT patients have fewer symptoms than TDT patients, it remains unclear whether these patients have a significant disease burden affecting their quality of life. β-TI is the most common of this group.

The severity of the clinic in NTDT patients depends on the degree of imbalance between the α-chains and other genetic factors that alter the natural course of the disease. The variety of mutations and subsequent varying degrees of α-/β-globin chain instability are the main determinants of the clinical status. Even within the same disease class, interactions between these alleles can cause a wide range of disease severity.

Determinants of disease severity in the β-thalassemia intermedia clinic include the type of β-thalassemia mutation, co-inheritance of α-thalassemia and determinants that increase fetal hemoglobin production (BCL11A and HBS1L-MYB), as well as the presence of complications such as hereditary diseases. The underlying cause of more severe chronic hyperbilirubinemia and the increase in gallstone formation observed in some patients may be variability in the function of the UDP glucuronosyltransferase-1 gene. It should not be forgotten that patients with hemoglobin E/β-thalassemia also show different phenotypic severity at certain stages of development 8-12. Patients with non-transfusion-dependent β-thalassemia (β-thalassemia intermedia or intermediate HbE-β-thalassemia) do not require transfusion. Transfused occasionally due to certain conditions (e.g., pregnancy, surgery, or acute infection) or frequently for a limited period of time (e.g., to promote growth or manage a disease during childhood) 7,11. Sometimes they are completely asymptomatic until adulthood. Clinical features may include pallor, mild to moderate jaundice, cholelithiasis, liver and spleen enlargement, moderate to severe bone changes, leg ulcers, extramedullary hyperplastic erythroid masses (Figure 2), osteopenia, osteoporosis, and a tendency to develop thrombotic complications 13. Cardiac involvement in thalassemia intermedia is mainly high output and is characterized by pulmonary hypertension. Systolic left ventricular function is usually preserved. Myocardial siderosis is rare. Without appropriate treatment, the incidence of comorbidities increases with age. Pseudoxanthoma elasticum, a disorder affecting the skin, eyes, blood vessels, and less commonly other sites, is characterized by the deposition of calcium and other minerals in elastic fibers and has been described in these patients 11, 12.

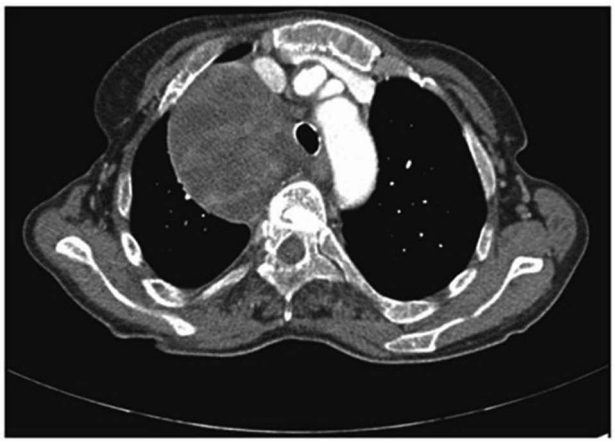


Figure 2: *Chest computed tomography (CT) showing a pulmonary extramedullary erythropoiesis mass (10x9x10 cm) in a woman with thalassemia intermedia who admitted with symptoms of acute respiratory insufficiency and right*

*heart failure and subsequently treated with radiotherapy. Other masses are also seen in the same hemithorax and bilateral paravertebral level in a 64-year-old patient (**13**)*

Bone complications include widening of the medullary space, osteoporosis (due in part to gonadal insufficiency), and fractures. Hypersplenism may develop in patients with thalassemia intermedia. Splenic sequestration has also been described. Cardiovascular complications are common and include congestive heart failure, acute pericarditis, chronic pericardial thickening, mitral and aortic valve regurgitation, and pulmonary hypertension.

There is an increased incidence of venous thromboembolism and portal vein thrombosis. The state of hypercoagulopathy is especially evident after splenectomy. Silent cerebral infarction may occur. Pulmonary hypertension can be attributed to both recurrent venous thromboembolism and interstitial fibrosis resulting from iron accumulation. Glomerular hyperfiltration is common and proteinuria occurs in a minority of patients. End-stage renal failure occurs in a small proportion of patients. Rarely, priapism has been described **(14)**. Cognitive impairment has been reported in adults with beta thalassemia intermedia **(12)**.

TRANSFUSION DEPENDENT THALASSEMIA

Patients with homozygous β0-thalassemia often live in the first 3-4 years of life. They present with severe anemia due to insufficient HbA synthesis. However, depending on the type of mutation and HbF production, the need for transfusion may be delayed until the age of two. Growth retardation, hepatosplenomegaly, hypersplenism, bone changes due to bone marrow growth, and thalassemic face (maxillary hyperplasia, frontal protrusion, nasal bridge collapse) develop in patients who do not receive adequate transfusion therapy (**15) (Picture 1-2)**.

The clinical spectrum of patients with homozygous β-thalassemia is highly variable. Many people face severe anemia early in life and remain transfusion-dependent throughout their lives. Such individuals are called thalassemia major. Some people with homozygous β-thalassemia present with milder anemia and may never require transfusion. Others have varying degrees of anemia and may require intermittent transfusions. Such individuals are defined as patients with thalassemia intermedia. Extramedullary hematopoiesis causes enlargement of the liver and spleen and paraspinal and pulmonary erythroid cell masses.

In the past, a hemoglobin level of 7 g/dL was used to distinguish between the two forms, but this criterion was confusing because the severity of anemia and associated developmental regression with splenomegaly may varied in individual patients at different times. On the other hand, Transfusion is based in part on socioeconomic issues as well as access to an adequate blood supply. The conclusion reached as a result of the examination of this subject is that β-thalassemias are due to the phenotypic diversity, the heterogeneity of mutations of the β-globin locus, the effects of many secondary and tertiary modifiers, and a wide range of environmental factors **(16)**. Transfusion desicion not show underlying disease severity, generally reflects physician or patient choice. In recent clinical studies, in the classification of B Thalassemia according to transfusion dependence, receiving at least 6 units of red blood cells in the last 6 months is defined as NTDT **(11)**.

Individuals with thalassemia major usually present between 6 and 24 months. The first presentation is usually due to developmental delay, infection attacks or abdominal enlargement. They then require regular red blood cell transfusions to survive. Affected babies fail to thrive and become increasingly pale. Abdominal swelling may occur due to feeding problems, diarrhea, agitation, recurrent fever attacks and splenomegaly. If prenatal diagnosis is not made in developed countries, it is diagnosed at this stage and a regular transfusion program is started. The classic clinical picture of TDT is currently seen only in some developing countries where resources for the implementation of long-term transfusion programs are not available. The most important features in individuals who are untreated or receive insufficient blood transfusion are growth retardation, pallor, jaundice, brown pigmentation on the skin, weak muscle structure, genu valgum, hepatosplenomegaly, leg ulcers, and skeletal changes caused by masses from extramedullary hematopoie-

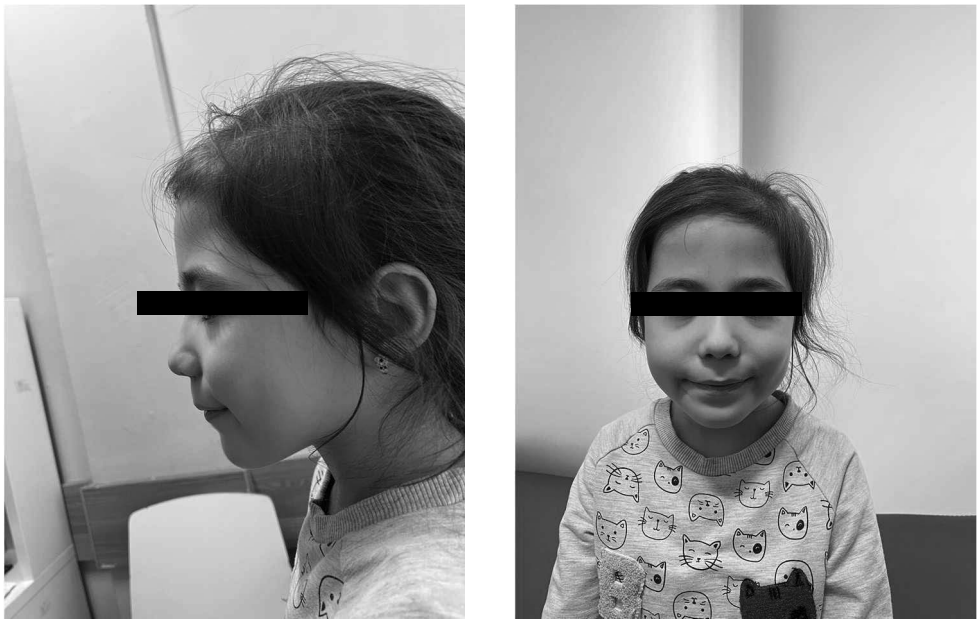
sis. These skeletal changes include deformations of the long bones of the legs, typical craniofacial changes, and osteoporosis. People who do not receive regular blood transfusions often die from high-output heart failure. If a regular transfusion program is initiated that maintains the minimum hemoglobin (Hb) concentration at 9.0 to 10.5 g/dl, ineffective erythropoiesis is prevented and growth and development tend to be normal for up to 10 to 12 years. However, complications due to iron overload may develop in transfused patients, depending on their compliance with chelation therapy **(17)**.

Additionally, although rare, cognitive disorders have been reported in these patients. In a study group consisting of β thalassemia major and intermedia and hemoglobin E/β thalassemia, there is an increased incidence of headaches associated with white matter lesions on magnetic resonance imaging **(18)**.

Many of the adverse effects of β thalassemia major can be largely prevented with an appropriate blood transfusion program. However, this leads to severe iron overload unless chelation therapy is given. Iron overload can lead to heart and liver damage, hypopituitarism (contributing to growth failure), hypogonadotropic hypogonadism with delayed puberty, diabetes, hypothyroidism and hypoparathyroidism (mostly subclinical), respectively. Adrenal insufficiency of central origin may be subclinical and occur only under stress. Cardiac damage may manifest as left ventricular dilatation with reduced ejection fraction or diastolic dysfunction with restrictive filling. There may be valve disease, atrial flutter or fibrillation, ventricular tachycardia and heart failure **(19)**.

Without treatment, children with homozygosity for β0 thalassemia usually die by age 3-4, whereas those with β+ homozygosity may survive into late childhood **(20)**.

As iron chelation regimens become less stringent, it becomes more feasible to start transfusion earlier, thus reducing the frequency of alloimmunization.



Picture 1-2: *Bone changes due to bone marrow growth, thalassemic face (maxillary hyperplasia, frontal protrusion, nasal bridge collapse.*

Hb E/ β -THALASSEMIA

Hemoglobin E (HbE) is an extremely common structural variant of hemoglobin that occurs at high frequency in many Asian countries. It is a variant of β-hemoglobin that is produced at a slightly lower rate and thus has the phenotype of a mild form of β-thalassemia. While its interactions with different forms of α thalassemia result in a wide range of clinical disorders, its co-inheritance with β thalassemia (a condition called hemoglobin E β thalassemia) is by far the most common form of severe β thalassemia in Asia. It constitutes approximately

50% of clinically severe β-thalassemia disorders **(21)**.

One of the most striking features of HbE β thalassemia is its remarkable clinical heterogeneity. At one end of the spectrum are patients whose clinical course is virtually indistinguishable from that of severe β-thalassemia major. On the other hand, there are patients who grow and develop normally without the need for blood transfusion, often with relatively low hemoglobin levels **(Figure 2)**.

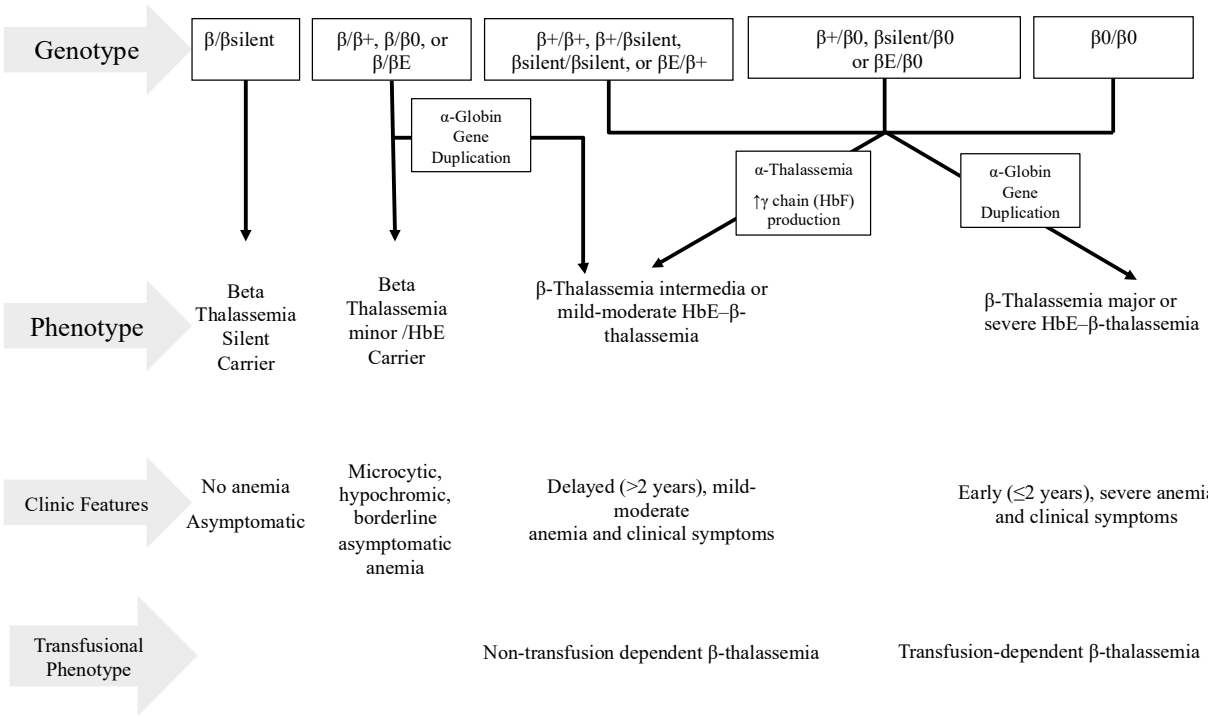


Figure 3: *Genetic and Clinical Features of β-Thalassemia (**7**).*

Babies with severe HbE β thalassemia at birth are asymptomatic because their HbF levels are high. At 6-12 months of age, as HbF production decreases and is replaced by HbE, anemia with splenomegaly develops. Signs of growth arrest appear in the first decade of life. Initial complaints vary from patient to patient and often several symptoms occur simultaneously. The most common is the development of a mass in the left upper quadrant and pallor. Over time and without transfusion, anemia, jaundice, hepatosplenomegaly, growth retardation and thalassemia develop. Lack of secondary sexual development is common, and chronic leg ulcers are sometimes also seen. These symptoms, secondary to reduced oxygen delivery to tissue, ineffective erythropoiesis, and iron overload, are similar to those in β-thalassemia major.

Patients with milder forms of HbE β thalassemia tend to grow and develop quite well and are fully active in early childhood. There may be some delay in the pubertal growth spurt and the emergence of secondary sexual characters. However, they usually reach a reasonable height and sexual maturity. More studies are needed to determine whether they develop the later complications described in older patients with other forms of thalassemia intermedia, such as kidney disease, pulmonary hypertension, cerebral infarctions, and others. Of course, some accumulate iron through increased absorption and may develop associated endocrine complications, including diabetes. There is undoubtedly phenotypic instability in the first years of life. In a study of HbE β thalassemia cases over the age of 15, at least 20 cases were observed to evolve from a mild phenotype to a more severe phenotype during the first 15 years of life (**7**).

REFERENCES

1. Bain BJ. α, β, δ and γ thalassaemias and related conditions in Haemoglobinopathy Diagnosis. Third edition.,2020, 85-185.
2. Aksu T, Unal S. Thalassemia Trend Pediatrics. 2021; 2(1): 1-7 | doi: 10.5222/TP.2021.10820
3. Khaing AA, Myint PP, Paiboonsukwong K, Win N, Fucharoen S, Sripichai O. Clinical Severity of β-Thalassemia Pediatric Patients in Myanmar. Hemoglobin. 2022 Jan;46(1):66-70. doi: 10.1080/03630269.2022.2025825.
4. Parisi S, Finelli C, Fazio A, De Stefano A, Mongiorgi S, Ratti S et al, Clinical and Molecular Insights in Erythropoiesis Regulation of Signal Transduction Pathways in Myelodysplastic Syndromes and β-Thalassemia. Int J Mol Sci. 2021 Jan 15;22(2):827. doi: 10.3390/ijms22020827.
5. Kattamis A, Kwiatkowski JL, Aydinok Y. Thalassaemia. Lancet. 2022 Jun 18;399 (10343):2310-2324.
6. Hokland P, Daar S, Khair W, S S, Taher A, Torti L, Hantaweepant C, Rund D. Thalassaemia—A global view. Br J Haematol. 2023;201:199–214.
7. Taher AT, Musallam KM, Cappellini MD. β-Thalassemias. N Engl J Med. 2021 Feb 25;384(8):727-743. doi: 10.1056/NEJM ra2021838.
8. Viprakasit V, Ekwattanakit S. Clinical Classification, Screening and Diagnosis for Thalassemia. Hematol Oncol Clin North Am. 2018 Apr;32(2):193-211. doi: 10.1016/j.hoc.2017.11.006.
9. Olivieri NF. The beta-thalassemias. N Engl J Med. 1999 Jul 8;341(2):99-109. doi: 10.1056/NEJM199907083410207. Erratum in: N Engl J Med 1999 Oct 28;341(18):1407.
10. Nienhuis AW, Nathan DG. Pathophysiology and Clinical Manifestations of the β-Thalassemias. Cold Spring Harb Perspect Med. 2012 Dec 1;2(12):a011726. doi: 10.1101/cshperspect.a011726.
11. Taher A, Musallam K, Capellini MM. Guidelines for the Management of Non-Transfusion Dependent β-Thalassemia. Published by Thalassemia International Federation. 3rd Edition, 2023, 1-129.
12. Musallam KM, Rivella S, Vichinsky E, Rachmilewitz EA. Non-transfusion-dependent thalassemias. Haematologica. 2013 Jun;98(6):833-44. doi: 10.3324/haematol.2012.066845.
13. Origa R. β-Thalassemia. Genet Med. 2017 Jun; 19(6):609-619. doi: 10.1038/gim.2016.173. Epub 2016 Nov 3. PMID: 27811859.
14. Mallat NS, Wehbe D, Haddad A, Cappellini MD, Marcon A, Koussa S, Abboud MR, Radwan A, Taher AT. Priapism, an emerging complication in β-thalassemia intermedia patients. Hemoglobin. 2014;38(5):351-4. doi: 10.3109/03630269.2014.960087.
15. T Sankaran VG, Nathan DG, Orkin SH. Thalassemias. In: Orkin SH, Nathan DG, Ginsburg D, Look AT, Fisher DE, Lux S (eds) Nathan and Oski's Hematology and Oncology of Infancy and Childhood. Eight Edition. Elsevier. PA, USA. 2015;715-769.
16. Nienhuis AW, Nathan DG. Pathophysiology and Clinical Manifestations of the β-Thalassemias. Cold Spring Harb Perspect Med. 2012 Dec 1;2(12):a011726. doi: 10.1101/cshperspect.a011726.
17. Galanello R., Origa R., Beta-thalassemia, Orphanet J Rare Dis. 2010; 5: 11,doi: 10.1186/1750-1172-5-11
18. Premawardhena A, Ranawaka U, Pilapitiya T, Weerasinghe G, Hapangama A, Hettiarachchi S et al. Headache: an important symptom possibly linked to white matter lesions in thalassaemia. Br J Haematol, 2019, 185, 541–548. doi: 10.1111/bjh.15825.
19. Kremastinos DT and Farmakis D (2011) Iron overload cardiomyopathy in clinical practice. Circulation, 124, 2253–2263, doi: 10.1161/CIRCULATIONAHA.111.050773.
20. Liang R, Liang S, Jiang NH, Wen X-J, Zhao J-B, Nechtman JF et al. (1994) α and β thalassaemia among Chinese children in Guangxi Province, P.R. China: molecular and haematological characterization. Br J Haematol, 86, 351–354. doi: 10.1111/j.1365-2141.1994.tb04738.x
21. Fucharoen S, Weatherall DJ. The hemoglobin E thalassemias. Cold Spring Harb Perspect Med. 2012 Aug 1;2(8):a011734. doi: 10.1101/cshperspect.a011734.

Correspondence Address:
Prof. Dr. Zeynep Karakaş
Istanbul Universty, Istanbul Medical Faculty, Pediatric Hematology/Oncology/İstanbul, Türkiye
E-mail: zkarakas@istanbul.edu.tr

CLINICS OF ABNORMAL HEMOGLOBIN

Assistant Proffesor Dr. Defne Ay Tuncel
Health Sciences University Adana City Training and Research Hospital
Pediatric Hematology Oncology Clinic Adana-Türkiye

ABSTRACT

Mutations caused by amino acid substitutions in hemoglobin chains result in defective hemoglobins. Abnormal hemoglobins are the most frequent hemoglobinopathies after thalassemias. The majority of them do not cause clinical symptoms, but anemia is discovered by chance and identified through screening tests. Clinical manifestations of common aberrant hemoglobins, such as homozygous hemoglobin S, C, D, and O-Arab, are visible. Electrophoresis and High Pressure Liquid Chromatography (HLPC) are used to make the diagnosis.

Keywords: Abnormal hemoglobin, sickle cell, thalassemia, oxygen affinity

INTRODUCTION

Hemoglobinopathies are frequent in the Mediterranean, Asia, India, and Africa. Hemoglobinopathies are caused by alpha, beta, gamma, and delta mutations in hemoglobin genes. As a result of these mutations, aberrant hemoglobins arise, causing structural alterations. Because most mutations are heterozygous, they do not cause clinical symptoms and are usually discovered by chance as a result of screening testing. Cyanosis in the neonatal era, as well as hemolytic anemia during fetal life and after birth, are clinical findings.

Classification of Clinically relevant genetic variants affecting hemoglobin

Sickle cell disease-hemoglobin polymerizes and becomes insoluble
Homozygous sickle cell disease (HbSS)
Hemoglobin SC disease
Sickle cell β thalassemia
Thalassemia-decreased production of one of the normal globin chains (alpha or beta), leading to an imbalance
Hemoglobin C disease - hemoglobin forms crystals
Hemoglobin M disease - hemoglobin iron becomes oxidized from ferrous to ferric state causing congenital methemoglobinemia
Structural variants can also have a thalassemic phenotype (Hb Lepore, Constant Spring, Hb E)
Unstable hemoglobin variants such as Hb Koln can cause congenital Heinz body hemolytic anemia
High oxygen affinity variants such as Hb Chesapeake can cause familial erythrocytosis
Low oxygen affinity variants such as Hb Kansas can cause familial cyanosis

Abnormal Hemoglobin Defects molecular mechanisms

Mechanisms	Examples
Single base substitution leading to amino acid replacement	Hemoglobin S Hemoglobin C Hemoglobin E
Two replacements in same subunit	Hemoglobin C-Harlem
Fusion hemoglobins	Hemoglobin Lepore (delta-beta) Hemoglobin Miyada (beta-delta) Hemoglobin Parchman (delta-beta-delta) Hemoglobin Kenya (gamma-beta)
Deletion or insertion	Hemoglobin Gun Hill
Insertion	Hemoglobin Grady Hemoglobin Catonsville
Deletion and insertion	Hemoglobin Montreal Hemoglobin Galicia
Termination codon mutation	Hemoglobin Constant Spring
Frameshift mutation	Hemoglobin Wayne Hemoglobin Cranston
N-terminal mutation leading to retention of initiator methionine	Hemoglobin Long Island Hemoglobin Marseille

Aberrant hemoglobins with a single amino acid change: These are structurally aberrant hemoglobins that develop when one amino acid is replaced by another due to a mutation in the globin genes.

Abnormal hemoglobins having two amino acid alterations in the same chain: The majority of aberrant hemoglobins with two amino acid mutations in the beta chain are unstable. There are at least six aberrant Hb in this group; one of the amino acids modified is the HbS mutation, while the second amino acid alteration occurs somewhere in the beta chain.

Fusion hemoglobins. They are hemoglobins made up of two distinct polypeptide chains.

Abnormal hemoglobins with simple amino acid loss: In healthy persons, alpha globin chains comprise 141 amino acids, while beta, gamma, and delta globin chains contain 146 amino acids. When globin gene mutations result in a premature stop codon, one or more aminosides are lost. The beta chain is responsible for the vast majority of aberrant hemoglobins.

Those that cause frameshift as a result of nucleotide loss or insertion: The removal or addition of one or two nucleotides induces a frameshift in the DNA sequence. Thalassemia is typically caused by frameshift mutations.

Hemoglobins having abnormally lengthy globin chains. Mutations in the alpha or beta globin genes cause the alpha chain to be longer than 141 amino acids and the beta chain to be longer than 146 amino acids.

ABNORMAL HEMOGLOBIN INHERITANCE

Autosomal co-dominant transmission is shown in abnormal hemoglobins. People with two separates aberrant Hb types, such as HbS and HbD, are said to have double heterozygozity. This disease has two distinct inheritance.

1. Double heterozygous abnormal hemoglobins are allelic (HbS and HbD are on the same chromosome). There is no HbA in patients with allelic HbS and HbD. Because the two aberrant Hbs are present in equal amounts, HbF may rise as a result.

2. Non-allelic abnormal hemoglobins in distinct chains [HbS (β chain), Hb Hopkins II (α chain)].

COMPONENTS OF ABNORMAL HEMOGLOBIN

Hemoglobinopathies associated with the beta chain:

In homozygous aberrant hemoglobin, HbA is non-existent, HbA2 is normal or slightly raised, and HbF is normal or slightly increased.

In heterozygous abnormal hemoglobin, because HbA synthesis is more than abnormal Hb, the HbA ratio is greater than abnormal Hb.

Aberrant hemoglobins linked to the alpha chain: Because healthy people have four alpha genes, those with alpha gene abnormalities have a higher rate of aberrant hemoglobin. As the number of faulty genes increases, so does the rate of aberrant hemoglobin.

ABNORMAL HEMOGLOBIN DEFECTS MOLECULAR MECHANISMS

BETA GLOBIN VARIANTS

Due to diminished beta globin production, beta thalassemia variations are detected in persons with inefficient erythropoiesis and microcytic anemia.

Hb C

Hb C with a high mean corpuscular hemoglobin concentration (MCHC) is reflecting red blood cell (RBC) dehydration a low mean corpuscular volume (MCV) and hexagonal crystals inside RBCs; HbC.

disease results in mild hemolytic anemia, jaundice, and splenomegaly. Sickling is not caused by Hb C abnormalities unless Hb S is also inherited.

HbC(β 6 Glu→ Lys) in investigations using electrophoretic methods, HbC has a similar look to HbA2. Typically, HPLC and column chromatography are used to make the diagnosis. In smaller populations in western Africa, HbC is present. Blood transfusions are available for those with severe anemia.

Hb C Harlem

This sickle cell mutation with a second point mutation (p.Asp73Asn;c.220G>A) make up Hb C Harlem. When coinherited with another beta globin variation, Hb C Harlem induces SCD and can polymerize when deoxygenated.

Hb D

Hb D-Punjab (β121Glu→Gln), commonly known as Hb D Los Angles, is the most widely available Hb D variation. When mixed with Hb S, Hb D-Punjab produces SCD. Hb D homozygosity is uncommon and only results in minor test abnormalities. Mild anemia may be present in homozygous people. Treatment or prenatal diagnosis are not required.

The clinical signs of HbSD are severe. Patients are now more likely to experience sickle cell anemia symptoms such as acute chest syndrome, stroke, painful crises, joint necrosis, and sequestration crises. Treatment should be similar to that for sickle cell anemia. Hydroxyurea may be advantageous for them. Patients with severe clinical symptoms should undergo bone marrow transplantation, and prenatal diagnosis should be encouraged to avert the condition.

HbE

Beta thalassemic phenotype-causing Hb E is moderately unstable to oxidative damage. Hypochromia, target cells, and significant microcytosis are seen in homozygotes along with mild anemia.

HbE (β26 Glu→Val) is one of the abnormal hemoglobins that is common in the world and causes significant health problems. HbE is common in southeast Asia especially in Eastern Thailand. Homozygous cases present with moderate hemolytic anemia and spleen enlargement. Since HbE heterozygotes are resistant to Plasmodium Falciparum, its prevalence worldwide is tried to be explained by this. This mutation also activates the cryptic mRNA

splice site in the beta globin gene. Thus, since the synthesis of theβ-E chain decreases, findings such as anemia and microcytosis observed in homozygous HbE patients indicate the development of a thalassemia phenotype. In treatment, blood transfusion should be given to those with severe anemia, and iron chelation should be started when hyperferritinemia develops.

HbE-Beta Thalassemia

The kind of HbE syndrome that manifests the most severe clinical signs is HbE-Beta Thalassemia. However, the phenotypic of the disease is modified by the kind of beta thalassemia mutation, HbF level, and relationship with alpha thalassemia. In beta-thalassemia and HbE double heterozygotes, the clinical course is particularly severe. Similar to thalassemia, patients experience hyperferritinemia and numerous organ damage as a result of blood transfusion. Blood transfusions, iron binders, and substances that raise HbF level can all be utilized as therapeutic options. When required, hydroxyurea is utilized. Patients with severe disease should undergo bone marrow transplantation, and prenatal diagnosis should be advised to prevent the disease.

Hb Lepore

The beta thalassemia phenotype is caused by the fusion gene Hb Lepore, which results from a recombination event between nearby delta globin and beta globin genes. The mRNA in the Hb Lepore fusion gene product is largely nonsteady. Patients experience a thalassemia phenotype as a result of the decreased Hb Lepore production. Thalassemia major or intermedia phenotype is caused by beta thalassemia carrier and Hb Lepore double heterozygosity. Prevention and treatment According to the clinical course (thalassemia major, intermediate), patients should be closely follow up and treated. When a patient's course is severe, prenatal diagnostics should be used. Patients with abnormal hemoglobin may therefore appear with a wide range of clinical symptoms. The truth will come out when patients with dominant thalassemia, unexplained erythrocytosis, cyanosis, hemolytic anemia of unclear cause, and erythrocytosis are examined for abnormal hemoglobin levels.

Hb O Arab

In homozygotes, Hb O-Arab results in a moderate case of compensated hemolytic anemia. Severe SCD is brought on by compound heterozygosity for Hb O- Arab and HbS.

ALPHA GLOBIN VARIANTS

Alpha thalassemia variants

The production of alpha globin is often decreased by variants in the alpha globin loci (HBA1 and HBA2), leading to alpha thalassemia.

Patients in the dominant thalassemia group suffer from severe deficiencies in protein synthesis in the globin gene's coding region. Thalassemia is more likely to be the cause than abnormal hemoglobin, according to clinical observations and straightforward hematological tests such complete blood count. As a result, even though their hemoglobin is abnormal, individuals are monitored with a diagnosis of thalassemia. The fact that it is dominant as opposed to autosomal recessive sets it apart from other thalassemias. Extremely unstable hemoglobins, particularly those with exon 3 mutations, arise in the heterozygous state.

Mutations that result in an increase in the length of the amino acid chain; premature stop codons that result in a reduction in the amount of amino acids. The beta thalassemia phenotype is caused by amino acid alterations in alpha globin that interfere with its ability to connect to the protein that stabilizes alpha globin.

In the bone marrow aspiration sample, there is erythroid hyperplasia, inefficient erythropoiesis, inclusion bodies in erythrocyte precursors, considerable hypochromism in peripheral blood smear, microcytosis, and basophilic stipling. The oxidation of HEM groups released after methemoglobin is formed and the presence of free radicals are thought to be the causes of inclusion development. The illness is also known as Inclusion Body-Thalassemia syndrome as a result of these characteristics. Unusually, one of the parents is. Both parents might occasionally be healthy. due to the fact that most of them are fresh (de novo) alterations.

Prevention and treatment: Since thalassemia is present in the majority of patients, transfusion is not

frequently required. Patients with a severe course or those who experience the onset of severe anemia should have blood transfusions. Chelation therapy should be applied to those with high ferritin levels

Hb Constant Spring

Four alpha genes exist. Hb an alpha globin chain termination variant known as Constant Spring is widespread in Southeast Asia.

Hb Barts and Hb H

A deadly intrauterine syndrome without fetal transfusion results from the deletion of all four genes, while three genes can be deleted or mutated to generate Hb H sickness. When there are relatively few alpha chains available, Hb Barts (gamma tetramers) and Hb H (beta tetramers) develop; these Hbs are not useful.

VARIANTS THAT CAN AFFECT ALPHA OR BETA GLOBIN

UNSTABLE HB S

It is often autosomal dominantly transmitted, and spontaneous mutation is common. Therefore, it should not be assumed that the condition is present just because the mother, father, or siblings do not have abnormal hemoglobin. Electrophoretic techniques cannot be used to demonstrate aberrant hemoglobin because hyperunstable hemoglobins degrade quickly. As a result, individuals are given the diagnosis of hemolytic anemia with undetermined origin. Clinical Results There is a wide variety of clinical and laboratory evidence of non-steady abnormal hemoglobins.

Some exhibit illness signs during the newborn period, while others do so at various ages; There are three distinct subgroups: reticulocytosis increases by up to 30% in excessive hemolysis, moderate or mild hemolysis in chronic hemolytic anemia, and intermittent hemolytic anemia. In the final category, hemolysis can be brought on by infections, drug use, and substances like phenosopyridine, sulfonamides, and nitrate water. This condition is frequently mistaken for erythrocyte G6PD enzyme deficiency.

Patients typically complain of pallor, jaundice, enlarged liver and spleen, dark urine, early gallstone development, and leg ulcers. They excrete urine that is dark in hue, ranging from brown to black. The dipyrroles released from HEM during the formation of the Heinz body are what give the pigment its black hue.

Hematological Findings

Chronic or sporadic hemolytic anemia is present in the majority of individuals. Common findings include reticulocytosis, polychromasia in peripheral blood smear, poikilocytosis, and normoblastemia. Microspherocytes and malformed red blood cells (bite cells) may be detected with acute hemolysis. Bite cells are formed as red blood cells travel through the spleen due to phagocytosis of Heinz bodies. Hemolytic anemia can still cause hypochromia. Hypochromia may result from blood loss when the Heinz body is developing, according to one theory.

Spleen sequestration may lead to the development of thrombocytopenia. About 25% of the total hemoglobin is non-steady aberrant hemoglobin connected to the beta chain. The abnormal Hb rate for alpha chain alterations (variant) is about 12%.

When beta thalassemia coexists with heterozygous abnormal Hb, the abnormal Hb rate exceeds 50%. When beta0 thalassemia and non-stationary Hbs like Hb Duart or Hb Köln are present, the abnormal hemoglobin rate is close to 100%.

Up to 2% of hyperunstable hemoglobins contain aberrant hemoglobin. The patient is identified as having hemolytic anemia of unknown origin because the abnormal hemoglobin cannot be seen during evaluation using traditional techniques due to the extremely low incidence.

What Steps Are Necessary to Make a Diagnosis?

Patients with undiagnosed hemolytic anemia should have their hemoglobin levels checked. The likelihood of non-stationary hemoglobin should be taken into account in individuals who experience acute pallor and hemolysis after contracting a virus, bacterial infection, using an oxidant, or using a sulfonamide. Patients go into an aplastic crisis when exposed to the Parvovirus B19. Hemolytic crises end on their own. Because they enhance hemoglobin denaturation, fever and brief acidosis produce hemolysis.

Search for Heinz bodies: The solubility of the molecule varies in some aberrant hemoglobins. Round (globular) inclusions start to build up in erythrocytes over time (Heinz Body). These shorten erythrocyte life expectancy and result in hemolytic anemia. Staining the peripheral blood smear with vivid cresyl blue or methylene blue allows for the demonstration of Heinz bodies. Test for heat stability, for one or two hours, the hemolysate is incubated at 50 °C. Precipitation happens when unstable hemoglobin is present. At 55 °C, normal hemoglobin starts to precipitate.

DNA sequence analysis needs to be used to analyze the globin genes.

Care and Monitoring

When hemolysis attacks are severe, blood transfusions should be done in addition to the administration of folic acid and the treatment of infections. Oxidant medications like acetaminophen should not be used. Splenectomy can end the need for transfusions in cases of severe hemolysis crises that occur often. Prenatal diagnosis is advised in the case of nonstationary hemoglobinopathies with a severe clinical course and a need for frequent blood transfusions.

OXIDIZED ABNORMAL HEMOGLOBINS: HEMOGLOBIN M DISEASE

This globin disorder is autosomal dominant. The divalent (Fe+2) HEM iron oxidizes into the ferric form (Fe+3) as a result of new amino acids being displaced by old ones in the alpha or beta globin chain as a result of mutation. Hemoglobin M shares characteristics with methemoglobin, such as cyanosis and a +3 iron value. HbM, on the other hand, is totally distinct from methemoglobin. The globin portion of methemoglobin is normal whereas the iron in hemoglobin is oxidized (Fe+3).

Because of a genetic error in hemoglobin M, HEM iron has a +3 value. In order to distinguish Hb M from methemoglobin, this leads to an aberrant spectrum. The amino acid Tyrosine replaces the proximal or distal Histidine that is often associated with HEM iron in Hb M. There have also been reports of substitution with different amino acids. The patients have brownish-purple skin and mucosa.

Cyanosis that starts at birth and those that start around one year of age both point to alpha gene mutations. The absence of dyspnea and clubbing aids in the differential diagnosis since patients may be mistaken for having congenital heart problems.

Hemoglobin M must be recognized from methemoglobin during the diagnostic process. Compared to HbA, Hb M has a distinct spectrum. Making the diagnosis relies heavily on spectrophotometric analysis. Hemoglobin electrophoresis has a restricted role; when potassium cyanide is given to the patient's hemolysate, red hue suggests methemoglobin. Because it is not distinguished from regular hemoglobin, the oxy form of hemoglobin in cellulose acetate is useless. The best diagnostic method is agar electrophoresis. Treatment and averting problems Treatment and prenatal diagnosis are not necessary because there are no other important clinical signs than cyanosis.

HIGH OXYGEN AFFINITY VARIANTS

Some of the changes in this group affect the Bohr effect, the placement of salt bonds, the binding of HbA to 2,3 DPG, and the Bohr effect, which increases hemoglobin's affinity for oxygen by upsetting the interactions within the HEM pocket. Higher oxygen-binding abnormal hemoglobins cause the O2 dissociation curve to shift to the left and bind more oxygen than HbA. As a result, with the same capillary oxygen pressure, they release less oxygen into the tissues. Erythropoietin is produced when there is mild tissue hypoxia, and more erythrocytes are produced as a result, leading to erythrocytosis.

Erythrocytosis results from tissue hypoxia because there is inadequate oxygen delivery to the tissues. Phlebotomy is typically not necessary in patients with erythrocytosis because these mechanisms increase oxygen supply to the tissues.

A family history of erythrocytosis, aberrant hemoglobin found during hemoglobin electrophoresis (a normal result should not rule out the disease), plasma and urine erythropoietin levels, and the Hb functional test (P50) all contribute to the diagnosis. The P50 value is low in aberrant hemoglobins that have a strong affinity for oxygen. A tool like a reverse phase HPLC must be used for this.

Prevention and treatment: Treatment and prenatal diagnosis are not necessary because there are no major clinical findings in the patients.

LOW OXYGEN AFFINITY VARIANTS

Oxygen binding is hampered in hemoglobins with poor oxygen affinity Hb Kansas (β 102 Arg→Thr). Because Hb Kansas cannot bind oxygen well, the oxygen dissociation curve swings to the right. The skin appears cyanotic in certain patients who have defective hemoglobin and poor oxygen affinity. Because alpha chains begin to be generated in the second trimester of fetal development, cyanosis is detected at birth due to aberrant hemoglobins caused by alpha gene abnormalities. After ruling out other causes of cyanosis, such as congenital heart disease, the potential of defective hemoglobin should be considered.

These hemoglobins release more O2 to the tissues than normal at low capillary PO2 pressure in the tissues. The release of more oxygen into the tissues has two outcomes.

Because more oxygen is delivered to the tissues in the first case, oxygen requirements can be met even at low hematocrit levels. Thus, despite the low hematocrit, patients appear to be totally healthy, and tissue oxygenation is normal.

Cyanosis is detected in the second condition because the amount of unsaturated (desturated) hemoglobin circulating in the capillaries and veins is greater than 5g/d L. Despite having cyanosis, the individuals have no complaints and appear clinically normal.

Diagnosis:

Methemoglobinemia, sulfhemoglobinemia, and Hb M should be investigated and confirmed to be normal. Normal venous blood (deoxy Hb) is violet in color, whereas the oxyhemoglobin of people with defective hemoglobin, which has a poor affinity for oxygen, is brilliant red. As a result, if the blood of a patient with a poor affinity for oxygen does not change color when oxygenated, disorders such as Methemoglobinemia, Sulfhemoglobinemia, or Hb M should be evaluated. Measuring p50 in whole blood is critical in diagnosis. Hb electrophoresis should be done; the band may not always be visible in electrophoresis. Cardiopulmonary illness should be avoided.

Treatment and prevention: Because the clinical course is mild, treatment and prenatal diagnosis are usually unnecessary.

REFERENCES

1. Hardison RC, Chui DH, Giardine B, et al. Hb Var: A relational database of human hemoglobin variants and thalassemia mutations at the globin gene server. Hum Mutat 2002; 19:225.
2. Lukens JN. Disorders of Red Cells. In Greer JP, foerster J, Paraskevas F, Glader B, Arber DA, Means RT(eds) Wintrobe's Clinical Hematology11th edition.Lippincott Williams&Wilkins,2004,1247-1500.
3. Piel FB,Weatherall DJ.The α- thalassemias.NEngl J Med 2014;271:1908.
4. Huisman TH. Percentages of abnormal hemoglobins in adults with a heterozygosity for an alpha-chain and/or a beta -chain variant.Am J Hematol 1983;14:393.
5. Gürgey A. Fonksiyon kazanan anormal hemoglobinler.Türk Pediatrik Hematoloji Dergisi.2009; 3:4-12.
6. Steinberg MH,Chui DH.HbC disorders.Blood 2013; 122:3698.
7. Kalle Kwaifa,I, Lai MI, Md Noor S. Non -deletional alpha thalassemia: A review.Orphanet J Rare Dis 2020; 15:166.
8. Piel FB,Hay SI, Gupta S, et al.Global burden of sickle cell anaemia in children under five,2010-2050; modelling based on demographics, excess mortality, and interventions.PLoS Med 2013;10:c 1001484.
9. Benz EJ.Hemoglobin variants associated with hemolytic anemia,altered oxygen affinity,and methemoglobiemias.Hematology Basic Principles and Practice.4th ed.2005.p645-53.
10. Lukens JN.Unstable hemoglobin disease. Wintrobe's Clinical Hematology,2th ed.2004.p.1313-8.
11. Hafsia R, Gouider E,Ben Moussa Set al. Hemoglobin O Arab: about 20 cases Tunis Med 2007; 85(8):637-40.
12. Hardison RC,Chui DH, Giardine B, et al. HbVar: Arelational database of human hemoglobin variants and thalassemia mutations at the globin gene server.Hum Mutat 2002; 19:225.
13. Olivieri NF, Pakbaz Z, Vichinsky E. HbE/ β - Talasemi: Basis of marked clinical diversity. Hematol Oncol Clin N Am. 2010;24:1055-1070.

Correspondence Address:
Assistant Proffesor Dr.Defne Ay Tuncel
Health Sciences University Adana City Training and Research Hospital
Pediatric Hematology Oncology Clinic Adana-Türkiye
E-mail: defneayinan@gmail.com

CHAPTER 4

SCREENING AND DIAGNOSIS METHODS OF THALASSEMIA AND HEMOGLOBINOPATHIES

CONVENTIONAL METHODS IN SCREENING AND DIAGNOSIS OF THALASSEMIA AND HEMOGLOBINOPATHIES

Funda Tayfun Küpesiz, Zeynep Öztürk
Akdeniz University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hematology and Oncology, Antalya, Türkiye

ABSTRACT

Laboratory diagnosis of thalassaemias and abnormal haemoglobins involve a group of tests including determination of red blood cell indices, haemoglobin HbA_2, Hb F and haemoglobin variants. Erythrocyte indices are essential in the laboratory diagnosis of thalassaemias and haemoglobinopathies. Erythrocytes are measured using an automated haematology analyser and are critical in demonstrating the low Hb and microcytosis observed in thalassaemia patients. However, erythrocyte indices alone are not enough to diagnose haemoglobinopathy and warrant haemoglobin variant analyses.

Currently, high performance liquid chromatography or capillary electrophoresis is used to analyse haemoglobin variants. Both methods provide both qualitative and quantitative analyses of haemoglobins. Cellulose acetate electrophoresis and the IEF method are not currently preferred due to laboratory workload and inaccurate analysis of low concentrations of haemoglobin. HPLC differentiates and reliably measures HbA, HbA_2, HbF and variant haemoglobins such as HbS, HbC, HbO^{-Arab}, D-Punjab and G-Philadelpia. However, many factors such as β-, α- and δ-gene mutations, iron metabolism, endocrinological mutations and some types of anaemia may affect HbA_2 level. HbA_2 has no known physiological function, but correct interpretation of HbA_2 values is important in thalassemia screening programmes. Therefore, HbA_2 values should always be interpreted with other findings such as erythrocyte indices, iron status and familial studies.

In addition to electrophoresis and chromatography, there are various methods used for the diagnosis of abnormal haemoglobins. These include in vitro dyes for the diagnosis of HbH, sickling test and sickle solubility test for the diagnosis of HbS, haemoglobin oxygen affinity test (p50) used to identify haemoglobin variants with low oxygen affinity, isopropanolol stability test to identify unstable haemoglobins.

Keywords: Haemoglobinopathy, thalassaemia, conventional methods, variant haemoglobin

BACKGROUND

Haemoglobinopathies are monogenic diseases that result in globin chain defects. If the mutation causes a quantitative change in globin chain synthesis, this results in thalassemias; if it causes a structural defect, it causes haemoglobinopathies such as sickle cell anaemia and variant haemoglobins.

Screening and diagnostic evaluation of thalassaemias and abnormal haemoglobins starts with a routine laboratory evaluation. The evaluation starts with a complete blood count, reticulocytes and peripheral smear to assess erythrocyte indices and is followed by a group of tests including haemoglobin electrophoresis/high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) to determine HbA_2, HbF and other haemoglobin variants.

In addition to electrophoresis and chromatography, there are various methods used for the diagnosis of abnormal haemoglobins. These include in vitro dyes for the diagnosis of HbH, sickling test and sickle solubility test for the diagnosis of HbS, hae-

moglobin oxygen affinity test (p50) used to identify haemoglobin variants with low oxygen affinity, isopropanolol stability test to identify unstable haemoglobins.

Evaluation of Complete Blood Count and Peripheral Smear

In β-thalassaemia major (β^0 thalassaemia), severe hypochromic microcytic anaemia (Hb 3-7 g/dL) is observed. Complete blood count shows markedly low haemoglobin **(1)** and mean corpuscular volume (MCV) (50-60 fl). Mean corpuscular haemoglobin (MCH) (12-18 pg) and Hb concentration (MCHC) per erythrocyte and erythrocyte count decreased. However, RDW, which shows erythrocyte cell distribution, is very high. The reticulocyte count could not increase as much as expected for the extent of anaemia. Peripheral smear evaluation reveals marked hypochromic microcytic erythrocytes, erythroblasts (nucleated erythrocytes), anisocytosis, poikilocytosis (fragmented erythrocytes, tear cells), polychromasia, basophilic stippling and target cells **(2-4)**.

In patients with β-thalassaemia intermedia (β^+ thalassaemia), erythrocyte indices in complete blood count and peripheral smear findings are similar to those in patients with thalassaemia major, but Hb value in patients with intermedia thalassaemia is usually between 7-10 g/dL.

People with β-thalassaemia minor (heterozygous β-thalassaemia) have a hypochromic microcytic anaemia that is borderline or slightly below normal for their age. The evaluation of erythrocyte indices reveals low MCV (<80 fl) and MCH (27 pg) and normal RDW. Increased erythrocyte count is a parameter that helps to differentiate iron deficiency anaemia. The peripheral blood smear evaluation shows that morphological changes in erythrocytes are milder than in sick individuals. While no erythroblasts and anisocytosis are observed in the smear, hypochromic microcytic erythrocytes, basophilic stippling and the presence of target cells are noted (3).

In carriers of alpha thalassaemia, if the carrier is α^+, the complete blood count is normal, but if the carrier is α^0, MCV and MCH are low. In patients with the coexistence of alpha-thalassaemia (-α/-α and --/αα) and β-thalassaemia carriage, MCV and MCH may be normal. Elevated HbA2 may help the diagnosis in these patients.

HbH usually includes a moderate (7-10 g/dL) hypochromic microcytic anaemia. MCV (50-65 fl), MCH 1 (5-20 pg) are low and blood smear may show marked anisocytosis, poikilocytosis, target cell, tear cell and more rarely erythroblasts and basophilic stippling. In these patients, staining with bright crezil blue or methylene blue may show HbH inclusion bodies.

In sickle cell haemoglobinopathies, complete blood count is normal in HbS carriers. In sickle cell patients with homozygous HbS mutation, Hb value is low whereas MCV and MCH are normal. Peripheral smear shows sickle and banana shaped erythrocytes.

Thus, complete blood count and peripheral smear, which are the first evaluation tests, are inadequate in making the diagnosis and analyses of haemoglobin variants take a step closer to the diagnosis. The diagnosis is confirmed by molecular gene analyses.

Haemoglobin Electrophoresis/High Performance Liquid Chromatography and Capillary Electrophoresis

Haemoglobins can be differentiated by various electrophoresis techniques. Conventional electrophoresis uses a cellulose acetate membrane and allows separation at alkaline pH, but this technique was later replaced by isoelectric focusing (IEF), which is based on the principle that haemoglobins migrate according to their isoelectric values. Normal haemoglobins including HbA, HbF and HbA2 and variant haemoglobins including HbS and HbC can be detected by isoelectric focusing technique. However, difficulties may arise in differentiating HbS from HbD or HbG and HbC from HbE or HbO^{Arab}. Cellulose acetate electrophoresis and isoelectric focusing (IEF) methods are not currently preferred due to laboratory workload and inaccurate analysis of low concentrations of haemoglobin.

Capillary electrophoresis is a technique that differentiates haemoglobin variants very well **(5)**. The separation of haemoglobin fractions occurs in a buffer capillary with an inner diameter of 50 microns. When the sample is injected at one end, the proteins migrate to the other end under high voltage conditions.

High performance liquid chromatography is an analytical chemistry method used to separate, identify and measure individual components in a mixture. Depending on the interaction between stationary phase, mobile phase and sample, haemoglobin variants are resolved in HPLC. High performance liquid chromatography differentiates and reliably measures HbA, HbA_2, HbF and variant haemoglobins such as HbS, HbC, HbO-Arab, D-Punjab and G-Philadelpia.

Currently, HPLC or CE is used to analyse haemoglobin variants. Both methods provide both qualitative and quantitative analyses of haemoglobins. High-performance liquid chromatography and CE have both been introduced as mandatory pre-marital screening tests in many countries because they allow the diagnosis of prenatal and postnatal haemoglobinopathy in a short time.

HbA_2 has no known physiological function, but measurement of HbA_2 values is essential in thalassaemia screening programmes. Various factors affect HbA_2. For example, glycolysed and chemically modified HbS may slightly (3.8-4.5%) increase the level of HbA_2 **(6, 7)**. In the presence of HbD, HbA_2 may be falsely low. Therefore, HbA_2 values should always be interpreted with other findings such as erythrocyte indices, iron status and familial studies. In the presence of hypochromic microcytic erythrocytes, increased HbA_2 levels are diagnostic for heterozygous β-thalassaemia. However, thalassaemia carriers may also have normal or borderline HbA_2. Therefore, detailed evaluation of HbA_2 is very important to avoid any mistake in the diagnosis.

Increased HbA_2

The classical phenotype of thalassaemia carriers shows increased HbA_2 (3.5-6.0%), increased erythrocyte count, low MCV (60-75 fL) and MCH (18-24 pg). Peripheral smear shows microcytosis, hypochromic and anisopoikilocytosis in erythrocytes. Elevated HbA_2 is the most common form in thalassaemia carriers and may be the result of transcriptional and sometimes post-translational effects in β-thalassaemia carriers **(8)**.

HbA_2 values above 7% in high performance liquid chromatography generally indicate the presence of another haemoglobin co-eluting with HbA_2. HbE and Hb Lepore variants are the two common mutations that elute on the band with HbA_2. Large deletions of several hundred bases adjacent to the β globin gene are also seen. In such a case, elevated HbA_2 (> 7%) and increased HbF indicate a phenotype **(9)**. These deletions affect the β-globin gene leading to an increase in δ- and γ-globin genes.

- Clinical findings of heterozygous HbE patients are usually normal and there is very little change in erythrocyte indices (MCV 80-90 fL and HbA_2 22-30%). While HbE levels on HPLC of these patients may be 30% and higher when co-inherited with a β-thalassaemia allele, they may decrease to 10-20% when co-inherited with an α-thalassaemia allele **(10)**.

- In patients with homozygous HbE, mild hypochrome microcytic anaemia and 80-90% HbA_2 may be observed. In β^+/HbE combined heterozygotes, electrophoresis shows HbA, whereas HbA is absent in β^0/HbE association.

- Patients with heterozygous Hb Leporrhoea have mild microcytic and hypochromic anaemia and a slightly elevated HbF (2-3%) and HbA_2 (10-15%) peak on HPLC **(11)**.

- Cases with homozygous Hb Leporea and β/Hb Leporea coexistence showed thalassaemia major phenotype **(12)**. Three variants of Hb Lepore have been detected by molecular analysis: Hb Lepore Boston, Hb Hollandia and Hb Baltimore. Haemoglobins including Hb Jeddah, HbD-Ouled Rabah, Hb Fortde France which are co-eluted with HbA_2 are very rare haemoglobin variants.

Borderline HbA_2

There are reports of β-thalassaemia carriers showing HbA_2 levels at the upper limit of the normal range **(1, 13, 14)**. An accurate diagnosis in these cases requires great effort.

- In 80% of patients without iron deficiency and with borderline HbA_2 levels, no β-globin gene defect was detected and only 20% had α-, β- and δ-globin gene mutations **(15)**. Low HbA_2 levels have been demonstrated in thalassaemia carriers with iron deficiency anaemia **(10, 16)**. Therefore, in patients with iron deficiency, if the percentage of HbA_2 is within the limit range, Hb

electrophoresis should be repeated after iron treatment.

- HbA_2 levels may also decline in cases that may cause post-translational modification, such as lead poisoning **(17)**.

- In β-thalassaemia carriers, co-inheritance of α or δ globin mutations can result in borderline normal HbA_2 levels. Thalassaemia carriers with a single α-gene deletion had higher MCV and MCH values and normal borderline HbA_2 ratios compared to carriers without α-gene deletion **(18)**. On the other hand, α globin gene triplication, some β globin gene variants and β thalassaemia mutations have also been reported to cause borderline HbA_2 levels in β thalassaemia carriers **(19)**. Kruppel-like factor 1 (KLF_1) mutations have also been demonstrated to lead to borderline HbA_2 levels **(20, 21)**.

- HbA_2 synthesis may increase in conditions that delay nuclear maturation of erythrocyte precursors, such as deficiencies in folic acid, vitamin B12 and use of anti-retroviral drugs **(22, 23)**. In patients with megaloblastic anaemia, HbA_2 levels have been shown to normalise with treatment **(22)**. If a thalassaemia carrier has megaloblastic anaemia due to B12/folic acid deficiency, HbA_2 levels may fall to borderline levels and normocytic anaemia may occur. The effect of thyroid hormones on HbA_2 has also been shown; mean HbA_2 levels are below normal in patients with untreated hypothyroidism, while HbA_2 levels are increased in patients with untreated hyperthyroidism **(24, 25)**.

Normal HbA_2

In healthy individuals, erythrocyte parameters, iron levels and haemoglobin types are normal.

- In case of iron deficiency in β-thalassaemia carriers, HbA_2 levels may be low and may be detected at normal values. Therefore, if microcytic erythrocytes are still present after iron treatment, DNA analysis for the β-globin gene is performed.

- If erythrocyte indices are low but Hb and iron parameters are normal, α-thalassaemia genotype is suspected. While α^0-thalassaemia leads to low erythrocyte indices and HbA_2 levels (2.2-2.8%), in α^+-thalassaemia, HbA_2 is at normal values despite a slight decrease in erythrocyte indices.

- If in patients with normal iron parameters and HbA_2 levels, erythrocyte cell indices are decreased and HbF levels are high, large deletions in the β-globin gene may be considered and this leads to δβ- or γδβ-thalassaemia.

- Patients with Hereditary Persistence of Fetal Haemoglobin (HPFH) have 3-30% HbF while erythrocyte parameters, HbA_2 and iron levels are normal. The clinical and haematological findings distinguish δβ-thalassaemia from those of hereditary inheritance of fetal haemoglobin. Hypochromia and microcytosis are seen in δβ-thalassaemia heterozygotes **(10)**. The association of β-thalassaemia and HPFH results in a silent or very mild phenotype, whereas the combined heterozygosity of δβ-thalassaemia and β-thalassaemia results in a severe thalassaemia phenotype. Co-inheritance of α-thalassaemia with HPFH causes hypochromia and microcytosis. Therefore, HPFH and δβ-thalassaemia can only be differentiated by molecular analysis.

Decreased HbA_2 (<2%)

- If HbA_2 is less than 2% in a person with normal erythrocyte indices and iron parameters, δ-genotype is considered **(26)**. When haemoglobin and iron parameters are normal but erythrocyte indices are low, co-heritance of δ- and α-thalassaemia is suspected and in such cases HbA_2 is lower (1.7±0.3) (10). In HbH the level of HbA_2 is below 2% **(27)**.

In conclusion, HbA_2 level may be affected by many factors such as β-, α- and δ-gene mutations, iron metabolism, endocrinological problems and some types of anaemia. Therefore, all factors should be taken into consideration to reduce the risk of error in haemoglobinopathy diagnosis and screening methods.

Sickling Test

A sickling test using sodium metabisulphite is performed when sickle cell syndrome is suspected. When sodium metabisulfite, a reducing agent, is added to the blood and deprived of oxygen, a rapid

sickling in the shape of the erythrocytes is observed. For this purpose, a drop of blood and a drop of 2% sodium metabisulphite solution is mixed on the slide, covered with a coverglass, the edges are sealed using wax/vaseline, kept at room temperature and examined under a microscope within 24 hours. Positive samples show typical sickle-shaped erythrocytes. If the sickling test is positive, then confirmatory tests such as haemoglobin electrophoresis should be performed for definitive diagnosis.

This test is used in the diagnosis of sickle cell anaemia and is also positive when HbS is above 25% and in the presence of other haemoglobins causing sickling (e.g., C-Harlem).

Sickle Resolution Test (Sickling Test)

This method is used to confirm sickle cell anaemia. It is used when a HbS band or peak is seen on electrophoresis or HPLC. The test is positive when haemoblobin S is greater than 8% **(4)**.

When anticoagulated blood is mixed with sickle buffer, the erythrocytes are haemolysed and haemoglobin is released. Haemoblobin S is insoluble in high concentration buffer and forms liquid crystals, resulting in a cloudy solution. If the medium does not contain HbS, the solution will be translucent or transparent. In patients with paraproteinaemia, patients who have undergone splenectomy and in the presence of multiple Heinz bodies, the test results of the sickle solubility test may be false positive.

Determination of Haemoglobin H (Demonstration of Inclusion Bodies)

When haemoglobin H (HbH) disease is suspected or when HPLC shows a peak that may be HbH, "HbH inclusions" need to be demonstrated. The test is based on the principle of exposing the HbH to a mild oxidant such as bright cresyl blue or methylene blue. A blood sample collected in an EDTA tube is mixed with 10 g/L bright crezil blue or 20 g/L methylene blue prepared in phosphate buffer at pH 7.4 in equal proportions and incubated at 37ºC for 1-3 hours. A peripheral smear is prepared without any additional staining and evaluated under a microscope. The HbH precipitate is evenly distributed in the erythrocytes as small, blue-stained inclusions, causing a golf ball-like appearance. In splenectomised patients, HbH inclusion bodies in erythrocytes exhibit Heinz bodies as well as golf ball appearance **(4)**.

Haemoglobin Oxygen Affinity Test (p50)

Variant haemoglobins show different levels of affinity for oxygen. As the release of oxygen to tissues is more difficult with haemoglobin with a high affinity for oxygen (low p50, left-shifted curve), erythropoietin synthesis is stimulated and individuals with this variant have erythrocytosis, which is often familial. Whereas variant haemoglobins with low oxygen affinity (high p50, right-shifted curve) release more oxygen to tissues, individuals with this variant may have a mild anaemia accompanied by cyanosis due to haemoglobin desaturation which is called familial cyanosis.

Haemoglobin electrophoresis may be inadequate to detect these variant haemoglobins. Partial pressure of oxygen in mmHg can be measured at 37ºC using the Hemox analyser. For testing, it is most convenient to use an erythrocyte haemolysate prepared from a freshly drawn blood sample.

Isopropanolol Stability Test

This test method is used for the detection of unstable haemoglobins. Unstable variant haemoglobins accumulate in the erythrocyte in the form of "Heinz bodies". This condition is called "Congenital Heinz Body Haemolytic Anaemia" and its inheritance is often autosomal dominant. Since circulating erythrocytes containing Heinz bodies are destroyed by the spleen, it may not always be possible to demonstrate these abnormal variants on haemoglobin electrophoresis. Measuring the stability of the Hb molecule in these patients is extremely useful for diagnosis.

In the isopropanol stability test, erythrocyte haemolysate is incubated with isopropanol to weaken the hydrophobic binding of globin chains. Thus, precipitation of the unstable Hb molecule is observed. A fresh blood sample should be taken for this test and the sample should be studied within a maximum of 24 hours.

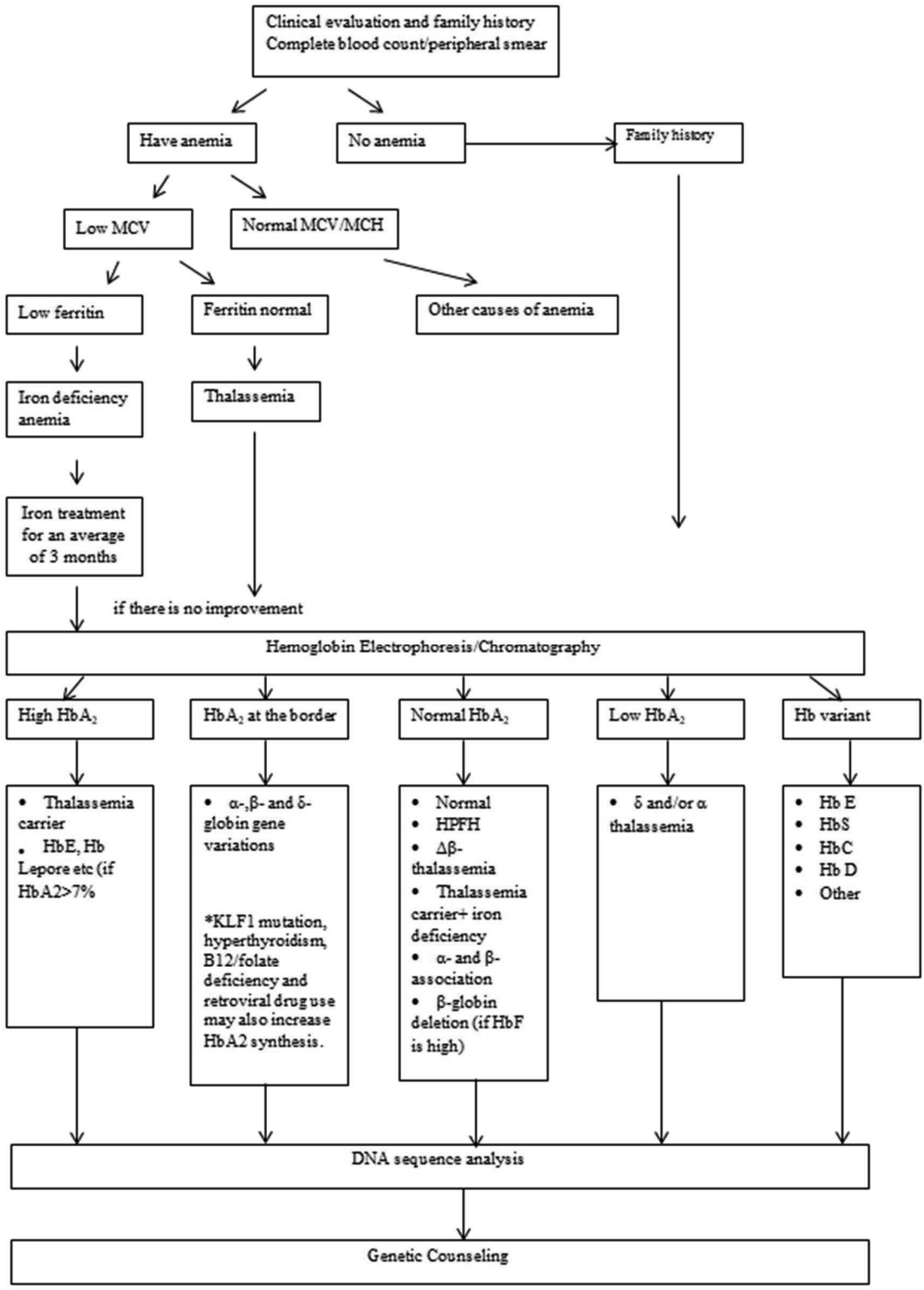


Figure 1: *Tests Required for the Identification of Thalassaemia and Haemoglobinopathies*

REFERENCES

1. Mosca A, Paleari R, Galanello R, et al. New analytical tools and epidemiological data for the identification of HbA2 borderline subjects in the screening for beta-thalassemia. Bioelectrochemistry. 2008; 73(2):137-40.
2. Meryem A. Kalıtsal Hemolitik Anemilerde Laboratuvar Tetkikleri. In: Özcan Bör HG, Şule Ünal, ed. Kalıtsal Anemiler. 1 ed. İstanbul: Galenos Yayınevi; 2021. p. 14-23.
3. Origa VR. Talaseminin Klinik Yönetim Rehberi. In: Aydınok Y TA, Ünal S, Kılınç Y, Malyalı D, ed. Genetik Temel, Patofizyoloji ve Tanı 3ed. Lefkoşa: Team up Creations Ltd; 2014. p. 259.

4. Bain BJ. Laboratory techniques for the identification of abnormalities. Haemoglobinopathy Diagnosis. 3 ed. London,UK: John Wiley & Sons Ltd.; 2020. p. 70.
5. Cotton F, Lin C, Fontaine B, et al. Evaluation of a capillary electrophoresis method for routine determination of hemoglobins A2 and F. Clinical chemistry. 1999; 45(2):237-43.
6. Whitten WJ, Rucknagel DL. The proportion of Hb A2 is higher in sickle cell trait than in normal homozygotes. Hemoglobin. 1981; 5(4):371-8.
7. Suh DD, Krauss JS, Bures K. Influence of hemoglobin S adducts on hemoglobin A2 quantification by HPLC. Clinical chemistry. 1996; 42(7):1113-4.
8. Menzel S, Garner C, Rooks H, et al. HbA2 levels in normal adults are influenced by two distinct genetic mechanisms. British journal of haematology. 2013; 160(1):101-5.
9. Padanilam BJ, Felice AE, Huisman TH. Partial deletion of the 5' beta-globin gene region causes beta zero-thalassemia in members of an American black family. Blood. 1984; 64(4):941-4.
10. Giambona A, Passarello C, Renda D, et al. The significance of the hemoglobin A(2) value in screening for hemoglobinopathies. Clinical biochemistry. 2009; 42(18):1786-96.
11. Schiliro G, Musumeci S, Pizzarelli G, et al. Haemoglobin Lepore Boston-Washington in Sicily: clinical, haematological, and biosynthetic studies. Journal of medical genetics. 1980; 17(3):179-82.
12. Efremov DG, Efremov GD, Zisovski N, et al. Variation in clinical severity among patients with Hb Lepore-Boston-beta-thalassaemia is related to the type of beta-thalassaemia. British journal of haematology. 1988; 68(3):351-5.
13. Galanello R, Barella S, Ideo A, et al. Genotype of subjects with borderline hemoglobin A2 levels: implication for beta-thalassemia carrier screening. American journal of hematology. 1994; 46(2):79-81.
14. Borgio JF, AbdulAzeez S, Al-Muslami AM, et al. KLF1 gene and borderline hemoglobin A(2) in Saudi population. Archives of medical science : AMS. 2018; 14(1):230-6.
15. Fortova H, Slavikova V, Musil F, et al. [Diagnosis of beta-thalassemia on the basis of HbA2 determination]. Vnitrni lekarstvi. 1995; 41(5):302-6.
16. Verma S, Gupta R, Kudesia M, et al. Coexisting iron deficiency anemia and Beta thalassemia trait: effect of iron therapy on red cell parameters and hemoglobin subtypes. ISRN hematology. 2014; 2014:293216.
17. Steinberg MH, Adams JG, 3rd. Hemoglobin A2: origin, evolution, and aftermath. Blood. 1991; 78(9):2165-77.
18. Musumeci S, Schiliro G, Pizzarelli G, et al. Thalassaemia of intermediate severity resulting from the interaction between alpha- and beta-thalassaemia. Journal of medical genetics. 1978; 15(6):448-51.
19. Rangan A, Sharma P, Dadu T, et al. beta-Thalassemia mutations in subjects with borderline HbA(2) values: a pilot study in North India. Clinical chemistry and laboratory medicine. 2011; 49(12):2069-72.
20. Paglietti ME, Satta S, Sollaino MC, et al. The Problem of Borderline Hemoglobin A2 Levels in the Screening for beta-Thalassemia Carriers in Sardinia. Acta haematologica. 2016; 135(4):193-9.
21. Lou JW, Li DZ, Zhang Y, et al. Delineation of the molecular basis of borderline hemoglobin A2 in Chinese individuals. Blood cells, molecules & diseases. 2014; 53(4):261-4.
22. Sahoo S, Sahu N, Das P, et al. Effect of megaloblastic anemia on hemoglobin A(2) and diagnosis of beta-thalassemia trait. Indian journal of pathology & microbiology. 2023; 66(2):327-31.
23. Colaco S, Nadkarni A. Borderline HbA(2) levels: Dilemma in diagnosis of beta-thalassemia carriers. Mutation research Reviews in mutation research. 2021; 788:108387.
24. Kuhn JM, Rieu M, Rochette J, et al. Influence of thyroid status on hemoglobin A2 expression. The Journal of clinical endocrinology and metabolism. 1983; 57(2):344-8.
25. Kendall AG, Bastomsky CH. Hemoglobin A2 in hyperthyroidism. Hemoglobin. 1981; 5(6):571-7.
26. Morgado A, Picanco I, Gomes S, et al. Mutational spectrum of delta-globin gene in the Portuguese population. European journal of haematology. 2007; 79(5):422-8.
27. Tamary H, Dgany O. Alpha-Thalassemia. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. GeneReviews((R)). Seattle (WA)1993.

Correspondence Address:
Funda Tayfun Küpesiz, MD
Akdeniz University, School of Medicine, Department of Pediatrics, Pediatric Hematology Oncology Subdivision, Antalya, Türkiye
Telephone Number: +902422494362
Fax Number: +902422496869
E-mail: fundatyfn@gmail.com

EMERGING POINT-OF-CARE DIAGNOSTIC METHODS FOR SCREENING AND MONITORING OF HEMOGLOBIN DISORDERS

Alara Tanlak[1], Mehmet Doğan Güneş[1], Melis Şahin[2], Ran An[3], Umut A. Gurkan[2,]*
[1] Middle East Technical University, Ankara, Türkiye
[2] Case Western Reserve University, Cleveland, Ohio, United States
[3] University of Houston, Houston, Texas, United States

ABSTRACT

Point-of-care diagnosis refers to quickly conducting medical tests for disease detection, typically near the patient. This approach enables healthcare professionals to promptly diagnose conditions during direct patient interaction, eliminating the need to await laboratory results. Point-of-care tests are applicable in various settings, including patient rooms, emergency rooms, clinics, and on-site conditions. They are widely adopted for delivering fast results and facilitating more efficient and quick treatment. For instance, glucose meters and pregnancy tests are among the most popular point-of-care devices used. Both the glucose meter and pregnancy tests can be used quickly in patients' own homes, without the need for a healthcare professional. Especially since the glucose meter allows measurements to be taken at the desired time, it can make the work of professionals much easier in the process of deciding on the treatment to be applied to patients. Genetic hemoglobin (Hb) disorders, such as sickle cell disease (SCD) and β-thalassemia are carried by nearly 7% of the world's population. Early screening and timely diagnosis are essential for the prevention and management of later clinical complications. However, in Africa, Southern Europe, the Middle East, and Southeast Asia, where SCD and β-thalassemia are most prevalent, the diagnosis and screening of β-thalassemia are still a challenge due to the cost and logistical burden of laboratory diagnostic tests. There are emerging point-of-care (POC) technologies such as point-of-care hemoglobin electrophoresis devices like Gazelle that can be used for screening, diagnosis, and treatment monitoring of hemoglobin disorders. These emerging technologies and their applications in screening and monitoring of hemoglobin disorders are reviewed in this chapter.

INTRODUCTION

Genetic hemoglobin (Hb) disorders, such as sickle cell disease (SCD) and β-thalassemia (β-Thal) are among the major causes of anemia globally **(1, 2)**. Inherited Hb disorders are carried by nearly 7% of the world's population, with most structural Hb variants having the recessive β-globin gene mutations, β^S or hemoglobin S (Hb S) and β^C or hemoglobin C (Hb C) **(3-5)**. SCD arises when these mutations are inherited homozygously (Hb SS or SCD-SS) or paired with another β-globin gene mutation, such as Hb C (Hb SC or SCD-SC). In SCD, sickle Hb (Hb S) polymerization occurs under hypoxia (reduced oxygen level). HbS). Hb S polymerization results in stiff and fragile RBCs prone to hemolysis causing chronic hemolytic anemia. The reduced number of RBCs compromises oxygen delivery, further exacerbating Hb S polymerization and sickling of Hb S-containing RBCs. SCD affects 100,000 Americans and millions worldwide **(6)**. In the U.S., SCD is estimated to cost more than 8 million dollars per patient over a 50-year lifespan **(7)**.

Thalassemia is a globin gene disorder that results in a diminished synthesis rate of one or more of the globin chains. Beta-thalassemia (β-Thal) syndromes, including β-Thal intermedia, β-Thal major and β-Thal trait, are a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis. Peripheral hemolysis contributing to anemia is more prominent in thalassemia intermedia than in thalassemia major and oc-

curs when insoluble alpha-globin chains induce membrane damage to the peripheral red cells. The resulting anemia causes the typical bone deformities. Prolonged and severe anemia and increased erythropoietic drive also result in hepatosplenomegaly and extramedullary erythropoiesis. About 1.5% of the global population (80 to 90 million people) are carriers of the beta Thalassemia **(8)**. Although the exact prevalence of β-Thal in the U.S. is unknown, it has increased by 7.5% over the past 5 decades **(9)**. Based on our current knowledge, there is no approved point of care (POC) diagnostics for SCD or β-Thal.

Childhood mortality caused by SCD has declined in the United States due to medical advances and preventive services. Despite this progress, life expectancy and quality of life for people living with SCD remain lower than for those without the disease **(10)**. In the U.S., SCD can reduce life expectancy by 25 to 30 years. Beta thalassemia can cause severe anemia which can result in serious complications. Thousands of people in the US have the most severe form of beta-thalassemia **(9)**. While newborn screening is in place in the U.S., many immigrants may have never been screened. The β-Thal trait cannot be detected in newborns and sickle trait status may be unknown when a person is considering parenthood. In addition, immigrants and people in emergent situations may have unknown hemoglobin variant status potentially impacting care.

MONITORING OF TRANSFUSION (TX) THERAPY IN SCD AND *B*-THALASSEMIA

Healthy red blood cell transfusion (with Hb A blood) is lifesaving and is proven to help prevent complications of SCD and β-Thal **(11, 12)**. Transfusions have the potential to avert strokes in children at high risk for both beta-thalassemia and sickle cell disease, and when administered after a stroke, they may also serve as a preventative measure against future occurrences **(13)**. Regular (monthly) blood transfusions are commonly used in approximately 10% of pediatric and 20% of adult patients with SCD. The primary objective of long-term transfusion is to maintain low percentage of Hb S in the blood **(14)**, aiming for less than 30% HbS percentage with a total Hb between 9-12 g/dL **(15)**.

If left untreated, iron overload is a significant and morbid complication of chronic transfusion therapy, with costly and cumbersome treatments. In one long-term study, HbS was allowed to increase to 50% pre-transfusion rather than 30%, which provided protection against recurrent strokes while reducing blood transfusion requirements and the rate of iron accumulation. In a clinical study, during the 1,023 patient months with a target Hb S level around 50%, no patients had a recurrent stroke **(16)**. After four years of intensive transfusion, reducing the transfusion frequency and allowing the Hb S concentration to rise to 50% may be a reasonable stroke prevention strategy in some **individuals (16, 17)**. All these transfusion programs require accurate continuous monitoring of Hb S levels.

Ongoing monitoring of HbS and HbA levels during transfusion care informs therapeutic and technical decisions about the length and frequency of transfusions **(18)**. Accurate blood transfusion administration limits excessive transfusion and reduces the risks associated with transfusions, such as alloimmunization, hemolytic reactions, and iron overload **(19)**. A malfunction of intravenous access for automated red cell exchange transfusion has been reported, resulting in poor post-exchange Hb A levels. This would be more easily detected with POC measurement of post-transfusion Hb A and Hb S levels. Coordinated real-time measurements of Hb S concentration before and following red cell exchanges and transfusions may improve treatment efficacy.

MONITORING OF HYDROXYUREA (HU) THERAPY IN SCD

HU is the most common drug used to treat SCD. Treatment with hydroxyurea changes hematologic phenotype in SCD by increasing total Hb levels, percentage of HbF (fetal hemoglobin), mean corpuscular volume (MCV), and decreasing leukocyte count and platelet count. In HbSS, HU improves clinical outcomes, and decreases the number of painful episodes, transfusions, hospitalizations, and mortality rates **(20, 21)**. The efficacy of hydroxyurea is attributed to its ability to induce HbF synthesis **(22)**. HU increases the overall HbF% and the percentage of red cells containing detectable HbF (F-cells) in children and adults **(21, 23, 24)**, thereby decreasing the tendency toward intracellular polymerization of HbS **(23, 25, 26)**. It is impera-

tive that the decision to initiate HU therapy, especially in children, is thoughtful, and is monitored and adjusted accordingly to achieve optimal results.

HbF level is an important biomarker for efficacy and adherence to the treatment **(25, 27)**. Greater treatment-related increases in HbF% may predict a more robust response to treatment in children **(28)**. HbF levels may also be associated with hematologic toxicity; patients with the greatest response in fetal Hb were more likely to have multiple episodes of hematologic toxicity **(29)**. Patients require frequent (monthly or bi-monthly) blood testing and monitoring once HU therapy is initiated. These safety labs comprise a complete blood count (CBC) including reticulocyte count; the next month's dose is not dispensed until that day's blood counts are available. The dose needed for maximal clinical benefit, which may or may not be the maximum tolerated dose (MTD) **(30)**, is generally identified within 6 to 8 months of initiating HU therapy but should be established and assigned only after the patient tolerates the dose for at least eight weeks. Because HbF response to hydroxyurea is dose-dependent, serially measured HbF levels help to establish MTD in individuals **(25)**. Clinical trials with escalation to MTD have reported a higher percentage of HbF and Hb as well as mean corpuscular volume **(31)**. Careful attention to the patient's response to treatment and the resulting individualized therapy can potentially improve clinical outcomes **(32)**. Increases in Hb and HbF are associated with clinical response to HU therapy and are sustained, especially in children **(23, 33, 34)**. Close monitoring and follow-up are vital to ensure adherence to treatment and appropriateness of dose.

ANEMIA AND HEMOGLOBINOPATHIES ARE INHERENTLY ASSOCIATED.

Anemia and SCD are inherently associated and prevalent in the same regions of the world **(35-38)**. In SCD, HbS polymerization results in stiff and fragile RBCs prone to intravascular hemolysis causing reduced Hb levels and chronic hemolytic anemia **(37)**. The reduced number of RBCs compromises oxygen delivery, which further exacerbates Hb S polymerization and sickling of Hb S-containing RBCs. Treatment of SCD and β-Thal requires close monitoring of blood Hb level and patient's total Hb level and anemia status **(11, 39)**. At least 3 million people suffer from chronic anemia in the US. Women of childbearing age, children, and the elderly are most at risk. Anemia is also more than 3.3 times more common among African Americans than Caucasians.

QUANTITATIVE, INTEGRATED POINT-OF-CARE SCREENING AND MONITORING FOR SCD, β-THAL, AND ANEMIA

The presence of Hb variants in the blood is the primary indicator of an inherited Hb disorder **(40)**, while blood Hb level (in g/dL) is used as the primary indicator of anemia **(41, 42)**. The current gold standard for Hb variant identification is High-Performance Liquid Chromatography (HPLC) and capillary electrophoresis. The current gold standard for anemia testing and Hb level measurement is a CBC using a hematology analyzer. All three tests require state-of-the-art laboratory infrastructure, including phlebotomists for venous blood draws, and skilled technicians. In addition, the tests are expensive and labor-intensive (>$30k for each device itself and $10-$30 total cost per test per sample **(43)**), often with relatively slow turnaround times, which may result in delays in patient feedback, provider decision-making, and treatment. As a result, a dire need for affordable POC tools has emerged at the point-of-need that are highly accurate and high throughput with fast turnaround times for screening and treatment monitoring for SCD, β-Thal, and anemia **(42, 44)**.

Several POC diagnostic systems for several hemoglobin variants such as Hb S have been described **(45-47)** based on testing methods such as the sickle cell solubility test and antibody-based lateral flow assays such as Sickle SCAN™ and HemoType SC™ **(48-50)**. However, based on our current knowledge, there is currently no POC test available for β-Thal detection. In a 2019 report, the World Health Organization (WHO) listed hemoglobin testing as one of the most essential in vitro diagnostic (IVD) tests for primary care use in low and middle-income countries **(42, 51)**. Furthermore, hemoglobin electrophoresis has recently been added to the WHO essential list of IVDs for diagnosing SCD and sickle cell trait **(52)**. Leveraging the WHO-recognized Hb electrophoresis test, a paper-based, miniaturized Hb electrophoresis platform was de-

veloped: Gazelle™ Hb Variant (**Fig. 1**) **(53-61)**. Gazelle has been tested in clinical studies in 4 different countries with more than 700 subjects and demonstrated the capability of identifying major Hb variants, including HbA, HbE, HbS, and HbF, in adults as well as in newborns with SCD, sickle cell trait, Hemoglobin C disorder, and Hemoglobin E Disorder **(53-60, 62-64)**.

Microchip electrophoresis technology implemented a customized image analysis algorithm to accurately quantify Hb A_2 for the first time, in addition to Hb A, Hb F, and Hb C/E. The Gazelle POC diagnostic platform was designed to detect levels of HbA, HbF, and HbA_2 and demonstrated high correlations with the results reported by laboratory gold standard high-performance liquid chromatography (HPLC), yielding a Pearson Correlation Coefficient of 0.99. These results suggest that Gazelle-Multispectral is potentially suitable for large-scale β-Thal testing.

SUMMARY AND CONCLUSIONS

In summary, quantitative, affordable POC microchip electrophoresis technologies provide a solution for screening and monitoring of hemoglobin disorders for the first time. The following features improve the usability and data analysis capabilities of these new emerging POC technologies: 1) animated on-screen instructions with step-by-step guidance for test operation procedures to minimize user errors; and 2) a data analysis algorithm that automatically reports Hb variant levels, predicted disease status, and treatment monitoring status. Overall, evidence in the literature suggests that the new POC microchip electrophoresis technology is potentially suitable for large-scale SCD and β-Thal screening and treatment monitoring studies in low-resource regions where the prevalence of hemoglobin disorders is high.

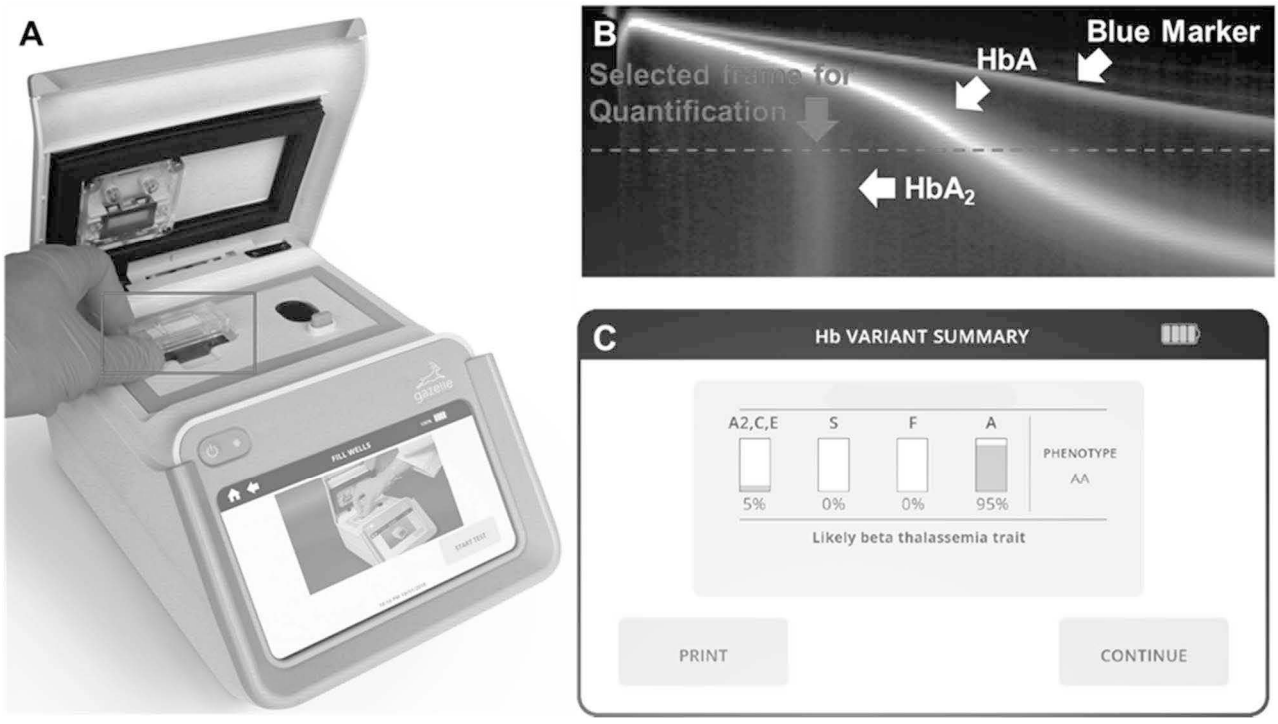


Figure 1: *Microchip electrophoresis platform for screening, screening, and monitoring of hemoglobin disorders, including sickle cell disease and beta-thalassemia. (A) Gazelle paper-based microchip electrophoresis using a disposable cartridge (red box) at the point of need. (B) Applying an internally integrated data analysis algorithm, the generated space-time plots based on the captured images are used for the identification and quantification Hb variants in real-time. (C) At the end of each test, the Gazelle algorithm automatically reports the identified and quantified Hb variant results and the patient phenotype. Quantitative results help with the monitoring of emerging therapies for hemoglobin disorders.*

REFERENCES

1. Kassebaum, N.J., R. Jasrasaria, M. Naghavi, et al., ***A systematic analysis of global anemia burden from 1990 to 2010***. Blood, 2014. 123(5): p. 615-24. PMC3907750
2. Barnett, R., ***Sickle cell anaemia***. Lancet, 2017. 389(10073): p. 998-998.
3. Modell, B. and M. Darlison, ***Global epidemiology of haemoglobin disorders and derived service indicators***. Bulletin of the World Health Organization, 2008. 86: p. 480-487.
4. Jamison, D.T., W. Bank, and D.C.P. Project, ***Disease Control Priorities in Developing Countries***. 2006: Oxford University Press.
5. Weatherall, D.J. and J.B. Clegg, ***Inherited haemoglobin disorders: an increasing global health problem***. Bull World Health Organ, 2001. 79(8): p. 704-12. PMC2566499
6. Wood, D.K., A. Soriano, L. Mahadevan, et al., ***A biophysical indicator of vaso-occlusive risk in sickle cell disease***. Sci Transl Med, 2012. 4(123): p. 123ra26. 3633235
7. Ballas, S.K., ***The cost of health care for patients with sickle cell disease***. Am J Hematol, 2009. 84(6): p. 320-2.
8. Khandros, E. and J.L. Kwiatkowski, ***Beta Thalassemia: Monitoring and New Treatment Approaches***. Hematology/Oncology Clinics, 2019. 33(3): p. 339-353.
9. Sayani, F.A. and J.L. Kwiatkowski, ***Increasing prevalence of thalassemia in America: Implications for primary care***. Ann Med, 2015. 47(7): p. 592-604.
10. National Academies of Sciences, E. and Medicine, ***Addressing Sickle Cell Disease: A Strategic Plan and Blueprint for Action***, ed. M. McCormick, H.A. Osei-Anto, and R.M. Martinez. 2020, Washington, DC: The National Academies Press. 522.
11. Chou, S.T., M. Alsawas, R.M. Fasano, et al., ***American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support***. Blood Advances, 2020. 4(2): p. 327-355.
12. Langhi, D., Jr., E.M.A. Ubiali, J.F.C. Marques, Jr., et al., ***Guidelines on Beta-thalassemia major - regular blood transfusion therapy: Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular: project guidelines: Associação Médica Brasileira - 2016***. Revista brasileira de hematologia e hemoterapia, 2016. 38(4): p. 341-345.
13. Steinberg, M.H., ***Sickle Cell Anemia, the First Molecular Disease: Overview of Molecular Etiology, Pathophysiology, and Therapeutic Approaches***. Thescientificworldjournal, 2008. 8: p. 1295-1324.
14. Kohne, E., ***Hemoglobinopathies Clinical Manifestations, Diagnosis, and Treatment***. Deutsches Arzteblatt International, 2011. 108(31-32): p. 532-U21.
15. Harmatz, P., E. Butensky, K. Quirolo, et al., ***Severity of iron overload in patients with sickle cell disease receiving chronic red blood cell transfusion therapy***. Blood, 2000. 96(1): p. 76-79.
16. Cohen, A.R., M.B. Martin, J.H. Silber, et al., ***A Modified Transfusion Program for Prevention of Stroke in Sickle-Cell Disease***. Blood, 1992. 79(7): p. 1657-1661.
17. Hurletjensen, A.M., I. Prohovnik, S.G. Pavlakis, et al., ***Effects of Total Hemoglobin and Hemoglobin-S Concentration on Cerebral Blood-Flow during Transfusion Therapy to Prevent Stroke in Sickle-Cell-Disease***. Stroke, 1994. 25(8): p. 1688-1692.
18. Stuart, M.J. and R.L. Nagel, ***Sickle-cell disease***. The Lancet, 2004. 364(9442): p. 1343-1360.
19. Yang, X., H.D. Reavis, C.L. Roberts, et al., ***Quantitative, Point-of-Care Immunoassay Platform to Guide and Monitor Sickle Cell Disease Therapy***. Analytical Chemistry, 2016. 88(16): p. 7904-9.
20. Brawley, O.W., Cornelius, L. J., Edwards, L. R., Gamble, V. N., Green, B. L., Inturrisi, C., James, A. H., Laraque, D., Mendez, M., Montoya, C. J., Pollock, B. H., Robinson, L., Scholnik, A. P., Schori, M., ***National institutes of health consensus development conference statement: Hydroxyurea treatment for sickle cell disease***. Annals of Internal Medicine, 2008. 148(12): p. 932-8.
21. Jodi B Segal, M., MPH, John J Strouse, MD, Mary Catherine Beach, MD, MPH, Carlton Haywood, MA, Catherine Witkop, MD, MPH, Haeseong Park, MD, MPH, Renee F Wilson, MSc, Eric B Bass, MD, MPH, Sophie Lanzkron, MD., *Hydroxyurea for the Treatment of Sickle Cell Disease*. February 2008, Agency for Healthcare Research and Quality: Rockville, MD.
22. Gulbis, B., D. Haberman, D. Dufour, et al., ***Hydroxyurea for sickle cell disease in children and for prevention of cerebrovascular events: the Belgian experience***. Blood, 2005. 105(7): p. 2685-2690.
23. Lanzkron, S., J.J. Strouse, R. Wilson, et al., ***Systematic review: Hydroxyurea for the treatment of adults with sickle cell disease***. Annals of Internal Medicine, 2008. 148(12): p. 939-+.
24. Zimmerman, S.A., W.H. Schultz, J.S. Davis, et al., ***Sustained long-term hematologic efficacy of hydroxyurea at maximum tolerated dose in children with sickle cell disease***. Blood, 2004. 103(6): p. 2039-2045.
25. Rodgers, G.P., G.J. Dover, C.T. Noguchi, et al., ***Hematologic Responses of Patients with Sickle-Cell Disease to Treatment with Hydroxyurea***. New England Journal of Medicine, 1990. 322(15): p. 1037-1045.
26. Rees, D.C., T.N. Williams, and M.T. Gladwin, ***Sickle-cell disease***. Lancet, 2010. 376(9757): p. 2018-2031.

27. McGann, P.T. and R.E. Ware, ***Hydroxyurea for sickle cell anemia: what have we learned and what questions still remain?*** Current Opinion in Hematology, 2011. 18(3): p. 158-165.
28. Ware, R.E., B. Eggleston, R. Redding-Lallinger, et al., ***Predictors of fetal hemoglobin response in children with sickle cell anemia receiving hydroxyurea therapy***. Blood, 2002. 99(1): p. 10-14.
29. Steinberg, M.H., Z.H. Lu, F.B. Barton, et al., ***Fetal hemoglobin in sickle cell anemia: Determinants of response to hydroxyurea***. Blood, 1997. 89(3): p. 1078-1088.
30. Halsey, C. and I.A.G. Roberts, ***The role of hydroxyurea in sickle cell disease***. British Journal of Haematology, 2003. 120(2): p. 177-186.
31. Ware, R.E., ***How I use hydroxyurea to treat young patients with sickle cell anemia***. Blood, 2010. 115(26): p. 5300-5311.
32. Heeney, M.M. and R.E. Ware, ***Hydroxyurea for Children with Sickle Cell Disease***. Hematology-Oncology Clinics of North America, 2010. 24(1): p. 199-+.
33. Maier-Redelsperger, M., M. de Montalembert, A. Flahault, et al., ***Fetal hemoglobin and F-cell responses to long-term hydroxyurea treatment in young sickle cell patients***. Blood, 1998. 91(12): p. 4472-4479.
34. Al-Anazi, K., ***Hydroxyurea therapy in patients with sickle cell disease***. Translational Medicine, 2015. 5: p. 145-150.
35. Semedo, R.M.L., M.M.A.S. Santos, M.R. Baião, et al., ***Prevalence of anaemia and associated factors among children below five years of age in Cape Verde, West Africa***. Journal of health, population, and nutrition, 2014. 32(4): p. 646-657.
36. Weatherall, D.J., ***The inherited diseases of hemoglobin are an emerging global health burden***. Blood, 2010. 115(22): p. 4331-6. PMC2881491
37. Kato, G.J., F.B. Piel, C.D. Reid, et al., ***Sickle cell disease***. Nature Reviews Disease Primers, 2018. 4: p. 18010.
38. Mburu, J. and I. Odame, ***Sickle cell disease: Reducing the global disease burden***. International Journal of Laboratory Hematology, 2019. 41: p. 82-88.
39. Tshilolo, L., G. Tomlinson, T.N. Williams, et al., ***Hydroxyurea for Children with Sickle Cell Anemia in Sub-Saharan Africa***. New England Journal of Medicine, 2019. 380(2): p. 121-131.
40. Prevention, C.f.D.C.a., ***Hemoglobinopathies: Current Practices for Screening, Confirmation and Follow-up***. 2015.
41. Northrop-Clewes, C.A. and D.I. Thurnham, ***Biomarkers for the differentiation of anemia and their clinical usefulness***. Journal of blood medicine, 2013. 4: p. 11-22.
42. An, R., Y. Huang, Y. Man, et al., ***Emerging point-of-care technologies for anemia detection***. Lab on a Chip, 2021. 21(10): p. 1843-1865.
43. Blank, K.R., M.A. Cascardi, and G.D. Kao, ***The utility of serial complete blood count monitoring in patients receiving radiation therapy for localized prostate cancer***. Int J Radiat Oncol Biol Phys, 1999. 44(2): p. 317-21.
44. An, R., Y. Man, S. Iram, et al., ***Point-of-Care microchip electrophoresis for integrated anemia and hemoglobin variant testing***. Lab on a Chip, 2021.
45. Arishi, W.A., H.A. Alhadrami, and M. Zourob, ***Techniques for the Detection of Sickle Cell Disease: A Review***. Micromachines, 2021. 12(5).
46. Ilyas, S., A.E. Simonson, and W. Asghar, ***Emerging point-of-care technologies for sickle cell disease diagnostics***. Clinica Chimica Acta, 2020. 501: p. 85-91.
47. Jaja, C., J. Edem-Hotah, J. Shepherd, et al., ***Analytic Characteristics and Performance of Novel Immunoassay Point-of-Care Tests for Early Diagnosis of Sickle Cell Disease A Systematic Review***. Point of Care, 2020. 19(3): p. 84-94.
48. Canning, D.M. and R.G. Huntsman, ***An assessment of Sickledex as an alternative to the sickling test***. Journal of clinical pathology, 1970. 23(8): p. 736-737.
49. Steele, C., A. Sinski, J. Asibey, et al., ***Point-of-care screening for sickle cell disease in low-resource settings: A multi-center evaluation of HemoTypeSC, a novel rapid test***. Am J Hematol, 2019. 94(1): p. 39-45. PMC6298816
50. Kanter, J., M.J. Telen, C. Hoppe, et al., ***Validation of a novel point of care testing device for sickle cell disease***. BMC Medicine, 2015. 13(1): p. 225.
51. Organization, G.W.H., ***First WHO Model List of Essential In Vitro Diagnostics***. 2019 ((WHO Technical Report Series, No. 1017)).
52. (WHO), W.H.O., ***Second WHO Model List of Essential In Vitro Diagnostics***. 2019(WHO/MVP/EMP/2019.05).
53. Hasan, M.N., A. Fraiwan, J.A. Little, et al., ***Hemechip: An Automated Portable Microchip Electrophoresis Platform for Point-of-Care Sickle Cell Disease Screening***. Blood, 2017. 130: p. 3519.
54. Ung, R., Y. Alapan, M.N. Hasan, et al., ***Point-of-Care Screening for Sickle Cell Disease By a Mobile Micro-Electrophoresis Platform***. Blood, 2015. 126(23): p. 3379-3379.
55. Hasan, M.N., A. Fraiwan, J.A. Little, et al. *A low-cost, mass-producible point-of-care platform for diagnosing hemoglobin disorders*. in *2017 IEEE Healthcare Innovations and Point of Care Technologies (HI-POCT)*. 2017.
56. Qua, K., S.M. Swiatkowski, U.A. Gurkan, et al., ***A retrospective case study of successful translational research: Gazelle Hb variant point-of-care diagnos-***

tic device for sickle cell disease. J Clin Transl Sci, 2021. 5(1): p. e207. PMC8727719
57. Fraiwan, A., M.N. Hasan, R. An, et al., ***International Multi-Site Clinical Validation of Point-of-Care Microchip Electrophoresis Test for Hemoglobin Variant Identification***. Blood, 2019. 134: p. 3373.
58. An, R., M.N. Hasan, Y. Man, et al. *Integrated Point-of-Care Device for Anemia Detection and Hemoglobin Variant Identification*. in *2019 IEEE Healthcare Innovations and Point of Care Technologies, (HI-POCT)*. 2019.
59. Hasan, M.N., A. Fraiwan, R. An, et al., ***Paper-based microchip electrophoresis for point-of-care hemoglobin testing***. Analyst, 2020. 145(7): p. 2525-2542.
60. An, R., M.N. Hasan, Y. Man, et al., ***Integrated Anemia Detection and Hemoglobin Variant Identification Using Point-of-Care Microchip Electrophoresis***. Blood, 2019. 134(Supplement_1): p. 378-378.
61. Hasan, M.N., R. An, A. Akkus, et al., ***Dynamic pH and Thermal Analysis of Paper-Based Microchip Electrophoresis***. 2021. 12(11): p. 1433.
62. An, R., A. Avanaki, P. Thota, et al., ***Paper-Based Microchip Electrophoresis Enabled First Point-of-Care Diagnostic Test for Beta-Thalassemia***. Blood, 2022. 140(Supplement 1): p. 7822-7823.
63. Hasan, M.N., R. An, A. Akkus, et al., ***Dynamic pH and Thermal Analysis of Paper-Based Microchip Electrophoresis***. Micromachines, 2021. 12(11): p. 1433.
64. An, R., Y. Huang, A. Rocheleau, et al., ***Multispectral imaging for MicroChip electrophoresis enables point-of-care newborn hemoglobin variant screening***. Heliyon, 2022. 8(12): p. e11778.

Correspondence Address:
Prof. Dr. Umut A. Gurkan
Case Western Reserve University, Cleveland, Ohio, United States
E-Mail: umut@case.edu

MOLECULAR DIADGNOSTIC METHODS IN HEMOGLOBINOPATHIES AND THALASSEMIA

Prof. Dr. Türker Bilgen
Department of Nutrition and Dietetics, School of Health, Tekirdağ Namık Kemal University, Tekirdağ-Türkiye
New York Medical College, Department of Pharmacology, NY-USA

ABSTRACT

Hemoglobinopathies and thalassemias are amongst the most common genetic diseases. Laboratory diagnosis is essential for the exact diagnosis of patients and carriers, disease management, genetic counseling, and prenatal diagnosis. Beyond red blood cells and Hb analyses, DNA analysis based molecular diagnostic tests are a necessity because of their inherited nature. On the other hand, because of the genetic heterogeneity, appropriate molecular test selection in the diagnosis of hemoglobinopathies and thalassemias depends on many factors such as clinical and hematological findings, previous genetic findings in family, population specific mutation spectrum, available laboratory resources, and laboratory's expertise.

Keywords: Molecular diagnosis, thalassemia, hemoglobinopathies

INTRODUCTION

Hemoglobin (Hb) is an oxygen-transporting heteromeric metalloprotein found inside the red blood cells (RBCs) **(1)**. Hb formed by the combination of different globin chains synthesized during embryonic, fetal and post-neonatal periods. There are four types of globin chains present named as alpha (α), beta (β), gamma (γ) and delta (δ) **(2, 3)**. According to a classification, the genetic disorders that affect hemoglobin are known as hemoglobinopathies. Structural Hb variants (abnormal Hb) and thalassemias are known as two distinguished subclasses of pathological conditions that cause hemoglobinopathies according to this approach **(4, 5)**. Furthermore, some researchers call structural Hb variants as hemoglobinopathies and impaired globin chain productions as thalassemias. Additionally, some structural Hb variant exhibit impaired globin chain productions just like as thalassemias **(6)**. Regardless from the classifications, DNA based molecular tests have great diagnostic value in the inherited hemoglobin disorders in human. The structural Hb variants are generally caused by single amino acid substitutions in the globin chains. Despite there are tens of clinically important ones, most structural variants are clinically silent and are discovered incidentally. On the other hand, the thalassemias result from defective globin peptide production. Thalassemias, as a group of inherited defects, is known as the world's most prevalent hemoglobin disorders. Thalassemias are classified into the α-, β-, δ β -, γ δ β -, δ - and γ - thalassemias according to which particular globin chain(s) are defectively synthesized **(7)**. The α- and β-thalassemias are accepted as much more important because of their incidence and clinical severity **(8, 9)**. Despite α-thalassemia is caused by deletion in approximately 95% of cases, 95% of β-thalassemias are due to point mutations that cause abnormal RNA transcription, processing or stability, or nonsense mutations resulting in production of abnormal proteins or nonsense-mediated RNA decay **(10)**. Although α- and β-thalassemias and abnormal Hbs are common in the countries in the Southwest Europe, the Mediterranean, the Middle East, the India and the Far East Asia, where malaria is endemic, they have also become an important health problem for the other parts of the world because of migrations **(11)**.

LABORATORY METHODS

Laboratory diagnosis of hemoglobinopathies and thalassemias requires several tests including RBCs indices, Hb analyses, and DNA analyses. RBCs analysis is a primary screening. Later, Hb analysis can be carried out by either electrophoresis or high-performance liquid chromatography (HPLC). These two techniques can provide both qualitative and quantitative analysis of Hb components that can

help to diagnosis of hemoglobinopathies and thalassemias. Eventually, because of their inherited nature, specific mutation(s) have to be detected and they can only be detected by DNA analysis for the accurate diagnosis of these conditions. Detection of the mutation(s) provides more accurate genetic counseling and a chance for prenatal diagnosis beyond to confirmation of the clinical diagnosis and better disease management. Most of the related mutations are single-base changes and minor insertions/deletions which are known as point mutations. The others are mainly large deletions or duplication. Various commonly used DNA analyses including allele specific polymerase chain reaction (PCR), real-time PCR and melting curve analysis, multiple ligation-dependent probe amplification (MLPA), DNA sequencing techniques, and reverse dot blot hybridization (RDBH) analysis have been used for mutation detection in molecular diagnosis of these diseases. All of these techniques have of course some advantages and disadvantages on diagnosis of hemoglobinopathies and thalassemias.

The method(s) to be used in molecular diagnosis of hemoglobinopathies and thalassemias must be selected by taking into account mutational spectrum in population encountered, previous experiences and infrastructural conditions of laboratory in addition to the patients clinical and hematological findings. Understanding the reasons for selection a specific method and molecular basis of the techniques used for diagnosis will be beneficial for accurate diagnosis of hemoglobinopathies and thalassemias. Therefore, methodological background of the techniques which have been commonly used for molecular diagnosis of hemoglobinopathies and thalassemias will be examined and discussed in this chapter.

DNA analyses were deeply affected by the invention and modifications of the PCR technique. Therefore, it is useful to take a closer look at to how PCR works and how it can be used during DNA analysis. PCR is a widely used technique for amplification of specific DNA sequences in DNA analyses. In diagnosis of hemoglobinopathies and particularly in α- and β-thalassemias, PCR can be employed to amplify and analyze the DNA segments containing the α-globin (HBA) and β-globin (HBB) genes. Various PCR-based methods, such as allele-specific PCR, real-time PCR, and gap-PCR can be used to detect specific mutations related to thalassemias and hemoglobinopathies. PCR technique followed by an electrophoresis step can itself be a diagnostic tool, but it is often used in combination with a downstream method such as DNA sequencing.

The PCR process involves 3 steps:

Denaturation: The first step involves heating the DNA sample to a high temperature (typically around 94-98°C). This causes the double-stranded DNA to denature into two single strands, breaking the hydrogen bonds between the complementary base pairs (A-T and G-C).

Annealing: After denaturation, the reaction mixture is cooled to a lower temperature, typically around 50-65°C. At this temperature, short DNA sequences called primers specifically bind (anneal) to the complementary sequences on each of the single-stranded DNA templates. Specific primers are essential because they provide a starting point for DNA polymerase to synthesize new DNA strands and thus limit the DNA fragments amplified.

Elongation: A thermostable DNA polymerase, such as Taq polymerase, adds nucleotides to the primers, synthesizing new DNA strands that are complementary to the templates. This step occurs at a temperature usually between 70-75°C, which is within the optimal range for the DNA polymerase used.

This three-stage cycle is repeated 30 to 40 times by a machine called a thermocycler and can be completed in a few hours. Eventually, PCR yields millions of copies of the DNA fragment of interested gene or DNA region.

Amplification Refractory Mutation System (ARMS)

Two primers identical in sequence except for the 3'-terminus bases, one of which is complementary to the wild-type and the other one complementary to the mutation, and a common reverse primer for the opposite strand are used in this technique. Usually, gel electrophoresis step is used following to ARMS-PCR **(12, 13)**. It is possible to perform this approach as real-time PCR combined with melting curve analysis which can reduces time required. Real-time PCR version of this test has become more attracted in recent years **(14)**. In the normal individual, PCR product should be seen only in the reaction the wild-type primer set. In a heterozygous individual, two different PCR products should be

seen for both wild-type and mutant primer set, and an individual with homozygous mutation will be negative with the normal primer, but positive with the mutant primer. There is a representative test result for ARMS-PCR assay shown on agarose gel electrophoresis in Figure 1 **(15)**.

ARMS-PCR has to be performed for each possible previously identified mutation for a new case. It is possible to screen multiple mutations with the well-designed multiplex PCR approach using this technique. Nonetheless, it should be noted that this technique is limited to screening of known mutations.

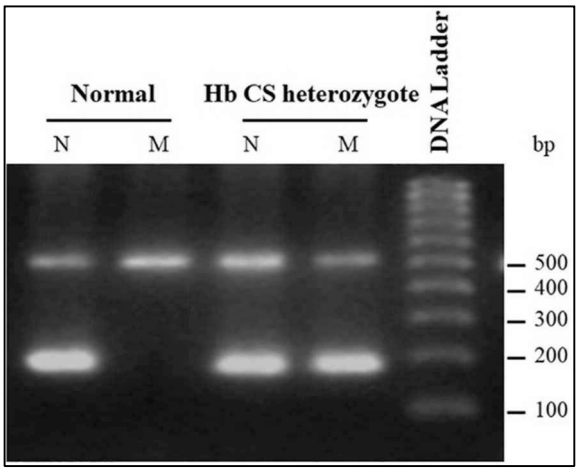


Figure 1: *ARMS-PCR results on agarose gel. A PCR band (lower) for normal individual can be seen only in the reaction employing the wild-type (N) primer set. Hb CS heterozygote will generate PCR band (lower) both with wild-type (N) and with mutant (M) primer set. Internal control band can be seen at 526 bp (upper PCR band at each lane) (15).*

High Resolution Melting Analysis (HRMA)

HRMA is a method used to identify DNA sequence variations by analyzing the melting curve of PCR-amplified DNA fragments. It can naturally be used to detect to known mutations, but also it enables researchers to detect unidentified genetic variants within PCR product. HRMA is a technique that detects mutations by monitoring the melting profile of PCR products using fluorescent dyes and real-time PCR system. Mutated DNA sequences melt at different temperatures compared to normal sequences, allowing to detection of specific mutations associated with hemoglobinopathies and thalassemias **(16, 17)**.

Gap-PCR

Gap-PCR is a specialized PCR technique used to detect large deletions or duplication/insertions. It involves designing primers that flank the suspected deletion or insertion site and then amplifying the DNA to identify size differences in the PCR products. A **representative samples of Turkish-type inversion/deletion (δβ)0 mutation detected by gap-PCR are shown in the Figure 2.** This technique is limited to screening of the known mutations. For the novel and unidentified mutations; MLPA or array-CGH techniques can be employed **(18)**.

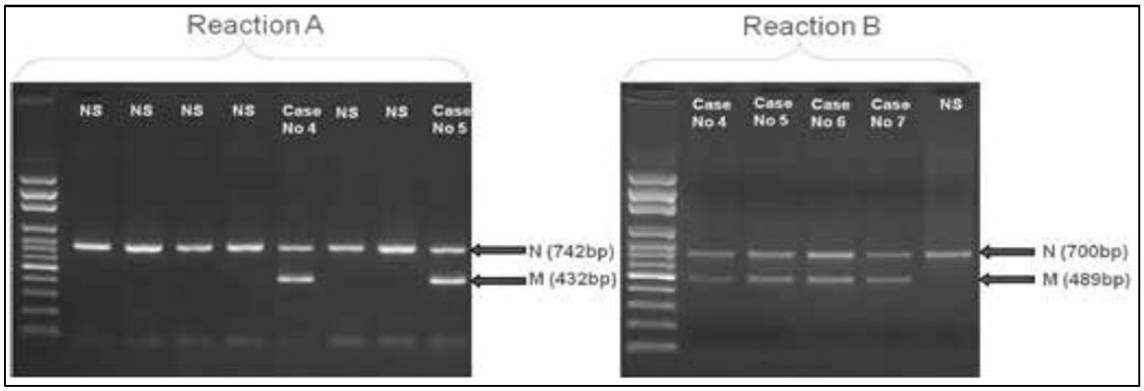


Figure 2: *A Representative samples of Turkish-type inversion/deletion (δβ)0 mutation detected by gap-PCR and agarose gel electrophoresis. For reaction A testing the upstream breakage of the mutation, the upper band (742 bp) corresponds to normal results and the lower band (432 bp) to the mutation. Case 4 and Case 5 are heterozygous as both have normal and mutation-related PCR fragments. For reaction B testing the downstream breakage of the mutation, the upper band (700 bp) corresponds to normal results and the lower band (489 bp) to the mutation. Cases 4, 5, 6, and 7 show both normal and mutation-related PCR fragments, confirming that they are heterozygous for this mutation. NS: Normal sample, N: normal, M: mutation (18).*

Restriction Fragment Length Polymorphism (RFLP) Analysis

RFLP analysis involves digesting amplified DNA with specific restriction enzymes that recognize and cleave DNA at particular sites. By comparing the resulting fragment sizes through gel electrophoresis, mutations can be identified associated with hemoglobinopathies and thalassemias. Although RFLP analysis can potentially be used in diagnosis of hemoglobinopathies and thalassemias, it is recently replaced by novel techniques which are more reliable and advantageous.

Reverse Dot Blot Hybridization (RDBH)

RBDH is a method used to detect diverse set of related mutations simultaneously. It is also known as strip assay. This approach allows us to simultaneous analyses of the common mutations regardless being a point or a large deletional/insertional mutation. This assay involves immobilizing specific DNA probes on a membrane (strip), then hybridizing them with the biotinylated patient's DNA by a PCR, followed by detection of the hybridized sequences that can be easily visible to the naked eye by enzymatic color reaction. The strips can be designed to population-specific mutation spectrum in different geographical regions of the world. Here is an example strip test designed for α-thalassemia by a company (ViennaLab Diagnostics) in Figure 3. Cases can be screened for the known 21 mutations of α-globin genes using this test strip. This assay can be performed in a laboratory with basic molecular genetics equipment. Interpretation and reporting the test results are relatively easy, on the other hand, these assays are limited to detection of the known mutations. This assay may need to be complimented in prenatal/postnatal diagnostic laboratories by other techniques allowing to detection of unidentified mutations and also the other mutations which are not included in the strip test **(7, 19)**.

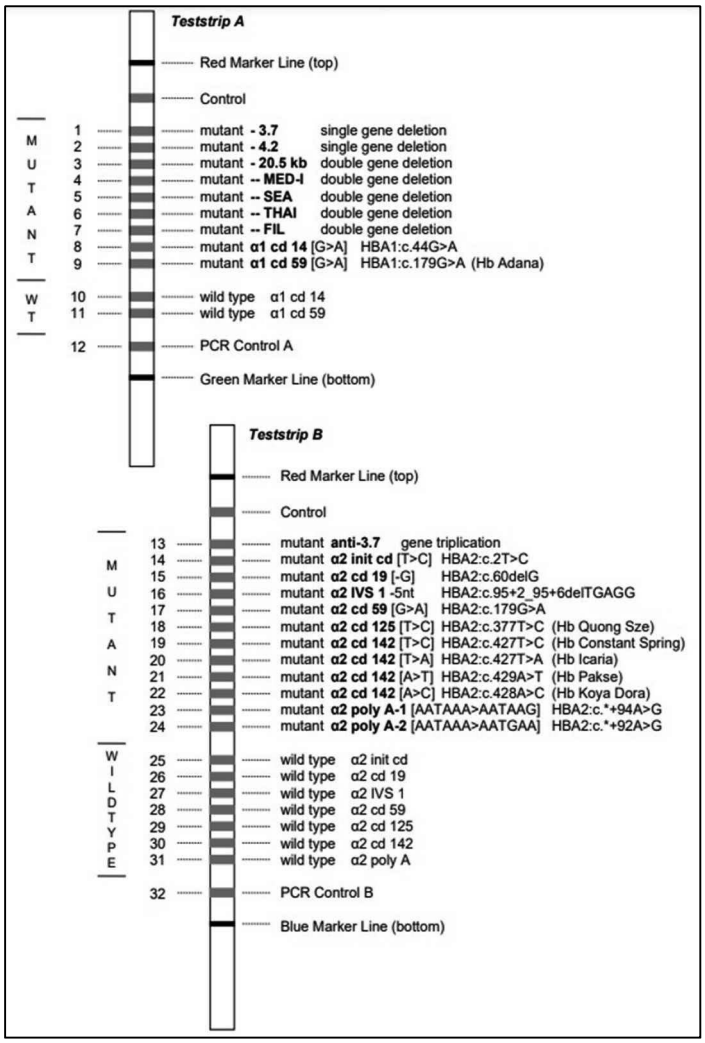


Figure 3: *A strip test by Reverse dot blot hybridization designed for α-thalassemia by the ViennaLab Diagnostics company.*

Multiplex Ligation-Dependent Probe Amplification (MLPA)

MLPA is a technique that can detect deletions or duplications in the α-globin or β-globin genes, which can be associated with thalassemias. This technique has been proven to find known and unknown deletions in unsolved cases after performing conventional techniques. The MLPA technique requires only a thermocycler and CE equipment **(20, 21)**. MLPA starts with DNA denaturation/hybridization steps. Following to denaturation step, DNA being hybridized with a mixture of MLPA probes. When the two probes within the MLPA probe mixture are hybridized to adjacent target sequences, the probes can be ligated to each other during the following ligation step. Only ligated probes can be amplified during the PCR reaction. The amplification products must be separated using CE. Using the specific software CE results are analyzed, and known and unknown deletions or duplications within the region of interest can be detected. MLPA is such a versatile technique having advantages in the determination of deletions or duplications, additionally, point mutations and also even methylation status can be detected by specifically designed MLPA tests. Representative MLPA analyze results using a specific software for the β-globin gene cluster showing a case heterozygous for the Turkish inversion-deletion $(\delta\beta)^{o}$-thalassemia mutation and a case heterozygous for the Sicilian $(\delta\beta)^{o}$-thalassemia mutation are shown in Figure 4 **(22)**.

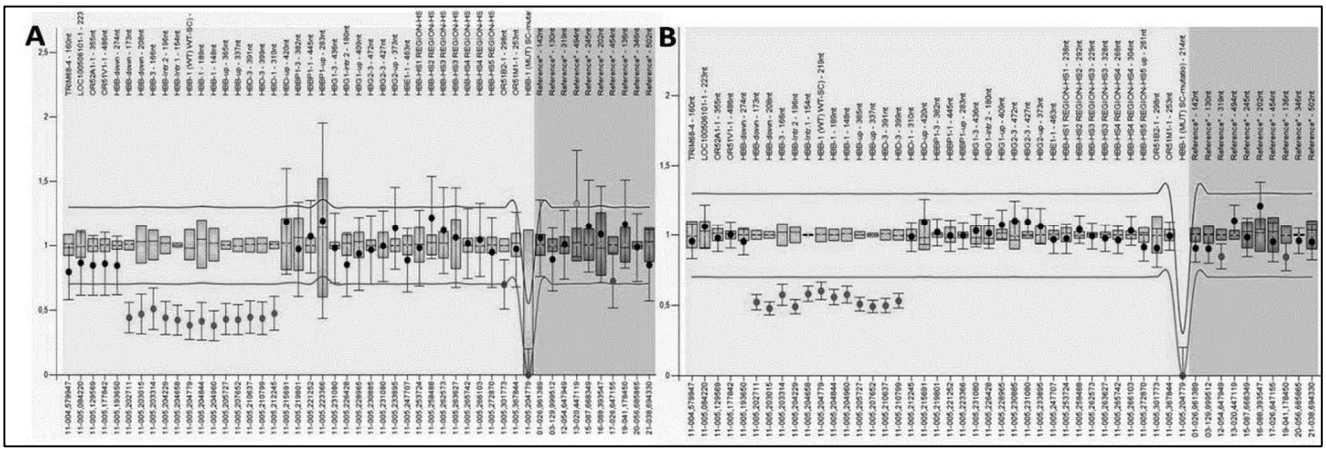


Figure 4: *MLPA results for the β-globin gene cluster. A) Heterozygous for the Turkish inversion-deletion $(\delta\beta)^{o}$-thalassemia mutation, B) Heterozygous for the Sicilian $(\delta\beta)^{o}$-thalassemia mutation (22).*

DNA Sequencing

Sanger sequencing and next-generation sequencing (NGS) or targeted large-scale sequencing techniques can be used to analyze the DNA sequences of the globin genes to identify specific point mutations or small deletions/insertions associated with hemoglobinopathies and thalassemias **(23)**. Sanger sequencing is much more widely used technique in hemoglobinopathies and thalassemias, while NGS and large-scale sequencing techniques are less common due to its high cost and limited to α-thalassemia **(6, 24)**. Nevertheless, NGS can offer advantages in solving of complex cases **(25)**. Sanger sequencing is a powerful and reliable DNA analyses method in genetic diagnostics. Representative Sanger sequence results showing two novel mutations of the β-globin gene in heterozygous form can be seen in Figure 5. The majority of β-thalassemias and hemoglobinopathies are due to nucleotide substitutions, small deletions or insertions, and it is assumed that only a minority of β-thalassemias are due to whole gene or large deletions. On the other hand, most α-thalassemias are due to deletion of one or more HBA genes, which can be detected by reverse dot blot hybridization, Gap-PCR, MLPA, and also array-comparative genomic hybridization (array-CGH) **(26)**. Except the large deletion/insertion mutations, direct DNA sequencing is a gold standard diagnostic method to detect both known and unknown mutations **(6)**. The clinical importance of most of the genetic variants within the α-globin and β-globin genes have been well understood. Because of the nature of this technique, there is still a little chance to find rare novel genomic variants which can create difficulties in in-

terpretation of test results. When a novel variant found, there is standard algorithms that can be used to predict its clinical relevance. Otherwise, direct DNA sequencing is a very convenient method especially for the β-globin gene analysis **(27-30)**. The direct DNA sequencing for α-thalassemia can be used as a complementary method because of the mutation profile and also difficulties in selective amplification of the α-globin genes mainly due to sequence similarities **(10)**.

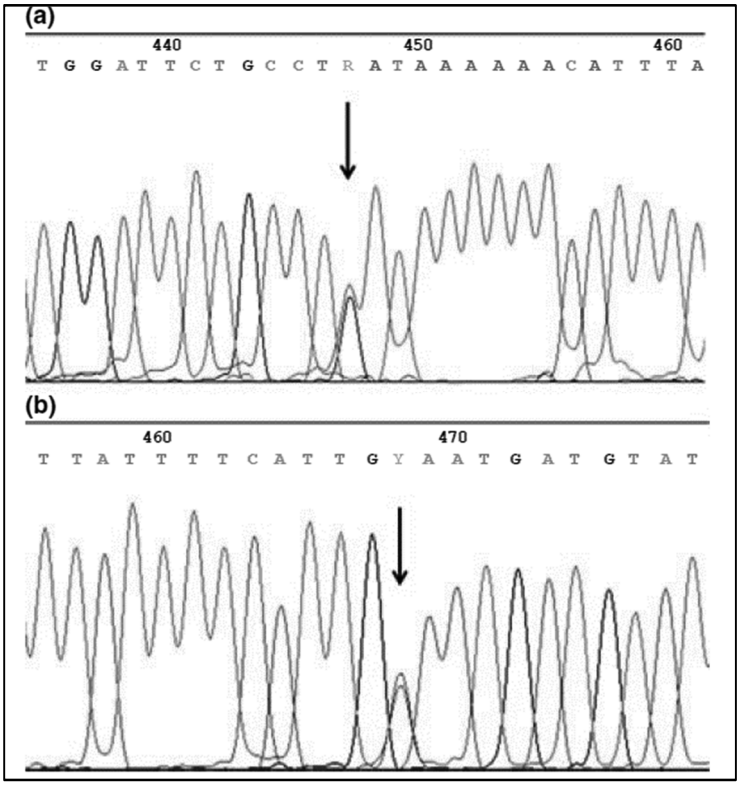


Figure 5: *DNA sequence analyses results showing two novel mutations of the β-globin gene in heterozygous form (29).*

Array Comparative Genomic Hybridization (Array-CGH)

Microarray-based comparative genomic hybridization (Array-CGH) is a method testing the copy number variation across the genome. According to the assay procedure; following to the DNA extractions from test and control samples, the test and control DNA samples are labeled with different fluorescent dyes of colors. Test and control DNAs are then mixed together and applied to a microarray to hybridize with the arrayed probes on the slide. Finally, the relative fluorescence intensities are quantified by an imaging system and the fluorescence ratio of the test and control hybridization signals is determined. Thus, the relative copy number of sequences interested in the test genome as compared to the control genome can be detected by the array-CGH technique **(31)**. Deletions in the β-globin gene cluster may cause β-thalassemia, δβ-thalassemia, $^{A}\gamma\delta\beta$-thalassemia, and hereditary persistence of fetal hemoglobin (HPFH) according to the lost region **(32)**. Some of them more recurrent, while some are sporadic. Because of the technical restrictions, their frequencies remain mysterious for most of the countries. They can be detected by gap-PCR if the deletion breakpoints are known. MLPA can also be used when the deletion breakpoints are known or unknown. In addition to the reverse dot blot hybridization test, similar strategies like gap-PCR and MLPA can be used for α-globin gene large deletions/duplications **(26)**. On the other hand, gene duplications which are known as more common in α-globin gene cluster are more difficult to detect. Array-CGH is such a useful technique for characterization of the large deletions and also duplications in thalassemias. For the unidentified large deletions and duplications found by MLPA or array-CGH, it is needed to design appropriate primers to amplify the region including the breakpoints, and determine the exact breakpoints by the Sanger sequencing technique.

Due to the clinical and the genetic heterogeneity in hemoglobinopathies and thalassemias, a various set of molecular test approaches including ARMS, HRMA, RDBH, Gap-PCR, MLPA, DNA Sequencing and array-CGH techniques have widely been accepted by geneticists as mainstay diagnostic tools in these group of diseases. In addition to the molecular tests mentioned above, ongoing research and advancements in diagnostic test technology continue to improve novel molecular diagnostic tests for hemoglobinopathies and thalassemias. Recently developed DNA analyses technologies like third-generation sequencing (TGS), droplet digital PCR or other modified PCR assays can be diagnostic tests in near future following to the validation studies that have to be performed **(23, 33-35)**.

Diagnostic and screening tests are two types of important medical tests used in healthcare. They differ in their objectives, timing, and the populations targeted. There exist both some diagnostic and screening tests playing important roles in diagnosis and prevention of hemoglobinopathies and thalassemias. Since hemoglobinopathies and thalassemias are known as recessive conditions, identification of the carriers is much of importance. In a general sense, DNA analysis based molecular methods are powerful diagnostic tests. DNA analyses offer high specificity and sensitivity in prenatal and postnatal diagnosis of hemoglobinopathies and thalassemias similar to other genetic diseases. They can determine the exact mutation(s), which is essential for providing appropriate clinical management of the disease, genetic counseling to affected individuals and their families, and prenatal diagnosis. Each molecular test confers specific advantages and disadvantages. Therefore, the appropriate molecular test choice in diagnosis of hemoglobinopathies and thalassemias depends on many factors such as clinical and hematological findings, previous genetic findings in family, population specific mutation spectrum, available laboratory resources, and laboratory's expertise.

REFERENCES

1. Burmester T, Hankeln T. Function and evolution of vertebrate globins. Acta Physiol (Oxf). 2014;211(3):501-14.
2. Lee JS, Cho SI, Park SS, et al. Molecular basis and diagnosis of thalassemia. Blood Res. 2021;56(S1):S39-S43.
3. Shafique F, Ali S, Almansouri T, et al. Thalassemia, a human blood disorder. Braz J Biol. 2021;83:e246062.
4. Steiner LA, Gallagher PG. Erythrocyte disorders in the perinatal period. Semin Perinatol. 2007;31(4):254-61.
5. Harteveld CL, Achour A, Arkesteijn SJG, et al. The hemoglobinopathies, molecular disease mechanisms and diagnostics. Int J Lab Hematol. 2022;44 Suppl 1(Suppl 1):28-36.
6. Sabath DE. The role of molecular diagnostic testing for hemoglobinopathies and thalassemias. Int J Lab Hematol. 2023;45 Suppl 2:71-8.
7. Ibrahim Keser, Türker Bilgen, Osman Alphan Kupesiz, et al.Investigation of Alpha Globin Gene Mutations by Complementary Methods in Antalya. Eastern Journal of Medicine. 2021;26(1):117-22.
8. Calzolari R, McMorrow T, Yannoutsos N, et al. Deletion of a region that is a candidate for the difference between the deletion forms of hereditary persistence of fetal hemoglobin and deltabeta-thalassemia affects beta- but not gamma-globin gene expression. EMBO J. 1999;18(4):949-58.
9. Taher AT, Cappellini MD. Management of non-transfusion-dependent thalassemia: a practical guide. Drugs. 2014;74(15):1719-29.
10. Sabath DE. Molecular Diagnosis of Thalassemias and Hemoglobinopathies: An ACLPS Critical Review. Am J Clin Pathol. 2017;148(1):6-15.
11. Williams TN, Weatherall DJ. World distribution, population genetics, and health burden of the hemoglobinopathies. Cold Spring Harb Perspect Med. 2012;2(9):a011692.
12. Purohit A, Singh K, Kalla AR, et al. Evaluation of beta-Thalassaemia Cases for Common Mutations in Western Rajasthan. Indian J Hematol Blood Transfus. 2021;37(4):684-8.
13. Jalil T, Yousafzai YM, Rashid I, et al. Mutational Analysis Of Beta Thalassaemia By Multiplex Arms-Pcr In Khyber Pakhtunkhwa, Pakistan. J Ayub Med Coll Abbottabad. 2019;31(1):98-103.
14. Suwannakhon N, Pangeson T, Seeratanachot T, et al. Noninvasive prenatal screening test for compound heterozygous beta thalassemia using an amplification refractory mutation system real-time polymerase chain reaction technique. Hematol Rep. 2019;11(3):8124.
15. Munkongdee T, Chen P, Winichagoon P, et al. Update in Laboratory Diagnosis of Thalassemia. Front Mol Biosci. 2020;7:74.
16. Islam MT, Sarkar SK, Sultana N, et al. High resolution melting curve analysis targeting the HBB gene mutational hot-spot offers a reliable screening approach for all common as well as most of the rare beta-globin gene mutations in Bangladesh. BMC Genet. 2018;19(1):1.

17. Tariq A, Khurshid S, Sajjad M. Evaluation of the High Resolution Melting Approach for Detection of beta-Thalassemia Gene Mutations. Hemoglobin. 2021;45(1):20-4.
18. Bilgen T, Altiok Clark O, Ozturk Z, et al. Gap-PCR Screening for Common Large Deletional Mutations of beta-Globin Gene Cluster Revealed a Higher Prevalence of the Turkish Inversion/Deletion (deltabeta)0 Mutation in Antalya. Turk J Haematol. 2016;33(2):107-11.
19. El-Fadaly N, Abd-Elhameed A, Abd-Elbar E, et al. Accuracy of Reverse Dot-Blot PCR in Detection of Different beta-Globin Gene Mutations. Indian J Hematol Blood Transfus. 2016;32(2):239-43.
20. Schouten JP, McElgunn CJ, Waaijer R, et al. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res. 2002;30(12):e57.
21. Lei YL, Sui H, Liu YJ, et al. Molecular and Hematological Characterization of a Novel Translation Initiation Codon Mutation of the alpha2-Globin Gene (ATG>ATC or HBA2: c.3G>C). Hemoglobin. 2019;43(4-5):241-4.
22. Bilgen T. Large Deletional Mutations of the Beta Globin Gene Turkiye Klinikleri J Med Genet-Special Topics. 2017;2(1):15-20.
23. Jiang F, Zhou J, Zuo L, et al. Utilization of multiple genetic methods for prenatal diagnosis of rare thalassemia variants. Front Genet. 2023;14:1208102.
24. Achour A, Koopmann TT, Baas F, et al. The Evolving Role of Next-Generation Sequencing in Screening and Diagnosis of Hemoglobinopathies. Front Physiol. 2021;12:686689.
25. Suhaimi SA, Zulkipli IN, Ghani H, et al. Applications of next generation sequencing in the screening and diagnosis of thalassemia: A mini-review. Front Pediatr. 2022;10:1015769.
26. Brancaleoni V, Di Pierro E, Motta I, et al. Laboratory diagnosis of thalassemia. Int J Lab Hematol. 2016;38 Suppl 1:32-40.
27. Keser I, Oztas A, Bilgen T, et al. First Observation of Hemoglobin Kansas [beta102(G4)Asn-->Thr, AAC>ACC] in the Turkish Population. Turk J Haematol. 2015;32(4):374-5.
28. Bilgen T, Canatan D, Delibas S, et al. A Novel Mutation in the Promoter Region of the beta-Globin Gene: HBB: c.-127G > C. Hemoglobin. 2016;40(4):280-2.
29. Bilgen T, Clark OA, Ozturk Z, et al. Two novel mutations in the 3' untranslated region of the beta-globin gene that are associated with the mild phenotype of beta thalassemia. Int J Lab Hematol. 2013;35(1):26-30.
30. Poon KS, Tan KM. Spectrum of pathogenic alterations identified after two decades of HBB gene sequencing for molecular diagnosis of beta-thalassaemias and haemoglobinopathies. Pathology. 2021;53(4):536-8.
31. Kashork CD, Theisen A, Shaffer LG. Prenatal diagnosis using array CGH. Methods Mol Biol. 2008;444:59-69.
32. Basak AN. The molecular pathology of beta-thalassemia in Türkiye: the Bogazici university experience. Hemoglobin. 2007;31(2):233-41.
33. Zhan L, Gui C, Wei W, et al. Third generation sequencing transforms the way of the screening and diagnosis of thalassemia: a mini-review. Front Pediatr. 2023;11:1199609.
34. Sawakwongpra K, Tangmansakulchai K, Ngonsawan W, et al. Droplet-based digital PCR for non-invasive prenatal genetic diagnosis of alpha and beta-thalassemia. Biomed Rep. 2021;15(4):82.
35. Chumworathayee W, Munkongdee T, Buasuwan N, et al. Diagnosis of alpha-thalassaemia by colorimetric gap loop mediated isothermal amplification. Sci Rep. 2023;13(1):9612.

Correspondance Address:
Prof. Dr. Türker Bilgen
Address: Department of Nutrition and Dietetics, School of Health, Tekirdağ Namık Kemal University, Tekirdağ-Türkiye 59100.
Current Address: New York Medical College, Department of Pharmacology, Basic Science Building, 15 Dana Road, Valhalla, NY-USA 10595.
Phone: +90 (282) 250 1139, +1 (914) 535 9851
Fax: +90 (282) 250 3127
E-mail: tbilgen@nku.edu.tr, tbilgen@nymc.edu

CHAPTER 5

TRANSFUSION

(THIS CHAPTER WAS WRITTEN BY THE TURKISH BLOOD CENTERS TRANSFUSION ASSOCIATION AND THE TURKISH BLOOD FOUNDATION)

THALASEMIA & SAFE BLOOD

N. Nuri Solaz[1] MD MSc., İlhan Birinci[2] MD. Clinical Microbiology Specialist,
1. Turkish Blood Foundation Istanbul-Türkiye
2. Turkish Red Crescent- Istanbul-Türkiye

ABSTRACT

Despite recent progresses in medicine "blood transfusion" is still essential for the thalassaemic patients. Safe, enough, and continuous blood supply are the basics of efficient blood transfusion. Although there have been different terminologies about the blood donor as the safe blood source; based on the international consensus "safe blood" should be based on VNRBD which is the toughest part of Blood Banking (BB). The most effective way to reach sufficient VNRBD status is establishing a special definition and body which is called "Blood Donor Recruitment & Retention; BDRR" and "Blood Donor Recruitment & Retention; Department; BDRRD". BDRRD should be considered and supported efficiently and continuously.

Keywords: Thalassemia, safe blood

REVIEW

Thalassemia patients have been dependent on blood transfusion. Although based on the progresses their treatment tools have improved still "safe blood" is essential for their survival and better life quality. During early days of thalassemia treatment by blood transfusion "blood" itself had considered mainly because it was not much known about the safety risks of blood. Family members and friends were the most common blood donors/givers for the patients at those days.

Post donation phases of blood preparation for transfusion have had very important progresses and dramatically decreased previous risks of blood transfusion but importance of volunteer nonremunerated blood donor (VNRBD) is still existing. At this point of view recruitment and retention (R&R) of VNRBD should be considered under an independent topic and handled seriously.

If we look at the historical evaluation of blood source, it will be a wise choice to start from the book "The Gift Relationship" which was written by Prof. Richard Titmus who was a socio-economist from London School of Economics **(1)**.

Titmus evaluated blood givers under 8 different types based on sociological view. Those are listed below;

Type – A (paid blood donor): one who is selling her/his blood based on market conditions.

Type – B (professional blood donor): one who is giving her/his blood either for money or other compensations such as free days, expensive promotional tools, etc. selling her/his blood based on market conditions.

Type – C (paid or directed blood donor): one who may accept remuneration for his blood, but she/he can give her/his blood free to the directed patient if the patient cannot afford remuneration.

Type – D (compensator blood donor): one who received blood and had a request to pay money or find blood donor for compensation either herself/himself or one she/he will find donation is called compensator blood donor.

Type – E (family credited blood donor): one who donates blood at certain intervals (at least 1 per year) for receiving blood either herself/himself or his family members. This system was used by so many organizations till the end of 1980ies even in Türkiye. It had a lot of problems and understood that its harms were much more than benefits and was quit.

Type – F (compulsory blood donor): one who cannot refuse to donate blood due to its social status.

Type – G (rewarded blood donor): one who donates blood due for the reward, which is not remuneration such as off days, holidays, free food, etc.

Type – H (voluntary public blood donor): one who donates blood only by his own will without any pressure, expectation neither remuneration nor else.

Another widely accepted terminology has prepared by World Health Organisation (WHO) which was based on "compensation". This terminology was consisted of 3 main types of blood donors.

1. Paid blood donor: one who gives blood for receiving remuneration

2. Exchange blood donor: one who gives blood for receiving blood for her/his patient

3. Volunteer blood donor: one who donates blood only by his own will without any pressure, expectation neither remuneration nor else.

By time being WHO terminology has had some revised and final terminology for the preferred blood donor has declared as "voluntary non-remunerated blood donor (VNRBD)"

Actually, Turkish Red Crescent (TRC) is using below listed donor terminology for their routine activities which is referenced from European (EU) Union documents **(2)**;

1) First time blood donor: one who never donated before donates blood.

2) Regular blood donor: one who donated blood at least twice during last 24 months, last blood donation should be done maximum 12 months after the previous blood donation and the interval between first and second blood donation should not be longer than 24 months.

3) Silent blood donor: one who donated at least once during last 24 months but did not donate blood last 12 months

4) Nonfunctional blood donor: one who donated at least once but did not donate during last 24 months

5) Return blood donor: one who donated blood in the past and donated once during last 12 months

The authors of this article also have created a terminology based on previous terminologies and their experiences. This terminology has 5 types of blood donor based on actual medical, technical, sociological, economic, and historical background. Those can be listed as below;

I- Based on the donation type: whole blood donor/apheresis donor (plasma/cells)

II- Based on the patient: allogeneic blood donor, autologous blood donor.

III- Based on sociological condition: civilian blood donor, military blood donor, family blood donor, exchange blood donor, directed blood donor, walking blood donor, immigrant blood donor, foreign blood donor, rewarded blood donor, volunteer non-remunerated blood donor (VNRBD), repeated VNRBD.

IV- Based on historical perspective: live blood donor, cadaver blood donor.

V- Others: one who gives her/his blood for money or easily convertible to money items.

Based on the international consensus "safe blood" should be based on VNRBD which is the toughest part of Blood Banking (BB).

The most effective way to reach sufficient VNRBD status is establishing a special definition and body which is called "Blood Donor Recruitment & Retention; BDRR" and "Blood Donor Recruitment & Retention; Department; BDRRD". Essentials of BDRR can be listed as below;

1. Concrete and sustainable policy for supporting VNRBD recruitment/retention by the **blood provider body, official bureaucracy, and politicians**

2. Establish a specific department which has branches at each regional blood centers

3. Dedicated, sufficient, and sustainable **FINANCIAL SOURCE** to BDOD for VNRBD recruitment/retention

4. Hire enough staffs whose educational, social, physical, etc. conditions are eligible for being "Blood Donor Recruiter" (BDR). These staffs are paid good enough to attract right people for this position

5. Specific training programme was created for these staffs and preferably organized as accommodated training courses

A model of BDRR activities can be planned by below listed steps;

1. Short term activities; evaluation actual situation, defining weak and strong points (SWOT analysis)

2. Mid-term activities; targeting annual number of blood donations, planning road steps to reach the target, creating necessary tools such as; conventional and social media advertisements, posters, flyers, innovating new attractive promotional gifts, planning blood mobile drives, paying visits to the authorized people of the targeted institutions (factories, labour unions, office buildings, universities, municipalities, ministries, etc.), collaborate with NGOs seriously, supply well designed and functional mobile blood collection vehicles, etc.

3. Long term activities; realizing sustainable and effective mobile blood drives, reaching the targeted number of blood donations, convincing the first-time donors to be repeated donors while keeping repeated donors satisfied, evaluating the quality and feasibility of BDOD activities, etc.

CONCLUSION

Despite recent progresses in medicine "blood transfusion" is still essential for the thalassaemic patients. Safe, enough, and continuous blood supply is the basic of efficient blood transfusion. Complying related regulations best transfusion comes from VNRBD. Supplying sufficient blood from VNRBD should be based an effective BDRR policy.

REFERENCES

1. The Gift Relationship, Vintage Books, 1972. Richard Morris Titmuss
2. Kort W, Veldhuizen I, et al.: Donor Management Manuel (Domaine Project) 2010;72-75

Correspondence Address:
*N. Nuri Solaz M.D. M.Sc.,
Turkish Blood Foundation, n.solaz26@yahoo.com
** İlhan Birinci M.D. Clinical Microbiology Specialist,
Turkish Red Crescent, ilhan.birinci@kizilay.org.tr

BLOOD BANK AND TRANSFUSION RELATIONSHIP IN THALASSEMIA

Prof. Dr. Meral Sönmezoğlu[1], Assoc. Dr. Berrin Uzun[2]

[1] Yeditepe University Hospital, Department of Infectious Diseases and Clinical Microbiology, Transfusion Center- İstanbul-Türkiye

[2] Izmir Ataturk Training and Research Hospital, Department of Medical Microbiology and Transfusion Center- İzmir-Türkiye

ABSTRACT

Thalassemia is a group of disorders of hemoglobin in which either globin chain synthesis is reduced or absent, and is termed as a quantitative disorder. This inherited disorder of hemoglobin is considered the commonest single-gene disorder globally with an autosomal recessive inheritance. It is increasingly prevalent in the Mediterranean, Asia and Sub-Saharan Africa along with other continents such as Europe, North America, and Australia due to population migration and therefore, has become a global health problem. These patients require regular blood transfusions to survive. Safe blood transfusion is a priority for these patients because of having transfusion-associated infections, formation of alloantibodies against donor's antigens, developing different grades of blood transfusion reactions. Therefore, thalassemia clinics should work in close relations with blood banks.

Regular transfusion with packed red cells is recommended to maintain a pre-transfusion hemoglobin threshold not exceeding 9.5 g/dl, which seems to be associated with adequate marrow inhibition and a relatively low iron burden. Transfusions should generally be given at an interval of three to four weeks. Transfusions should be scheduled in advance and maintained at a fixed schedule. This enables patients and families to establish routines and will improve quality of life. If cardiac insufficiency is present, higher pre-transfusion hemoglobin levels (10 to 12 g/dL) should be maintained with smaller volume transfusions given every one to two weeks.

Keywords: Thalassemia, transfusion, annual blood requirement

INTRODUCTION

Thalassemia syndromes are a heterogeneous group of hereditary disorders caused by genetic lesions that lead to decreased synthesis of one or more globin subunits **(1)**. Beta-thalassemia is one of the most important hemoglobinopathies worldwide, resulting in little or no β-globin chain synthesis (**1, 2)**. Mutation in the beta globin gene results in impaired beta globin production, ineffective erythropoiesis, reduced red blood cell (RBC) survival, and chronic hemolytic anemia (**3)**. If left untreated, β thalassemia major is fatal within the first decade of life due to its complex pathophysiology leading to a wide range of clinical manifestations **(2)**. Traditional treatment of beta thalassemia major is based on regular blood transfusion from early childhood **(1)**. These patients are dependent on transfusion from early childhood and throughout life **(4)**. Survival of patients with transfusion-dependent thalassemia major has improved significantly over the past few decades as better treatments have become available **(5)**. Current clinical management for patients with thalassemia depends on regular transfusions followed by iron chelation therapy **(2)**.

THE ROLE OF RED BLOOD CELL TRANSFUSIONS IN THALASSEMIA

The focus of contemporary management of thalassemia is health-related quality of life throughout the lifespan. With the introduction of regular blood transfusion in the treatment of thalassemia, thalassemia ceased to be a fatal disease in childhood and turned into a chronic disease. Despite numerous advances, many patients around the world still have limited access to regular and safe blood transfusions, especially in countries with a high population

of affected individuals. The reasons why safe blood cannot be supplied on time include the scarcity of voluntary unpaid blood donors, low awareness of thalassemia, the absence of national blood policies in many countries, and the fragmentation of blood services **(6)**. As can be seen, first of all, blood must be supplied on time and meet the demand. In this case, each country must regularly find regular donors and provide blood for use as required by its own health policies.

Thalassemia children face various problems in case of insufficient transfusion, but repeated blood transfusions bring them the risk of transfusion-transmitted infections (TTI). TTIs such as HIV, Hepatitis B, and Hepatitis C may also occur. Therefore, chronic blood transfusion in thalassemia patients is a double-edged sword **(7)**. Screening tests for transfused blood components are being conducted in many countries in terms of transfusion-transmitted infections. The screening tests required are specified in the second module of the World Health Organization's Safe Blood and Blood Products Guide **(8)**. In Türkiye, Hepatitis B surface antigens, hepatitis C antibody, HIV antibody and syphilis antibodies are used as obligatory screening tests **(9)**. Generally, countries can conduct additional tests to these screening tests, depending on their endemic situation in terms of infectious agents. In addition, in accordance with the thalassemia patient follow-up guide, hepatitis B, hepatitis C and HIV screening should be performed in thalassemia patients immediately after diagnosis and annual HBV, HCV and HIV serology follow-up is required **(10)**.

Blood should be collected safely from carefully selected voluntary, unpaid donors and processed, tested, stored and distributed in dedicated centers with well-established quality assurance programs. Blood should be administered by trained personnel with close monitoring of patients **(11)**. In order to provide safe blood, national legislation and guidelines should be prepared and their use should be made mandatory. The American Society of Hematology issued a “Strong Blood Supply Statement” in September 2022 that seeks to address clinical management and research on establishing and maintaining federally funded monitoring, redistribution, and hemovigilance systems **(12)**. It has been emphasized to expand the eligibility criteria and public awareness by focusing on the recruitment of more diverse donors to the donor pool. In Türkiye, the Ministry of Health published national guidelines with the European Union's "Technical Support for Strengthening the Blood Supply System" project. The standards are based on the "Appropriate Clinical Use of Blood and Patient Blood Management" guidelines **(2)**.

The goal of transfusion in thalassemia patients is to use the safest product with the fewest side effects to suppress ineffective erythropoiesis (IE) and extramedullary hematopoiesis (EMH). Transfusion-dependent thalassemia patients (TDT) require ≥8 transfusions per year **(13)**. Clinical criteria for initiating transfusion for TDT patients are pre-transfusion target hemoglobin (Hgb) levels, transfusion frequency, and red blood cell (RBC) product selection, and many guidelines on these topics have been published in many countries, e.g. the United States, Canada, Italy, the United Kingdom, and the International Thalassemia Federation, with a consensus **(14)**.

Transfusions should be applied at hemoglobin values and frequencies determined within the guidelines. For thalassemia patients, transfusion frequency is typically every 2-4 weeks to maintain pre-transfusion hemoglobin between 9.0 and 9.5-10.5 g/dL. It has been shown in many studies that this target supports normal growth, allows normal physical activity, and adequately suppresses IE while minimizing iron accumulation **(10, 15, 16)**. In addition, erythrocyte suspensions are 50% to 80% of the minimum acceptable donor hematocrit (Hct) value. Determining the volume to be transfused becomes difficult due to the different anticoagulant storage solutions used. In randomized controlled studies, using a formula based on the Hct value of the PRBC unit significantly reduced the number of donor exposures and the need for annual erythrocyte transfusion **(17)**. For these reasons, the use of erythrocyte suspensions with high hematocrit values should be considered in thalassemia patients.

The number of red blood cell suspensions to be transfused should be determined based on the patient's weight and condition before transfusion, and whether there is any degree of hypersplenism or an unexpected or unexplained response to a previous transfusion. In smaller, younger patients, transfusion can be calculated as mL/kg. **(13)**. Again, most

patients should receive a full unit to limit donor exposure **(12)**.

Apheresis allows two units versus one to be collected from a donor in a single collection for use in the same patient **(18)**. Although there is no supporting data regarding the use of erythrocyte suspension obtained by erythrocytopheresis (automatic red cell exchange) showing that it provides as much benefit as it does in sickle cell patients, it can be considered in terms of limiting donor exposure **(19, 20)**. It reduces exposure to donor-derived infections and alloantibody formation. However, the need for the donor to have a higher initial hematocrit level and the donor's inability to donate frequently are limiting factors. Additionally, the apheresis process is more invasive for the donor and requires more coordination to ensure that both units of apheresis go to the same patient **(18)**. It also costs higher than whole blood donation. Its use should be limited to exceptional cases **(19, 20)**.

STROGE OF DONOR RED CELL UNITS

Erythrocyte suspensions are stored in anticoagulant preservative solutions to prevent clotting and preserve metabolic functions. All storage solutions contain sodium citrate, citric acid and glucose and components can be stored for 21 days. Some of them additionally contain adenine, guanosine, and phosphate, and the storage period is extended to between 35 and 42 days **(21)**. All of these storage solutions reach >75% of transfused cells at an average post-transfusion survival time of 24 hours. However, actual half-life is not routinely tested. 2,3-BPG (previously known as 2,3-DPG) is degraded during storage, however, the rapid repletion of 2,3-BPG after transfusion generally compensates for the loss of function during storage **(22)**. Units stored for less than 2 weeks should be used for TST patients whenever possible **(13)**. In order to ensure adequate oxygenation and reduce the frequency of transfusion in thalassemia patients, the use of the freshest possible blood should be preferred. SAG-M supplement solution is added to erythrocyte suspensions to extend the storage period of blood. It is recommended that the erythrocyte suspension to be used in transfusion in thalassemia patients be at most 7 days old if prepared with CPD-A, and 14 days at most if prepared with other solutions (e.g., SAG-M). However, if the appropriate blood component is not available and causes an unacceptable delay in transfusion, blood that has been stored for a longer period of time can also be used **(10)**.

The number of leukocytes per erythrocyte suspension unit, must be reduced below 1x10 **(6)** per liter, which is the critical threshold to prevent many transfusion-related reactions caused by leukocytes **(10, 23)**. These adverse reactions include febrile nonhemolytic transfusion reactions (FNHTRs), human leukocyte antigen (HLA) alloimmunization, and transfusion-transmitted infections such as cytomegalovirus (CMV). Filtration of leukocytes will also reduce the transmission of Creutzfeldt-Jakob disease in countries where it is common. Although there are multiple methods for leukocyte reduction, filtration of blood before storage is the preferred method used **(24)**. Routine leukofiltration of erythrocyte suspensions is a very important method in preventing transfusion-related adverse reactions that are frequently encountered in both thalassemia and other transfused patients.

BLOOD PRODUCTS FOR SPECIAL PATIENT POPULATIONS

CMV negative blood products are indicated for pregnant patients who are CMV negative or whose CMV status is unknown **(25)**. This criterion also applies to thalassemia patients.

Washing of red blood cell suspensions (RBCs) is indicated in patients with Immunoglobulin A (IgA) deficiency (preformed IgA antibody) and in patients with recurrent, severe urticarial allergic reactions (despite premedication). It removes plasma proteins that cause allergic reactions through the washing process. Washed RBCs should be transfused within 24 hours. Since a significant amount of erythrocytes will be lost during the washing process, it will cause Hgb to be lower in patients after transfusion **(18)**. For this reason, washed RBCs should not be used in thalassemia patients except in cases with a clear indication.

Irradiation is indicated only in patients with T-cell dysfunction (e.g., patients receiving treatment for Hodgkin lymphoma or bone marrow transplantation). It should not be applied routinely due to decreased erythrocyte recovery after transfusion and increased intracellular potassium flow rate **(18)**.

Therefore, irradiated products should not be used in thalassemia patients except when indicated.

Thalassemia patients who need frequent blood transfusion are also at high risk of infection **(26)**. In Türkiye, the National Blood and Blood Components Preparation, Use and Quality Assurance Guide determines the microbiological screening and verification tests that must be applied to blood donors and blood components, and the working methods and quality control requirements of these tests **(27)**. With the creation and use of national guidelines on blood banking in every country and the use of new technological developments and better screening tests, transfusion-transmitted infection rates have decreased significantly **(26)**. However, in order to reduce these risks in thalassemia patients who receive frequent blood transfusions, hepatitis B, hepatitis C and HIV screenings should be performed before starting transfusion treatment and annual screenings should be repeated **(10)**.

Frozen erythrocyte suspensions can be used for patients who have unusual erythrocyte allo-antibodies or are known to be deficient in common erythrocyte antigens before forming allo-antibodies **(11)**. Cryopreserved erythrocytes are frozen within 7 days of collection, stored at -60 to 80◦C in an electric freezer using a high-glycerol-based cryopreserver, or at -140 to 150◦C in vapor-phase liquid nitrogen when using the low-glycerol method. There is no clear evidence on how long these units can be stored (the US National Health Service stores them for up to 30 years). Shelf life depends on whether the erythrocytes are washed in an open (1 day) or closed system (7 days) and re-suspended in a saline or storage solution. After thawing and washing, approximately 20% of the erythrocyte count is lost.[18] The use of frozen erythrocytes may be considered in thalassemia patients when indicated.

Neocyte transfusions (transfusion of blood rich in young erythrocytes) may slightly reduce transfusion requirements, but increase the number of donor exposures and costs **(18)**.

ADVERSE REACTIONS

Although blood transfusion is a life-saving treatment for thalassemia patients, it also carries many risks, one of which is platelet and erythrocyte alloimmunization **(28, 29)** and poses a significant clinical challenge in the management of thalassemia patients **(3, 30)**. Exposure to different blood groups through repeated transfusions produces erythrocyte antibodies (allo-antibodies and/or auto-antibodies) **(1)**. Alloantibodies can complicate the process of finding compatible blood products with appropriate cross-matching. The simultaneous presence of auto-antibodies may further complicate these dangers **(30)**. However, the development of anti-erythrocyte alloantibodies and auto-antibodies limits safe transfusions, as it significantly shortens the in-vivo survival of transfused erythrocytes **(3, 30)**. Although erythrocyte auto-antibodies occur less frequently, they can also cause hemolysis and difficulties in cross-match testing. Allo-immunization against erythrocyte antigens increases the need for transfusion and can significantly complicate transfusion therapy. Some allo-antibodies cause hemolysis and can cause hemolytic transfusion reactions. Mostly clinically insignificant antibodies are formed **(1)**.

Alloimmunization, varies greatly between centers depending on the degree of homogeneity of the donor and recipient populations **(11)**. The rates of allo-immunization and auto-immunization observed in patients with transfusion-dependent thalassemia major are highly variable and different from the general population. However, most reports are based mostly on pediatric patients with transfusion-dependent thalassemia major, and less data are available on rates in adult patients **(3)**. Antibodies of clinical importance in terms of transfusion are those that shorten erythrocyte survival after transfusion. These are Rh, Kell, Duff and Kidd antibodies **(31)**. Of the alloantibodies, anti-E, anti-c and anti-Kell constitute 90% of the antibodies found in thalassemia patients. In addition, warm or cold antibodies of undefined specificity may also develop. Expanded erythrocyte antigen typing for at least ABO, CcDEe and Kell should be performed in screening in all patients with thalassemia **(11, 32)**. In accordance of National Thalassemia Diagnosis and Monitoring Guide of Türkiye, the patient's blood group is determined by scanning ABO, Rh subgroups (C,c,D,e,E) and Kell antigen in a wide panel, and before all transfusions the blood group, subgroup and Kell grouping of component are determined, full matched blood components should be selected and cross-matching should be done **(10)**.

It is noteworthy that the risk of autoimmunization increases in allo-immunized patients. The mecha-

nism of this has not been fully explained. The possible mechanism is that alloantibodies bind to transfused erythrocytes, causing confusion in the epitope of the antigen and stimulating autoantibody production **(31)**. Due to the direct relationship between transfusion frequency and antibody formation, antibody screening and identification should be performed before starting transfusion treatment in patients who will be frequently transfused, such as thalassemia patients **(31)**.

CRITERIA FOR INITIATING TRANSFUSION THERAPY

Before each transfusion of thalassemia patients, a screening and indirect antiglobulin test for new antibody development should be performed at the transfusion center. The window for antibody screening and transfusion should be <72 hours if possible, although in some centers this has been extended to less than 7 days. Historical records of antigen testing, antibody formation and any reactions should be kept and shared with the patient and other centers if the patient receives transfusion in different places **(11)**. It may be considered to create cards such as ID cards for patients or to store and use this information with smartphone applications as technology develops. However, a study on patient knowledge about transfusion complications in thalassemia found that patients supported the development of a national registry system rather than using an ID card or smartphone application to store and distribute transfusion data **(33)**.

Before starting each transfusion, patients should be informed about the transfusion, given the opportunity to ask questions, an informative explanation should be made, and it should be ensured that the patient is sufficiently informed and their consent for transfusion should be obtained **(12, 27)**. In addition, they should be educated about the risks and symptoms of transfusion reactions and when and how to report them **(12)**. If the patient is under 18 years of age, informed consent should be obtained from the legal guardian **(10)**.

The patient and the blood and blood component to be transfused must be identified correctly and it must be ensured that the blood is given to the correct person. In order to prevent incorrect blood transfusions, it is important that this comparison be made separately by both the physician and the nurse administering the transfusion. It is a necessity to follow the steps in national guidelines to ensure accurate transfusion monitoring **(10, 27)**.

It is recommended to transfuse blood with a 150-200 µm filter set to reduce the leukocytes. Warming the blood is generally not recommended. Only, transfusion of young children and those with hemodynamic instability, and in presence of cold antibodies, blood can be warmed with appropriate devices during transfusion **(10)**.

Transfusion should be performed at a transfusion rate of 5 ml/kg per hour, with a total transfusion time not exceeding 3-4 hours. Monitoring should be done without leaving the patient's side during the first 15 minutes of transfusion. Forms prepared in accordance with national guidelines should be used for transfusion monitoring. Hemoglobin values should be monitored before and at least 2 hours after transfusion. In addition, all documents, including the date of all transfusions of thalassemia patients, the patient's pre- and post-transfusion hemoglobin values, the amount of blood given, any reactions that develop, and informed consent, should be recorded **(10)**.

In addition, thalassemia patients have to periodically come to the hospital to receive transfusion. Most of them come from outside the city and have a shortage of accommodation. In addition, psychologically, they want to stay in the hospital for less time and finish their work as soon as possible. In order to achieve this, blood components with the above-mentioned properties should be kept ready and the functioning should be adjusted for this purpose. Creating a hospital algorithm specific to patients with thalassemia will ensure that the system operates uninterruptedly, independent of individuals.

There is lack of studies describing newer and novel transfusion parameters useful in blood bank for thalassemia major patients about the annual need of transfusion. Novel parameters which includes Annual blood requirement and its derivatives Annual pure red cell requirement, Annual iron load and Daily iron loading were calculated for each patient as per the formula. Annual blood requirement was calculated as per guidelines as below for each patient. Annual blood requirement (mL/Kg) = Volume of blood transfused per 4 weeks (mL) x Total number of transfusions in one year /Weight of the patient (Kg) **(34)**.

CONCLUSION

Transfusion dependent thalassemia major patients need comprehensive care. Transfusion record of all thalassemia major patient needs to be maintained regularly in the hospital blood bank for transfusion requirements of patient. It is imperative to maximize the survival of the transfused red cells, minimize the occurrence of transfusion reactions, and prevent allo-immunization as much as possible.

Therefore, the following steps are recommended;

- Obtain a red cell genotype (preferred) or extended red cell phenotype for all thalassemia patients.
- Transfuse phenotypically matched RBCs for the following red cell antigens: Rh (D, C, c, E, e) and K.
- Transfuse leukocyte-reduced RBCs.
- Transfuse hemoglobin sickle negative RBCs.
- Transfusion of fresher RBCs may be preferred.
- If patients are to receive a transfusion at another institution, blood bank to blood bank communication is recommended to ensure historical transfusion medicine testing, blood product administration, and transfusion reaction information is relayed.
- Irradiation is not routinely required.

REFERENCES

1- Sadeghian MH, Keramati MR, Badiei Z, et al. Alloimmunization among transfusion-dependent thalassemia patients. Asian J Transfus Sci. 2009 Jul;3(2):95-8. doi: 10.4103/0973-6247.53884. PMID: 20808654; PMCID: PMC2920480.

2- Grech L, Borg K, Borg J. Novel therapies in β-thalassaemia. Br J Clin Pharmacol. 2022 Jun;88(6):2509-2524. doi: 10.1111/bcp.14918. Epub 2021 Jun 4. PMID: 34004015.

3- Pazgal, I., Yahalom, V., Shalev, B. et al. Alloimmunization and autoimmunization in adult transfusion-dependent thalassemia patients: a report from a comprehensive center in Israel. Ann Hematol 99, 2731–2736 (2020). https://doi.org/10.1007/s00277-020-04104

4- Rund D, Rachmilewitz E. Beta-thalassemia. N Engl J Med. 2005 Sep 15;353(11):1135-46. doi: 10.1056/NEJMra050436. PMID: 16162884.

5- Borgna-Pignatti C, Cappellini MD, De Stefano P,et al. Cardiac morbidity and mortality in deferoxamine- or deferiprone-treated patients with thalassemia major. Blood. 2006 May 1;107(9):3733-7. doi: 10.1182/blood-2005-07-2933. Epub 2005 Dec 22. PMID: 16373663.

6- Shah FT, Sayani F, Trompeter S, et al. Challenges of blood transfusions in β-thalassemia. Blood Rev. 2019 Sep;37:100588. doi: 10.1016/j.blre.2019.100588. Epub 2019 Jul 6. PMID: 31324412.

7- Tiwari BR, Ghimire P , Kandel SR , M. et al. Seroprevalence of HBV and HCV in blood donors: A study from regional blood transfusion services of Nepal. Asian J Transf Sci - Vol 4, Issue 2, July 2010.

8- Guidelines and Principles for Safe Blood Transfusion Practice - Module 2: Screening for HIV and Other Infectious Agents. 2009

9- National blood and blood components preparation, use and quality assurance guide, 2016.

10- General Directorate of Health Services, Turkish Ministry of Health, Thalassemia Diagnosis and Monitoring Guide, 2022.

11- Boudreaux J. Transfusion management in thalassemia. Ann NY Acad Sci. 2023; 1–7. Volume1527;1, September 2023: 42-48. https://doi.org/10.1111/nyas.15049

12- Statement on a strong blood supply. https://www.hematology.org/advocacy/policynews-statements-testimony-and-correspondence/policystatements/2021/statement-on-a-strong-blood-supply. American Society of Hematology. (2022).

13- Cappellini, M. D., Farmakis, D., Porter, J., et al. (2021). Guidelines for the management of transfusion dependent thalassaemia (TDT). 4th ed. Version 2.0. Thalassaemia International Federation.

14- Langhi,D, Ubiali, E. M. A., Marques, J. F. C., Verissimo, M.D. A., Leggett, S. R., Silvinato, A., & Bernardo, W. M. (2016). Guidelines on beta-thalassemia major - Regular blood transfusion therapy: Associacao Brasileira de Hematologia, Hemoterapia e Terapia Celular: Project guidelines: Associacao Medica Brasileira - 2016. Revista Brasileira de Hematologia e Hemoterapia, 38(4), 341–345.

15- Cazzola,M., Stefano, P. D., Ponchio, L., Locatelli, F., Beguin, Y., Dessi, C., Barella, S., Cao, A., & Galanello, R. (1995). Relationship between transfusion regimen and suppression of erythropoiesis in beta-thalassaemia major. British Journal of Haematology, 89(3), 473–478.

16- Cazzola, M., Borgna-Pignatti, C., Locatelli, F., Ponchio, L., Beguin, Y., & Stefano, P. (1997). A moderate transfusion regimen may reduce iron loading in beta-thalassemia major without producing excessive) expansion of erythropoiesis. Transfusion, 37(2), 135–140.

17- Kaur, M., Kaur, R., Sood, T., Jindal, G., Kaur, P., & Mittal, K. (2022). Efficacy of packed red blood cell transfusions based on weight versus formula in tha-

lassemic children: An open-label randomized control trial. Transfusion, 62(4), 791–796.
18- AABB Standards for Blood Banks and Transfusion Services. 2022
19- Friedman, D. F., Jawad, A. F., Martin, M. B., Horiuchi, K., Mitchell, C. F., & Cohen, A. R. (2003). Erythrocytapheresis to reduce iron loading in thalassemia. Blood, 102(11), 121.,
20- Berdoukas, V. A., Kwan, Y. L., & Sansotta, M. L. (1986). A study on the value of red cell exchange transfusion in transfusion dependent anaemias. Clinical & Laboratory Haematology, 8, 209–220.
21- Kan bileşenlerinin hazırlanması (preparation of blood components). XXV. National Blood Banks and Transfusion Course Book. P:53, 2023.
22- Cohn, C. S., Delaney, M., Johnson, S. T., & Katz, L. M. AABB technical manual (20th ed.) 2020.
23- Klein, H. G., Spahn, D. R., & Carson, J. L. (2007). Red blood cell transfusion in clinical practice. Lancet, 370; (9585):415–426.
24- Schiroli, D., Merolle, L., Quartieri, E., Chicchi, R., Fasano, T., De Luca, T., Molinari, G., Pulcini, S., Pertinhez, T. A., Di Bartolomeo, E., Biguzzi, R., Baricchi, R., & Marraccini, C. (2021). Comparison of two alternative procedures to obtain packed red blood cells for β-thalassemia majör transfusion therapy. Biomolecules, 11(11), 1638.
25- AABB Standards for a Patient Blood Management Program (3rd ed.) 2021.
26- Pediatric Hematology and Oncology, 28:6, 479-485,DOI:10.3109/08880018.2011.568595.
27- Ulusal Kan Ve Kan Bileşenleri Hazırlama, Kullanım ve Kalite Güvencesi Rehberi
28- Prati D. Benefits and complications of regular blood transfusion in patients with beta-thalassaemia major. Vox Sang 2000;79:129-37.
29- Lo SC, Chang JS, Lin SW, Lin DT. Platelet alloimmunization after long-term red cell transfusion in transfusion-dependent thalassemia patients. Transfusion 2005;45:761-5.
30- Stower, H. A therapy for β-thalassemia. Nat Med 26, 649 (2020). https://doi.org/10.1038/s41591-020-0906-4
31- Dhawan HK, Kumawat V, Marwaha N, Sharma RR, Sachdev S, Bansal D, Marwaha RK, Arora S. Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients: Study on 319 patients. Asian J Transfus Sci. 2014 Jul;8(2):84-8. doi: 10.4103/0973-6247.137438. PMID: 25161344; PMCID: PMC4140069.
32- Turkiye Klinikleri J Hem Onc-Special Topics. 2010;3(1):50-7
33- Patterson, S., Singleton, A., Branscomb, J., Nsonwu, V., & Spratling, R. (2022). Transfusion complications in thalassemia: Patient knowledge and perspectives. Frontiers inMedicine, 9, 772886.
34- Guidelines for the Clinical Management of Thalassaemia. 2nd Revised ed., No. 9, Nicosia (CY): Thalassaemia International Federation; 2008

Correspondence Address:
Prof. Meral Sönmezoglu, MD
Infectious Diseases and Clinical Microbiology Department, Yeditepe University
Blood Bank Director
Yeditepe University Hospital, Transfusion Center
Kosuyolu Mah Kosuyolu Cad No 168 Kadikoy Istanbul-Türkiye
E-mail: msonmezoglu@yeditepe.edu.tr
Assoc. Dr. Berrin Uzun MD
Izmir Ataturk Training and Research Hospital, Department of Medical Microbiology and Transfusion Center-İzmir-Türkiye
E-mail: berrinuzun@gmail.com

GENETICS OF BLOOD GROUPS AND RARE BLOOD GROUPS IN THALASSAEMIA

Dr. Levent Tufan Kumaş[1] MD, Dr. Salih Haldun Bal[2] MD
1. Blood Bank Services, Labmed, Acıbadem Health Group, İstanbul/Türkiye
2. Dr. Raşit Durusoy Blood Bank, Faculty of Medicine, Bursa Uludag University, Bursa/Türkiye

ABSTRACT

Thalassemias are a heterogeneous group of diseases that are inherited in an autosomal recessive manner and are characterized by hypochromic, microcytic anemia that develops as a result of defective synthesis of one or more of the hemoglobin chains. A significant portion of patients defined as transfusion-dependent thalassemia (TDT) patients require frequent and continuous blood transfusion. Transfusion practices, as is known, involve many risks of complications. One of the most important difficulties encountered in thalassemia patients during the transfusion process is alloantibodies against foreign RBC antigens. In alloimmunized patients, treatment effectiveness will decrease as a result of increased RBC destruction after transfusion. Additionally, RBC autoantibodies may develop in addition to alloantibodies in TDT patients. To date, 45 blood group systems and 360 blood group antigens assigned to these systems have been identified. Current guidelines recommend transfusion of blood matched for clinically important antigens such as C, c, E, e and K, in addition to ABO and RhD blood groups, in thalassemia patients. In fact, expanded antigen typing is also recommended in allo- and/or autoimmunized patients. However, various difficulties are encountered in serological antigen typing (phenotyping) in patients who have recently transfused or have developed allo-/autoantibodies. Therefore, in such patients, molecular blood group typing (genotyping) seems to be a solution that has recently been proposed and become widespread. It should also be noted that a large donor database with typed RBC antigens is required in order to find blood components with the required antigenic properties. For this purpose, particularly high-throughput molecular methods are used. Another problem that is rarely encountered is providing the rare blood component needed to a patient who is negative for a high incidence antigen. For his purpose the International Rare Donor Panel (IRDP) was established in 1965 under a joint initiative of the World Health Organization (WHO) and ISBT to facilitate the rapid availability and exchange of rare blood between countries.

Keywords: Thalassemia, blood groups, molecular typing for red blood cell antigens, rare donor panel

REVIEW

Thalassemias are a heterogeneous group of diseases that are inherited in an autosomal recessive manner and are characterized by hypochromic, microcytic anemia that develops as a result of defective synthesis of one or more of the hemoglobin chains. The most common types are α and β thalassemias. Beta thalassemia is a group of hereditary disorders with high clinical heterogeneity, characterized by defective production of β-globin chains. It is caused by decreased or no synthesis of hemoglobin beta chains due to mutation in the beta gene of hemoglobin (chromosome 11). The severity of the disease is related to the nature of the mutation, and no genetic interaction with any blood group system has been reported to date. So far, 45 blood group systems and 360 blood group antigens assigned to these systems have been identified. Table 1 shows the genes encoding these blood group systems and their chromosomal locations (https://www.isbtweb.org/resource/tableofbloodgroupsystems.html).

The term rare blood group describes the condition of being negative for a high-prevalence antigen

with a frequency of less than 1/1000, or being negative for more than one high-incidence antigen, which occurs rarely. Examples of rare blood groups include Rh_{null}, Bombay (Oh) and Jr(a-). Because some blood types occur at different frequencies in different ethnic groups, finding a blood donor with the exact same blood type can be a major challenge in populations containing mixed ethnic groups. It is therefore important to have international cooperation and an accurate database of rare donors. The "ISBT International Rare Donor Panel" is managed by the International Blood Group Reference Laboratory – NHS Blood and Transplant and uses rare blood lists provided by ISBT WP "rare donor" member organizations to ensure that patients requiring life-saving rare blood components have access to these products (https://www.isbtweb.org/isbt-working-parties/rare-donors.html). The International Rare Donor Panel (IRDP) was established in 1965 under a joint initiative of the World Health Organization (WHO) and ISBT to facilitate the rapid availability and exchange of rare blood between countries. The panel currently includes information on rare donors from the 27 countries involved in the organization, as well as frozen blood component inventories from blood banks around the world. The IRDP is coordinated by the RedCell Reference department of the IBGRL, UK (Bristol). (https://ibgrl.blood.co.uk/services/international-rare-donor-panel/). Like all other rare blood group patients who need blood, the needs of thalassemia patients with such a condition are met by this organization.

Thalassemia patients have a clinical spectrum ranging from severe anemia to asymptomatic individuals. The most serious complications relate to anemia caused by ineffective erythropoiesis and destruction of red blood cells (RBCs). Patients need blood transfusion throughout their lives, starting from infancy. This makes them susceptible to alloimmunization. Despite all the improvements in transfusion protocols, alloimmunization still remains among the important complications. The main reason for this is the phenotypic differences between donors and recipients. Therefore, matching blood donors and patients in terms of RBC antigens reduces this risk. In alloimmunized patients, the risk of hemolytic reaction increases and it becomes difficult to obtain RBCs with matched antigenic phenotype for transfusion. Another important problem for thalassemia patients is autoimmunization. It is thought that the development of autoantibodies in patients with hemoglobinopathies may lead to autoimmune hemolytic anemia (AIHA), which may reduce the in vivo survival of transfused RBCs **(1)**. It has been reported that auto- and alloantibodies sometimes develop together, and the risk of autoantibody development is higher in alloimmunized patients **(2–4)**. The presence of autoantibodies makes it difficult to identify possible alloantibodies and poses a risk for transfusion safety. In these cases, extended antigen matching stands out as a safety strategy. However, as explained in detail below, serological antigen typing (phenotyping) is inadequate in this group of patients who receive blood frequently and continuously, so molecular typing (genotyping) methods stand out as a necessity.

There are many publications in the literature about the possibilities of allo- and autoimmunization in thalassemia patients. In a study from India, allo-antibody was detected in 5.64% of thalassemia patients receiving regular transfusion, autoantibody was detected in 28.2%, and auto-antibody and allo-antibody were detected together in 2.8% of the patients. It has been demonstrated that 52% of allo-antibodies develop against the Rh blood group system and 35% against the Kell blood group system **(2) (Table 2, Figure 1)**. In a multicenter study conducted in Hong Kong, it was reported that the alloimmunization rate was 18.3% and the autoimmunization rate was 4.7%, and auto- and alloantibodies were found to be present together in 1.8% of the cases **(4) (Table 2)**. In this study, it was reported that Chinese patients were more prone to develop antibodies against Miltenberger antigens, while they did not develop antibodies against Kell and Duffy blood group antigens **(4)**. Similarly, anti-Mia antibodies were also seen in analyzes in Thailand and Singapore, but no antibodies against Kell blood group antigens were detected. In a study conducted in eight medical centers across Thailand, the allo-immunization rate was calculated as 15.6% **(5)** while in another study, the allo-immunization rate was found to be 16.9% and the auto-immunization rate was 2.3% **(3)**. In Singapore, the allo-immunization rate is calculated as 29% **(6) (Table 2)**. There are significant differences in data from the Asian conti-

nent, especially between India and other regions. Significantly different allo-immunization rates are also found in analyzes for the Arabian peninsula. While the allo-immunization prevalence was found to be 9.3% in Oman **(7)**, it was found to be 35.57% in Saudi Arabia **(8)**. In two other studies conducted in Saudi Arabia with similar results, the frequency of allo-immunization was found to be 11.8% and 13.2%, and the frequency of auto-immunization was 1.7% and 3.8% **(9, 10) (Table 2)**. Interestingly, in a retrospective analysis conducted in the United States, the prevalence of Rh allo-immunization was found to be 32.5% in thalassemia patients receiving RhD, C, E and K matched transfusions **(11) (Table 2, Figure 1)**. As can be seen from these results, allo- and auto-antibody development rates in thalassemia patients vary between patients and societies. Even different results can be obtained between cities in one region of the same country. The prevalence of allo-immunization, which is 11.8% in total in the Al-Ahsa region of Saudi Arabia, was calculated as 13.2% and 20.2% in the cities of the region, Jizan and Jeddah, respectively **(9)**. As can be seen in Table 2 and Figure 1, alloantibodies develop most frequently against Rh antigens, especially E antigen. Unlike others, anti-Mia, anti-Dia and anti-P1 antibodies have been detected in Far Eastern countries. While anti-K antibodies were detected at level close to anti-E antibodies in other countries, they were not observed in the Far East. Although this result may be due to the different distribution of blood group antigens among societies, what could be the reason 236how236hi differences in immunization frequencies?

Variables that predispose to immunization have been evaluated in various studies, and it has been reported that allo-immunization may be related to transfusion age, and auto-immunization may be related to the number of transfusions and splenectomy. The same study showed that receiving the first transfusion after the age of 2 increased the risk of allo-immunization **(2)**. There are studies showing that variables such as age at first transfusion ≥3 years, advanced age, female gender, history of splenectomy, amount and frequency of RBC transfusions are important factors associated with alloimmunization **(3, 5, 7, 12, 13)**. However, there are also studies showing that there is no relationship between allo-immunization and age **(14)**, or between allo-immunization and gender and splenectomy history **(7)**. The results are contradictory. In fact, it has been found that there is a difference in the alloimmunization 236how236his236n between transfusion-dependent thalassemia (TDT) patients, who need regular transfusions throughout life, and non-transfusion-dependent thalassemia (NTDT) patients, who need intermittent transfusions for reasons such as anemia exacerbations, pregnancy, and preparation for surgery. NTDT patients have been shown to have a higher risk of alloimmunization than TDT patients **(6, 15, 16)**. In summary, it has not been clearly determined what the predisposing factor(s) are. Regardless of the predisposing factors, prevention of allo-immunization is the main goal in this group of patients receiving regular transfusion. 236how236his purpose, the best way is to perform RBC antigen typing and give antigen matched blood (at least for Rh and Kell antigen) before the first transfusion **(2)**. More ideally, matched RBCs would be transfused with extended phenotyping between patient and donor **(9)**. While preparing an extended panel, the ethnic characteristics of each society should be taken into consideration, and in addition to blood group antigens such as ABO, RhD, RhC, Rhc, RhE, Rhe, K, other antigens such as Mia, Dia and P1 should be added **(3, 5, 6, 11) (Table 2)**. Particularly for patients who develop auto-antibodies, further extended panels can be used by adding antigens such as Fya, Fyb, Jka, Jkb, S, s, etc. On the other hand, there are researchers who suggest that it would be beneficial to start transfusion therapy early after diagnosis to prevent alloimmunization **(2)**.

In a study conducted in Thailand evaluating the relationship of antigen (ABO, RhD, RhC, Rhc, RhE, Rhe, Mia) matched RBC transfusions with alloimmunization, it was shown that antigen-matched transfusions provided low alloimmunization rates, even without an extended panel. It was reported that the frequency decreased from 16.9% to 3.5% **(3)**. Despite this, the anti-E and anti-Mia antibodies that developed were evaluated as naturally occurring antibodies. But the underlying cause of this situation may be antigenic variations in the society. A retrospective analysis conducted in the United States evaluated

Rh alloimmunization in thalassemia patients who received ABO, RhD, RhCE, RhE, and K-matched RBCs. In this analysis, the Rh genotype of the patients and the race and Rh phenotypes of the donors of the 3 transfusions before antibody detection were examined. Interestingly, it was determined that patients developed anti-D, anti-C and anti-E antibodies despite receiving RBCs that did not express the relevant antigen. Rh antibodies were not associated with patients with variant RH, although 25% of patients had a different RH genotype. But it was found that black donors, who are known to have a high frequency of RH variants, donated 63% of the products used in the 3 transfusions before anti-Rh detection. 237how237his reason, it was reported that it would be useful to investigate the role of donor RH genotypes on alloimmunization **(11)**. A study in patients with sickle cell anemia identified RH genotypes (R0r′ and R1R0) restricted to the population in Iran. In addition to differences in RH genotypes, it has been reported that 90% of alloantibodies developed after blood transfusions from other ethnicities are against Rh antigens **(14)**. These two studies indicate that ethnic differences between patients and blood donors may be the reason for allo-immunizations despite antigenically matched transfusions. However, contrary to this view, there are also studies showing that there is no relationship between ethnicity and allo-immunization, as in the analysis conducted in Singapore **(6)**.

The priority in the follow-up of patients who have to receive regular transfusions, such as thalassemia patients, is to prevent the development of allo-antibodies. 237how237his reason, it is useful to perform extended phenotyping before the first transfusion, as mentioned above. However, phenotyping may not be possible in patients receiving regular transfusion and/or immunization. Donor RBCs from previous transfusions pose problems for phenotyping, and molecular techniques are preferred for antigen typing in such patients. Because while DNA-based tests directly reveal the alleles coding for antigens on the erythrocyte surface, they can also identify variant alleles and weak phenotypes. This has been shown in many studies comparing phenotyping and genotyping. Although some studies achieved similar results with both methods, generally incompatible results were obtained. This shows that phenotyping will not be sufficient, especially in patients who have recently been transfused. In the study by Sonker et al. İn which Kell, Kidd and Duffy blood group systems were tested serologically and using SSP-PCR, genotyping and phenotyping results showed complete concordance in normal blood donors, but showed 24% discordance in thalassemia patients. The frequency of alloimmunization in thalassemia patients was found to be 8%, and inconsistency between genotyping and phenotyping was found in half of alloimmunized cases **(17)**. In another study, thalassemia patients without alloantibodies were transfused with a match containing ABO, RhD, RhC, Rhc, RhE, Rhe, K and k antigens for one year. The following year, the same patients received RBC transfusions with a more extended match, including Fya, Fyb, Jka, Jkb, M, N, S, and s antigens. Matches were made by phenotyping and DNA-based genotyping. It has been shown that patients do not develop new alloantibodies after two years of RBC transfusions with both antigen matching protocols **(1)**. Mediterranean Hemopathic Syndrome (MHS) patients, who were shown to be negative for alloantibodies, were divided into two groups and phenotyping and genotyping were performed. Group-1 consisted of newly diagnosed patients with no history of transfusion, and group-2 consisted of previously diagnosed patients receiving transfusion treatment. While insignificant differences between genotyping and phenotyping were observed in group-1, significant inconsistencies were noted in group-2 patients. Discrepancies were noted to be evident in Jka, Jkb and Rhc antigens **(18)**. In summary, studies 237how that the fact that the patient has received transfusion or developed alloantibodies makes phenotyping insufficient and the importance of genotyping. Antigen-compatible RBCs were given on the basis of phenotype, comparison was made in alloimmunized patient samples with a history of delayed hemolytic transfusion reaction, and incompatibility between phenotypes and genotypes was determined in 90% of the samples. One sample was phenotyped as Rhcc and two samples were phenotyped as RhCc, while all were genotyped as RH*C/C. Two samples were phenotyped as RhEe but genotyped as RH*e/e. Three samples were phenotyped as Jk(a+b+) but genotyped as homozygous for JK*B. One sample

was phenotyped as Fy(a+b-) and genotyped as homozygous for FY*B **(19)**. In another study, blood group phenotype and genotype were determined in alloimmunized thalassemia patients using the serological method and PCR-SSP, respectively. The genotypes of patients with discordance between phenotype and genotype were re-evaluated by RFLP-PCR and confirmed by DNA sequencing. Discordance between phenotype and genotype results was found in 42% of the patients. However, complete agreement was found between the three genotyping methods. The most inconsistency was detected in the Rh (24%) and Duffy (23%) systems. The main discrepancy was in the FY*B/FY*B allele, which was serologically detected as Fy(a+b+) **(20)**. In addition, all 19.5% of cases with undetermined phenotype due to mixed field reactions were resolved by molecular genotyping **(20)**. In allo-immunized thalassemia patients in which M, N, S and s antigens were evaluated, phenotyping was done by hemagglutination method, and genotyping was done using SSP-PCR and DNA sequencing methods. Discrepancies were observed in 21% of patients with the S/s allele and 2% of patients with the M/N allele. However, full agreement was found between SSP-PCR and DNA sequencing results **(21)**. In another study conducted in alloimmunized thalassemia patients, phenotyping and genotyping (Multiplex-ASO-PCR and T-ARMS-PCR) were performed for Rh, Kell, Kidd and Duffy blood groups, and phenotype/genotype incompatibility was evaluated by sequencing. Genotype/phenotype mismatch was detected in 2.1% of the cases. 9.3% of these were detected in the Rh, 34.8% in the Duffy and 26.1% in the Kidd blood group system. Sequencing has demonstrated that the results of molecular methods are accurate **(22)**. In a cohort of thalassemia and sickle cell anemia patients, RHCc, RHEe, K, k Fya, Fyb, Jka and Jkb antigens were tested by tube agglutination, allele-specific PCR or RFLP technique and the results were compared. In more than 80% of cases, molecular and serological test results differed **(23)**. In the alloimmunized thalassemic patient in whom Kidd blood group antigens were evaluated, phenotyping was done by routine serological methods, genotyping was done by SSP-PCR, and DNA sequencing and PCR-RFLP were used to confirm the SSP-PCR results. A 16% discrepancy was found between phenotype and genotype. In 8% of the cases, the phenotype is Jk(a+b+) and genotype is JK*A/JK*A, in 7% of the cases, the phenotype is Jk(a+b+) and the genotype is JK*B/JK*B, in 1% of the cases the phenotype is Jk(a+b-) while the genotype was determined as JK*A/JK*B, and in 1% of cases, the phenotype was determined as Jk(a-b+) and the genotype was determined as JK*A/JK*B **(24)**. In a study evaluating C, c, D, E, e, Fya, Fyb, Jka, Jkb, K, k, M, N, S, s antigens, genotypes and phenotypes were found to be fully compatible among group O regular blood donors, while discrepancies were detected in 77% of thalassemia patients. The maximum discrepancy was detected in the Rh system c and E antigens **(25)**. In summary, molecular genotyping enables the determination of the true antigen profile in multiple transfused thalassemia patients and the resolution of cases with an undetermined phenotype due to mixed field reactions. In addition to these effects, genotyping, which helps to better manage transfusion therapy, is also useful for creating secure databases and donor registry for these patient groups **(26, 27)**. In addition, the consistency of the methods used in genotyping such as SSP-PCR, PCR-RFLP, Multiplex-ASO-PCR and Tetra-Primer-ARMS-PCR, and DNA sequencing makes it easier to decide on method selection.

Table 1: *Blood group systems*

No.	System name	System symbol	Gene name(s)*	Number of antigens	Chromosomal location	CD numbers
001	ABO	ABO	*ABO*	4	9q34.2	
002	MNS	MNS	*GYPA, GYPB, (GYPE)*	50	4q31.21	CD235a CD235b
003	P1PK	P1PK	*A4GALT*	3	22q13.2	CD77
004	Rh	RH	*RHD, RHCE*	56	1p36.11	CD240
005	Lutheran	LU	*BCAM*	28	19q13.2	CD239
006	Kell	KEL	*KEL*	38	7q33	CD238
007	Lewis	LE	*FUT3*	6	19p13.3	
008	Duffy	FY	*ACKR1*	5	1q21-q22	CD234
009	Kidd	JK	*SLC14A1*	3	18q11-q12	
010	Diego	DI	*SLC4A1*	23	17q21.31	CD233
011	Yt	YT	*ACHE*	6	7q22	
012	Xg	XG	*XG, CD99*	2	Xp22.32	CD99†
013	Scianna	SC	*ERMAP*	9	1p34.2	
014	Dombrock	DO	*ART4*	10	12p13-p12	CD297
015	Colton	CO	*AQP1*	4	7p14	
016	Landsteiner-Wiener	LW	*ICAM4*	4	19p13.2	CD242
017	Chido/Rodgers	CH/RG	*C4A, C4B*	9	6p21.3	
018	H	H	*FUT1; FUT2*	1	19q13.33	CD173
019	Kx	XK	*XK*	1	Xp21.1	
020	Gerbich	GE	*GYPC*	13	2q14-q21	CD236
021	Cromer	CROM	*CD55*	20	1q32	CD55
022	Knops	KN	*CR1*	13	1q32.2	CD35
023	Indian	IN	*CD44*	6	11p13	CD44
024	Ok	OK	*BSG*	3	19p13.3	CD147
025	Raph	RAPH	*CD151*	1	11p15.5	CD151
026	JohnMiltonHagen	JMH	*SEMA7A*	8	15q22.3-q23	CD108
027	I	I	*GCNT2*	1	6p24.2	
028	Globoside	GLOB	*B3GALNT1*	3	3q25	

029	Gill	GIL	*AQP3*	1	9p13	
030	Rh-associated glycoprotein	RHAG	*RHAG*	6	6p12.3	CD241
031	FORS	FORS	*GBGT1*	1	9q34.13-q34.3	
032	JR	JR	*ABCG2*	1	4q22.1	CD338
033	LAN	LAN	*ABCB6*	1	2q36	
034	Vel	VEL	*SMIM1*	1	1p36.32	
035	CD59	CD59	*CD59*	1	11p13	CD59
036	Augustine	AUG	*SLC29A1*	4	6p21.1	
037	Kanno	KANNO	*PRNP*	1	20p13	
038	SID	SID	*B4GALNT2*	1	17q21.32	
039	CTL2	CTL2	*SLC44A2*	2	19p13.2	
040	PEL	PEL	*ABCC4*	1	13q32.1	
041	MAM	MAM	*EMP3*	1	19q13.33	
042	EMM	EMM	*PIGG*	1	4p16.3	
043	ABCC1	ABCC1	*ABCC1*	1	16p13.11	
044	Er	ER	*PIEZO1*	5	16q24.3	
045	CD36	CD36	*CD36*	1	7q21.11	CD36

Table 2: *Allo/auto-immunization prevalence and antibody specificities among thalassemia patients by country*

(%)	Alloantibody prevalence	Autoantibody prevalence	Anti-C	Anti-D	Anti-E	Anti-c	Anti-e	Anti-C^w	Anti-K	Anti-Kp^a	Anti-Mi^a	Anti-M	Anti-N	Anti-S	Anti-JK^a	Anti-JK^b	Anti-Le^a	Anti-Le^b	Anti-Lu^a	Anti-Fy^a	Anti-Fy^b	Anti-Xg	Anti-Di^a	Anti-P_1	Anti-V
India(2)	5,6	28,2	13,0	13,0	17,0			9,0	35,0							9,0						4,0			
Hong Kong(4)	18,3	4,7			39,3	13,1					30,9				6,6										
Thailand(3)	16,9	2,3	0,8		39,5	15,3	0,8				19,4	1,6		1,6	2,4	2,4	5,6	1,6			0,8		0,8	2,4	
Thailand(5)	15,6		2,8		28,2	11,3	1,1				15,8	0,3	0,3	2,8	2,8	1,4	6,0	2,1			1,8		3,9	2,1	
Singapur(6)	29,0		6,0		31,0	16,0	4,0	2,0			12,0			8,0	12,0						2,0		4,0		
Umman(7)	9,3		12,0	12,0	24,0	12,0	8,0		24,0	8,0															
S. Arabia(8)	35,6		8,7	6,5	45,6	2,1	2,1	2,1	19,5						2,1		2,1		2,1	4,3	2,1				
S. Arabia(9)	11,8	1,7	14,0	7,0	21,8	1,8		7,0	33,3					1,8	3,5				1,8	1,8					
S. Arabia(10)	13,2	3,8		25,0					12,5	12,5					25,0										
USA(11)	32,5		30,8	38,5	15,4		7,7																		7,7
Iran(20)			7,0	11,0	21,0	5,0	1,0	2,0	28,0	7,0				4,0	4,0	2,0					1,0				
Iran(22)	8,5		17,0		45,0	23,0	4,0	7,0	23,7																

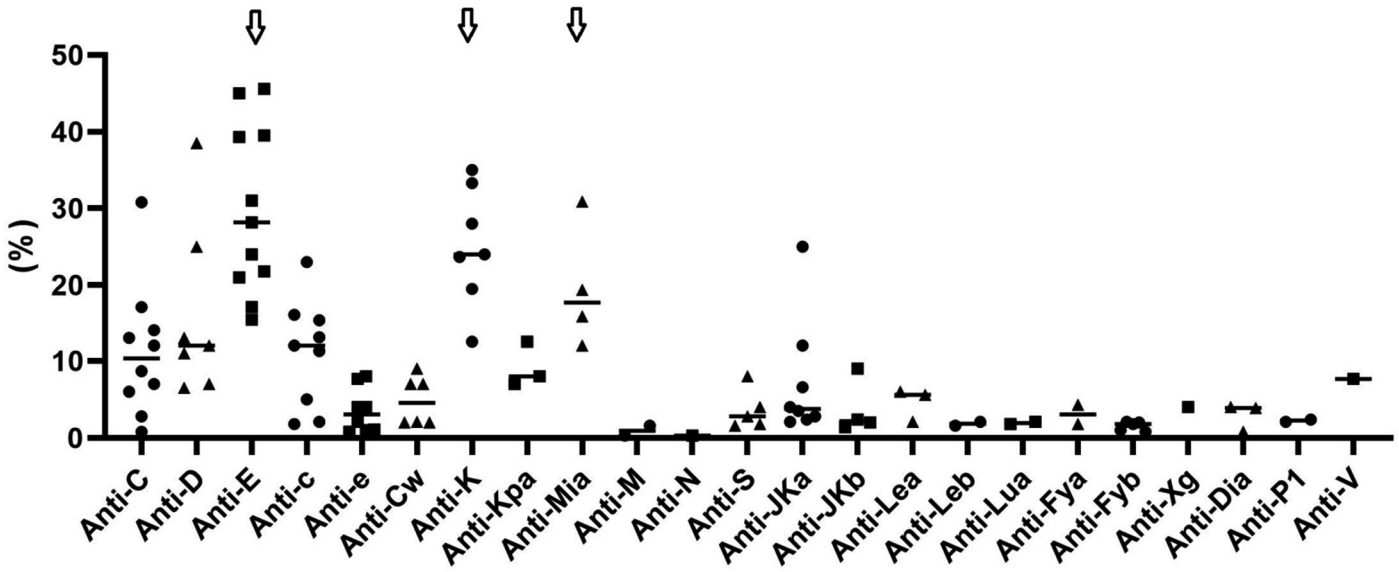


Figure 1: *Prevalance of allo-antibodies*

REFERENCES

1. Belsito A, Costa D, Signoriello S, et al. Clinical outcome of transfusions with extended red blood cell matching in β-thalassemia patients: A single-center experience. Transfus Apher Sci. 2019 Feb 1;58(1):65–71.
2. Dhawan HK, Kumawat V, Marwaha N, et al. Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients: Study on 319 patients. Asian J Transfus Sci. 2014;8(2):84.
3. Romphruk A V., Simtong P, Butryojantho C, et al. The prevalence, alloimmunization risk factors, antigenic exposure, and evaluation of antigen-matched red blood cells for thalassemia transfusions: a 10-year experience at a tertiary care hospital. Transfusion. 2019 Jan 1;59(1):177–84.
4. Cheng CK, Lee CK, Lin CK. Clinically significant red blood cell antibodies in chronically transfused patients: a survey of Chinese thalassemia major patients and literature review. Transfusion. 2012 Oct 1;52(10):2220–4.
5. Teawtrakul N, Songdej D, Hantaweepant C, et al. Red blood cell alloimmunization and other transfusion-related complications in patients with transfusion-dependent thalassemia: A multi-center study in Thailand. Transfusion. 2022;62:2039–47.
6. Ang AL, Lim CY, Ng WY, et al. Non-transfusion dependent thalassemia is independently associated with higher alloimmunization risk than transfusion dependent thalassemia and would benefit the most from extended red cell antigen-matching. Transfusion. 2021 Sep 1;61(9):2566–77.
7. Al-Riyami AZ, Al-Muqbali A, Al-Sudiri S, et al. Risks of red blood cell alloimmunization in transfusion-dependent β-thalassemia in Oman: a 25-year experience of a university tertiary care reference center and a literature review. Transfusion. 2018 Apr 1;58(4):871–8.
8. Hindawi S, Badawi M, Elfayoumi R, et al. The value of transfusion of phenotyped blood units for thalassemia and sickle cell anemia patients at an academic center. Transfusion. 2020 Feb 1;60 Suppl 1(S1):S15–21.
9. Kuriri FA, Ahmed A, Alanazi F, et al. Red Blood Cell Alloimmunization and Autoimmunization in Blood Transfusion-Dependent Sickle Cell Disease and β-Thalassemia Patients in Al-Ahsa Region, Saudi Arabia. Anemia. 2023;2023.
10. Halawani AJ, Mobarki AA, Arjan AH, et al. Red Cell Alloimmunization and Autoimmunization Among Sickle Cell Disease and Thalassemia Patients in Jazan Province, Saudi Arabia. Int J Gen Med. 2022;15:4093–100.
11. Waldis SJ, Uter S, Kavitsky D, et al. Rh alloimmunization in chronically transfused patients with thalassemia receiving RhD, C, E, and K matched transfusions. Blood Adv. 2021 Feb 9;5(3):737–44.
12. Franchini M, Forni GL, Marano G, et al. Red blood cell alloimmunisation in transfusion-dependent thalassaemia: a systematic review. Blood Transfus. 2019 Jan 1;17(1):4–15.
13. Al-Riyami AZ, Daar S. Red cell alloimmunization in transfusion-dependent and transfusion-independent beta thalassemia: A review from the Eastern Mediterranean Region (EMRO). Transfus Apher Sci. 2019 Dec 1;58(6).
14. Jalali Far MA, Oodi A, Amirizadeh N, et al. The Rh blood group system and its role in alloimmunization rate among sickle cell disease and sickle thalassemia patients in Iran. Mol Genet genomic Med. 2021 Mar 1;9(3):1–9.
15. Azarkeivan A, Ansari S, Ahmadi MH, et al. Blood transfusion and alloimmunization in patients with thalassemia: multicenter study. Pediatr Hematol Oncol. 2011 Sep;28(6):479–85.
16. Abdelrazik AM, Elshafie SM, El Said MN, et al. Study of red blood cell alloimmunization risk factors in multiply transfused thalassemia patients: role in improving thalassemia transfusion practice in Fayoum, Egypt. Transfusion. 2016 Sep 1;56(9):2303–7.
17. Sonker A, Dubey A, Mohan Y. A comparison of serological phenotyping and molecular genotyping for Kell, Kidd, and Duffy antigens in multi-transfused thalassemia patients. Asian J Transfus Sci. 2023 Jan 1;17(1):53.
18. Eldin EN, Khaled SAA, Abdelhameed NO, et al. Genotyping versus phenotyping of non-ABO erythrocyte antigens in patients with the Mediterranean hemopathic syndromes: Effect of transfusion therapy. PLoS One. 2021 Jul 1;16(7).
19. Castilho L, Rios M, Pellegrino J, et al. Blood group genotyping facilitates transfusion of β-thalassemia patients. J Clin Lab Anal. 2002 Jan 1;16(5):216–20.
20. Sarihi R, Oodi A, Dadkhah Tehrani R, et al. Blood group genotyping in alloimmunized multi-transfused thalassemia patients from Iran. Mol Genet genomic Med. 2021 Jul 1;9(7):1–11.
21. Gholamrezazade A, Amirizadeh N, Oodi A. Genotyping analysis of the MNS blood group system of thalassemia patients with alloantibodies in Iran. Transfus Apher Sci. 2021 Feb 1;60(1).
22. Gholami MS, Shahidi M, Tabibian S, et al. Genotyping of blood groups in alloimmunized patients with β-thalassemia major by T-ARMS-PCR and multiplex-aso-pcr. Transfus Apher Sci. 2021 Feb 1;60(1):102984.
23. Shah A, Patel P, Patel K, et al. Comparison of serology and molecular detection of common red cell antigens in multitransfused thalassemia major and sickle cell disease patients. Transfus Apher Sci. 2020;59:102599.

24. Jalali SF, Oodi A, Azarkeivan A,et al. Kidd Blood Group Genotyping for Thalassemia Patient in Iran. Indian J Hematol Blood Transfus. 2020 Jul 1;36(3):550–5.
25. Kulkarni S, Choudhary B, Gogri H, et al. Molecular genotyping of clinically important blood group antigens in patients with thalassaemia. Indian J Med Res. 2018 Dec 1;148(6):713.
26. Kulkarni S, Choudhary B, Gogri H, et al. Red cell antigen phenotypes in blood donors & thalassaemia patients for creation of red cell antigen-matched inventory. Indian J Med Res. 2020 Sep 1;152(3):273–9.
27. Portegys J, Rink G, Bloos P, et al. Towards a Regional Registry of Extended Typed Blood Donors: Molecular Typing for Blood Group, Platelet and Granulocyte Antigens. Transfus Med Hemotherapy. 2018 Oct 1;45(5):331.

Correspondence Address:
Dr. Levent Tufan Kumaş MD
Blood Bank Services, Labmed, Acıbadem Health Group, İstanbul/Türkiye
Mail: tufan.kumas@acibademlabmed.com.tr
Tel:+90 532 2575823

Dr.Salih Haldun Bal MD
Dr. Raşit Durusoy Blood Bank, Faculty of Medicine, Bursa Uludag University, Bursa/Türkiye
Tel: +90 533 651 63 22
E: Mail:haldun@uldag.edu.tr

TRANSFUSION PRINCIPLES IN THALASSEMIA

Burcu Belen Apak,MD[1], Nil Banu Pelit, MD[2]
[1]Başkent University Medical Faculty, Department of Pediatric Hematology and Oncology, Ankara, Türkiye
[2] Acıbadem Cord Blood Bank, Director, İstanbul, Türkiye

ABSTRACT

Transfusion is one of the most basic treatments for thalassemia, as chronic anemia has serious consequences for individuals with thalassemia. In addition to correcting anemia, transfusion provides control of ineffective erythropoiesis, increasing oxygen delivery to tissues, growth and development in children, and a healthy life free from complications in adults. Although the disease genotype (homozygous or combined heterozygous β-thalassemia mutations/deletions) is suggestive for distinguishing between thalassemia major/intemedia, clinical weight determines the transfusion decision. If the patient has a pause in weight gain or height growth, facial bone changes and/or hepatosplenomegaly develops, or when hemoglobin (Hb) < 7 g/dl measured 2 times with 1-2 week intervals, the patient is started on regular transfusion. It is recommended to perform at least ABO, RhD, Kell, C, E typing before the first transfusion. Full cross-match and antibody screening should be performed before each transfusion. In cases with a history of previous antibody development, transfusion should be continued with the antigen-negative erythrocyte suspension corresponding to the antibody. Pre-transfusion Hb value should be kept at a level of 9.5-10.5 g/dl (mean 9.5 g/dl), especially in children in the growth-development age. Pre-transfusion Hb values can be targeted higher (11-12 g/dl) in patients with cardiac complications. The interval between transfusions can be every 1-1.5 months in young children and then every 2-4 weeks. It is recommended to transfuse 10-15 ml/kg. It is recommended to use leukodepleted erythrocyte suspension, washed erythrocyte suspension should be used in cases with severe allergic reaction. Red cell suspensions stored with CPD-A and other solutions should be transfused within 1 week and 2 weeks respectively. Recording the number and volume of erythrocyte concentrate given annually is important for the detection of blood consumption rate, iron accumulation and signs of hypersplenism.

Keywords: Thalassemia, transfusion, erythrocyte suspension

INTRODUCTION

Beta thalassemia is an autosomal recessive, inherited hemoglobin disorder caused by a genetic defect in the β-globulin chain in the hemoglobin structure and is the most common hereditary anemia treated with blood transfusions. Beta-thalassemia syndromes constitute the most frequent inherited anemias managed with chronic red blood cell transfusions **(1, 2)**. Despite transfusion therapy transformed the disease from a severe, fatal anemia; into a chronic disease where complications due to transfusions dysequilibrium between are now the leading cause of morbidity and mortality (3). With optimal management of transfusional hemosiderozis today, we can expect a healthy life extending to seventh decade in thalassemia patients **(4)**. Transfusion is crucial as chronic anemia has serious consequences for individuals with thalassemia. In children, low hemoglobin levels are associated with decreased growth rate and physical activity, enlarged spleen, delayed puberty, cognitive impairement and osteopenia **(5, 6)**. In adults, anemia of thalassemia also leads to reduced work capacity, fatigue, cognitive impairement, osteopenia, hypersplenism and decreased quality of life **(7, 8)**.

THE ROLE OF RED BLOOD CELL TRANFUSION IN THALASSEMIA

Improvement of anemia

Transfusion is crucial as chronic anemia has serious consequences for individuals with thalassemia. In

children, low hemoglobin levels are associated with decreased growth rate and physical activity, enlarged spleen, delayed puberty, cognitive impairement and osteopenia **(5, 6)**. In adults, anemia of thalassemia also leads to reduced work capacity, fatigue, cognitive impairement, osteopenia, hypersplenism and decreased quality of life **(7, 8)**.

Ineffective eritropoesis control

In β-thalassemia patients, there is a dysequlibrium between α and β chains, causing aggregation of α chains in developing red blood cells, 60-80% of erythroid progenitors die at polychromatic stage **(9)**. Erythropoietin drives the expansion of erythroid precursors and together with shortened half life of red blood cell (Rbc) survival, hepatosplenomegaly, skeletal deformities of face and skull and fragile bones ocur. Furthermore, supressed production of hepcidin increases absorbtion of iron from intestine and release of it from body stores **(10)**.

Improve in oxygen in tissues

Hemoglobin A is decreased or absent in β-thalassemia, accompanied by an increase in Hb F. The predominance of Hb F with high oxygen affinity makes oxygen delivery to tissues less effective. Differences in adaptation to anemia among patients can be present due to variability of Hb composition and modifying factors **(11)**. Each patient's need and frequency of transfusion should be determined on individual basis in order improve oxygen transport in tissues.

TRANSFUSION RECOMMENDATIONS FOR B-THALASSEMIA

In homozygous beta thalassemia cases, there is a large spectrum of phenotypes; from the thalassemia major clinic, which leads a transfusion-dependent life in relation to the clinical severity (phenotype) and the severity of the genetic defect (genotype), to the thalassemia intermedia clinic (non-transfusion-dependent thalassemia), where growth and development can be achieved with transfusion or intermittent transfusions. Guidelies for the management of thalasemia and transfusion recommendations have been published by Thalassemia International Federation and other Professional organizations as well as by Ministry of Health in our country as Guidelines for Clinical Use of Blood **(12, 15)**.

Decision to start transfusion

The decision to about when to start red cell transfusions in a child with β-thalassemia is an important one. The decision to start transfusion in a β-thalassemia child is taken is based on clinical severity and likely transfusion dependency by the β-globulin gene phenotype. Although the disease genotype (homozygous or combined heterozygous β-thalassemia mutations/deletions) is suggestive for distinguishing between thalassemia major/intemedia, clinical picture determines the transfusion decision. Pediatric patients should be monitored monthly in order to observe whether they present with thalassemia major or intermedia phenotype after diagnosis.

How is the decision for regular transfusion made through monthly assessment?

After diagnosis, monthly follow-up is required for determining the clinical phenotype of patients. During this follow-up, the patient should be evaluated in terms of history, physical examination, vital signs, growth development, and hepatosplenomegaly due to thalassemia, as well as bone deformities on the face **(14, 15)**. Generally, in the absence of accompanying conditions such as folate deficiency, which can exacerbate anemia severity, febrile illness, blood loss, or glucose-6-phosphate dehydrogenase (G6PD) deficiency, regular transfusion program is initiated when Hb < 7 g/dl is observed twice at intervals of 1-2 weeks. On the other hand, stagnation in weight gain or height growth, bone changes on the face and/or hepatosplenomegaly, or Hb < 7 g/dl also necessitate the inclusion of the patient in the transfusion program. Generally, it is recommended to start regular transfusion program for cases in need before the age of 3 (since the risk of alloimmunization increases after this age). Patients in the clinic of thalassemia major typically require initiation of a transfusion program before this age. If the transfusion program has been initiated due to bone changes and growth stagnation (more common in cases of thalassemia intermedia), the patient can be included in the 'wean off' group after the closure of bones and completion of growth

development, but should be closely monitored for thalassemia complications **(14)**. Laboratory tests that need to be performed before starting regular transfusion are provided below **(Table 1)**.

Table 1: *Tests to be performed before the first transfusion in thalassemia patients*

Related laboratory	Examinations
Hematology	Repeated Hb measurements, G6PD*** scan, parent-patient hemoglobin electrophoresis
Transfusion Center	Major blood group (ABO-RhD) and erythrocyte phenotype examination from the patient (EeCc,cw, Kell, Jka,Jkb, Fya, Fyb, P, Lea, Leb,M,N,S) (14,15), If possible, erythrocyte genotype examination (14)
Biochemistry	Basal biochemical values (ALT ˜, AST˜˜, LDH§, total, direct, indirect bilirubin, urea, creatinine), basal ferritin level
Microbiology	Hepatitis B surface antigen, Hepatitis C antibody, HIV* antibody
Tissue typing laboratory	Mother, father, child HLA** tissue groups
Medical Genetics	Patient's thalassemia gene mutation

*HIV: human immunodeficiency virus, **HLA: human leukocyte antigen, ***G6PD: Glucose 6 Phosphate Dehydrogenase, ˜˜ AST: Aspartate aminotransferase, ˜Alanine aminotransferase, §LDH: Lactate dehydrogenase

Recommended blood products for thalasemia patients

Procurement of Blood Products

In regions where thalassemia is prevalent, the absence of voluntary and altruistic blood donors, underdeveloped disease awareness, lack of a national blood policy, limited economic resources, and difficulties in accessing reliable blood supply create significant problems in transfusion treatments. β-thalassemia patients are categorized into two groups: Those dependent on transfusion (TDT) and those non-dependent (NTDT). It is stated that an average of 26,000 TDT patients are born annually, with over 90% of births occurring in middle and low-income countries. It is reported that approximately 12% of TDT patients born each year can receive regular transfusions, and around 22,500 TDT patients die annually due to the inability to obtain blood. Transfusion is an expensive procedure. While 1 unit of erythrocyte suspension costs 200-250 $ in North America and European countries, it is around 100-150 $ in our country. It is reported that transfusion constitutes 47% of the cost of a thalassemic patient, while chelation therapy constitutes 43% **(16)**. In recent years, the frequency of occurrence has increased in developed countries due to migrations, but the number of cases is considerably lower compared to the general population. However, migrations make it difficult to obtain blood in this patient group. While suitable blood can be accessed in their own countries due to similar antigenic structures, alloimmunized patients may find it challenging to obtain antigen-negative blood from different ethnic groups. Therefore, North American and European countries have intensified their efforts on standard testing and treatment methods, even if the incidence rate of TDT is low **(17, 18)**. Canatan and colleagues initiated the "blood mother, blood father" project in 1998 with the aim of extending the life expectancy and improving the quality of life of TDT patients **(19)**.

Hemoglobin target, volume, rate

The frequency and intensity of transfusion therapy is determined by the patient's pre-transfusion hemoglobin level. It is recommended that the pre-transfusion hemoglobin value should be maintained at a level of 9.5-10.5 g/dl (mean 9.5 g/dl), especially in children in the growth-development age, and

the post-transfusional hemoglobin value should not exceed 15 g/dl **(12, 15, 21)**. The interval between transfusions can be every 1-1.5 months in young children and then every 2-4 weeks. It is recommended to transfuse 10-15 ml/kg (maximum 20 ml/kg) as the amount of transfusion **(15)**. In general, the goal of transfusion is to prevent the development of splenomegaly and to ensure appropriate growth, and it is known that keeping 9.5-10.5 g/dl of pre-transfusion hemoglobin prevents splenic growth and the development of skeletal anomalies (21,22). In milder cases such as thalassemia intermedia and HbEβ thalassemia, transfusion can be performed in cases such as intermittent and infection that causes a decrease in basal hemoglobin levels. Most of these cases can be transfused at a lower density, keeping the pre-transfusion hemoglobin value of 9-10 g/dl.

If the hemoglobin is below 5 g/dl or if the patient has signs of heart failure, hemoglobin can be gradually increased by giving a dose of 5 ml/kg in order to avoid volume overload. Recording the number and volume of erythrocyte concentrate given annually is important for the detection of blood consumption rate, iron accumulation and signs of hypersplenism. In Table 2, the Target Hemoglobin Value, Volume, and Infusion Rate are provided.

Table 2: *Recommendations for hemoglobin target, volume and rate*

1. Target hemoglobin
• β thalassemia major: Pre-transfusion hemoglobin: 9.5-10.5 gr/dl (mean 10 gr/dl)
• E β thalassemia: Pre-transfusion hemoglobin: 9-10 gr/dl
2. Frequency of transfusion:
• Every three weeks in most older children and aduts with β thalassemia major
• Every 4 weeks in younger children with β thalassemia major and in most children and adults with E β thalassemia
• It is preferable to change the volume of blood instead of the interval of transfusion to maintain Hb target
3. Volume of transfusion
• Children: Transfuse 4 ml/kg per gram increase in Hb desired. The calculation uses post-transfusion hemoglobin of 13 gr/dl on 3 week and 14 gr/dl on 4 week schedule
• Adults: 2-4 units per transfusion. Generally; 3units if pre-transfusion Hb<10 gr/dl, and 2 units if pre-transfusion Hb≥10 gr/dl
4. Other volume considerations:
• Patients with intact spleen have higher transfusion needs; Splenectomy is not recommended unless under exceptional circumstances
• Adults with body weight >60 kg may need 4 units on some transfusions
• Higher Hb target or transfusion more frequent than every 3 weeks are needed in rare circumstances
✓ Congestive heart failure
✓ Pulmonary hypertension
✓ Symptomatic extramedullary hematopoietic masses
✓ Occurence of fatigue or bone pian in pre-transfusion period
5. Rate of transfusion
• Children: 5 ml/kg/hour
• Adults: 200-300 ml/hour, based on tolerence
• Congestive heart failure: Reduce volume and rate based on cardiac function

Properties of erythrocyte concentrate

Regular blood transfusions bring lifelong risks to patients with thalassemia. These are transfusion reactions, long-term donor exposure, associated alloimmunization, and transfusional hemosiderosis. Many parameters of the donor (such as donor age, gender, hemoglobin concentration) determine the efficiency of transfusion **(23)**. In order to prevent febrile non-hemolytic transfusion reactions, a laboratory type leukocyte filter and reduced leukocyte concentrate should be used. Shiroli and colleagues investigated leukocyte reduction procedures, both of which are used as standard methods, with the aim of reducing transfusion side effects. For this purpose, methods of leukocyte reduction from whole blood and from erythrocyte suspension were compared. It was determined that products filtered from whole blood had higher Hb content and RBC count, while when leukocytes were filtered from erythrocyte suspension, potassium, iron, and storage lesions were found to be lower. Proinflammatory cytokines were found to be similar in amount, but the leukocyte count was slightly higher in the first method. It was suggested that conducting a clinical study based on this preclinical research would be appropriate **(24)**.

When citrate phosphate dextrose adenine (CPD-A) is used as an anticoagulant, the hematocrit of the product is 65-80% and the volume is 250-300 ml. Additional solutions that provide longer shelf life are often used, in this case the hematocrit is 55-65% and the volume is 300-350 ml **(23)**. Although transfusion can be performed by premedication with antihistamines and steroids in cases with allergic reactions, transfusion with washed erythrocyte suspension is recommended in cases with severe allergic/anaphylactic reactions **(15, 25)**. Shelf life of erythrocyte concentrate is also important because of the effect of storage on erythrocyte lifespan. Although fresh erythrocyte suspension is desired whenever possible, erythrocyte suspensions that have a storage time of less than 14 days and have not been irradiated before may be preferred **(26)**. Red cell suspensions stored with CPD-A should be transfused within 1 week, and red cell suspensions stored with other supplemental solution should be transfused within 14 days. It is known that irradiation causes radiation-related damage to the erythrocyte membrane, and accordingly, erythrocytes will be destroyed more quickly by the spleen and have a shorter life **(27)**.

Immunological compatibility of the erythrocyte suspension

Patients with thalassemia who receive frequent transfusions have the potential to develop antibodies against donor-derived erythrocyte antigens that are not found on their own erythrocytes, except for ABO-RhD antigens. This condition is called alloimmunization. Due to the risk of alloimmunization, it is aimed to give C,E,c,e,Kell compatible erythrocyte suspension in addition to ABO-RhD when transfusing these patients **(14, 15)**. Antibody development is frequently observed against the Rh antigen system and the Kell antigen system **(28, 29)**. Therefore, although administration of ABO-RhD-Kell compatible erythrocyte suspension is a general approach, there is no consensus that transfusion performed in this way can completely prevent alloimmunization **(13, 30, 31)**. However, since there is a possibility of developing more than one alloantibody in a patient who develops alloantibodies once, it is recommended to give a wide range of erythrocyte suspensions such as Rh, K, Jk, Fy, MNS in the transfusion of these individuals **(2, 15, 32)**. In cases with a history of previous antibody development, transfusion should be continued with the antigen-negative erythrocyte suspension corresponding to the antibody in order to prevent acute and delayed hemolytic transfusion reactions **(33)**. The reason for this is that alloantibody production is triggered in case of encountering the same antigenic structure again. In transfusions with both major blood group antigens (ABO-RhD) and broad antigenic compatibility (ABO-RhD-C, E, c, e, Kell), full cross-match and 'antibody screening' should be performed before each transfusion. It is recommended to perform at least ABO, RhD, Kell, C, E typing before the first transfusion. In cases where antibody screening is positive, 'antibody identification' should be performed **(15)**. Since multiple antibodies may develop in cases with previously identified antibodies, 'antibody screening' should be repeated before each transfusion. The development of donor registration systems with erythrocyte genotyping will be beneficial in terms of providing erythrocyte suspension to cases with multiple antibodies **(33)**. Antibodies developed in patients, transfusion reactions and annual transfusion need should be recorded for each patient. Table 3 summarizes the properties of the erythrocyte suspension to be used in thalassemia patients.

Table 3: *Properties of erythrocyte suspension to be used in thalassemia patients (33)*

1. Leukoreduced erythrocyte suspension: Pre-storage leukoreduction
2. Storage: Additive solution (hematocrit 55-60%) or CPD-A (Htc 70-75%)
3. Age of unit: Less than 2 weeks when possible
4. Washed erythrocyte suspension: For patients with severe allergic reactions
5. Irradiation of erythrocyte suspension is unnecessary
6. Phenotypic matching: Recommended minimum antigen matching ✓ Patients lacking alloantibody: Match to Rh/Kell ✓ Patients with one or more alloantibody: Match to Rh/Kell/Jk/Fy/S

Erythrocyte suspensions used in specific patient groups

Washed erythrocyte suspension

In cases with recurrent severe allergic reaction or IgA deficiency, a washed erythrocyte suspension can be given to remove the plasma proteins in the product. The washed erythrocyte suspension can be prepared with "continuous flow cell washing devices" or manually. Transfer bags are used in the manual washing process. During the procedure, 10-20% of the erythrocytes are also destroyed. Washed erythrocyte suspensions should be used within 24 hours as they are prepared with open systems, otherwise they should be destroyed. With the washing process, the liquid that the erythrocytes will feed on is also removed. Such products, which have a short shelf life due to the risk of contamination, should be prepared just before transfusion **(34)**. It is necessary to monitor the post-transfusional Hb level, as erythrocyte loss will occur with washing.

Cryopreserved (Frozen) erythrocyte suspension

A cryoprotective liquid (a protective liquid that prevents crystallization while the cell is frozen) is used for the cryopreservation of erythrocytes. For this purpose, glycerol is most often used. Within a maximum of 7 days after donation, erythrocytes are frozen in different concentrations of glycerol (20-40%) in a deep freezer (at -80 °C) or in liquid nitrogen (at -120/-140 °C). When it is desired to be used, it is dissolved in a double boiler containing warm water at 37 °C. With a closed automated system, glycerol is removed from the environment (deglycerolized) and made ready for transfusion. Although the storage period varies according to the national guidelines of the countries, it is between 10-30 years. The erythrocyte suspension prepared by this method can be used especially in cases with rare erythrocyte antibodies.

Red cells obtained by donor apheresis

It is a component obtained by erythrocyte apheresis method from a single donor using automatic cell separator devices. Depending on the preparation method and the device used, it is possible for erythrocytes prepared with this technology to be predictable, reproducible and standardized. Depending on the preparation method and the device used, the content of platelets, leukocytes and plasma may vary. It can be used to reduce the risk of alloimmunization and infection transmission and to provide less donor exposure **(34)**.

Neocyte transfusion

It is the separation of a young fraction of erythrocytes at an early age, but this method may also increase donor exposure **(35)**.

In conclusion, appropriate transfusion in thalassemia is one of the basic principles in the treatment of the disease, and long and quality life is possible for patients when appropriately performed in the light of evidence-based guidelines.

REFERENCES

1. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008;86(6):480-7.
2. Lal A, Wong TE, Andrews J et al. Transfusion practices and complications in thalassemia. Transfusion. 2018;58(12):2826-2835.
3. Telfer P, Constantinidou G, Andreou P et al. Ann N Y Acad Sci. 2005;1054:273-82.
4. Gupta V, Aggarwal P. Complications in Transfusion-Dependent Thalassemia. Indian Pediatr. 2022: 15;59(12):911-912.

5. Olivieri NF, Brittenham GM. Management of the thalassemias. Cold Spring Harb Perspect Med. 2013;3(6):a011767.
6. Berdoukas V, Modell B. Transfusion-dependent thalassaemia: a new era. Med J Aust. 2008; 188(2):68-9.
7. Mohamed N, Jackson N. Severe thalassaemia intermedia: clinical problems in the absence of hypertransfusion. Blood Rev. 1998;12(3):163-70.
8. Taher AT, Musallam KM, Cappellini MD et al. Optimal management of β thalassaemia intermedia. Br J Haematol. 2011;152(5):512-23
9. Sorensen S, Rubin E, Polster H et al. The role of membrane skeletal-associated alpha-globin in the pathophysiology of beta-thalassemia. Blood. 1990;75(6):1333-6.
10. Camaschella C, Nai A. Ineffective erythropoiesis and regulation of iron status in iron loading anaemias. Br J Haematol. 2016;172(4):512-23.
11. Weatherall DJ. Pathophysiology of thalassaemia. Baillieres Clin Haematol. 1998; 11(1):127-46.
12. Capellini MD, Framakis D, Porter J, Taher A editors. 2021 Guidelines for the Managfement of Transfusion Dependent Thalassemia (TDT): Nicosia: Thalassemia International Federation;2021
13. Compernolle V, Chou ST, Tanael S et al; International Collaboration for Transfusion Medicine Guidelines. Red blood cell specifications for patients with hemoglobinopathies: a systematic review and guideline. Transfusion. 2018;58(6):1555-1566.
14. https://ukts.org/3d-flip-book/standards/ accessed in 14.7.23
15. https://shgmkanhizmetleridb.saglik.gov.tr/Eklenti/37459/0/kanin-uygun-klinik-kullanimi-rehberi-kukk--23-mayis-2020pdf.pdf accessed in 14.7.23
16. Shah FT, Sayani F, Trompeter S et al. Challenges of blood transfusions in β-thalassemia. Blood Rev. 2019 ;37:100588.
17. Lal A. Challenges in chronic transfusion for patients with thalassemia. Hematology Am Soc Hematol Educ Program ;2020(1):160-166.
18. Lal A, Wong T, Keel S. The transfusion management of beta thalassemia in the United States; Transfusion. 2021; 61: 3027–3039
19. Canatan, Duran; Özsancak, Ahmet. A new donor system for the patients with thalassemia: "Blood mother and blood father". Asian Journal of Transfusion Science;2010; 4(2):109-111
20. Piga A, Serra M, Longo F et al. Changing patterns of splenectomy in transfusion-dependent thalassemia patients. Am J Hematol. 2011 Sep;86(9):808-10
21. Taher A, Vichinsky E, Musallam K et al. Guidelines for the Management of Non Transfusion Dependent Thalassaemia (NTDT) [Internet]. Weatherall D, editor. Nicosia (Cyprus): Thalassaemia International Federation; 2013. PMID: 24672826.
22. Roubinian NH, Plimier C, Woo JP et al. Effect of donor, component, and recipient characteristics on hemoglobin increments following red blood cell transfusion. Blood. 2019 Sep 26;134(13):1003-1013.
23. Cabibbo S, Fidone C, Antolino A et al. Clinical effects of different types of red cell concentrates in patients with thalassemia and sickle cell disease. Transfus Clin Biol. 2007 Dec;14(6):542-50.
24. Schiroli D, Merolle L, Quartieri E, Chicchi R, Fasano T, De Luca T, Molinari G, Pulcini S, Pertinhez TA, Di Bartolomeo E, Biguzzi R, Baricchi R, Marraccini C. Comparison of Two Alternative Procedures to Obtain Packed Red Blood Cells for β-Thalassemia Major Transfusion Therapy. Biomolecules. 2021;11(11):1638.
25. Tobian AA, King KE, Ness PM. Transfusion premedications: a growing practice not based on evidence. Transfusion. 2007 Jun;47(6):1089-96.
26. Moroff G, Holme S, AuBuchon JP et al. Viability and in vitro properties of AS-1 red cells after gamma irradiation. Transfusion. 1999 Feb;39(2):128-34.
27. Reverberi R, Govoni M, Verenini M. Deformability and viability of irradiated red cells. Ann Ist Super Sanita. 2007;43(2):176-85.
28. Thompson AA, Cunningham MJ, Singer ST et al; Thalassemia Clinical Research Network Investigators. Red cell alloimmunization in a diverse population of transfused patients with thalassaemia. Br J Haematol. 2011 Apr;153(1):121-8.
29. Singer ST, Wu V, Mignacca R et al. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly asian descent. Blood. 2000 Nov 15;96(10):3369-73.
30. Vichinsky EP. The prevention and management of alloimmunization in sickle cell disease: the benefit of extended phenotypic matching of red blood cells. Immunohematology. 2012;28(1):20-3.
31. Schonewille H, van de Watering LM, Brand A. Additional red blood cell alloantibodies after blood transfusions in a nonhematologic alloimmunized patient cohort: is it time to take precautionary measures? Transfusion. 2006 Apr;46(4):630-5.
32. Schonewille H, Haak HL, van Zijl AM. RBC antibody persistence. Transfusion. 2000 Sep;40(9):1127-31.
33. Lal A, Wong T, Keel S et al. The transfusion management of beta thalassemia in the United States. Transfusion. 2021 Oct;61(10):3027-3039.
34. Kan bileşenlerinin hazırlanması. Türkiye Kan Merkezleri ve Transfüzyon Derneği 20. Temel Kurs Kitabı,2017, S:57-60
35. Spanos T, Ladis V, Palamidou F et al. The impact of neocyte transfusion in the management of thalassaemia. Vox Sang. 1996;70(4):217-23.

Correspondence Address:
Prof. Dr. Burcu Belen Apak
Baskent University Ankara Hospital Pediatrics Outpatient Department
Şehit Temel Kuğuluoğlu Sok No:24 Bahçelievler/Ankara
E-mail: draidabb@gmail.com
Tel: 0 532 581 45 51

TRANSFUSION CHARACTERISTICS IN THALASSEMIA PATIENTS WITH STEM CELL TRANSPLANTATION

Hülya Bilgen MD, PhD[1], Mehmet Yay[2] MSc, Bio
[1] İstanbul Medipol University Hospital Transfusion Center Istanbul-Türkiye
[2] Erciyes University Hospitals Blood Transfusion Center- Kayseri-Türkiye

ABSTRACT

Transfusion medicine plays a vital role in the supportive care of patients receiving therapy for hematopoietic stem cell transplants (HSCT) with thalassemia who are intended not to have blood transfusion . Indications for transfusion in this population are similar to other patients being treated with HSCT; however, special considerations must be made in regards to pretransfusion testing, ABO compatibility, product modifications, and anticipated challenges while patients undergo engraftment. Additionally, infusion of hematopoietic stem cells requires acute understanding of product collection, modification, and potential side effects. As these patients often require numerous platelet transfusions, platelet refractoriness may be encountered. Hematopoietic stem cell transplantation (HSCT) is a curative therapy for thalassemia patients and requires human leukocyte antigen (HLA) matching, which is a major barrier to donor selection. Rigorous matching is associated with favorable outcomes. ABO and Rh matching statuses are not considered before HSCT, but crossing the ABO and Rh barrier has caused complications after HSCT and difficulties in selecting optimal transfusion products. In this chapter, we discuss the various aspects of transfusion support before, during, and after HSCT.

Keywords: Thalassemia, Stem Cell Transplantation, ABO incompatibility

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is used to treat Thalassemia. The duration and specificity of transfusion support for patients receiving HSCT depends on the disease, the source of the stem cells, the preparative regimen applied prior to transplantation, and patient factors during the post-transplantation recovery period **(1)**. Hematopoietic progenitor cells (HPCs) for allogeneic transplantation come from 3 sources: apheresis derived, mobilized peripheral blood (HPC-A), bone marrow (HPC-M), and umbilical cord (HPC-C). Pediatric patients receive more HPC-M, whereas adults receive more HPC-A. Use of HPC-C is more in pediatric patients **(2)**. Patients undergoing HSCT remain dependent on red blood cell (RBC) and platelet transfusions until the engraftment of these cell lines. The platelet line is considered engrafted when a patient's count is at least 20,000/μL after 3 consecutive days without platelet transfusion **(3)**. RBC engraftment is more difficult to assess and may be defined by the appearance of 1% reticulocytes in the peripheral blood **(4)** or on the day of the last RBC transfusion, with no transfusion given the following 30 days **(5)**. Typically, neutrophil engraftment is defined as an absolute neutrophil count of more than 500/μL across 3 consecutive days. Engraftment is influenced by many factors, including the relationship of the donor to the patient, the stem cell source, and the dose of CD34+ cells in the transplantation. In general, engraftment time is shortest with HPC-A and greatest when HPC-C is used **(6)**; however, considerable patient-to-patient variability exists. One study that compared HPC-C and HPC-A noted roughly equivalent neutrophil recovery times but found a longer time to platelet and RBC engraftment for HPC-C **(5)**. These prolonged engraftment times translated into higher transfusion rates for RBCs and platelets.

BASIC REQUIREMENTS OF TRANSFUSION IN HSCT WITH THALASSEMIA

1. Leukocyte-reduced erythrocyte and platelet components, usually used to decrease the occurrence of febrile, non-hemolytic transfusion reactions, and also to decrease both the incidence of allo-immunization to HLA antigens and the risk of transfusion-transmitted CMV infections to CMV-seronegative recipients are recommended for HSCT patients.

   Red blood cell products contain about 2×10^9 leukocytes, or 2×10^6 leukocytes are present in platelet products **(8)**. Prestorage leukoreduced products could be administered, or a leukocyte reduction filter could be applied during transfusion. Leukocyte reduction through centrifugation or filtration reduces more than 99.9% of leukocytes, which results in a leukocyte count of less than 1 $\times10^6$ /unit. Transfusion-transmitted infections, such as HTLV-1, HTLV-2, CMV, herpesvirus, Epstein-Barr virus, and Trypanosoma cruzi could be prevented or reduced. Febrile non-hemolytic transfusion reactions and alloimmunization to leukocyte antigens including HLA could be reduced **(8)**. The disadvantages of leukocyte reduction are extra cost and time, a 2% loss of RBCs, and a 10% loss of platelets. However, the benefit is higher than the disadvantages; therefore, leukocyte-reduced products are highly recommended for patients before, during, and after HSCT **(9)**.

2. CMV-seronegative blood products for CMV seronegative recipients who have received a CMV seronegative hematopoietic stem cell graft **(10)**.

3. Gamma irradiation of all transfused erythrocyte and platelet components to prevent Transfusion Associated Graft Versus Host Disease (TA-GVHD) **(11)**. Packed RBC, leukocyte-reduced RBC, packed platelet, single donor-derived platelet, fresh frozen plasma, cryoprecipitate, and granulocytes are some of the most commonly transfused products. Most of the products are irradiated with gamma rays or X-ray irradiators (25–50 Gy) to prevent GVHD and leukoreduction (leukocyte $<1\times10^6$ /unit), except for granulocyte products. The British Society of Hematology recommends that irradiated components should be continued until all the following criteria are met **(12)**.

   1. >6 months have elapsed since the transplant date;
   2. The lymphocyte count is $>1.0\times109$ /L;
   3. The patient is free of active chronic GVHD;
   4. The patient is off all immunosuppression. As patients might have GVHD, administration of immunosuppressants, different HSCT conditions, underlying diseases, and previous treatments, usually, lifetime use of irradiated blood administered as immunological reconstitution status is difficult to confirm **(12)**.

But currently, no data exist to justify lifetime use of irradiated blood products after transplantation but most centers recommend that all blood products be irradiated because no test that shows complete immunologic reconstitution is available **(13)**.

TRANSFUSION STRATEGY DUE TO ABO COMPATIBILITY BETWEEN AND THE RECEIPT

The human major histocompatibility complex, also called human leukocyte antigens (HLA), has been placed into 3 regions on chromosome 6p21: class I (telomeric), class II (centromeric), and class III **(14)**. This genomic region is critical to engraftment, prediction of clinical outcomes, and balancing the potential harm from graft-versus-host disease (GVHD) and the benefit from the graft-versus-leukemia effect. In contrast to HLA, mismatching of the ABO blood group system is not a barrier to HSCT **(15)**. The genes encoding ABO carbohydrate glycosyltransferases are located on chromosome 9q34, far from the genes encoding HLA, and are, therefore, inherited independently **(16, 17)**. As a consequence, HLA-matched allogeneic stem cell donors have some degree of ABO incompatibility in approximately 25% to 50% of transplantations **(18, 19)**. Although not as significant as the degree of HLA match, graft source, risk of infection, and donor age and gender, clinical outcomes in ABO incompatible HCT are generally considered inferior

to those in ABO-compatible HCT, with mixed or undefined results in overall survival, acute and chronic GVHD, and engraftment of platelets and granulocytes (20). Given that both ABO-identical and ABO-incompatible HSCT require extensive transfusion support,

ABO compatibility has important consequences for clinical outcomes as well as blood management in the pretransplantation (phase I), transplantation (phase II), and postengraftment (phase III) time periods (21). Transfusion strategy differs according to the phases of transplantation and showed in table 1 **(22)**.

ABO incompatibility is classified as either major, minor, or bidirectional. Major ABO-incompatible HSCT (e.g., from a type A, type AB, or type B donor to a type O recipient) is characterized by the presence of antidonor blood group antibodies in recipient plasma. Minor ABO incompatibility (e.g., from a type O donor to a type A, type B, or type AB recipient) is characterized by the passive transfer of incompatible blood group antibodies from the donor to the recipient. In bidirectional ABO incompatibility (e.g., type A donor to a type B recipient), both major and minor incompatibilities are present **(23, 24, 25)**.

In HSCT, complications due to ABO mismatch arise from incompatibility due to antibodies and antigens present in the graft and recipient blood, as well as other cells of the donor and recipient immune system.

The major complications of major ABO-incompatible transplantation are hemolysis of red cells at the time of graft infusion, delayed red cell engraftment, and pure red cell aplasia (PRCA). All of these complications occur because of cross-reactivity between donor blood group antigens and recipient antibodies (isohemagglutinins). Stem cell products, collected from either peripheral blood apheresis collection, bone marrow, or cord blood, contain varying quantities of donor red blood cells that can potentially destroyed by corresponding blood group antibodies produced by the recipient immune system. The quantity of infused red cells depends on the graft source and cell processing issues, such as cryopreservation, and may result in an acute hemolytic transfusion reaction during transplantation **(23, 24, 25)**.

We may try to prevent the reactions by manipulations in the graft or the donor. It is important to note that there are no regulations regarding the volume of RBCs allowed in an HPC product. One strategy to prevent hemolysis of a HSCT graft is to deplete RBCs from HPC products before transplantation **(21)**. Unfortunately, this process can also reduce the overall progenitor cell content of the HPC product, making this strategy less appealing in circumstances where there are few progenitor cells to spare, as may occur with cord blood transplantation **(21)**.

Another approach to prevent complications of major ABO-incompatible HSCT is to reduce the titer of incompatible recipient isohemagglutinins. This can be achieved by therapeutic plasma exchange or, if available, the use of immunoadsorption columns **(26)**. Although pretransplantation (recipient) isohemagglutinin reduction may be associated with decreased immunohematologic complications in this setting, there is no consensus in the literature with respect to the most efficient strategy for isohemagglutinin reduction in major ABO-mismatched HSCT. In some studies, patients with PRCA had significantly higher pretransplantation isohemagglutinin titers compared with those who did not develop PRCA. Studies have shown the successful use of donor-type secretor plasma for the purpose of isohemagglutinin reduction before HCT. Although use of donor-type nonsecretor plasma has been previously used for this purpose, use of secretor plasma donors (i.e., donors who secrete A/B antigens into plasma and other body fluids) maximizes the chance for (recipient) anti-A and/or B isohemagglutinin reduction after infusion in comparison to nonsecretor plasma. This is because the A/B antigens present in secretor plasma will deplete anti-A/B isohemagglutinins directly by antigen antibody binding, as opposed to nonsecretor plasma, which is reliant on plasma expansion for dilution of anti-A/B isohemagglutinin titers **(27)**. Slow infusion of donor-type RBCs to deplete (recipient) anti-A and/or B isohemagglutinins before HCT has also been used for this purpose, although even with concurrent rigorous hydration and pre-administration of antihistamine medication, significant RBC-related transfusion reactions may still occur, including fever, rigors, hematuria, and frank hemolysis **(28)**. Risk of hemolytic-type reactions can be avoided with use of secretor plasma rather than RBC transfusions. Especially in patients at

high risk for delayed engraftment and those who have received multiple RBC transfusions, selection of ABO compatible HCT donors is optimal. However, ABO incompatibility generally exerts less impact on outcome than other donor recipient relationships, such as graft source, degree of HLA matching, status of exposure to CMV and other infectious disease, and most likely, donor age, gender, and parity. Overall, major ABO mismatch does not seem to have a consistent effect on other major outcomes after allogeneic HSCT, such as incidence of acute or chronic GVHD, relapse rate, and overall survival, regardless of the stem cell source **(29)**.

Table 1: *Transfusion strategy in ABO mismatch transplantation phases*

ABO incompatibility	Recipient	Donor	Phase I	Phase II				Phase III
			All product	RBC	PLT 1st	PLT 2nd	Plasma	All product
Major	O	A	Recipient	O	A	AB, B, O	A	Donor
	O	B	Recipient	O	B	AB, A, O	B	Donor
	O	AB	Recipient	O	AB	A, B, O	AB	Donor
	A	AB	Recipient	A	AB	A, B, O	AB	Donor
	B	AB	Recipient	B	AB	B, A, O	AB	Donor
Minor	A	O	Recipient	O	A	AB, B, O	A	Donor
	B	O	Recipient	O	B	AB, A, O	B	Donor
	AB	O	Recipient	O	AB	A, B, O	AB	Donor
	AB	A	Recipient	A	AB	A, B, O	AB	Donor
	AB	B	Recipient	B	AB	B, A, O	AB	Donor
Bidirectional	A	B	Recipient	O	AB	B, A, O	AB	Donor
	B	A	Recipient	O	AB	A, B, O	AB	Donor

This table was modified based on previous studies

The overall clinical and laboratory management of ABO incompatible HPC transplantation is complex **(Table 2)**. During the initial transplantation evaluation, potential ABO discrepancies between the recipient and donor require a systematic approach. Although the Foundation for the Accreditation of Cellular Therapies requires ABO typing of both the donor and recipient, there is ultimately no selection guidance, other than requiring resolution and documentation before issuing the HPC product **(29)**. A good transfusion policy, conditioning regimen, cellular processing protocol, and post-transplantation immunosupression treatment plan needs to be established by the individual institution to address major, minor, and bidirectional mismatching. It is very important that the clinical teams and blood bank remain in communication to collectively anticipate and manage post transplantation immunohematologic events, and especially to correctly manage potentially catastrophic immune hemolysis and avoid the inappropriate transfusion of donor incompatible RBCs in patients due to minor ABO incompatibility.

Table 2: *Suggested Approach to ABO-Incompatible HSCT Transplantation*

Evaluation
Phase I, pretransplantation conditioning Donor and recipient laboratory analysis
Evaluate ABO/Rh status, presence or absence of antibodies
1. Two independent peripheral blood samples for ABO/Rh typing and antibody screen
a. Determination of clinical significance or insignificance of all non-ABO minor RBC antibodies (e.g., anti-K versus anti-N)
b. Determination of ABO-incompatibility type: major, minor, bidirectional, or none
2. Communication with clinical teams regarding transfusion support and risk for hemolysis
HPC acquisition and potential manipulation
Confirmation of ABO-incompatibility and stem cell dose. If product contains transplant dose (or approximate dose), HPC product manipulation may not be warranted given anticipated CD34 loss with product modification.
1. Major mismatch: RBC depletion
2. Minor mismatch: plasma depletion
3. Bidirectional: consider both product modifications in appropriate clinical context
Management
If donor erecipient ABO-discrepancy is identified, immediate notification and documentation with clinical team, transfusion medicine service, and stem cell processing laboratory. Transplant conditioning and post-transplant immunosuppression regimen will determine risk profile for persistence of recipient plasma cell isohemagglutinin production.
Major mismatch:
1. Monitor for acute RBC hemolysis (DAT, LDH, haptoglobin, reticulocyte count, isohemagglutinin titers, AST/ALT, bilirubin, peripheral blood smear)
2. Evaluation for potential pure red blood cell aplasia
Minor mismatch:
1. Based on product modification (plasma reduction), risk stratify potential for acute and delayed hemolysis
a. Day 5 to 15, monitor for passenger lymphocyte syndrome with daily CBC
b. Keep hemoglobin level at least 9 g/dL, with donor compatible RBC transfusions
Bidirectional:
1. Monitor for both major and minor incompatibility-related adverse events
2. Ensure adequate supply of blood type AB plasma products and O RBCs

DAT, direct antiglobulin test; LDH, lactate dehydrogenase; AST/ALT, aspartate aminotransferase/alanine aminotransferase ratio

RH-D COMPATIBILITY

RhD incompatibility should also be considered. If RhD antigens differ between donors and recipients, the HSCT would be a RhD blood group antigen mismatch. The presence of anti-RhD antigens in donors or recipients before HSCT is incompatible with the RhD blood group antigen **(30)**. A minor RhD mismatch can be defined as a negative donor with a RhD-positive recipient. A major RhD mismatch can be defined as RhD-positive donors with RhD-negative recipients. Several studies showed that alloimmunization of RhD or anti-D production occurred in 9% of minor RhD mismatch HSCT,

whereas 1% occurred in major RhD mismatch HSCT RhD blood group mismatch (30). RhD-negative blood is recommended for transfusion unless both the donor and recipient have RhD blood antigens.

GRANULOCYTE TRANSFUSIONS

Granulocyte transfusion (GT) can be administered to patients undergoing chemotherapy or HSCT for infection control. GT can cause logistic problems and difficulties in recruiting designated donors. The indications for GT are as follows **(31, 32)**.

1. Proven or probable bacterial or fungal infection with fever for 24–48 hours with persistent morbidity;
2. No response to antimicrobials, defined as failure to reach neutrophil recovery ($<1.0\times10^9$ /L);
3. Absolute neutropenia ($<0.5\times10^9$ /L);
4. Expected recovery of bone marrow function

CONCLUSION

Transfusion support for HSCT in thalassemia is an essential part of supportive care and should be performed considering the patient and donor ABO blood group results. Transfusion should be carefully planned during HSCT to maximize transfusion effects and minimize adverse events. Local transfusion guidelines, hospital transfusion committees, and patient management should be considered for transfusions.

REFERENCES

1. Angelucci E, Matthes-Martin S, Baronciani D, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. Haematologica 2014; 99:811.
2. Pasquini MC, Wang Z. Current use and outcome of hematopoietic stem cell transplantation: CIBMTR summary slides, 2013. http://www.cibmtr. org.
3. Simon TB, Snyder EL, Solheim BG, et al, eds. Rossi's Principles of Transfusion Medicine. 4th ed. Oxford: Wiley-Blackwell; 2009
4. Canals C, Muñiz-Díaz E, Martinez C, et al. Impact of ABO incompatibility on allogeneic peripheral blood progenitor cell transplantation after reduced intensity conditioning. Transfusion. 2004;44(11):1603-1611.
5. Solh M, Brunstein C, Morgan S, et al. Platelet and red blood cell utilization and transfusion independence in umbilical cord blood and allogeneic peripheral blood hematopoietic cell transplants. Biol Blood Marrow Transplant. 2011;17(5):710-716.
6. Danby R, Rocha V. Improving engraftment and immune reconstitution in umbilical cord blood transplantation. Front Immunol. 2014;5:68.
7. Bakker E, Pearson PL, Meera Khan P, et al. Orientation of major histocompatibility (MHC) genes relative to the centromere of human chromosome 6. Clin Genet. 1979;15:198-202.
8. Shapiro MJ. To filter blood or universal leukoreduction: what is the answer? Crit Care 2004;8(Suppl 2):S27-30.
9. Dong Wook Jekarl , Jae Kwon Kim , Jay Ho Han, et.al Transfusion support in hematopoietic stem cell transplantation.Blood Res 2023;58: 1-7.
10. Lipson SM, Shepp DH, Match ME, et al. Cytomegalovirus infectivity in whole blood following leukocyte reduction by filtration. Am J Clin Pathol 2001; 116:52.
11. Christou G, Iyengar A, Shorr R, et al. Optimal transfusion practices after allogeneic hematopoietic cell transplantation: a systematic scoping review of evidence from randomized controlled trials. Transfusion. 2016;56:2607–14.
12. Foukaneli T, Kerr P, Bolton-Maggs PHB, et al. Guidelines on the use of irradiated blood components. Br J Haematol 2020;191: 704-24.
13. Schrezenmeier H, Körper S, Höchsmann B , et al. Transfusion support 8chapter 23) EBMT handbook Carreras E, Dufour C, Mohty M, et al., editors.Cham (CH): Springer; 2019; 163-164.
14. Campbell RD, Trowsdale J. Map of the human MHC. Immunol Today. 1993;14:349-352
15. Sieff C, Bicknell D, Caine G, et al. Changes in cell surface antigen expression during hemopoietic differentiation. Blood. 1982;60:703-713
16. Yamamoto F. Review: ABO blood groups system-ABH oligosaccharide antigens, anti-A and anti-B, A and B glycosyltransferases, and ABO genes. Immunohematology. 2004;20:3-22.
17. Kominato Y, Hata Y, Matsui K, et al. Transcriptional regulation of the human ABO histo-blood group genes is dependent on the N box upstream of the proximal promoter. Transfusion. 2004;44: 1741-1749.
18. Rowley SD, Donato ML, Bhattacharyya P. Red blood cell-incompatible allogeneic hematopoiet-

ic progenitor cell transplantation. Bone Marrow Transplant. 2011;46:1167-1185.
19. Kimura F, Sato K, Kobayashi S, et al. Impact of ABO-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan Marrow Donor Program. Haematologica. 2008;93:1686-1693.
20. Benjamin RJ, McGurk S, Ralston MS, et al. ABO incompatibility as an adverse risk factor for survival after allogeneic bone marrow transplantation. Transfusion. 1999;39:179-187
21. Rowley SD. Hematopoietic stem cell transplantation between red cell incompatible donor-recipient pairs. Bone Marrow Transplant. 2001;28: 315-321
22. Dong W J, Jae Kwon Kim, Jay Ho Han , et al . Transfusion support in hematopoietic stem cell transplantation. Blood Res 2023;58: 1-7.
23. Worel N, Kalhs P. AB0-incompatible allogeneic hematopoietic stem cell transplantation. Haematologica 2008;93:1605–7.
24. Kimura F, Sato K, Kobayashi S, Ikeda T, Sao H, Okamoto S, et al. Impact of AB0-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan Marrow Donor Program. Haematologica 2008;93:1686–93.
25. Daniel-Johnson J, Schwartz J. How do I approach ABO-incompatible hematopoietic progenitor cell transplantation? Transfusion 2011;51:1143–9.
26. Buckner CD, Clift RA, Sanders JE, et al. ABO-incompatible marrow transplants. Transplantation. 1978;26:233-238.
27. Curley C, Pillai E, Mudie K, et al. Outcomes after major or bidirectional ABO-mismatched allogeneic hematopoietic progenitor cell transplantation after pretransplant isoagglutinin reduction with donor-type secretor plasma with or without plasma exchange. Transfusion. 2012; 52:291-297.
28. Stussi G, Halter J, Bucheli E, et al. Prevention of pure red cell aplasia after major or bidirectional ABO blood group incompatible hematopoietic stem cell transplantation by pretransplant reduction of host anti-donor isoagglutinins. Haematologica. 2009;94:239-248.
29. Kim JG, Sohn SK, Kim DH, et al. Impact of ABO incompatibility on outcome after allogeneic peripheral blood stem cell transplantation. Bone Marrow Transplant. 2005;35:489-495.
30. Chan JY, Tokessy M, Saidenberg E, Giulivi A, Tay J, Allan DS. Rh D alloimmunization in allogeneic HSCT. Bone Marrow Transplant 2013;48:459-60.
31. Estcourt LJ, Stanworth S, Doree C, et al. Granulocyte transfusions for preventing infections in people with neutropenia or neutrophil dysfunction. Cochrane Database Syst Rev 2015;2015:CD005341
32. INF276/5 – Clinical Guidelines for the use of Granulocyte Transfusions NHS Blood and transplant accessed 20.09.2023

Correspondance Address:
Dr.Hülya Bilgen
Medipol Mega Hastaneler Kompleksi
TEM Avrupa Otoyolu Göztepe çıkışı No.1 Bağcılar/İstanbul
Tel: 90-5337760187
ORCID: 0000-0002-7424-8688
E-Mail: hbilgen@medipol.edu.tr

IMMUNOHEMATOLOGIC PROBLEMS IN TRANSFUSION

Mahmut Bayık[1], F. Yüce Ayhan[2]
[1] Turkish Blood Foundation, Istanbul, Türkiye
[2] Dr. Behcet Uz Pediatric Diseases and Surgery Research and Training Hospital, Izmir, Türkiye

ABSTRACT

As a result of multipl transfusions, it is challenging to cope with immunohematological problems in transfusion-dependent patients. Cross-match incompatibilites related to alloimmunization, clinical hemolysis associated with autoimmunization or perplexity in blood grouping due to chimeric red cells because of previous transfusions are major problems.

Extendend phenotyping in blood grouping previous to transfusion therapy would be helpful to overcome the trouble in patients with hemoglobinopathies such as beta thalassemia or sickle cell disease. Furthermore, implementation of molecular methods for blood grouping in these patients would also provide a solution.

Keywords: Thalassemia, sickle cell disease, alloimmunization, autoimmunization

IMMUNOHEMATOLOGIC PROBLEMS IN TRANSFUSION

Since they expose multiple transfusions of red blood cell (RBC) concentrates, it is a challenge to maintain the patient blood management (PBM) program in transfusion dependents patients. As a result of alloimmunization, blood transfusion centers could be distressed for supplying compatible blood components because of the cross-match incompatibilites and the perplexities in blood typing **(1-3)**.

It was reported that the prevalence of the alloimmunization in thalassemic patients differs between 3% and 47,5% in various studies. The association between the number of transfusions and alloimmunization was investigated in various studies and the upward movement of the cumulative incidence of alloimmunization in repetitive transfusions was demonstrated; it was estimated as 6,5% at 40 units while it was 1,0% at 5 units. Even if a complete serologically matched RBC transfusion could be held, alloimmunization may be continued to be a risk in transfusion dependent patients in relation with the inflammatory profile of the patients or the number of RBC units **(3-6)**.

Besides alloimmunization, hemolysis is another serious problem for patients with hemoglobinopathies. It was stated that, autoantibodies directed against patient's red cells appear more less than the alloantibodies in thalassemic patients, but they can cause autoimmune hemolytic anemia (AIHA) that may increase the transfusion rates in these patients. The frequency of auto antibodies in patients with beta thalassemia differ between 6,5% to 37% in various reports. The development of autoantibodies in thalassemic patients is a consequence of a complex process associated with multiple factors such as antigenic diversity between red blood cells of blood donors and patients, and the immune status of the patients. The immunomodulatory effect of the blood transfusions may also have a role in autoimmunization **(7-9)**.

Although the serologic methods are simple, easy to use in the immunohematology laboratory, it would be unable to get reliable results in patients with recent transfusions or presented autoantibodies and, alloantibodies **(6)**.

In this matter, a challenge would be raised not only for the supply of the compatible blood components for transfusion dependent recipients but also for increased costs as a consequence of repetitive immunohematological tests. Therefore, to identify the immunohematological problems accurately and to create algorithms and flowcharts for problem solving are vital for providing safe transfusions in these patients.

CROSS -MATCH INCOMPALITIES

Cross matching is essential both in getting the greatest benefit from the transfusion therapy and in minimizing the risks associated with the blood transfusion by proofing the compatibility of blood components for transfusion to patients. It is an expectable situation to have trouble in selection of compatible RBC concentrates in multitransfused patients because of the presence of auto/alloantibodies. In these cases, it would be necessary to find out the root cause and a problem-solving approach must be applied for resolution.

In general, when the cross-match is found out incompatible with the ABO group specific donor unit it is advisable to retest the patient's sample for blood grouping and to repeat the crossmatch with the same unit and also two additional units. If the results are incompatible with all units, it would be necessary to exclude the clerical and technical errors at first. Indirect anti-human globulin test (DAT) positivity in donor units must also be considered. Consequently, it should be investigated that the incombatibility is whether related to alloimmunization or associated with the autoantibodies of the patient **(10-12)**.

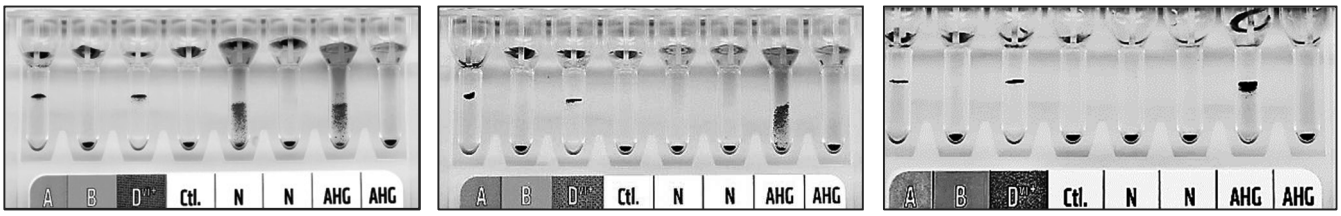


Figure 1: *Cross-match incompatibity in consecutive tests*

ALLOIMMUNIZATION

Although, the development of alloimmunization is common in patients with hemoglobinopathies, a varying range for alloimmunization have been reported in different studies. It was reported between 5% to 75% in sickle cell patients, while ranging between 3% to 47,5% in thalassemics. The most common alloantibodies detected in thalassemia and sickle cell disease patients are against RHCE and Kell blood group antigens. Besides these, it was reported that the antibodies against Kidd, Duffy and MNS blood group antigens are also associated with alloimmunization in thalassemia **(1, 3, 5, 13)**.

It should be well comprehended that the structures and properties of blood groups antigens and antibodies against them to cope with the problems in antibody detection and identification. Two main types of biochemical determinants of red cell antigens may specify the nature of alloantibody response. While the blood group antigens that express protein determinants mostly induce the development of IgG class antibodies, IgM class antibodies principally would be stimulated by the antigens with carbohydrate determinants. Although, a reactive test result in antibody screeing test which is performed with limited red cell panels would indicate the presence of alloantibodies, it is necessary to identify the antibody by using extended multipl cell panels for clinical evaluation of the patient. It is relatively simple to make decision in test samples that contain a single antibody, but it would be troublesome if multiple alloantibodies exist. In additon, alloantibodies against low-frequency blood group antigens stand out with incompatibility in cross-matching, but as a result of using test cells without expression of the low-frequency antigens in screening and identification process would reveal no antibodies in indirect Coombs tests. In case of failure in identifying the alloantibodies, alternative methods and additional procedures should be performed. The anti-human globulin reagent using for antibody detection and identification contains IgG, and the enzyme treating the test cells is usually papain. Different reagents and enzymes would be necessary for identification of certain alloantibodies **(14-16)**.

AUTOIMMUNIZATION

It was reported that the occurrence of autoantibodies against red blood cell antigens is higher in thalassemic patients than normal population. Autoantibodies which are significant with regard to development of the autoimmune hemolytic anemia are also in worsening the prognosis of the disease. The presence of autoantibodies which is indicated by a

positive direct Coombs test don't associate always with the hemolysis of red cells in vivo. In thalasemic patients, red cell destruction progress is associated with alloimmunization as well as other factors, presence of beta thalassemia intermediate or family history for autoimmunization, number of transfusions, the age of the patient, and splenektomy. Autoimmunization was also stated as the strongest risk factor for delayed hemolytic transfusion reactions due to alloimmunization in patients with sickle cell disease **(5-8)**.

The presence of autoantiodies would be revealed by a positive direct Coombs test (DAT) which is most likely without red cell destruction. But autoimmune haemolytic anaemia (AIHA) can be seen in particular cases. In contrast to alloantibodies, autoantibodies don't target to a specific antigen on the red cells and, they can be classified as warm or cold autoantibodies in regard to their optimal thermal reactivities. While the warm autoantibodies are mostly IgG and react at 37oC, the thermal range for cold autoantibodies is between 4-37oC and they are usually due to IgM. It is also important to investigate the presence of complement. In warm AIHA complement can play role in particular but in cold AIHA spesifically C3d is common on the surface of red cells. If cold agglutinins accompanied with the presence of IgG, a mixt AIHA should be considered. In rare cases of atypical AIHA in which autoantibodies are mostly IgA or warm reactive IgM types, DAT will reveal negative results. An extended direct Coombs test (Figure) which can detect IgA and IgM autoantibodies need to be performed to clarify the clinical situation **(8, 17-19)**.

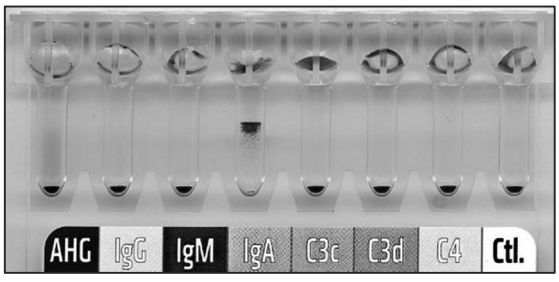


Figure 2: *Extended Direct Antiglobulin Test*

DIFFEERNTION OF AUTOANTIBODIES AND ALLOANTIBODIES

Autoantibodies that can react with the red cells of both patient's own and transfused may delay the recogniton of clinically significant alloantibodies. Furthermore, they may give the impression of a specific alloantibody and raised the issue of trouble in decision-making of transfusion therapy. Most of the autoantibodies are pan-agglutinins which have no specify to red cell antigens. But some of them can direct to specific targets, particularly the antigens of Rh blood group system **(20)**.

It is critical to differentiate underlying, masked alloantibodies from the autoantibodies to improve the safety of blood transfusions. Adsorption techniques provide suitable tools for the differentiation by removal of autoantibodies. Although it is preferable to use patient's own red cells for autoantibody removal (autologous adsorption), when it is unuseful in such cases, as anemia, pregnancy or history of recent transfusions, allogeneic adsorption would be more proper. But the allogeneic adsorption in which a set of red cells with known phenotypes are used for autoantibody removal would have some limitations because of adsorbing the alloantibodies against high frequency antigens. Since, there are a variety of reagents to use in these techniques, the alternatives of the methodology should be determined on the basis of patient types, transfusion urgency, and laboratory resources of the facility. Implementation of adsorption, elution and inhibition techniques at the immunohematology laboratories will help to patient blood management in these patients **(17, 20)**.

INDISTINCSTNEES ON BLOOD GROUP PHENOTYPES OTHER THAN ABO AND RH D

It was stated that extended antigen-matched blood components would be more useful for prevention of alloimmunization in transfusion of patients with certain hematological disorders However, unless determination of extended blood group phenotype has done at first admission of the patient, it would be a challenge to resolve the patient's blood group antigen profile for red blood cell antigens other than ABO and RhD because of the previous multiple transfusions. To obtain undetermined results in blood grouping would not be surprising because of the different phenotypes of red blood cells originated from the recipient and the donor **(21)**.

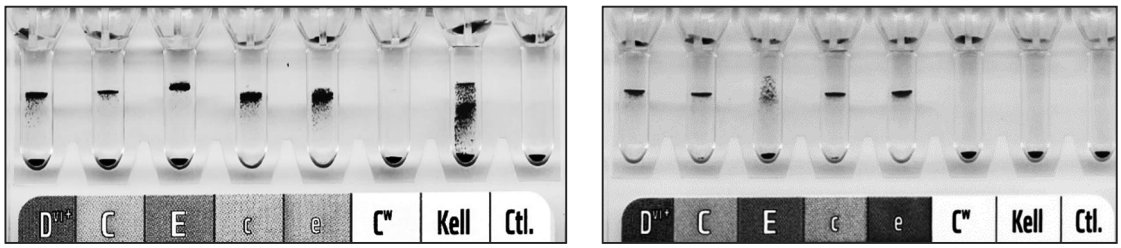


Figure 3: *Double RBC population in RHCE & Kell antigen determination*

CONCLUSION & RECOMMENDATIONS

Serological methods which are widely used in immunohematological investigations have limitations in transfusion dependent patients with hemoglobionopathies. Besides ABO and RhD determination, an extended phenotyping that will include other irregular red blood cell antijens should be performed at the intial admission of those patients. In the guidelines of Thalassemia International Federation, it is recommended to determinate the extended blood group antigen profile of the patients before the beginning of transfusion therapies. At least major RHCE antigens (C,c,E,e) and Kell (K) antigen should be determined and those patients should be transfused with matched blood components for ABO, DCcEe and K antigens. Even if blood transfusions will made with full antigen-matched blood components, the development of the alloimmunization would not be suprising. In these cases which are usually associated with the variants of RHD and RHCE antigens, it would be challenge to find a compatible blood component to transfuse particularly in certain ethnic groups **(1, 3, 5, 21, 22)**.

Therefore, the approach of extended determination of red cell antigens is necessary especially in patients with thalassemia and sickle cell disease. It should be also taken the same approach for blood donors who will donate blood for support of transfusion therapy in these patients. Growing diagnostic resources on genotyping will improve the transfusion safety by providing full-matched blood components and reduce the alloimmunization rates in these patients in future **(1, 3, 5)**.

REFERENCES

1. Aydinok Y. Treatment of thalassaemia: a review of the new approaches on transfusion safety and the novel therapeutics. ISBT Science Series.2020; 15(1):102-109.
2. Belsito A, Magnussen K, Napoli C. Emerging strategies of blood group genotyping for patients with hemoglobinopathies. Transfus Apher Sci. 2017;56(2):206-213.
3. Van Buren NL, Gorlin JB, Corby SM, et al. How do I incorporate red cell genotyping to improve chronic transfusion therapy? Transfusion. 2020; 60(1): 16-25.
4. Putzulu R, Piccirillo N, Orlando N, et al. The role of molecular typing and perfect match transfusion in sickle cell disease and thalassaemia: an innovative transfusion strategy. Transfus Apher Sci. 2017; 56(2): 234-237.
5. Kulkarni S, Maru H. Extended phenotyping of blood group antigens: Towards improved transfusion practices. Global J Transfusion Med. 2020; *5*:120 - 125.
6. Hirani R, Tarafdar S, Mondy P, et al. Understanding the demand for phenotyped red blood cell units and requests to perform molecular red blood cell typing for Australian patients. Transfus Apher Sci. 202; 60(1):102968.
7. Thatikonda KB, Kalra M, Sachdeva P, et al. Uncommon association of hypertriglyceridemia and autoimmune haemolytic anaemia in an infant with transfusion dependent thalassemia. Pediat Hematol Oncol J. 2021; 6(2): 110-112.
8. Khaled MB, Ouederni M, Sahli N, et al. Predictors of autoimmune hemolytic anemia in beta-thalassemia patients with underlying red blood cells autoantibodies. Blood Cells Mol Dis. 2019; 79:102342.
9. Pazgal I, Yahalom V, Shalev B, Raanani P, et al. Alloimmunization and autoimmunization in adult transfusion-dependent thalassemia patients: a report from a comprehensive center in Israel. Ann Hematol. 2020; 99(12):2731-2736.
10. Bhattacharya P, Samanta E, Afroza N, et al. An approach to incompatible cross-matched red cells: Our experience in a major regional blood transfusion center at Kolkata, Eastern India. Asian J Transfus Sci. 2018; 12(1):51-56.
11. Andıç N. Practical Solutions for Problems in Blood Grouping and CrossmatchingTurk J Haematol. 2022; 39(1):55-60.
12. Sidhu M, Shah S. Evaluation of incompatible crossmatch. Global J Transfusion Med. 2020; 5(1), 68-68.

13. Linder GE, Chou ST. Red cell transfusion and alloimmunization in sickle cell disease. Haematologica. 2021 Jul 1;106(7):1805-1815.
14. Daniels G. Human blood groups: Introduction. Human Blood Groups, 3rd edn (ed. by G. Daniels). 2013:1-10.
15. Zwazinga JJ, van Ham SM. Essential immunology for transfusion medicine. In: Murphy MF, Pamphilon DH, Heddle NM (eds).PracticalTransfusion Medicine. 4th ed. Oxford, John Wiley&Sons Ltd.; 2013: 9-20.
16. Tormey CA, Hendrickson JE. Transfusion-related red blood cell alloantibodies: induction and consequences. Blood. 2019 Apr 25;133(17):1821-1830.
17. Sekhar Das S, Mukherjee S, Chowdhury S. Immunohematological evaluation of red cell alloimmunization and statistical assessment of various adsorption techniques in warm autoimmune hemolytic anemia. Transfus Apher Sci. 2023 Jul 26:103769. Transfusion and Apheresis Science, 26:103769
18. Thatikonda KB, Kalra M, Pallavi S, et al. Uncommon association of hypertriglyceridemia and autoimmune haemolytic anaemia in an infant with transfusion dependent thalassemia. Pediatr Hematol Oncol J 2021; 6(2): 110-2.
19. Fattizzo B, Giannotta JA, Serpenti F, et al. Difficult Cases of Autoimmune Hemolytic Anemia: A Challenge for the Internal Medicine Specialist. J Clin Med. 2020 Nov 27;9(12):3858.
20. Agrawal S, Chowdhry M, Gajullupalli SP. Autoantibodies mimicking alloantibodies: A case series unveiling the dilemmas of transfusion. Asian J Transfus Sci. 2023 Jan-Jun;17(1):58-62.
21. Cruz BR, de Souza Silva TC, de Souza Castro Bet al. Molecular matching for patients with haematological diseases expressing altered RHD-RHCE genotypes. Vox Sang. 2019;114(6):605-615.
22. Dezan MR, Oliveira VB, Conrado MCAV, et al. Variant genotypes associated with reduced expression of RhCE antigens among Brazilian blood donors. Transfusion. 2021;61(6):1923-1931.

Correspondence Address:
Mahmut Bayık- Turkish Blood Foundation, Istanbul, Türkiye
mahmutbayik@gmail.com
F. Yüce Ayhan-Dr. Behcet Uz Pediatric Diseases and Surgery Research and Training Hospital, Izmir, Türkiye
E-mail: yayhan@yahoo.com

TRANSFUSION-TRANSMITTED INFECTIONS AND THEIR COMPLICATIONS IN THALASSEMIA

Dr. Ramazan Uluhan, Assoc. Prof. Dr. Yasemin Heper***
**Medical Science University Zeynep Kamil Women and Children Diseases Training and Research Hospital, Clinical Microbiology Laboratory and Blood Bank, Istanbul, Türkiye*
***Bursa Uludag University Medical Faculty, Department of Infectious Diseases and Clinical Microbiology and Blood Bank, Bursa, Türkiye*

ABSTRACT

Thanks to advances in transfusion medicine, the risk of transfusion transmissible infections have decreased significantly, especially in developed countries. However, all practices for preventing those infections in blood banks still have vulnerabilities. In this section, these vulnerabilities are discussed in order for the physician to understand the reasons of the risks of infection transmission through transfusion. The conditions that may occur due to transfusion-transmitted infections are explained, and the precautions that can be taken are mentioned. Since the risk of infection cannot be reset, patients who receive frequent transfusions, such as Thalassemia patients, are at higher risk. The physician making the transfusion decision should be able to evaluate the risk of infection within his own conditions and design the precautions he can take. It is important for the physician to know which infections may develop in the patient, their course, how to diagnose them and what to do if detected, not only for the patient but also for the public health.

Keywords: Transfusion transmitted infections, thalassemia, transfusion, bacterial contamination, donor screening tests

INTRODUCTION

Innovations such as stem cell and gene therapy have freed some Thalassemia patients from receiving regular transfusions. Obtaining neocyte concentrates through apheresis has enabled the transfusion intervals to be extended. Although transfusions decreased in these patients compared to the past, Thalassemia patients are still a group who receive frequent transfusions due to various reasons such as the inability of some countries and/or patients to access these treatments. As expected, due to frequent transfusion, they constitute a special risk group in terms of undesirable effects of transfusion **(1)**. Therefore, transfusion in Thalassemia patients requires some special approaches (leukocyte filtration, Rh subgroup and Kell compatible transfusion, iron chelation, etc.). Although the majority of transfusion reactions can be prevented with appropriate approaches, the risk of infection transmission through transfusion has not been eliminated despite all developments. Since the risk of infection for each blood component is specific to that component, the risk of infection increases with the number of transfused blood component. As a result, the incidence of some transfusion-transmitted infections (TTI), especially Hepatitis C, is significantly higher in Thalassemia patients than the general population and other patient groups that do not receive regular transfusions **(2–4)**. Physicians dealing with Thalassemia should be well aware of which infections these are and how patients should be monitored. Early diagnosis of an infection transmitted through transfusion is important for both patient and public health.

HOW DOES INFECTIONS GET TRANSMITTED BY TRANSFUSION?

All kinds of microorganisms (viruses, bacteria, fungi, parasites and even prions) can be transmitted through transfusion, but only those that can survive or grow in the blood bag under storage conditions pose a problem. For example, citrate, which is present in the solution inside the blood bag for antico-

agulation and providing the necessary substances for the vitality of cells, has antiseptic properties for some microorganisms. After the blood is placed in the bag, leukocytes and antibodies continue to eliminate at least some microorganisms for several hours. The temperature that blood components are stored is not suitable for all microorganisms. Microorganisms that overcome these conditions have the potential for contamination and transmission **(5)**. Important microorganisms that can be transmitted by transfusion are shown in Table 1.

Table 1: *Microorganisms that can be transmitted by transfusion*

VIRUSES	BACTERIAS	PARAZITES	**RICKETTSIAS**	**PRIONS**
CMV	Campylobacter spp	*Babesia microti*	*Rickettsia rickettsii*	vCJD PrP
EBV	Pseudomonas spp	Plasmodium spp	*Coxiella burnetti*	
HAV	Acinetobacter spp	*Toxoplasma gondii*		
HBV	Klebsiella spp	*Trypanasoma cruzi*		
HDV	Salmonella spp	Leishmania spp		
HCV	Serratia spp	Filariasis		
HGV	Staphyloccoccus spp			
HEV	Streptococcus spp			
HTLV 1-2	*Treponema pallidum*			
HHV-6	*Borrelia burgdorferi*			
HHV-8	Brucella spp			
HIV1/2				
Parvovirus-B19				
West Nile virus				
Zika virus				
Chikungunya virus				
SEN virus				
TT virus				
Dengue virus				

Infections can be transmitted in two ways by transfusion **(5)**:

1- When the donor donates blood, the microorganisms in his/her blood can pass into the blood bag: Although the donor evaluation process is more detailed and developments in screening tests have significantly reduced this risk compared to previous decades, it has not been eliminated. A person with any symptoms of a disease is not accepted for donation, but asymptomatic infections pose a risk. Brucellosis, salmonellosis, campylobacter and spirochete infections (e.g., syphilis), recurrent fever, Lyme disease and rickettsioses are bacterial infections that can be asymptomatic. Additionally, there may be a temporary and insignificant bacteremia that develops from an unimportant focus in the donor (tooth infection, acne, etc.). Although there are some bacterial or protozoal infections that are asymptomatic, these are relatively rare. The main problem in this type of transmission are viruses.

2- Contamination during the preparation, storage (in the blood center), transportation or transfusion of the blood product: Except the contamination of blood bags or sets in the factory during the production phase, the microorganisms are transmitted to the blood bag from outside. The risk of contamination during the preparation, storage and transportation in modern blood centers that comply with standards is not very high. But the slightest unnoticed injury of the blood bag may lead to contamination and, over time, to the proliferation of microorganisms may cause a septic reaction. Here are especially bacteria's responsible. Rarely, fungi can also be transmitted this way.

PREVENTION PRACTICES AND RISKS

Practices that aim reducing the risk of transmission of an infection from a blood component are already routinely carried out in blood banks that comply with standards **(6–8)**. However, it should not be forgotten that not all blood banks around the world can provide the same standard of service. Therefore, the risk of an infection through transfusion is closely related to where the patient lives **(9–11)**. The development level of the country and blood bank is not the only factor affecting this. The epidemiology of infections are also important. While the frequency of some infections varies according to geographical regions (such as Hepatitis B, Hepatitis C, HIV), some are limited to certain geographical regions (such as Malaria, West Nile, Zika, Chagas disease).

In order to prevent TTI's, the first thing that comes to mind are the screening tests performed in blood banks. Although both the test technologies and the specificity and sensitivity of the tests have improved greatly, there is still no test that eliminate the risk totally of an infection by transfusion. Moreover, although many infections can be transmitted by transfusion, not all of them are screened, only the most important ones are.

Which infections will be screened is decided according to many factors such as the importance of that infection for the public and the individual, its frequency, treatment possibilities and treatment costs, whether there is a suitable screening test for blood banks, and the cost of the test. It may vary by country and time. While HBV, HCV, HIV and Syphilis are generally screened all over the world, for example, in some countries, HTLV I-II, Chagas disease, Malaria, West Nile Virus etc. can also be screened routinely or during epidemic periods. More examples can be given. For example, HIV screening started in 1985 and HCV screening started in 1996. The importance of West Nile, Chikungunya and Zika viruses and prions in transfusion has been understood much more recently **(12–18)**. New transfusion-transmissible pathogens may emerge at any time **(19)**. A physician who have patients that receive frequent transfusions needs to know which TTIs may be present in that country, and which infections are screened and by which method.

The methods used in screening tests affect the results. Tests with the highest sensitivity should be used. Many infections, especially HBV, HCV and HIV infections, have a seronegative period and/or window period, which they cannot be detected by tests. When a donor arrives during this period, the tests will be negative, blood components obtained from that donor will be used and patients may get infected. The duration of the seronegative/window period varies depending on the testing method. When testing antibodies, this period is the longest because antibody production takes time, and is shorter in antigen tests. It is the shortest in PCR tests (NAT: nucleic acid amplification technology), based on the detection of the DNA or RNA of the microorganism. As expected, NAT reduces the risk of infection transmission, but it should be noted that no method can reset this risk. Moreover, it is possible that some mutants or variants cannot be detected by the test. The cost of tests and the laboratory conditions required are also different. Testing methods used in blood banks may vary depending on the conditions of the countries. While NAT is widely used in addition to serological tests (antigen and antibody tests) in developed countries, low-sensitivity test systems are still used in some countries **(20–22)**. It is also known that in some parts of the world, transfusions are still performed without any screening tests. The physician making the transfusion decision should be aware of the situation in the country.

A lot of data can be found in the literature about the probability of transmitting a specific infection (such as HIV or HBV or HCV) or any screened/ unscreened infection through transfusion. This probability differs significantly depending on the country, even the region, the testing method used, the prevalence of that infection in the society, the immune status of the society to that infection, and changes over time, also **(10)**. Therefore, it is not possible to give a general probality.

In our country, as in many countries, screening tests used in blood banks are determined by national guidelines and are updated when necessary. In our country, those that are compulsorily screened A lot of data can be found in the literature about the probability of transmitting a specific infection (such as HIV or HBV or HCV) or any screened/ unscreened infection through transfusion. However, this probability differs significantly depending on

the country, even the region, the testing method used, the prevalence of that infection in the society, the immune status of the society to that infection, and changes over time. are HBV, HCV, HIV and *T. pallidum*, which is the causative agent of syphilis. Our mandatory tests are currently HBsAg for HBV, Anti-HCV (±HCVAg) for HCV, p24+Anti-HIV I-II for HIV, *Treponema pallidum* total Ab for Syphilis. NAT has not yet been made mandatory. However, the Turkish Red Crescent additionally uses NAT for HCV, HBV and HIV since few years.

Although the first thing that comes to mind are screening tests, the process actually starts with evaluating the suitability for donation of the blood donor. Blood is not taken from a person who already looks sick. However, TTIs are infections that have a subclinical or asymptomatic course, long incubation period, latent infection, carrier status, etc., which the donor candidate appears healthy, so they can be easily missed. These features are more common in viral infections. Donors are questioned not only for the presence of an active infection, but also for risky behavior (e.g., sexual life, drug use, etc.) or travel history in terms of various infections such as HIV, HBV, HCV, Syphilis, Malaria, Zika etc. If there is a risky situation, they are not accepted as blood donors, are not even taken into screening tests. This is due to the possibility of being seronegative or in the window period. However, the effectiveness of the donor evaluation (questioning) depends on providing accurate information. Whether correct information is given or not, which includes personal questions, depends entirely on the person's statement. As society's level of awareness about blood donation increases, the rate of answering questions correctly and the habit of donating blood increases. In such societies, the donor population is a non-remunerated volunteer population called "regular donors". The probability of their screening tests being positive or being seronegative/in the window period is significantly lower than those who donate blood only once or are first time donors. In countries where there is a shortage of blood donors, as in our country, a small number of donors are volunteers and regular donors, and since the majority of them are first-time donors, the risk of infection is higher. In particular, directing patient relatives and acquaintances to donate (referred donors) is considered riskier due to the greater tendency to hide special circumstances during the questioning process. Ultimately, although it is not desired, referral donors can often be resorted due to the shortage of volunteer donors and sometimes for cultural reasons. As can be understood, it is important for countries to establish and implement voluntary and regular blood donor recruitment policies not only to avoid blood shortages and to meet blood needs easily, but also to reduce the risk of TTIs. Another issue that should not be forgotten is transfusion ethics **(23)**: The patient and the donor must not know each other in order to avoid a relationship of interest. Redirected-referred donors also violates this universal principle. On the other hand, it is known that the risk of infection (and alloimmunization) increases as the number of donors increases whom a patient receives blood. For this reason, there have been some practice examples to reduce these risks by ensuring that Thalassemia patients constantly receive blood from the same donors **(24)**. However, it should not be forgotten that transfusion ethics should be taken into consideration in such practices.

Since some microorganisms that are not screened (such as CMV, EBV, HHV-6, HHV-8, and even Toxoplasma and Leishmania species) are found in leukocytes, applying leukoreduction to blood components almost completely reduces the possibility of their transmission. The most effective method for leukoreduction is leukocyte filtration and should be done especially during the preparation of the blood component in the blood bank. In some countries, it is mandatory that all blood components be leukoreduced at the production stage (universal leukoreduction). Where this cannot be done, bedside leukocyte filters can also be used during transfusion, although their effectiveness is lower. The physician must know whether the blood component is prepared as leukoreduced and by what method. Some leukoreduction methods other than filtration (such as top and bottom bags) are less effective. There is no need to use a bedside leukocyte filter when transfusing a blood component that has already undergone leukocyte filtration at the blood bank.

If the blood component contains a viral agent, an acute reaction in the patient is not expected during or immediately after transfusion. At the end of the incubation period, the disease specific to that agent emerges.

BACTERIAL CONTAMINATION

Not very uncommonly, blood components may also be contaminated with bacteria. Bacteria may grow in contaminated blood and cause a septic reaction that may cause mortality. The clinical picture due to transfusion of contaminated blood may vary from asymptomatic or mild infection findings to septic shock, disseminated intravascular coagulation, and even death, depending on the type and amount of bacteria, whether they produce endotoxin, the patient's immune status, and whether or not taking antibiotics. For these reasons, in studies conducted by taking cultures of blood components, the frequency of contamination is high, mostly in Platellet suspensions (PS), while the frequency of septic reactions is much lower **(25, 26)**.

The ability of the bacterium to grow depends on its ability to withstand in the blood bag and storage conditions. Many different types of bacteria have been reported in the literature to cause bacterial contamination. Although it is not a rule, Gram positives generally contaminate the blood component from outside during the blood collection process from the donor skin, and Gram negatives during the preparation, storage, transportation or transfusion of the component. However, Gram negatives such as Salmonella, Yersinia, Brucella species come from the donor through bacteremia. Detection of the microorganism gives an idea about the possible route of contamination and, if necessary, can guide corrective-preventive actions.

PSs are most risky, as they are stored at room temperature and many microorganisms can grow at this temperature. Bacteria that can grow in erythrocyte suspensions (ES) at +4-6 C^{o} may cause problems also. All reported septic reactions due to ESs have been reported with ESs older than 15-17 days, because it takes time for the bacteria to reach the amount that will cause a septic reaction.

Blood components are prepared under aseptic conditions and stored under special conditions. Disinfection of the phlebotomy area is extremely important during blood donation. Ensuring asepsis of the phlebotomy area and placing the first 15-20 ml of the blood into a separate small satellite bag to prevent bacteria that may be present on the skin from entering the main blood bag, significantly reduces bacterial contamination arising from the donor skin **(27)**.

Transport of blood components also requires special conditions and attention. The blood bag should not be damaged in any way. Some basic transfusion principles that every physician and nurse performing transfusion should know, such as delivering the blood component for transfusion to the clinic immediately before transfusion (not keeping it waiting in the clinics) and completing the transfusion as soon as possible according to the component, are important to prevent contamination and its harm.

Visual control of the blood component (such as color, clot, turbidity, signs of hemolysis) is also important to detect the contaminated product and should be done carefully both at the exit from the blood center and at the admission to the clinic. If there is even the slightest visual doubt, that blood should never be transfused and must immediately sent back to the blood center. Blood centers will examine this and investigate the cause.

There are some technics and commercial systems (cultures, tests, etc.) to detect bacterial contamination in blood banks, especially for PSs **(28–32)**. However, the lack of a method that is suitable for use in every blood center and every blood component and that detects contamination with a complete reliability prevents their widespread use.

A septic reaction resulting from transfusion of contaminated blood is an acute condition that may result in mortality. Septic reactions developing with Gram negatives have a more severe course due to the presence of endotoxins. Due to severe anemia, iron overload, splenectomy and changes in their immune system, severe infections may develop in Thalassemia patients, especially with *Yersinia enterocolitica, Klebsiella pneumoniae, Pseudomonas aeruginosa, Vibrio vulnificus, Acinetobacter baumannii, Streptococcus intermedius, Staphylococcus aureaus, Escherichia coli* and Salmonella species.

A septic reaction requires urgent diagnosis and treatment. If the patient develops symptoms such as fever, chills, tachycardia, or hypotension during or after transfusion, a septic reaction should be considered and the blood center should be contacted immediately. While the differential diagnosis process is being initiated for other reactions with similar findings, especially the acute hemolytic reaction, the blood bag which the reaction occurred should be sent to the blood center immediately along with the

transfusion set. Blood cultures should be taken from a peripheral vein from the other arm of the patient, not from the transfused arm. At the blood center, a Gram stained preparation will be prepared from the content of the bag and multiple cultures (aerobic + anaerobic cultures at +4 C°, +22 C°, +37 C°) will be taken **(32)**.

Growth of the same agent from the bag and the patient is very valuable for diagnosis, but it is not necessary. If the possibility of a septic reaction is high (for example, if it is a PS or an ES older than 14 days, if a hemolytic reaction is excluded), broad-spectrum antibiotics should be started immediately without waiting for culture results. The blood bank should investigate the cause of contamination and, if necessary, take action to prevent recurrence.

Rarely, contaminations with fungi (Candida and other yeasts, Aspergillus and Penicillium species) may also occur. Clinical findings may be milder or have a later onset than bacteria. It is possible to see and recognize mushrooms with painted preparations made from bags.

TRANSFUSION TRANSMISSIBLE INFECTIONS

HIV infection

HIV is the first thing that comes to mind about transfusion-transmitted infections. Transmission of HIV through transfusion has caused dramatic consequences and created sensations. HIV has created a milestone in transfusion medicine and has led to radical changes in blood donor evaluation, screening tests and transfusion approaches.

After infected with HIV, it usually takes 3 weeks - 3 months for Anti-HIV to be detected, but rarely it can take up to 6 months. While antibodies are not detectable, 2-3 weeks after infection, the p24 antigen becomes positive and remains positive for about 3 weeks. These two tests (p24+Anti-HIV) are run together in blood banks to shorten the seronegative period. HIV-RNA can be detected by PCR starting from the 10th day.

The incubation period for HIV is 2-12 weeks. At the end of this period, flu-like symptoms (fever, headache, sore throat, intense muscle and joint pain, swollen lymph nodes, abdominal pain, diarrhea, rash, etc.) occur in approximately half of the patients. During this period, it is often thought to be an insignificant viral infection and is overlooked. It may also remain completely asymptomatic. If missed, the patient may present with AIDS within a few years. Today, patients can live their normal lives with expensive but very effective anti-retroviral treatments, especially if they are started before the development of AIDS. It is extremely important not to miss a HIV infection due to its contagiousness. There is no vaccine, but in case of a risky contact, drug prophylaxis is given and continued for 28 days. Prophylaxis should be started as soon as possible after exposure (preferably within hours), maximum in 72 hours. The patient receiving prophylaxis should be monitored for at least 6 months for the development of HIV infection. In transfusion practice, prophylaxis is only possible if the patient is accidentally given blood known to contain HIV and this is immediately recognized.

If a Thalassemia patient develops flu-like symptoms outlined above, HIV infection should also be considered. If the anti-HIV test result is negative, it should not be forgotten that antibodies may not be detected due to the early stage, and in case of doubt, HIV-RNA should also be requested. Due to the asymptomatic course, it would be appropriate to screen transfusion depended Thalassemia patients for HIV from time to time, taking into account the transfusion dates and incubation period.

Hepatitis B

It can be transmitted despite screening tests due to the window period in the natural course of this infection, some variants that could not be detected by tests, and low level antigenemia/viremia that is below the sensitivity limits of the tests. If only HBsAg tests are performed in this risk is higher than if Anti-HBc (IgM+IgG) and/or NAT (HBV-DNA) tests are additionally performed. In countries where the incidence of HBV infection is low, Anti-HBc is also studied and positive donors are rejected **(20, 21)**. Anti-HBc IgG remains positive lifelong in everyone exposed to HBV, but only a minority of them are known to be infectious. For this reason, Anti-HBc is not screened in countries where the prevalence of HBV is high, as it will lead to a significant loss of blood donors. In countries where Hepatitis B vaccines are included in routine child-

hood vaccinations, as in our country, both the risk of encountering an infectious donor and get infected by transfusion are significantly reduced.

In case of HBV transmission, disease occurs in the patient within 4 weeks to 6 months. The clinical picture is not always an acute hepatitis with icterus. It can be subclinical or even asymptomatic and can easily be transmitted to others. If it is missed, chronic hepatitis, cirrhosis, and hepatocellular carcinoma may develop. For this reason, Thalassemia patients should be vaccinated as early as possible by checking their Anti-HBs levels to determine whether they are immune or not. It should be kept in mind that, although rare, Hepatitis B may develop despite vaccination, due to the existence of vaccine-escape variants. Since the infection may be asymptomatic and variants that can evade the vaccine, it would be appropriate to screen patients for Hepatitis B from time to time, taking into account transfusion dates and the incubation period.

In case of a risky contact, prophylaxis with hyperimmune gamma globulin can be applied within the first 48 hours (maximum 7 days). However, in transfusion practice, this is only possible if the patient is accidentally given blood that is known to carry HBV and this is immediately recognized.

Since Hepatitis D virus can only infect in the presence of HBsAg, screening for HBsAg in blood banks indirectly prevents HDV transmission.

Hepatitis C

Although the person is infected with HCV and contagious, it takes 5-12 weeks for Anti-HCV to be produced. This long seronegative period poses a risk in transfusion. While infection is detected earliest in 5 weeks with Anti-HCV, this period decreases to 1 week if additionally, HCVcorAg test is used, and to 4-5 days with HCV-RNA. The fact that HCV is largely asymptomatic causes it to be overlooked in both the donor and the infected patient. The incubation period is 2 weeks–6 months. The rate of chronicity in infected people is very high (75-85%), and in some of those cirrhosis and hepatocellular carcinoma may developed. There is no vaccine or appropriate prophylaxis. Today, radical treatment with antivirals is possible, but it is stated that only 10% of the cases worldwide are detected and treated because they are missed due to the asymptomatic course. Since there is no vaccine and it is asymptomatic, Thalassemia patients need to be screened for HCV from time to time. The incidence of transfusion-associated HCV in Thalassemia patients, especially in some countries, is significantly higher than in the general population **(2–4)**.

Hepatitis A

In Hepatitis A, viremia occurs from 2 weeks before to 2-3 months after the transaminase elevation and lasts for an average of 3 months. There is no latent infection and chronisity. Asymptomatic course is rare in blood donation ages (adults), meaning the probability of being missed is low. Excluding developed countries, the susceptible population that can be infected through transfusion is extremely small, as the infection is acquired almostly always in the childhood (often asymptomatic) and became lifelong immune. This significantly reduces the possibility of transmission through transfusion. However, in developed countries where herd immunity is low, the risk of a donation is higher during the 2-week viremia period before the onset of symptoms, as the infection is more common in adulthood in these countries. The susceptible population that can be infected through transfusion is also larger, but the Hepatitis A vaccine can eliminate this risk. Before the first transfusion, the immune status of Thalassemia patients should be evaluated by checking Anti-HAV, and if negative, they should be vaccinated.

Cytomegalovirus (CMV) infection

CMV can cause serious problems in immunocompromised patients and in babies born to seronegative mothers. Today, the increasing population of immunosuppressed patients has increased the importance of this virus. CMV progresses as reactivation in previously infected, and as primary infection in unexposed individuals. The level of seropositivity in society varies greatly from country to country. For example, this rate for blood donors in our country is 95%, but it is much lower in developed countries. In high prevalence countries, it is almost impossible to find CMV negative blood for a susceptible patient, so such an effort is not undertaken. However, in countries where the rate of susceptible individuals is high and the rate of infected is low, blood components can be tested for CMV for pa-

tients in the risk group. Since CMV is carried within leukocytes, leukoreduction of blood components (especially by filtration in the blood bank) significantly prevents the possibility of transmission **(33)**. High-risk patients (e.g., bone marrow transplant) may be given ganciclovir and gammaglobulin prophylaxis in addition to leukoreduced blood.

Other viruses

EBV, Parvovirus B19, HHV-6 and 8, HTLV 1-2 and others cause their own clinical manifestations at the end of the incubation period, but it should not be forgotten that some of them have oncogenic potential. They do not have any vaccine or prophylaxis, but leukoreduction of blood components significantly prevents the transmission of most of them. Knowing the patient's serological status before transfusion is only valuable in terms of differential diagnosis and to clear the association with transfusion in a case with some clinical findings. Viruses such as Zika, Dengue, Chikungunya and West Nile are limited to epidemic periods and/or certain geographies (12–18). Physicians living in risky areas need to be aware of these infections and follow the recommendations. The medical significance of some viruses, such as TT and SEN virus, is unknown.

Syphilis

Although *Treponema pallidum*, the causative agent of syphilis, is considered to survive for 24-72 hours in blood components stored in cold and for a very short time in gas-permeable platelet bags, it has been shown that they can survive for a longer time **(34)**. Antibodies become positive shortly after infection, often during the chancre period (IgM in the 2nd week, IgG in the 4th week), and IgG remains positive for life. In our country, *Treponema pallidum* total Ab test (i.e., IgM+IgG) are used in blood banks. In practice, when Syphilis comes to the period when blood contamination can occur, the tests almost always become positive. For these reasons, the probability of missing a donor and transmission through transfusion is low in centers where sensitive screening tests are performed, but the risk may be higher if VDRL/RPR is used **(35, 36)**. There is no chancre in syphilis via transfusion. The patient will present with nonspecific findings, especially fever, rash, hepatitis, central nervous system and eye findings. It is easy to diagnose when brought to mind that the patient could be infected with *Treponema pallidum* via transfusion.

Malaria

Malaria may not always have the typical clinical findings (periodic chills and fever attacks) and especially in subclinic-asymptomatic patients, it can be carried in the blood for 2-40 years, depending on the *Plasmodium* species. This situation poses a risk especially in endemic areas. It is transmitted only through ESs (and whole blood). The survival time in the blood component is approximately 20 days for *P falciparum* and 1 week for others. Since the screening test suitable for blood banks is problematic, it is screened only in endemic regions with different methods that vary from country to country, and blood donors are evaluated for malaria with additional criteria **(37–40)**. But there is still a possibility of transmission. The incubation period is often 7-30 days. If typical chill and fever attacks are present, the diagnosis is easy. But the patient may also present with long-lasting irregular fevers and nonspecific symptoms. Malaria should be kept in mind in patients with such symptoms. Chemoprophylaxis can also be applied to risky patients who will receive transfusion in endemic areas.

Other parasitic diseases

Babesia microti, the causative agent of **Babesiosis**, is transmitted within erythrocytes like malaria and poses a risk in north-eastern America. It may be severe in splenectomized and immunosuppressed patients. It can survive for three weeks in ESs. **Chagas disease** is endemic in Central and South American countries, and its causative agent, *Trypanasoma crusi*, is screened in blood banks in some countries. The agent remains viable for 4 days in PSs and 2 days in ESs. *Leishmania*, the causative agent of **Kala-Azar**, can cause also asymptomatic infections. It is emphasized that the risk increases due to migration, and newly screening tests are discussed. Leukoreduction reduces the risk of transmission. **Toxoplasmosis** can be transmitted during primary infection, where it can be asymptomatic. The risk of transmission varies depending on the seropositivity rate of the community. Leukoreduction also reduces the risk of transmission of *Toxoplasma gondii*. **Filariasis** agents can also be

found in the blood and cases of transmission by transfusion have been reported in endemic areas.

Prion diseases

Since it is known that some prion diseases are transmitted through tissue and organ transplantations, the variant Creuzfeld-Jakob disease (vCJH - Mad cow disease), which emerged in England in the 1980s and was later seen in different countries, was examined in experimental animals to see whether it could be transmitted by transfusion, and it was found that it could. Since clinical findings appear years after the infection, people who received blood from donors who donate while appearing healthy and were later diagnosed with vCJH were monitored, it was observed that some of them also became ill and were subjected to transfusion **(41–43)**. For this reason, some countries (e.g., USA) do not accept blood from people who have been in these countries during the years of the vCJD epidemic. It always result in death, there is no cure and no screening test. Because prion proteins are carried within leukocytes, leukoreduction can prevent transmission. Although there is no vCJD left at the moment, it is an example of the fact that new pathogenes can emerge at any time.

IS IT POSSIBLE TO STERILIZE BLOOD COMPONENTS?

Since the risk of infection cannot be eliminated despite all practices, "pathogen inactivation/reduction methods" (PI) have been developed based on the idea of sterilizing the blood component **(44)**. PI is basically based on destroying nucleic acids in the blood with the help of chemical and/or physical agents. Thanks to the absence of nucleic acid in plasma, erythrocytes and platelets, only microorganisms (viruses, bacteria, fungi, protozoa) and leukocytes, which are unwanted cells, are eliminated by PI, but prions are not affected because they do not contain nucleic acid. Theoretically, such an ideal technique would not require either evaluation of the donor for an infection risk, screening tests, or applications to detect bacterial contamination. Since it also inactivates leukocytes, there will be no need for leucoreduction and irradiation to prevent transfusion-related Graft versus Host Disease. Moreover, it will be extremely advantageous both in routine times abd in crisis periods (e.g., an epidemic, disaster), and also in countries with limited resources. However, today there is no cost-effective, ideal method that can be easily applied to every blood component in every blood center, but promising studies are continuing.

There have been methods/commercial systems that have been approved and used for blood banks for many years for fresh frozen plasma (FFP) and, relatively recently, for PSs **(45)**. However, ESs and whole blood are problematic in PI **(46)**. Although PI for FFP is widely used in some countries (mandatory in some), existing PI methods, especially for PSs, have not yet become widespread for various reasons, especially because of the cost. Unfortunately, there is no approved system yet for the ESs that Thalassemia patients specifically need, but studies are ongoing **(47)**. It can be predicted that PI will become increasingly widespread in the future.

RESPONSIBILITIES OF THE PHYSICIAN

Due to the risks of transfusion, it is obvious that the indication for transfusion must be given meticulously and accurately. The physician's first responsibility begins here.

Physicians treating Thalassemia patients who are receiving transfusions should be able to question the safety of the blood components where they supply them, and should also know what can be transmitted to the patient in the region, the symptoms of these infections and how to diagnose them. It should not be forgotten that some infections may be subclinical or even asymptomatic. As mentioned above, patients should be screened for such infections from time to time. Since some infections can be contagious even if the patient is asymptomatic (such as HIV, HBV, HCV), the issue is also important for public health. On the other hand, in some infections, early diagnosis and treatment are also important for the patient.

Vaccinating Thalassemia patients who will receive transfusion against vaccinable infections (e.g., Hepatitis B, Hepatitis A, etc.) before the first transfusion will provide prevention of infection and facilitate the differential diagnosis of an infection that may be associated with transfusion. In terms of infections such as EBV, CMV, Parvovirus, etc., which do not have a vaccine but can be transmitted

by transfusion, it is also useful to screen the patient before the first transfusion to see if he/she has encountered these microorganisms, for similar reasons.

If the physician detects a possible TTI, he/she should also initiate the hemovigilance process. Hemovigilance is the reporting of all undesirable situations that occur in all processes related to transfusion: Starting from the evaluation of the blood donor, preparation, testing, stocking, transportation of the blood component, transfusion to the patient, until the emergence of any undesirable situation that is thought to be related to transfusion in the patient during and after transfusion, and the surveillance of all. The main purpose of this process is to detect risky situations and prevent their recurrence (48,49). How hemovigilance will work has been determined by guidelines in many countries, including our country. Physicians performing transfusion should know the concept of hemovigilance, in which situations they should initiate this process and how to initiate it. If a physician reports a suspicion of a TTI hemovigilance officers/nurses will take the necessary steps to determine whether the infection detected in the patient can be associated with the transfused blood and its donor (such as tracing from the patient to the donor, or from the donor to the patient if necessary). For example, when the donor is traced back to a patient who is thought to have HIV (or HBV or HCV) through an ES transfusion, it may be revealed that the donor was in the window period by the donation. The FFP obtained from the same donation can be easily traced to find out where it is, and whether it has been used. If it is not used, it can be recalled immediately to prevent another patient from being infected. Even if it is transfused, the patient to whom it is administered can be diagnosed as soon as possible. This is only possible if the physician initiates the hemovigilance. As can be seen, the physician who thinks that a patient has a TTI has a responsibility towards other patients and public too.

Although the main goal is to prevent Thalassemia from the very beginning by providing Thalassemia screening, genetic testing and counseling in risky areas and groups, developments in Thalassemia treatment are promising the possibility that all Thalassemia patients will be able to avoid transfusion in the future. However, it is a fact that as long as Thalassemia patients continue to receive transfusions, the risk of transfusion-transmitted infections will continue, at least for now.

REFERENCES

1. Shah FT, Sayani F, Trompeter S, et al. Challenges of blood transfusions in β-thalassemia. Blood Rev. 2019 Sep 1;37. Available from: https://pubmed.ncbi.nlm.nih.gov/31324412/
2. Waheed U, Saba N, Wazeer A, et al. A Systematic Review and Meta-Analysis on the Epidemiology of Hepatitis B and Hepatitis C Virus among Beta-Thalassemia Major Patients in Pakistan. J Lab Physicians. 2021 Sep;13(3):270–6. Available from: https://pubmed.ncbi.nlm.nih.gov/34602793/
3. Ehsan H, Wahab A, Anwer F, et al. Prevalence of Transfusion Transmissible Infections in Beta-Thalassemia Major Patients in Pakistan: A Systematic Review. Cureus. 2020 Aug 27;12(8). Available from: https://pubmed.ncbi.nlm.nih.gov/32874814/
4. Riaz M, Abbas M, Rasool G, et al. Prevalence of transfusion-transmitted infections in multiple blood transfusion-dependent thalassemic patients in Asia: A systemic review. Int J Immunopathol Pharmacol. 2022 Apr 20;36. Available from: https://pubmed.ncbi.nlm.nih.gov/35452334/
5. Uluhan R, Heper Y, editors. Ulusal Kan Merkezleri ve Transfüzyon Tıbbı Kursu XXIII. İstanbul: Türkiye Kan Merkezleri ve Transfüzyon Derneği; 2021.
6. Busch MP, Bloch EM, Kleinman S. Prevention of transfusion-transmitted infections. Blood. 2019 Apr 25;133(17):1854–64.
7. Godbey EA, Thibodeaux SR. Ensuring safety of the blood supply in the United States: Donor screening, testing, emerging pathogens, and pathogen inactivation. Semin Hematol. 2019 Oct 1;56(4):229–35. Available from: https://pubmed.ncbi.nlm.nih.gov/31836028/
8. Neuberger J, Brailsford SR, Mallinson G, et al.Challenges for the maintaining the microbiological safety of the UK blood supply. Clin Med. 2023 Mar 1;23(2):151–6. Available from: https://pubmed.ncbi.nlm.nih.gov/36806203/
9. Puerto-Meredith S, Singogo E, Chagomerana M, et al. Systematic review of prevalence and risk factors of transfusion transmissible infections among blood donors, and blood safety improvements in Southern Africa. Transfus Med. 2023; Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/tme.12988
10. Rasool G, Khan AM, Malik IR, et al. Reported Seroprevalence of Transfusion-Transmitted Infections in Voluntary and Replacement Donors. Transfus Med Rev. 2023 Apr 1;37(2).
11. Javed SO, Saleem A, Sahito AM, et al. Transfusion Transmitted Infections: A Present-Day Danger for

Pakistan. Am J Trop Med Hyg. 2022 May 1;106(5):1311–4.
12. Appassakij H, Silpapojakul K, Promwong C, et al. The Potential Impact of Chikungunya Virus Outbreaks on Blood Transfusion. Transfus Med Rev. 2020 Jan 1;34(1):23–8. Available from: https://pubmed.ncbi.nlm.nih.gov/31303361/
13. Gimenez-Richarte A, De Salazar MO, Arbona C, et al. Prevalence of Chikungunya, Dengue and Zika viruses in blood donors: a systematic literature review and meta-analysis. Blood Transfus. 2022 Jul 1;20(4):267–80. Available from: https://pubmed.ncbi.nlm.nih.gov/34694219/
14. Giménez-Richarte Á, Ortiz de Salazar MI, Giménez-Richarte MP, et al. Transfusion-transmitted arboviruses: Update and systematic review. PLoS Negl Trop Dis. 2022;16(10). Available from: https://pubmed.ncbi.nlm.nih.gov/36201547/
15. Frank C, Schmidt-Chanasit J, Ziegler U, et al. West Nile Virus in Germany: An Emerging Infection and Its Relevance for Transfusion Safety. Transfus Med Hemother. 2022 Aug 20:192–203. Available from: https://pubmed.ncbi.nlm.nih.gov/36159956/
16. Garzon Jimenez RC, Lieshout-Krikke RW, Janssen MP. West Nile virus and blood transfusion safety: A European perspective. Vox Sang. 2021 Nov 1;116(10):1094–101. Available from: https:// pubmed.ncbi.nlm.nih.gov/33900632/
17. Kiely P, Seed CR, Gambhir M, et al. West Nile virus transfusion-transmission risk in Australia associated with a seasonal outbreak in the United States. Transfusion. 2022 Nov 1;62(11):2291–6. Available from: https://pubmed.ncbi.nlm.nih.gov/36120961/
18. Nagy A, Csonka N, Takács M, et al. West Nile and Usutu virus seroprevalence in Hungary: A nationwide serosurvey among blood donors in 2019. PLoS One. 2022 Apr 1;17(4). Available from: https://pubmed.ncbi.nlm.nih.gov/35395048/
19. Mowla SJ, Drexler NA, Cherry CC, et al. Ehrlichiosis and Anaplasmosis among Transfusion and Transplant Recipients in the United States. Emerg Infect Dis. 2021 Nov 1;27(11):2768–75. Available from: https://pubmed.ncbi.nlm.nih.gov/34670661/
20. van de Laar TJ, Hogema BM, Molenaar-de Backer MW, et al. Blood donor screening in the Netherlands: Universal anti-HBc screening in combination with HBV nucleic acid amplification testing may allow discontinuation of hepatitis B virus antigen testing. Transfusion. 2021 Jul 1;61(7):2116–24. Available from: https://pubmed.ncbi.nlm.nih.gov/33899233/
21. Fiedler SA, Oberle D, Chudy M, et al. Effectiveness of blood donor screening by HIV, HCV, HBV-NAT assays, as well as HBsAg and anti-HBc immunoassays in Germany (2008-2015). Vox Sang. 2019 Jul 1;114(5):443–50. Available from: https://pubmed.ncbi.nlm.nih.gov/31012114/
22. Wu D, Feng F, Wang X, et al. The impact of nucleic acid testing to detect human immunodeficiency virus, hepatitis C virus, and hepatitis B virus yields from a single blood center in China with 10-years review. BMC Infect Dis. 2022 Dec 1;22(1). Available from: https://pubmed.ncbi.nlm.nih.gov/35321684/
23. Garraud O, Tissot JD. Transfusion, history and ethics: Hundred years after WWI battlefield operations. Transfus Clin Biol. 2019 Feb 1;26(1):1–2.
24. Canatan D, Özsancak A. A new donor system for the patients with thalassemia: "Blood mother and blood father." Asian J Transfus Sci. 2010 Jul 1;4(2):109–11. Available from: https://pubmed.ncbi.nlm.nih.gov/20859510/
25. Levy JH, Neal MD, Herman JH. Bacterial contamination of platelets for transfusion: strategies for prevention. Crit Care. 2018 Oct 27;22(1). Available from: https://pubmed.ncbi.nlm.nih.gov/30367640/
26. Ahmad Y, Heroes AS, Hume HA, et al. Bacterial contamination of blood products in Africa. Transfusion. 2021 Mar 1;61(3):767–80. Available from: https://pubmed.ncbi.nlm.nih.gov/33469916/
27. Tsegaye W, Bitew A, Gize A. Bacterial Contamination and Susceptibility Pattern Among Blood and Blood Components Using Divergent and Non-Divergent Collection Methods at Armed Forces Comprehensive Specialized Hospital, Addis Ababa, Ethiopia. Infect Drug Resist. 2022;15:1677–86. Available from: https://pubmed.ncbi.nlm.nih.gov/35422636/
28. Kundrapu S, Srivastava S, Good CE, et al. Bacterial contamination and septic transfusion reaction rates associated with platelet components before and after introduction of primary culture: experience at a US Academic Medical Center 1991 through 2017. Transfusion. 2020 May 1;60(5):974–85. Available from: https://pubmed.ncbi.nlm.nih.gov/32357261/
29. Kamel H, Ramirez-Arcos S, McDonald C. The international experience of bacterial screen testing of platelet components with automated microbial detection systems: An update. Vox Sang. 2022 May 1;117(5):647–55. Available from: https://pubmed.ncbi.nlm.nih.gov/35178718/
30. Schmidt M, Ramirez-Arcos S, Stiller L, et al. Current status of rapid bacterial detection methods for platelet components: A 20-year review by the ISBT Transfusion-Transmitted Infectious Diseases Working Party Subgroup on Bacteria. Vox Sang. 2022 Aug 1;117(8):983–8. Available from: https:// pubmed.ncbi.nlm.nih.gov/35412655/
31. Martin IW, Cohn CS, Delaney M, et al. Limitations of current practices in detection of bacterially contaminated blood products associated with suspected

septic transfusion reactions. Transfusion. 2021 Aug 1;61(8):2414–20. Available from: https://pubmed.ncbi.nlm.nih.gov/34181247/
32. Ruby KN, Khan J, Martin IW, et al. Application of Standardized Residual Component Culture Criteria for Suspected Septic Transfusion Reactions Would Increase the Component Culturing Rate at a Single Academic Medical Center. Am J Clin Pathol. 2022 Aug 1;158(2):216–20. Available from: https:// pubmed.ncbi.nlm.nih.gov/35311936/
33. Shigemura T, Yanagisawa R, Komori K, et al. Prevention of transfusion-transmitted cytomegalovirus infection using leukoreduced blood components in patients receiving seronegative umbilical cord blood transplantation. Transfusion. 2019 Oct 1;59(10):3065–70. Available from: https:// pubmed.ncbi.nlm.nih.gov/31322734/
34. Drews SJ. Prevention of transfusion-transmitted syphilis by blood operators: How much is enough when transfusion-transmission has not been identified for decades? Transfusion. 2021 Nov 1;61(11):3055–60. Available from: https:// onlinelibrary.wiley.com/doi/full/10.1111/trf.16696
35. Attie A, de Almeida-Neto C, S. Witkin S, et al. Detection and analysis of blood donors seropositive for syphilis. Transfus Med. 2021 Apr 1;31(2):121–8. Available from: https://pubmed.ncbi.nlm.nih.gov/33480044/
36. Jayawardena T, Hoad V, Styles C, et al. Modelling the risk of transfusion-transmitted syphilis: a reconsideration of blood donation testing strategies. Vox Sang. 2019 Feb 1;114(2):107–16. Available from: https://pubmed.ncbi.nlm.nih.gov/30565234/
37. Ahmadpour E, Foroutan-Rad M, Majidiani H, et al. Transfusion-Transmitted Malaria: A Systematic Review and Meta-analysis. Open forum Infect Dis. 2019 Jun 5;6(7):1–8. Available from: https:// pubmed.ncbi.nlm.nih.gov/31334300/
38. Rocha D, De Melo GC, Carneiro JMH, et al. Use of a NAT-based assay to improve the surveillance system and prevent transfusion-transmitted malaria in blood banks. Malar J. 2020 Jul 31;19(1). Available from: https://pubmed.ncbi.nlm.nih.gov/32736625/
39. Antwi-Baffour S, Kyeremeh R, Amoako AP, et al. The Incidence of Malaria Parasites in Screened Donor Blood for Transfusion. Malar Res Treat. 2019;2019. Available from: https://pubmed.ncbi.nlm.nih.gov/31885856/
40. Niederhauser C, Galel SA. Transfusion-Transmitted Malaria and Mitigation Strategies in Nonendemic Regions. Transfus Med Hemother. 2022 Aug 15;49(4):205–17. Available from: https://pubmed.ncbi.nlm.nih.gov/36159954/
41. Saá P. Is sporadic Creutzfeldt-Jakob disease transfusion-transmissible? Transfusion. 2020 Apr 1;60(4):655–8. Available from: https://pubmed.ncbi.nlm.nih.gov/32246483/
42. Salamat MKF, Stewart P, Brown H, et al. Subclinical infection occurs frequently following low dose exposure to prions by blood transfusion. Sci Rep. 2022 Dec 1;12(1). Available from: https://pubmed.ncbi.nlm.nih.gov/35764688/
43. Salamat KFM, Blanco ARA, McCutcheon S, et al. Preclinical transmission of prions by blood transfusion is influenced by donor genotype and route of infection. PLoS Pathog. 2021 Feb 18;17(2). Available from: https://pubmed.ncbi.nlm.nih.gov/33600501/
44. Gehrie EA, Rutter SJ, Snyder EL. Pathogen Reduction: The State of the Science in 2019. Hematol Oncol Clin North Am. 2019 Oct 1;33(5):749–66. Available from: https://pubmed.ncbi.nlm.nih.gov/31466602/
45. Seltsam A. Pathogen inactivation of cellular blood products-An additional safety layer in transfusion medicine. Front Med. 2017;4 (DEC):1–6.
46. Allain JP, Goodrich R. Pathogen reduction of whole blood: utility and feasibility. Transfus Med. 2017;27(5):320–6.
47. Drew VJ, Barro L, Seghatchian J, et al. Towards pathogen inactivation of red blood cells and whole blood targeting viral DNA/RNA: Design, technologies, and future prospects for developing countries. Blood Transfus. 2017;15(6):512–21.
48. Haass KA, Sapiano MRP, Savinkina A, et al. Transfusion-Transmitted Infections Reported to the National Healthcare Safety Network Hemovigilance Module. Transfus Med Rev. 2019 Apr 1;33(2):84–91. Available from: https://pubmed.ncbi.nlm.nih.gov/30930009/
49. Vuk T, Politis C, Laspina S, et al. Thirty years of hemovigilance - Achievements and future perspectives. Transfus Clin Biol. 2023 Feb 1;30(1):166–72. Available from: https://pubmed.ncbi.nlm.nih.gov/36216308/

Correspondence Address:
Doç. Dr. Yasemin Heper
Bursa Uludağ Üniversitesi Tıp Fakültesi, Bursa, Türkiye
Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Anabilim Dalı ve Kan Merkezi Sorumlusu
E-Mail: yheper@uludag.edu.tr

CHAPTER 6
IRON LOAD AND CHELATION

IRONLOAD AND PHYSIOPATHOLOGY IN THALASSEMIA

Assoc. Prof. Dr. Sultan Aydın M.D.
University of Health Sciences, Antalya Education and Research Hospital, Thalassemia Center Antalya -Türkiye

ABSTRACT

Iron overload occurs either as a result of red blood cell transfusions or increased absorption of iron through the gastrointestinal (GI) tract. Also, the major cause of iron overload in transfusion dependent thalassaemia (TDT) is blood transfusion therapy, while more important in non-transfusion dependent thalassaemia (NTDT) is increased GI absorption. Iron accumulation is toxic to many tissues, causing heart failure, cirrhosis, liver cancer, growth retardation and multiple endocrine abnormalities.

Keywords: Iron load, physiopathology, thalassemia

BACKGROUND

Thalassemia is an inherited disease with multiple genetic forms, including α-thalassemia, β-thalassemia, hemoglobin E/β-thalassemia, and others. Molecular defects in the gene encoding α-globin on chromosome 16 or the β-globin gene on chromosome 11 result in abnormal hemoglobin synthesis. Increased ineffective erythropoiesis, chronic hemolytic anemia, and early apoptosis of erythroid cells with abnormal nuclei as a result of the imbalance of α/β chains that develop as a result of excessive α-globin synthesis and impaired β-globin synthesis are responsible for secondary pathophysiological mechanisms. Thalassemia disorders have a broad spectrum with different clinical phenotypes, complications, and treatment strategies. According to the degree of transfusion dependence, thalassemic disorders are divided into transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT) according to the new classification. While iron overload results from increased intestinal iron absorption as a result of ineffective erythropoiesis, it may also be secondary to regular transfusions used in relation to the severity of the disease **(1)**. As a result of iron overload, iron accumulation in various organs causes complications in thalassemia patients.

PATHOPHSIOLOGY OF IRON OVERLOAD IN TDT AND NTDT

The human body lacks a physiological mechanism to eliminate iron overload resulting from blood transfusion. Each unit of transfused packed red blood cells contains 200 to 250 mg of elemental iron. In TDT, transfusional iron usually amounts to 0.3 to 0.6 mg/kg per day, and the monthly transfusion rate is assumed to be 2 to 4 U of packed red blood cells. Transfused elderly red blood cells are phagocytosed by reticuloendothelial macrophages. As a result, cellular iron is released into the plasma to bind to transferrin **(2)**. After the iron bound to transferrin reaches saturation, the increased free circulating iron not bound to transferrin is easily transported to the liver (hepatocytes), heart (cardiac myocytes) and endocrine organs via calcium channels. Accumulation of iron in different organs leads to different clinical complications of iron overload. Reactive oxygen species produced as a result of the metabolism of non-transferrin-bound iron contribute to cellular dysfunction, apoptosis and necrosis in target organs.

Transferrin is the main iron transport protein and can bind 2 Fe^{3+} molecules. Transferrin then binds to transferrin receptor 1 (TfR1) and transferrin receptor 2 (TfR2). Transferrin is then taken in by endocytosis, and then Fe^{3+} is released from transferrin in the lysosomal acidic environment. It is reduced to Fe^{2+} by cytochrome B and then reaches the cytoplasm via divalent metallic transporter 1 (DMT1). TfR2 is expressed in the liver and intestine, while TfR1 is expressed in the liver, myocardium, and all tissues containing erythroid precur-

sors. The affinity of TfR1 to iron is approximately 25 times higher than that of TfR2 **(3)**. Through these pathways, TDT causes iron accumulation in various organs of patients, leading to cardiac siderosis, which is the most important clinical complication and an important cause of mortality such as arrhythmias and heart failure. At the same time, hepatic and endocrine dysfunction due to iron overload are also common in TDT patients.

The main pathogenesis of iron accumulation in NTDT patients is increased iron absorption from the gastrointestinal tract. According to recent studies in NTDT patients, it was noticed that iron overload affects the liver rather than the myocardium differently in patients who are not dependent on regular red blood cell transfusion. Observational studies have demonstrated the absence of cardiac siderosis even in patients with severe liver iron overload. It remains unclear whether this is attributable to the mechanism of iron overload in NTDT or to slower iron loading. Iron overload in NTDT is known to be associated with increased intestinal iron absorption caused by hepcidin suppression and erythron expansion. Each 1 mg/g dry weight (kW) increase in LIC is associated with a higher likelihood of thrombosis, pulmonary hypertension, hypothyroidism, osteoporosis, and hypogonadism in NTDT **(3)**. NTDT patients with iron overload are also more likely to develop renal dysfunction. Hepcidin synthesis in the liver normally binds to ferroportin, which provides iron transport, and suppresses iron release from erythroid precursors, hepatocytes, basolateral membranes of hepatocytes and macrophages **(2)**. Therefore, hepcidin suppression secondary to ineffective erythropoiesis leads to upregulation of the transport of absorbed iron through the basolateral membrane of enterocytes into the systemic circulation. Erythroferrone, a 340 amino acid soluble protein produced by bone marrow and spleen erythroid precursors, acts directly on the liver, leading to inhibition of hepcidin production. Thus, it causes iron accumulation **(5)**.

REFERENCES

1. Camaschella C, Nai A. Ineffective erythropoiesis and regulation of iron status in iron loading anaemias. Br J Haematol. 2016; 172(4): 512-523.
2.Coates TD. Physiology and pathophysiology of iron in hemoglobin-associated diseases. Free Radic Biol Med. 2014; 72: 23-40
3.Cappellini MD, Cohen A, Porter J, Taher A, and Viprakasit V Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT). 3rd ed. Nicosia, Cyprus: Thalassaemia International Federation; 2014.
4. Taher AT, Musallam KM, Wood JC, Cappellini MD. Magnetic resonance evaluation of hepatic and myocardial iron deposition in transfusion-independent thalassemia intermedia compared to regularly transfused thalassemia major patients. Am J Hematol. 2010; 85(4): 288-290.
5. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat Genet. 2014; 46(7): 678-684.

Correspondence Address:
Assoc. Prof. Dr. Sultan Aydın MD
University of Health Sciences, Antalya Education and Research Hospital, Thalassemia Center Antalya-Türkiye
E-mail: drsultanaydin@hotmail.com

IRON LOAD DETECTION METHODS

Prof. Dr. Sule Unal Cangul

Hacettepe University, Department of Pediatric Hematology, Ankara, Türkiye

ABSTRACT

Thalassemia patients are at risk for iron overload through frequent transfusions or gastrointestinal iron hyperabsorption. The iron accumulation needs to be quantified and monitored, in order to chelate these patients adequately. The recent advancements in iron accumulation monitroing improved the survival of these patients. In this chapter, the tools used in monitoring iron accumulation are summarized. These tools include serum ferritin as a more widely available option to cardiac T2* and hepatic R2 MRI methods, which requires to be done by validated centers.

Keywords: Iron load, ferritin, T2*MRI

BACKGROUND

Iron overload is one of the life-threatening complications encountered in patients who are on chronic transfusion programme. Additionally, the patients with a condition of ineffective eryhtopoiesis, such as thalassemia intermedia and congenital dyserythropoietic anemia have increased gastrointestinal absorption of iron and those patients with inherited forms increased iron absorption, namely hereditary hemochromatosis have iron overload risks **(1)**.

Determination of iron overload is crucial in order to make a decision on when to start iron chelation, to monitor iron loading and to change the chelation doses or chelator types accordingly. Not only the availability of iron chelators, especially the oral chelators, but the availability of iron deposition methods improved the survival in thalassemia patients **(2)**.

Estimation of iron intake can be calculated through the amount of transfusion in transfused patients with the following formula:

> **Fe intake** (mg/kg/year)=(transfusion volume (ml/year) x Hematocrit x 1.08)/Body weight (kg)

Unless the patient is on iron chelation, the value calculated as Fe intake can be used to estimate the change in liver iron concentration (LIC) through Angelucci relationship **(1, 3)**.

> Change in LIC (mg/g dry weight)=Fe intake/10.6

However, since most patients require iron chelation, addition methods are required to monitor iron loading in these patients.

SERUM FERRITIN

As a relatively cheap and widely available tool, serum ferritin is still the only iron load detection method in most of the countries. However, although serum ferritin is sensitive to detect iron loading, the specificity is poor. Serum ferritin usually correlates with total body iron stores. However, the correlation is not linear. Different patients with the same serum ferritin levels may have different LIC values and cardiac iron concentrations **(1)**. Besides, serum ferritin levels are usually lower in patients with thalassemia intermedia, compare to patients with thalassemia major who have the same LIC values; reflecting the major iron accumulating cell in non-transfusion dependent thalassemia (NTDT) as hepatocytes; compared to macrophages in transfusion dependent thalassemia (TDT) **(4)**.

Serum ferritin is important to determine the trends of iron loading and the decision of iron chelation treatment should not rely on single test values. Since serum ferritin levels are affected with infection and inflammation due to being an acute phase

reactant, single high values should not prompt physician to initiate iron chelation or increase the doses **(5, 6)**. This also precludes the use of serum ferritin reliably in patients with sickle cell anemia who have a chronic inflammation **(6)**.

On the other hand, serum ferritin levels can be elevated in patients with liver disease. Liver disease is not uncommon in TDT or NTDT patients and therefore consistently high levels in serum ferritin levels do not always indicate inadequate chelation or patient incompliance **(5)**.

Despite these limitations, serum ferritin levels between 500 and 1000 ng/ml in TDT patients usually reflect a mild iron loading, whereas serum ferritin values above 3000 ng/ml usually reflects severe iron loading **(1)**. The relationship between serum ferritin values and total body iron have alse been reported to vary according to the chelator used **(7)** and the duration of chelation use **(8)**. Maintenance of sserum ferritin levels below 2500 ng/ml in TDT patients; even lower according to other studies (under 1000 ng/ml) have been recommended in order to derease the morbidity and mortalities related to iron **(5, 9, 10)**.

NON TRANSFERRIN BOUND IRON(NTBI) OR LABILE PLASMA IRON (LPI)

When the transferrin saturation exceeds 85%, NTBI is present in the circulation and LPI is a type of NTBI which can enter Fenton reactions and may pass across the cellular membranes **(1)**. Since the amount of NTBI and LPI reflect the toxic iron, it might be reasonable to determine their amounts. However, the availability of these methods are limited and the techniques measuring them are not standardized. Additionally, the use of NTBI and LPI on chelation decisions is limited **(5)**.

LIC measurement:

LIC reflects the total body iron levels and measurement of LIC in order to estimate the total body iron loading, does not have the limitations of serum ferritin, such as being affected with inflammation. On the other hand, LIC is not reliable to predict cardiac iron loading in patients using iron chelation **(5)**.

The available methods to measure LIC include biopsy, magnetic resonance imaging (MRI) and SQUID.

- Although, liver biopsy is an invasive method, has the advantage of giving histopathologic information related to the chronic liver disease due to iron loading or transfusion related viral hepatitis. On the other hand, the amount of liver tissue obtained might be inadequately obtained rendering the quantification of iron or the amount of iron might not be homogenous due to accompanying cirrhosis **(11)**.
- As a non-invasive tool MRI is being more commonly used in many centers and is a validated tool with acceptable interrater reliability. The preferred method is use of R2 or R2* values obtained from 1.5 Tesla MRI machines. The half-life of a spin-echo image is known as T2 (in milliseconds) and the half-life for a gradient echo is named as T2* (in milliseconds). The higher the tissue iron, the smaller the T2 or T2* values are **(5)**.
  On the other hand, R2 and R2* values (Hertz) are the reciprocals of T2 and T2* values, multiplied with 1000. R2 based FerriScan® have been reported to have over 85% sensitivity and specificity upto an LIC of 15 mg/g dry weight **(5, 12)**. An LIC over 3 mg/g dry weight is consistent with hepatic iron loading. However, since the softwares for R2/R2* based MRI measurements of LIC are not widely available; most of the centers still do not have to opportunity to measure LIC with MRI.
- Another tool for LIC measurement is superconducting quantum interference device (SQUID) which requires liquid helium and thus very expensive and the least available method **(5)**.

CARDIAC IRON CONCENTRATION MEASUREMENT

Since obtaining endomyocardial biopsies in order to measure cardiac iron load is dangerous, the need for non-invasive methods in cardiac iron load determination is more important. For this purpose, cardiac

T2* MRI is used as a gold-standard method with T2* values below 20 ms indicating cardiac iron accumulation having a risk of lower left ventricular ejection fraction values **(13)**. Cardiac T2* values correlate with the risk of heart failure within 12 months and arrythmias in these patients. The cardiac iron loading does not correlate with the LIC. Additionally, cardiac T2* MRI measurement requires validated center measurement, otherwise different results might be obtained in different centers **(5)**. Technically the measurement requires patient's compliance and need for breatholding.

Hepatic iron loading is expected to occur in TDT or NTDT patients; however cardiac iron loading is usually not encountered among NTDT patients and cardiac iron overload monitorization is not required for patients with NTDT.

In conclusion, better monitorization of iron loading and increasing the availability of validated non-invasive tools will improve the patient's survival and decrease the iron related morbidities of these patients through appropriate chelation accordingly.

REFERENCES

1. Wood JC. Guidelines for quantifying iron overload. Hematology Am Soc Hematol Educ Program. 2014;2014:210-5.
2. Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. J Cardiovasc Magn Reson. 2008;10:42.
3. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, Galimberti M, Polchi P, Lucarelli G. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med. 2000;343:327-31.
4. Taher AT, Porter JB, Viprakasit V, Kattamis A, Chuncharunee S, Sutcharitchan P, Siritanaratkul N, Origa R, Karakas Z, Habr D, Zhu Z, Cappellini MD. Defining serum ferritin thresholds to predict clinically relevant liver iron concentrations for guiding deferasirox therapy when MRI is unavailable in patients with non-transfusion-dependent thalassaemia. Br J Haematol. 2015;168:284-90.
5. Porter JP. Iron overload: pathophysiology, diagnosis, and monitoring. In: Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT), 3rd edition. Eds: Maria Domenica Cappellini, Alan Cohen, John Porter, Ali Taher, and Vip Viprakasit. Nicosia (CY): Thalassaemia International Federation; 2014.
6. Adamkiewicz TV, Abboud MR, Paley C, Olivieri N, et al. Serum ferritin level changes in children with sickle cell disease on chronic blood transfusion are nonlinear and are associated with iron load and liver injury. Blood. 2009;114:4632–4638.
7. Ang AL, Shah FT, Davis BA, Thomas A, et al. Deferiprone Is Associated with Lower Serum Ferritin (SF) Relative to Liver Iron Concentration (LIC) Than Deferoxamine and Deferasirox- Implications for Clinical Practice. Blood 2010;116:4246–4246.
8. Fischer R, Longo F, Nielsen P, Engelhardt R, et al. Monitoring long-term efficacy of iron chelation therapy by deferiprone and desferrioxamine in patients with -thalassaemia ma- jor: application of SQUID biomagnetic liver susceptometry. British Journal of Haematology 2003;121:938–948.
9. Olivieri NF, Nathan DG, MacMillan JH, Wayne AS, et al. Survival in medically treated patients with homozygous beta-thalassemia. The New England Journal of Medicine. 1994; 331:574–578.
10. Borgna-Pignatti C, Rugolotto S, Stefano PD, Zhao H, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica. 2004;89:1187–1193.
11. Villeneuve JP, Bilodeau M, Lepage R, Côté J, et al. Variability in hepatic iron concen- tration measurement from needle-biopsy specimens. Journal of Hepatology 1996;25:172–177.
12. St Pierre TG, Clark PR, Chuaanusorn W. Fleming AJ, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. Blood. 2005;105:855–861.
13. Anderson LJ, Holden S. Davis B, Prescott E, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. European Heart Journal2001; 22:2171–2179.

Correspondence Address:
Prof. Dr. Sule Unal Cangul
Hacettepe University, Department of Pediatric Hematology, Ankara, Türkiye
E-mail: suleunal@hacettepe.edu.tr

CONVENTIONAL CHELATION THERAPY

Prof. Dr. Yesim Aydinok
Ege University School of Medicine, Department of Pediatric Hematology & Oncology, Izmir, Türkiye

ABSTRACT

Iron chelation therapy is the mainstay of managing transfusion-dependent and non-dependent thalassemia syndromes. Iron chelation therapy remains the crucial standard of care in transfusion-dependent other anemias, including sickle cell disease. Conventional chelation agents can achieve the objectives of iron chelation therapy in the case of their administration by considering their ability to access different iron pools, appropriate chelator regimens in individual patients, and chelator dose titration based on iron burden, thereby avoiding inadequate or over-chelation. Compliance with chelation in a particular patient is essential in response to prescribed chelation therapy. Suboptimal chelation might be associated with intolerability, toxicity, resistance, or non-compliance with the given chelator regimen. Timely identification of the problem leading to suboptimal therapy and taking appropriate action may improve compliance and provide optimum iron balance, preventing organ dysfunctions and allowing complication-free survival.

INTRODUCTION

Iron chelation therapy (ICT) is essential to prevent or treat hemosiderosis in patients with transfusion-dependent and non-transfusion-dependent thalassemia (TDT & NTDT) and sickle-cell disease (SCD) on chronic transfusion programs. Because of the different characteristics of iron burden and the ICT principles, chelation therapies in TDT, NTDT, and SCD will be discussed separately.

Objectives of iron chelation therapy in thalassemia and sickle cell disease

Optimizing ICT depends on achieving a neutral or negative iron balance. Maintenance of body iron levels requires iron excretion by ICT, like the amount of iron acquired from transfusion and gastrointestinal absorption. However, to achieve a negative iron balance, the iron excreted with ICT must be more than the amount acquired from transfusion and gastrointestinal absorption.

Prospective studies demonstrated that besides baseline body iron burden, consideration of ongoing transfusional iron intake is essential in selecting the chelator dose for achieving a desired iron balance **(1)**. Higher chelator doses per kg body weight (b.w.) per day are required to achieve a negative iron balance in patients with significant iron overload (IOL). However, close monitoring of the iron load is essential for timely individual dose titration to achieve an ultimate therapeutic goal by avoiding iron deprivation **(2)**.

The therapeutic target of ICT in TDT patients is to control labile plasma iron (LPI), thereby preventing the expansion of the labile cell iron (LCI) pool that may promote the generation of reactive oxygen species, which chemically damage cells leading to organ dysfunctions **(3)**. It is assumed that preventing the primary iron accumulation in hepatocytes should avoid secondary iron distribution to endocrine tissues and the heart in chronically transfused patients **(4)**. However, clinical observations suggest that once transferrin is entirely saturated, high transfusion iron intake exceeding its utilization by endogenous erythropoiesis appears as a key mechanism for generating LPI that is readily uptake by cardiomyocytes **(5)**. Although the prospective cardiac risk increases with severe hepatic siderosis, there is no safe liver iron threshold for avoiding cardiac iron loading in patients with TDT **(6)**. Once iron accumulates in the heart, in contrast to the liver, cardiac iron clearance by ICT is a slow process relying on the ability of chelators to access a tiny

fraction of LCI available for chelation at any moment, and chronic control of LPI is also essential to prevent further tissue iron loading **(6, 7)**.

The clinical observations suggest that LPI was absent in most NTDT patients without previous transfusions, while elevated liver iron concentrations (LIC) and iron turnover markers **(8)**. In fact, despite significant hepatic siderosis, none of the NTDT patients showed evidence of cardiac iron loading in contrast to patients with TDT **(9)**. Therefore, the objective of ICT in NTDT is to maintain liver iron at safe levels to prevent liver damage and related morbidities.

Patients with non-transfused SCD do not develop systemic IOL because inflammation increases hepcidin synthesis, which decreases iron absorption and enhances iron retention within the reticuloendothelial system. In a retrospective analysis, the only predictor of iron-induced organ damage in SCD was the duration of chronic transfusion **(10)**. It has been demonstrated that patients with sickle cell disease (SCD) often require blood transfusion starting in early childhood. Multiple blood transfusions on a chronic basis lead to excessive iron accumulation, especially in adults with SCD, associated with morbidity and mortality that justify ICT **(11)**. Although clinical observations showed no evidence of cardiac siderosis in SCD patients with elevated LIC, it may not exclude the risk of cardiac iron loading at long-term follow-up **(12)**.

Overview of iron chelator agents in clinical use

Three iron chelators are available to prevent and remove toxic iron accumulation **(Table 1)**. Deferoxamine (DFO) has been a reference standard therapy since the 1980s and was the only approved chelator until 1999 when the European Union (EU) granted marketing approval for oral chelator Deferiprone (DFP) specifically for patients with thalassemia major (TM) when DFO is inadequate, intolerable, or unacceptable. The Food Drug Administration (FDA) approved DFP in 2011 for treating transfusional IOL in adult and pediatric patients ≥ 3 years old. European Medical Agency (EMA) updated the indication of DFP as when monotherapy with any iron chelator is ineffective or when prevention or treatment of life-threatening consequences of iron overload (mainly cardiac overload) justifies rapid or intensive correction (EMA/473109/2019). The FDA in 2005 and EMA in 2006 approved Deferasirox (DFX) dispersible tablet (DT) as a once-daily oral chelator for treating patients with transfusional iron overload older than two years of age- as first-line therapy. DFX is approved for the treatment in NTDT patients aged ≥10 years. Subsequently, DFX film-coated tablet (FCT) was approved in patients with beta-thalassemia major aged six years and older by EMA and above two years by FDA **(13)**.

Deferoxamine

Because of the rapid metabolism of DFO in plasma (t1/2=20-30 min), DFO must be administered by prolonged subcutaneous infusion at least 8-10 hours per day. The recommended starting dose of DFO in pediatric patients at serum ferritin (SF) levels below 2000 ug/L is 20-25 mg/kg/day, 5-7 days a week, and the DFO dose is permitted up to 40 mg/kg/day until linear growth is completed **(14)**. However, an observational study demonstrated that a DFO dose of 25–30 mg/kg/d, five days a week in chelation naïve TM patients aged below six years, required a dose increment of up to 40 mg/kg/d during the first year and up to 50 mg/kg/d after that to maintain targeted SF trends. This practice will unlikely result in chelator-induced toxicity if DFO doses are carefully titrated against SF levels **(15)**. A therapeutic index was implemented to avoid the toxic effects (neurotoxicity and growth retardation) of DFO chelation in which the mean daily dose of DFO (mg/kg) divided by individual SF level (µg/L) should not be greater than 0.025 **(16)**. While LPI chelated with DFO is predominantly excreted by kidneys, LCI pools into hepatocytes efficiently chelated by DFO is eliminated via the biliary-fecal route **(17, 18)**. Although penetrance of high molecular weight, hydrophilic DFO delays access into the LCI pools in cells and organelles, clinical data demonstrated the benefit of 50-60 mg/kg DFO 24 hours of continuous intravenous infusion at reversal of siderotic heart failure and improving myocardial T2* **(19)**. The observation of a rapid return of LPI levels after cessation of DFO in high-risk patients with severe

iron overload justifies its prolonged subcutaneous or intravenous infusion of up to 24 hours **(20)**.

Adverse effects: Local reactions at the injection site is the most common adverse effect (AE) of subcutaneous DFO infusions. Ocular and auditory disturbances, including a decrease in visual acuity, visual defects, impaired color or night vision, retinal pigmentary abnormalities, bilateral neurosensorial high-frequency hearing loss, and growth suppression are associated with inappropriately high doses of DFO at relatively low iron burden, which suggest the implementation of DFO dose by considering therapeutic index (see above) to avoid neurotoxicity and short stature. The patients using DFO chelation should have an auditory and ocular examination every year, and auxologic assessments of growing children should be done at three monthly intervals. Timely detected DFO toxicity and appropriate DFO dose reduction likely reverse neurotoxicity and resume growth velocity. *Yersinia enterocolitica*, *V. vulnificus,* and *Mucorales* can utilize DFO as a siderophore to increase their pathogenicity. Therefore, temporarily discontinuing DFO during a febrile illness until establishing that the episode is not associated with one of those microorganisms or taken under control is recommended **(14)**.

Deferiprone

Moderate plasma half-life (t1/2=2-3 hours) of oral chelator DFP supports a widely used 75 mg/kg/day regimen at three divided doses up to 100 mg/kg. Randomized prospective studies demonstrated that standard doses of DFP may not establish a negative iron balance in some patients with significant iron overload **(21)**. Although higher doses of DFP in well-chelated patients by liver criteria prevented liver iron accumulation, it was still less effective than DFO **(22)**. By contrast to the less impressive effect on hepatocellular iron, small, lipophilic DFP can rapidly access intracellular LCI pools and extract iron **(23)**. A prospective randomized study confirmed in vitro observation for DFP against DFO by demonstrating more remarkable improvement in cardiac T2* and increased LVEF for high-dose DFP than standard subcutaneous DFO infusion 5 to 7 days a week in TM patients with asymptomatic cardiac siderosis **(22)**. Monitoring daily LPI levels demonstrated that LPI values significantly exceeded the mean threshold between DFP doses.[7] DFP is excreted predominantly by urine. Recently, DFP twice-daily tablets have been designed to provide the same total daily exposure as DFP three times daily. AEs reported with twice-daily DFP were no different from those previously reported with three-times-daily DFP **(24)**.

Adverse effects: Agranulocytosis is the most severe, life-threatening, and not dose-dependent AE of DFP, occurring in 1.7% of 642 DFP-treated TM and 1.5% of 192 SCD patients in clinical trials. DFP can also cause neutropenia, which may foreshadow agranulocytosis. Because most agranulocytosis events develop during the first year of DFP therapy, complete blood count (CBC) monitoring is recommended weekly during the first six months and two weekly after that up to one year. The patients on DFP therapy should be aware to immediately interrupt DFP and contact their physician if they experience any symptoms indicative of infection. Because the risk of recurrence is as high as 75% among individuals who develop agranulocytosis, DFP should be ceased permanently with a history of this complication.[25] Gastrointestinal disturbances, including nausea, vomiting, and abdominal pain, are the commonest AE related to DFP use, occurring in up to 33% of patients, typically at the beginning of therapy, and a gradual increase in dose may help prevent the problem. Most patients usually resolve these symptoms within a few weeks without discontinuing treatment. Arthralgia and arthritis may develop anytime during the treatment in 15% of patients and sometimes may persist and require discontinuation of therapy. The transient increase in liver enzymes returns to baseline without discontinuation or decreasing the dose of DFP in most cases. Zinc deficiency may be observed because of increased urinary zinc excretion by DFP **(26)**.

Deferasirox

The pharmacokinetic characteristics of DFX support once-daily dosing in the range of 10-40 mg/kg for the dispersible tablet (DT) and 7-28 mg/kg for the granule or film-coated tablet (FCT) formulations. Although the standard daily dose for the

maintenance of iron balance is 20 mg/kg for the DT and 14 mg/kg for the FCT forms, DFX dose titrations of 5–10 mg/kg/day up to 40 mg/kg and 3-7 mg/kg/day up to 28 mg/kg for DT and FCT, respectively, should be considered based on SF trends and safety parameter **(27)**. Although once-daily DFX supports 24-hour coverage for LPI **(7)**, an experimental study suggested that twice-daily dosing of DFX might provide a more homogenous suppression of labile iron species and extract more cardiac iron than once-daily DFX **(28)**. Dividing the once-daily DFX dose into two doses might increase trough concentrations and the period during which drug concentrations remain at efficacious levels. This strategy can be rational for heavily iron-loaded patients with inadequate response to high DFX doses or intolerance to once-daily DFX **(29)**. Although in-vitro studies suggested the superiority of DFX compared to DFO at accessing intracellular LCI pools **(23)**, clinical data showed that DFX was only as efficient as DFO at penetrating cardiac cells and extracting the LCI pool **(30)**.

Adverse effects: Skin rash is a dose-dependent, mostly mild to moderate, and generally transient side effect despite continued therapy. Dose interruption, modifications, and short-term low-dose oral steroids can be considered if severe **(27)**. Gastrointestinal side effects, including nausea, vomiting, abdominal pain, and diarrhea, are common, occurring in 26% of patients during a large DFX study **(2)**. Some patients may develop gastric or duodenal ulcus with hemorrhage. Severe gastrointestinal symptoms may be lower with DFX FCT than with DFX DT **(27)**. A nonprogressive increase in serum creatinine is observed in one-third of patients on DFX. In the same large cohort study, a significant increase in serum creatinine above the upper limit of normal was observed in 3.6% of patients in whom DFX dose reductions, interruptions, and discontinuations because of this dose-dependent side effect occurred in 33.1%, 10.9%, and 1.7% of patients, respectively **(2)**. Renal tubular damage (Fanconi syndrome) resulting in metabolic acidosis, hypokalemia, hypophosphatemia, hypouricemia, glycosuria, phosphaturia, and aminoaciduria is a severe adverse effect of DFX **(27)**.

Iron chelation in patients with TDT

Starting and maintaining ICT in TDT: The patients with β-thalassemia major, severe HbE/β-thalassemia, and non-deletional HbH disease require regular red cell transfusions. Transferrin saturation exceeds the normal range after 4–6 transfusions in patients on chronic transfusion programs, and thereby, iron overload becomes evident very early in the transfusion history. ICT has traditionally been suggested to start after 10–20 transfusions once SF exceeds 1000 µg/l. LIC criteria for initiating ICT is over 3 mg Fe/g dry weight (d.w.). The suggested maintenance SF and LIC levels to avoid iron damage and chelator toxicity are 500-1000 µg/l and 3-5 mg Fe/g d.w., respectively. Despite a close correlation between SF and LIC levels, both weakly correlate with cardiac iron. The assessment of myocardial iron by cardiac T2* MRI (cT2*) can be deferred in well-chelated children with TDT until 8–10 years of age when they can undergo MRI without anesthesia. However, cardiac siderosis may occur in younger children with high transfusional iron burden and poor chelation history. The clinical studies demonstrated that cT2* >20 ms indicates safe iron levels from the cardiac perspective.

Principles of ICT in TDT: Clinical studies demonstrated that standard chelator doses can maintain iron balance, but higher doses are required to achieve negative iron balance in TDT patients **(31, 32)**. A careful dose tailoring strategy on chelation therapy based on decreasing trends in SF and/or LIC levels is essential to avoid chelator toxicity **(33)**. However, when a chelator is absent in the circulation, TDT patients with a high transfusion iron intake relative to iron utilization by endogenous erythropoiesis have a potential for tissue iron accumulation **(5)**. Therefore, stopping iron chelation therapy is not recommended in TDT patients, even at normalized iron status.

The principles of iron chelation therapy include timely initiation, considering patients' preferences, close monitoring, and continuous adjustment of chelator doses to maintain optimum iron balance. Prospective studies assessing the efficacy of iron chelation regimens have highlighted the importance of the rate of transfusional iron intake, the existing hepatic and extrahepatic (cardiac) iron burden, and the chelator dosing and regimen for appropriate

management of iron overload in TDT patients **(13)**. Nevertheless, iron-induced morbidities and early cardiac deaths still exist due to inadequate control of iron overload **(34)**.

Adherence to ICT in TDT: Suboptimal chelation might be associated with intolerability, toxicity, resistance, or non-compliance with the given chelator regimen. Poor adherence to demanding parenteral use of DFO 5 to 7 times a week may be overcome by oral chelators. Still, oral chelation may not always be associated with higher adherence. Patients are highly likely to experience adverse effects from all the medications available for chelation that may result in non-adherence to therapy. The DFX DT bioavailability fluctuates based on the ingredients of the food, and drug exposure increases with the higher fat content of the meal. Therefore, DFX DT is recommended for taking ≥30 minutes before the meal, which might increase intolerability and adherence. DFX FCT formulation allowed with food has reduced gastrointestinal side effects and palatability complaints resulting from DFX DT formulation. The forgetfulness of the chelator dose may also impact tissue iron loading. Adherence to a once-daily DFX might be better than DFP's three-times-a-day regimen. The modified-release formulation of DFP, which is given twice daily, might promote better adherence. Combination chelation therapies might increase the complexity of the treatment, resulting in compliance problems. The close monitoring of IOL and communication with the patient timely identify compliance problems that provide switching to a different iron chelator to improve adherence **(35)**.

Optimizing ICT in TDT: Although DFO chelation has markedly improved prognosis in TDT patients since the 1980s when it became a standard treatment for transfusional iron overload **(36)**, iron-induced cardiac disease, including heart failure and arrhythmia, remained the leading cause of death because of compliance issues with its administration **(37)**. The introduction of oral chelators in clinical use and the documentation of organ-specific siderosis by MRI technologies and appropriate intensification of iron chelation treatment markedly improved survival in patients with TDT **(38)**. Although the standard practice for DFO administration is 8–12 h of subcutaneous infusion by infusion pump given overnight, at least five times a week continued to be a gold standard chelation regimen, because of the cumbersome administration, it has not been the first choice of patients and physicians as a chelation regimen in the modern era.

Efficacy of iron chelation therapies in TDT: Although prospective randomized studies demonstrated a significant reduction in mean SF and LIC levels with either standard DFO (50 mg/kg 5 d/week) or DFP monotherapy (75 mg/kg/d) in TM patients **(21, 39, 40)**, Aydinok et al., stated that LIC increased in a substantial proportion of patients with standard DFP therapy while decreased in all but stabilized below 7 mg Fe/g d.w. in one patient with DFO chelation **(21)**. In a randomized study, higher doses of DFP (100 mg/kg/d) in well-chelated patients by liver criteria but having asymptomatic cardiac siderosis prevented liver iron accumulation, although it was still less effective than standard DFO infusion (43 mg/kg, 5-7 days a week). However, improvement in cT2* and increase in LVEF was more remarkable for high-dose DFP than standard subcutaneous DFO infusions **(22)**. The combination chelation therapy has been established to allow negative iron balance, which may not be achieved by DFP monotherapy **(41)**. It was suggested that DFP and DFO might act differently on unloading liver and cardiac iron. Probably due to the higher ability of DFO to interact with hepatocellular iron, DFO facilitates the biliary excretion of liver iron. In contrast, DFP enters LCI pools in myocytes and extracts iron **(42)**. A randomized placebo control study demonstrated that combination therapy of standard doses of DFP and DFO combination has been more effective than standard DFO monotherapy in decreasing liver iron, removing cardiac iron, and improving LVEF **(43)**.

DFX monotherapy at a once-daily dose of 20 mg/kg/d and 30 mg/kg/d led to maintenance and a significant reduction in LIC, respectively **(44)**. However, higher tolerable doses of DFX up to 40 mg/kg/d are required to allow faster removal of iron overload **(45)**. A randomized study in severely iron-overloaded patients with cardiac siderosis (cT2* 6-20 ms) demonstrated non-inferiority of DFX 40 mg/kg/d compared to DFO (40.7 mg/kg/d normalized to a 7-day regimen) in cardiac iron removal

during which LVEF remained unchanged **(30)**. An insight gained from DFX studies was that cardiac iron clearance was less in patients with a higher LIC and increased once liver iron was lowered **(46)**. Dividing the once-daily DFX dose into two doses might provide a more homogenous suppression of labile iron species and extract more cardiac iron than the once-daily DFX **(28)**. DFX/DFO combination was evaluated in patients with severe liver and cardiac siderosis (mT2* 6–10 ms; LVEF ≥56%), followed by an optional switch to DFX monotherapy when achieving cT2*>10 ms. DFX/DFO combination achieved faster removal of liver iron compared to DFX monotherapy regimens. Although continuous improvement in myocardial siderosis was obtained, and none of the patients developed cardiac failure during the two-year study, the DFX/DFO combination has not shown an advantage over DFX monotherapy in cardiac iron removal rate **(47)**.

Combination chelation in TDT: Although the principle of chelation therapy preferentially should rely on a monotherapy regimen, it may have resulted in a suboptimal therapy due to intolerability, toxicity, resistance, or non-compliance with the given chelator regimen. The patients may fail to achieve iron balance at conventional doses of chelators or may not tolerate higher doses. Combination therapy can be an option for achieving a negative iron balance and would permit a flexible approach to improve compliance and efficacy of the chelation. All three chelator combinations with infinite dosing regimens simultaneously or sequentially on the same day or alternating the drugs on different days can be administered. We have already discussed the clinical efficacies of combination regimens of DFP/DFO and DFX/DFO above **(43, 47)**. However, combining two oral chelators would be more appealing to the patients. A pharmacokinetic study has demonstrated an additive effect of sequential DFX and DFP administration on total iron excretion **(48)**. DFX and DFP might be administered at the highest tolerable doses on the same or alternating days. The clinical experiences confirmed the safety and efficacy of combining DFX and DFP in heavily iron-loaded subjects and were reviewed elsewhere **(49)**.

Chelation therapy in patients with heart failure: The clinical experiences in patients with severe cardiac siderosis (cT2*<6ms) and those with cardiac dysfunction related to myocardial IOL suggested the use of either a combination regimen of DFO and DFP **(50)** or continuous (uninterrupted) intravenous DFO infusion via indwelling catheter **(51)**. American Heart Association strictly recommended immediately commencing 24 hours per day continuous intravenous iron chelation treatment with DFO 50 mg/kg/d and the introduction of DFP 100 mg/kg/d as soon as possible besides cardiac medication **(52)**.

Iron chelation in patients with NTDT

NTDT comprises several thalassemia syndromes that do not require regular blood transfusions for survival, including beta-thalassemia intermedia (TI), deletional HbH disease, and moderate Hb E/beta-thalassemia. Increased iron accumulation resulting from increased gastrointestinal iron uptake and infrequent blood transfusions underlies some of the disease complications or contributes in some way to their severity and may require iron chelation. Gastrointestinal iron absorption in NTDT patients who receive no or only occasional transfusions leads to a continuous but slow accumulation of iron in parenchymal tissues, particularly in the hepatocytes, which becomes evident after 10 and 15 years old in TI and HbH, respectively, and needs to be monitoring. However, siderotic cardiac disease does not seem to be a concern in NTDT.

The experiences with DFO to control iron burden in NTDT are limited. Although clinical experiences have reported that NTDT patients generally well tolerated DFX and DFP, all investigational studies were small, open-label, and single-arm cohorts, limiting their applicability in whole NTDT populations until randomized, double-blind, placebo-controlled trials of DFX **(53)**. DFX approved for the treatment of IOL in NTDT ≥10 years old.

The insights from DFX studies allowed for proposing an algorithm for managing iron overload in NTDT. ICT is started when LIC ≥5 mg Fe/g d.w. and/or SF ≥800 µg/L to reduce levels to LIC of 3 mg Fe/g d.w., or SF of 300 µg/L. Assessment of LIC is advised at SF levels between 300 and 800 µg/L for a more precise decision to start ICT. Starting doses of chelators in NTDT should not be as

high as those administered to TDT patients. The recommended DFX DT and DFX FCT doses for NTDT patients with LIC 5-7 mg Fe/g d.w. are 10 and 7 mg/kg/d, respectively. Higher body iron burden depicted by LIC above 7 mg Fe/g d.w. may require higher DFX doses of up to 20 mg/kg/d for DFX DT and 14 mg/kg/d for DFX FCT **(54, 55)**. The corresponding DFP doses might be 50 mg/kg/d up to 75 mg/kg/d. Dose tailoring by monitoring SF at three monthly intervals is needed for optimizing chelation. ICT should be stopped at LIC of 3 mg Fe/g d.w. or SF of 300 μg/L, as safety data are unavailable to support continued chelation below this level, and re-started once iron parameters reach the threshold **(33)**.

Iron chelation in patients with SCD

Many patients with SCD may require intermittent or chronic red blood cell transfusions. Consequently, SCD patients may display significant systemic and hepatic iron overload. However, extrahepatic iron loading is relatively uncommon, and cardiac T2* values are usually normal, even in patients with severe liver iron burden. Moreover, iron overload toxicity is substantially less in patients with SCD than in patients with thalassemia, even though both populations have equal exposure to blood transfusion and LIC. Nevertheless, monitoring and managing iron overload status remain crucial in routine care for SCD **(12)**.

Because the relation of SF to total iron in individual patients with SCD is poor, only the trends over repeated SF measurements can present a more accurate iron burden. However, LIC monitoring is an optimum measure for managing ICT if available. Cardiac T2* MRI assessment is recommended in patients with a severe liver iron overload, etc. LIC>20 mg Fe/g d.w. All three chelators and their combinations are effective at controlling iron. The principles, including dosing regimens of ICT, are similar to TDT patients **(56)**.

The results of the use of DFO in SCD are comparable to other indications and underline the difficulties with chronic compliance **(57)**. In a randomized 1-year trial of DFX versus DFO in 195 patients with pediatric SCD patients, DFX and DFO were equally effective in reducing the iron burden, and the decrement was related to chelator dose. The safety profile of DFX was not different from previous reports on other populations with transfusional iron overload. In particular, no unexpected adverse events on renal function were noted with DFX in the 1-year core study and five-year follow-up period, suggesting acceptable safety with long-term use under close observation **(58)**. However, DFX may not be well tolerated in older SCD patients, in which complications of the underlying disease are already fully installed. The use of DFX in patients with abnormal renal function, which is not unusual in SCD patients, cannot be recommended **(59)**. The non-inferiority of DFP vs. DFO in 228 patients with SCD, 38% were adults, was shown in a 1-year randomized study. The efficacy and safety profiles of DFP were consistent with those seen in patients with TDT **(60)**. The choice of the ideal iron chelator for this population should include an evaluation of comorbidities and organic dysfunctions and the need to find pharmacogenetic safety markers in this group of patients.

Summary thoughts

The efficacy and safety profiles, advantages, and limitations of conventional iron chelators have been demonstrated with well-designed clinical studies. The choice of ICT and dosing regimen are shaped based on insights gained from these experiences. However, compliance with the given chelator(s) is the fundamental element of optimal iron chelation. Therefore, the patient/family preferences, adverse effects, and lifestyle should be considered when choosing chelator(s). In particular, adverse effects of any chelator emerge as a significant issue for chelator adherence, requiring careful identification. The patients can achieve the most appropriate chelation, which they may comply with if the problem underlying non-compliance is understood correctly and their physician takes the required action. Close monitoring of SF levels, assessing tissue iron burden by MRI, and considering changing body weight in growing children and adults allow the management of iron overload with appropriate chelator dosing regimens and avoid inadequate or over-chelation.

REFERENCES

1. Cohen AR, Glimm E, Porter JB. Effect of transfusional iron intake on response to chelation therapy in β-thalassemia major. *Blood*. 2008; 111(2):583-587. doi:10.1182/blood-2007-08-109306
2. Cappellini MD, Porter J, El-Beshlawy A, et al. Tailoring iron chelation by iron intake and serum ferritin: The prospective EPIC study of deferasirox in 1744 patients with transfusion-dependent anemias. *Haematologica*. 2010;95(4):557-566. doi:10.3324/haematol.2009.014696
3. Cabantchik ZI. Labile iron in cells and body fluids: Physiology, pathology, and pharmacology. *Front Pharmacol*. 2014;5 MAR. doi:10.3389/fphar.2014.00045
4. Di Tucci AA, Matta G, Deplano S, et al. Myocardial iron overload assessment by T2* magnetic resonance imaging in adult transfusion dependent patients with acquired anemias. *Haematologica*. 2008;93(9):1385-1388. doi:10.3324/haematol.12759
5. Garbowski MW, Evans P, Vlachodimitropoulou E, Hider R, Porter JB. Residual erythropoiesis protects against myocardial hemosiderosis in transfusiondependent thalassemia by lowering labile plasma iron via transient generation of apotransferrin. *Haematologica*. 2017;102(10):1640-1649. doi:10.3324/haematol.2017.170605
6. Noetzli LJ, Carson SM, Nord AS, Coates TD, Wood JC. Longitudinal analysis of heart and liver iron in thalassemia major. *Blood*. 2008;112(7):2973-2978. doi:10.1182/blood-2008-04-148767
7. Zanninelli G, Breuer W, Cabantchik ZI. Daily labile plasma iron as an indicator of chelator activity in Thalassaemia major patients. *Br J Haematol*. 2009;147(5):744-751. doi:10.1111/j.1365-2141.2009.07907.x
8. Porter JB, Cappellini MD, Kattamis A, et al. Iron overload across the spectrum of non-transfusion-dependent thalassaemias: role of erythropoiesis, splenectomy and transfusions. *Br J Haematol*. 2017;176(2):288-299. doi:10.1111/bjh.14373
9. Fullmer A, Cortes J, Kantarjian H, Jabbour E. ASH 2009 meeting report - Top 10 clinically oriented abstracts in acute leukemia. *Am J Hematol*. 2010;85(4):277-280. doi:10.1002/ajh.21644
10. Marsella M, Borgna-Pignatti C. Transfusional iron overload and iron chelation therapy in thalassemia major and sickle cell disease. *Hematol Oncol Clin North Am*. 2014;28(4):703-727. doi:10.1016/j.hoc.2014.04.004
11. Ballas SK. Iron overload determines morbidity and mortality in adult patients with sickle cell disease. *Semin Hematol*. 2001;38(1, Suppl 1):30-36. doi:10.1053/shem.2001.20142
12. Badawy SM, Liem RI, Rigsby CK, Labotka RJ, DeFreitas RA, Thompson AA. Assessing cardiac and liver iron overload in chronically transfused patients with sickle cell disease. *Br J Haematol*. 2016;175(4):705-713. doi:10.1111/bjh.14277
13. Aydinok Y. Iron Chelation Therapy as a Modality of Management. *Hematol Oncol Clin North Am*. 2018;32(2):261-275. doi:10.1016/j.hoc.2017.12.002
14. https://www.novartis.com/us-en/sites/novartis_us/files/desferal.pdf. Published online 2022.
15. Aydinok Y, Unal S, Oymak Y, et al. Observational study comparing long-term safety and efficacy of Deferasirox with Desferrioxamine therapy in chelation-naïve children with transfusional iron overload. *Eur J Haematol*. 2012;88(5):431-438. doi:10.1111/j.1600-0609.2012.01769.x
16. Porter JB, Jaswon MS, Huehns ER, East CA, Hazell JWP. Desferrioxamine ototoxicity: evaluation of risk factors in thalassaemic patients and guidelines for safe dosage. *Br J Haematol*. 1989;73(3):403-409. doi:10.1111/j.1365-2141.1989.tb07761.x
17. Hershko C, Rachmilewitz EA. Mechanism of Desferrioxamine-Induced Iron Excretion in Thalassaemia. *Br J Haematol*. 1979;42(1):125-132. doi:10.1111/j.1365-2141.1979.tb03704.x
18. Porter JB, Rafique R, Srichairatanakool S, et al. Recent insights into interactions of deferoxamine with cellular and plasma iron pools: Implications for clinical use. In: *Annals of the New York Academy of Sciences*. Vol 1054. New York Academy of Sciences; 2005:155-168. doi:10.1196/annals.1345.018
19. Davis BA, Porter JB. *Long-Term Outcome of Continuous 24-Hour Deferoxamine Infusion via Indwelling Intravenous Catheters in High-Risk-Thalassemia*.; 2000.
20. Cabantchik ZI, Breuer W, Zanninelli G, Cianciulli P. LPI-labile plasma iron in iron overload. *Best Pract Res Clin Haematol*. 2005;18(2 SPEC. ISS.):277-287. doi:10.1016/j.beha.2004.10.003
21. Aydinok Y, Ulger Z, Nart D, et al. A randomized controlled 1-year study of daily deferiprone plus twice weekly desferoxamine compared with daily deferiprone monotherapy in patients with thalassemia major. *Haematologica*. 2007;92(12):1599-1606. doi:10.3324/haematol.11414
22. Pennell DJ, Berdoukas V, Karagiorga M, et al. Randomized controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. Published online 2006. doi:10.1182/blood
23. Glickstein H, El R Ben, Link G, et al. Action of chelators in iron-loaded cardiac cells: Accessibility to intracellular labile iron and functional consequences.

Blood. 2006;108(9):3195-3203. doi:10.1182/blood-2006-05-020867
24. Badawy SM, Kattamis A, Ezzat H, et al. The safety and acceptability of twice-daily deferiprone for transfusional iron overload: A multicentre, open-label, phase 2 study. *Br J Haematol*. 2022;197(1):e12-e15. doi:10.1111/bjh.17999
25. Tricta F, Uetrecht J, Galanello R, et al. Deferiprone-induced agranulocytosis: 20 years of clinical observations. *Am J Hematol*. 2016;91(10):1026-1031. doi:10.1002/ajh.24479
26. Cohen AR, Galanello R, Piga A, De Sanctis V, Tricta F. Safety and effectiveness of long-term therapy with the oral iron chelator deferiprone. *Blood*. 2003;102(5):1583-1587. doi:10.1182/blood-2002-10-3280
27. Kattamis A, Aydinok Y, Taher A. Optimising management of deferasirox therapy for patients with transfusion-dependent thalassaemia and lower-risk myelodysplastic syndromes. *Eur J Haematol*. 2018;101(3):272-282. doi:10.1111/ejh.13111
28. Otto-Duessel M, Aguilar M, Nick H, Moats R, Wood JC. Comparison of twice-daily vs once-daily deferasirox dosing in a gerbil model of iron cardiomyopathy. *Exp Hematol*. 2007;35(7):1069-1073. doi:10.1016/j.exphem.2007.04.001
29. Lu MY, Wang N, Wu WH, et al. Simultaneous Determination of Plasma Deferasirox and Deferasirox-Iron Complex Using an HPLC-UV System and Pharmacokinetics of Deferasirox in Patients with β-Thalassemia Major: Once-daily Versus Twice-daily Administration. *Clin Ther*. 2015;37(8):1751-1760. doi:10.1016/j.clinthera.2015.05.506
30. Pennell DJ, Porter JB, Piga A, et al. A 1-year randomized controlled trial of deferasirox vs deferoxamine for myocardial iron removal in b-thalassemia major (CORDELIA) on behalf of the CORDELIA study investigators. *Blood*. 2014;123(10):1447-1454. doi:10.1182/blood-2013-04- 497842
31. Fisher SA, Brunskill SJ, Doree C, Chowdhury O, Gooding S, Roberts DJ. Oral deferiprone for iron chelation in people with thalassaemia. *Cochrane Database Syst Rev*. 2013;2013(8). doi:10.1002/14651858.CD004839.pub3
32. Taher A, El-Beshlawy A, Elalfy MS, et al. Efficacy and safety of deferasirox, an oral iron chelator, in heavily iron-overloaded patients with β-thalassaemia: the ESCALATOR study. *Eur J Haematol*. 2009;82(6):458-465. doi:10.1111/j.1600-0609.2009.01228.x
33. Aydinok Y, Kattamis A, Viprakasit V. Current approach to iron chelation in children. *Br J Haematol*. 2014;165(6):745-755. doi:10.1111/ bjh.12825
34. Jobanputra M, Paramore C, Laird SG, McGahan M, Telfer P. Co-morbidities and mortality associated with transfusion-dependent beta-thalassaemia in patients in England: a 10-year retrospective cohort analysis. *Br J Haematol*. 2020;191(5):897-905. doi:10.1111/bjh.17091
35. Kwiatkowski JL. Clinical Challenges with Iron Chelation in Beta Thalassemia. *Hematol Oncol Clin North Am*. 2023;37(2):379-391. doi:10.1016/j.hoc.2022.12.013
36. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica*. 2004;89(10):1187-1193.
37. Modell B, Khan M, Darlison M. *Survival In-Thalassaemia Major in the UK: Data from the UK Thalassaemia Register*. Vol 355.; 2000.
38. Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*. 2008;10(1):1-8. doi:10.1186/1532-429X-10-42
39. Peng CT, Wu KH, Wu SF, et al. Deferiprone or deferoxamine vs. combination therapy in patients with β-thalassemia major: A case study in Taiwan. In: *Hemoglobin*. Vol 30. ; 2006:125-130. doi:10.1080/03630260500455581
40. El-Beshlawy A, Manz C, Naja M, et al. Iron chelation in thalassemia: Combined or monotherapy? The Egyptian experience. *Ann Hematol*. 2008;87(7):545-550. doi:10.1007/s00277-008-0471-2
41. Wonke, Wright, Hoffbrand. Combined therapy with deferiprone and desferrioxamine. *Br J Haematol*. 1998;103(2):361-364. doi:10.1046/j.1365-2141.1998.01002.x
42. Link G, Konijn AM, Breuer W, Cabantchik ZI, Hershko C. Exploring the “iron shuttle” hypothesis in chelation therapy: Effects of combined deferoxamine and deferiprone treatment in hypertransfused rats with labeled iron stores and in iron-loaded rat heart cells in culture. *J Lab Clin Med*. 2001;138(2):130-138. doi:10.1067/mlc.2001.116487
43. Tanner MA, Galanello R, Dessi C, et al. A randomized, placebo-controlled, double-blind trial of the effect of combined therapy with deferoxamine and deferiprone on myocardial iron in thalassemia major using cardiovascular magnetic resonance. *Circulation*. 2007;115(14):1876-1884. doi:10.1161/CIRCULATIONAHA.106.648790
44. Cappellini MD. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients

with beta-thalassemia. *Blood*. 2006;107(9):3455-3462. doi:10.1182/blood-2005-08-3430

45. Taher A, Elalfy MS, Al Zir K, et al. Importance of optimal dosing ≥30mg/kg/d during deferasirox treatment: 2.7-yr follow-up from the ESCALATOR study in patients with β-thalassaemia. *Eur J Haematol*. 2011;87(4):355-365. doi:10.1111/j.1600-0609.2011.01662.x
46. Wood JC, Kang BP, Thompson A, et al. The effect of deferasirox on cardiac iron in thalassemia major: impact of total body iron stores. *Blood*. 2010;116(4):537-543. doi:10.1182/blood-2009-11-250308
47. Aydinok Y, Kattamis A, Cappellini MD, et al. Effects of deferasirox-deferoxamine on myocardial and liver iron in patients with severe transfusional iron overload. *Blood*. 2015;125(25):3868-3877. doi:10.1182/blood-2014-07-586677
48. Lin CH, Chen X, Wu CC, et al. Therapeutic mechanism of combined oral chelation therapy to maximize efficacy of iron removal in transfusion-dependent thalassemia major–a pilot study. *Expert Rev Hematol*. 2019;12(4):265-272. doi:10.1080/17474086.2019.1593823
49. Aydinok Y. Combination chelation therapy. 2023;(Lci):1-9. doi:10.1111/nyas.15052
50. Tanner MA, Galanello R, Dessi C, et al. Combined chelation therapy in thalassemia major for the treatment of severe myocardial siderosis with left ventricular dysfunction. *J Cardiovasc Magn Reson*. 2008;10(1). doi:10.1186/1532-429X-10-12
51. Anderson LJ, Westwood MA, Holden S, et al. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: A prospective study using T2* cardiovascular magnetic resonance. *Br J Haematol*. 2004;127(3):348-355. doi:10.1111/j.1365-2141.2004.05202.x
52. Pennell DJ, Udelson JE, Arai AE, et al. Cardiovascular function and treatment in β-thalassemia major: A consensus statement from the american heart association. *Circulation*. 2013;128(3):281-308. doi:10.1161/CIR.0b013e31829b2be6
53. Taher AT, Viprakasit V, Musallam KM, Cappellini MD. Treating iron overload in patients with non-transfusion-dependent thalassemia. *Am J Hematol*. 2013;88(5):409-415. doi:10.1002/ajh.23405
54. Taher AT, Porter J, Viprakasit V, et al. Deferasirox reduces iron overload significantly in nontransfusion-dependent thalassemia: 1-Year results from a prospective, randomized, double-blind, placebo-controlled study. *Blood*. 2012;120(5):970-977. doi:10.1182/blood-2012-02-412692
55. Taher AT, Cappellini MD, Aydinok Y, et al. Optimising iron chelation therapy with deferasirox for non-transfusion-dependent thalassaemia patients: 1-year results from the THETIS study. *Blood Cells, Mol Dis*. 2016;57:23-29. doi:10.1016/j.bcmd.2015.11.002
56. Coates TD, Wood JC. How we manage iron overload in sickle cell patients. *Br J Haematol*. 2017;177(5):703-716. doi:10.1111/bjh.14575
57. Lucania G, Vitrano A, Filosa A, Maggio A. Chelation treatment in sickle-cell-anaemia: Much ado about nothing? *Br J Haematol*. 2011;154(5):545-555. doi:10.1111/j.1365-2141.2011.08769.x
58. Vichinsky E, Bernaudin F, Forni GL, et al. Long-term safety and efficacy of deferasirox (Exjade®) for up to 5years in transfusional iron-overloaded patients with sickle cell disease. *Br J Haematol*. 2011;154(3):387-397. doi:10.1111/j.1365-2141.2011.08720.x
59. Ribeiro LB, Soares EA, Costa FF, Gilli SCO, Olalla Saad ST, Benites BD. The challenges of handling deferasirox in sickle cell disease patients older than 40 years. *Hematol (United Kingdom)*. 2019;24(1):596-600. doi:10.1080/16078454.2019.1657667
60. Kwiatkowski JL, Hamdy M, El-Beshlawy A, et al. Deferiprone vs deferoxamine for transfusional iron overload in SCD and other anemias: a randomized, open-label noninferiority study. *Blood Adv*. 2022;6(4):1243-1254. doi:10.1182/bloodadvances.2021004938

Correspondence Address:
Prof. Dr. Yesim Aydınok
Ege University School of Medicine, Department of Pediatric Hematology & Oncology, Izmir, Türkiye
E-mail: yesim.aydinok@yahoo.com

Table 1: *Overview of iron chelating agents*

Chelator-iron (III) complex, MW, charge	Hexadentate, 1:1 559, charged	Bidentate, 3:1 139, uncharged	Tridentate, 2:1 373, uncharged
Usual dose, route	20–60 mg/kg/day, s.c., i.v.	75–100 mg/kg/day p.o.	^{DT}10–40, ^{FCT}7–28 mg/kg/day p.o.
Administration	8–12 h, 5–7 days/week	2^{SR}–3^{IR} times daily	once daily
Half-life	20–30 min	2–3 h	8–16 h
Excretion	Urinary, fecal	Urinary	Fecal
Hydro/lipophilic	Hydrophilic	Lipophilic	Lipophilic
Depletion of LPI	yes (continuous infusion)	yes (rebound between doses)	yes
Removal of hepato-cellular iron	yes	less impressive	yes
Experimental; Accessing LCI pool	slow penetrance	rapidly penetrate and bound	rapidly penetrate and bound
Clinical; Extracting LCI (cardiomyocytes)	benefits of 24h i.v.	higher than standard DFO	comparable to DFO
Dose-dependent adverse effects	Local reactions, Hearing loss, Retinopathy, Growth retardation	GI symptoms (nausea, vomiting, abdominal pain), Arthritis/Arthropathy, Low plasma zinc	GI symptoms (nausea, vomiting, abdominal pain, diarrhea), Skin rash, Serum creatinine increase/Proteinuria
Not dose-dependent adverse effects	Hypersensitivity, Yersinia infections	Transaminitis, Neutropenia, Agranulocytosis, Increased appetite	Transaminitis, Fanconi syndrome
Limitations	Poor adherence to demanding parenteral use	May fail to achieve iron balance	Intolerability

Abbreviations: DT, dispersible tablet; FCT, film coated tablet; SR; slow release, IR; immediate release; DFO, deferoxamine; GI, gastrointestinal; LPI; labile plasma iron; LCI, labile cell iron; MW, molecular weight; i.v., intravenous; p.o., by mouth; s.c., subcutaneous

CHAPTER 7
THALASSEMIA INTERMEDIA

THE CLINIC OF THALASSEMIA INTERMEDIA

Prof. Dr. Mehran Karimi MD
Professor of pediatric Hematologist-Oncologist, American Hospital Dubai, Dubai, UAE

ABSTRACT

Beta-thalassemia intermedia (β-TI) is a genetic variant of beta-thalassemias with a clinical disorder whose severity falls between thalassemia minor and thalassemia major. Different genetic defects are detected in B-TI patients and based on severity of disease, clinical complications like skeletal deformities, growth retardation, splenomegaly, extramedullary hematopoiesis, heart failure and thrombosis may be present in untreated patients. Timely diagnosis and management are essential in these patients for prevention of later clinical complications. Diagnosis of B-TI is based on clinical manifestations and laboratory data. There are some treatment strategies like modulation of gamma globulin chain production with hydroxyurea, transfusion, splenectomy, novel pharmacological agents attempted to ameliorate B-TI by targeting erythropoiesis impairments via different mechanisms that include activin II receptor traps (Luspatercept), Janus-associated kinase 2 (JAK2) inhibitors (Ruxolitinib), Iron metabolism regulation (mini Hepcidin), gene editing/therapy, and stem cell transplantation. Iron chelation therapy is also needed in many of these patients even if they are not transfused. The aim of this manuscript is to review the clinical manifestations and unmet treatments needs in B-TI.

Keywords: Clinical manifestations, Beta-thalassemia intermediate, management

INTRODUCTION

Beta-thalassemia is a common genetic disorder worldwide which is caused by decreased synthesis of beta-globin chain subunits and subsequent alpha/beta-globin chain imbalance. Accumulation and deposition of free excessive alpha globin chains on the red cells and their precursor's cell membrane caused generation of reactive oxygen species. It damages cellular lipids and proteins in the red cells and their precursors, so that the destruction of these cells by macrophages leads to ineffective erythropoiesis and hemolytic anemia **(1, 2)**. Ineffective erythropoiesis, chronic hemolytic anemia, and iron overload are the main pathophysiologic complications in beta-thalassemia intermedia (β-TI). Beta-thalassemias are diverse groups of disease based on a wide spectrum of clinical phenotypes. The clinical phenotypes usually include asymptomatic forms of beta-thalassemia minor to severe transfusion dependent of beta-thalassemia major (β-TM). It results from homozygous or compound heterozygous forms of beta-gene mutations, is a severe hemolytic anemia that is usually presented in the first year of life and necessitates regular transfusions and close follow up. β-TI is a form of disease which lies between thalassemia minor and TM based on its clinical phenotypes. It is usually clinically milder than TM and causes mild to severe hemolytic anemia. Although TI is a non-transfusion dependent thalassemia (NTDT) form, some patients occasionally need blood transfusions. They also require careful medical attention like TM to improve quality of life **(3, 4)**.

NTDT is classified into 5 subgroups including B-TI, Sickle/thalassemia, Hb H disease, Hb sickle/D and Hb E/B thalassemia but the first three diseases are most seen in the middle east region.

EPIDEMIOLOGY OF B-TI

Recent estimates suggest that approximately 68,000 children are born with various forms of thalassemia each year, nearly 23,000 of these children will have B-TM. A smaller, ill-defined number of these individuals will have a milder, non-

transfusion-dependent condition. As the thalassemia challenge is considered a hazard to worldwide health, it is supposed that the global outbreak of the disease may change significantly. The epidemiology of B-TI is also altering; its reason could be changes in migration patterns **(5, 6)**.

DEFINITION AND CLINICAL MANIFESTATIONS OF B-TI

The term TI is clinically descriptive of beta-thalassemic patients whose clinical manifestations are not as mild as thalassemia minor or as severe as TM **(5)**. TI was initially described by Rietti Greppi Micheli in 1955. He described a thalassemic patient with clinical phenotype between thalassemia minor and TM **(7)**.

Therefore, the patient's clinical status evaluation is more important for controlling the disease's detrimental effects and better apprehending the mysterious aspects of its pathophysiology for extending patients' longevity and quality of life **(6)**.

TI is associated with a wide clinical spectrum presentation from mild to severe hemolytic anemia and can be divided into two subgroups:

1- Some patients are mildly affected leading to mild clinical problems until adult life. These patients maintain hemoglobin levels between 7 and 11 gr/dL and are usually transfusion independent or rarely require blood transfusions.

2-Patients with more severe anemia who generally present at ages 2–6 years old. They frequently develop clinical symptoms such as skeletal deformities and growth retardation **(5, 8-9)** if they do not manage appropriately including occasional blood transfusion. Sometimes they may need regular blood transfusions. TI and TM have some overlap in their clinical presentations but differentiation of the two disorders is essential for optimal care and prevention of their later complications. TI can present with pallor, jaundice, anemia, splenomegaly, or skeletal deformities during childhood or later. Diagnosis of TI is usually made after the age of 2 years with initial Hb levels of 7 gr/dL or more in patients with beta-thalassemia who are free of infection and have adequate folic acid. One of their parents is also an atypical carrier of beta-thalassemia such as normal or borderline HbA2 or isolated increased HbF (usually up to 10%) **(5-8)**.

The patients are usually presented with microcytic-hypochromic anemia (low MCV and low MCH) and the peripheral smear shows mild to severe microcytosis, hypochromia, anisopoikilocytosis, polychromasia, target cell, basophilic stippling, and nucleated RBC (NRBC). Hb electrophoresis includes: HbA: up to 80%; HbA2: normal or up to 7%, HbF: > 10%. Serum iron, serum ferritin and transferrin saturation may be increased.

Differential diagnosis between TI and TM is essential because the first step for management of patients with TI is usually not transfusion; but the first choice of TM management is blood transfusion. Table 1 shows some differentiating parameters between TI and TM **(5)**.

Most TI patients are homozygotes or compound heterozygotes for beta-thalassemia. Less commonly only one beta-globin gene is affected **(10, 11)**. The different phenotypes of patients with TI arise from different gene defects that cause a mild to severe alpha/beta-globin chains imbalance **(Table 1)**.

Homozygous or compound heterozygous of beta-thalassemia with heterocellular hereditary persistence of fetal hemoglobin, some forms of delta/beta-thalassemia and Xmn-I polymorphism are related to higher level of gamma-globin chain production and ameliorate the clinical course of betathalassemia **(5, 12)**.

Table 1: *Differentiation Between Thalassemia Intermedia and Thalassemia Major* [5]

Clinical manifestations	Thalassemia intermedia	Thalassemia major
Age of presentation (years)	>2	< 2
Splenomegaly	Moderate to severe	Mild in case of optimal management
Transfusion	Nondependent or occasionally	Dependent
Pallor, jaundice	Usually yes	No or mild if optimal management
Skeletal deformities	Usually yes	No or mild if optimal management
Type of mutation: - Coinheritance of alpha-thalassemia, - Hereditary persistence of fetal hemoglobin, - Delta-thalassemia, - XmnI polymorphism	Yes	No
Genetic and molecular characteristics of parents	One or both are atypical carriers of beta-thalassemia minor*	Both are typical carriers of beta-thalassemia minor
Hemoglobin levels (g/dL)	≥6–7	<6–7
Mean cell volume (MCV)	Decreased	Normal, if optimal management
Nucleated red blood cells (NRBC)	Increase	Normal, if optimal management
White blood cell (WBC)	Increase (they are NRBC, not real WBC)	Normal, if optimal management

***** Normal or borderline HbA2 or isolated increased HbF (usually up to 10%) or normal HbA2/normal HbF but low MCV/MCH

MANAGEMENT OF TI

1-Transfusion Therapy

Blood transfusion is not a routine treatment plan in all TI patients, but it is an essential treatment option in some situations **(Table 2)**. Occasional transfusion should be done in pregnancy, surgery, and infections. More frequent transfusions should be considered in patients with a declining hemoglobin level in parallel with profound enlargement of the spleen, patients with growth failure, poor performance at school, diminished exercise tolerance, and secondary sexual developmental delay in correlation with bone age **(5)**. Moreover, signs of bone changes, and poor quality of life should prompt initiation of blood transfusion therapy. Transfusion may also be considered for the primary prevention, management or secondary prevention of patients having thrombotic or cerebrovascular disease, patients with pulmonary hypertension (PHT) with or without secondary heart failure, extramedullary hematopoiesis (EMH), and leg ulcers. Alloimmunization to red cell antigens in TI patients is more common than in TM and may be related to the later age at which transfusions are started. Alloimmunization is also more common in splenectomized patients **(5, 13-14)**. Pretransfusion red cell antigen matching, particularly for the Rh and Kell/E systems, is recommended since Anti-E and anti-K antibodies are the most common antibodies relevant to alloimmunization **(6)**.

Table 2: *Indications of Blood Transfusion in Thalassemia Intermedia (5)*

Regular transfusion	Occasional transfusion
Pulmonary hypertension (PHT)	Decrease exercise tolerance
Congestive heart failure (CHF)	Cord compression
Persistent symptomatic severe anemia (Hb < 6–7 gr/dL) leading to persistent growth retardation/failure	Thrombosis
Progressive skeletal deformities	Pregnancy
Progressive pathologic fracture	Post infection severe anemia (Hb < 6–7 gr/dL)
Patient/legal guardian request	Leg ulcer

2-Iron Chelation Therapy

Iron overload in TI is frequently derived from increased intestinal iron absorption and/or transfusion therapy. Chronic hemolysis, ineffective erythropoiesis, and hypoxia may lead to increased intestinal iron absorption through suppression of the regulatory protein hepcidin **(15)**. Until recently, the cutoff for starting chelation was an LIC of 7 and above but a recent study showed that complications were more likely to occur at an LIC of 7 Fe/g dry weight and above, thus the need to start chelation patients earlier **(16)**. Data from the first and largest randomized clinical trial of the iron chelator deferasirox in 166 patients (THALASSA) **(44)** showed that deferasirox therapy causes a significant reduction in liver iron concentration (LIC) compared to patients on placebo, following 1 year therapy in patients above the age of 10 and with a baseline iron concentration of more than 5 mg Fe/g dry weight. The Thalassemia International Federation (TIF) recommends initiating iron chelation therapy corresponding to a ferritin level of above 800 ng/mL and an LIC of 5 mg Fe/g dry weight or above. Suspension of therapy should be initiated when serum ferritin level is 300ng/ml corresponding to an LIC level of 3 mg Fe/g dry weight or less. The TIF also recommends that all patients above the age of 10 be frequently evaluated for iron overload by LIC at 1–2-year intervals along with serial measurements of serum ferritin every 3 months **(5)**. If the serum ferritin level is stable and low (<500.0 ng/mL), it is not necessarily due to regular iron chelator drug therapy **(6)**.

3-Hydroxyurea (HU) therapy

Increased gamma-globin chain production might ameliorate some of the major clinical manifestations in TI patients by increasing the production of HbF and reducing the alpha/beta-globin chain imbalance **(5, 6)**. One of the safe and effective known drugs to enhance gamma-globin chain production is HU. The results show that a significant number of transfusion-dependent TI patients became transfusion free or needed only occasional transfusions as well as significant increases of Hb levels in not transfused patients with a dose of 8-15 mg/kg/day **(6, 17-18)**.

Long-term observation reveals no significant adverse effects in these patients who are on HU such as malignancy, infertility, or bone marrow suppression when used at a dose of 8–15 mg/kg/day **(19-21)**.

4-Splenectomy

splenomegaly is one of the common manifestations of B-TI. Splenectomy in patients with B-TI is sometimes necessary due to complications such as frequent infection, bleeding stemming from chronic leukopenia or thrombocytopenia, pain mediated by splenomegaly, and reducing the risk of spleen rupture. It should be considered that surgical intervention is not possible for patients less than 5 years old. However, splenectomy has side effects, including thrombosis, sepsis, and infection. However, our plan is to preserve the spleen as much as possible in B-TI patients. Using appropriate preventive measures, antibiotics, given immunization, and

thromboprophylaxis to restrain the thrombosis events in patients with B-TI after splenectomy are recommended **(5, 6, 22)**.

5- Bone marrow transplantation (BMT)

It is not usually recommended in patients with TI, although if these patients are transfusion dependent, it may be considered. Therefore, patients with severe B-TI may need BMT similar to patients with B-TM, and it is an appropriate intervention if a suitable donor is available **(6)**.

6- Advanced therapeutic approaches

- Gene editing/ therapy by inducing Hb F:

Heredity persistence of fetal Hb (HPFH) is associated with an intense pancellular level of Hb F in adulthood, which is considered as a 'benign' condition in which patients with HPFH mutations present milder forms of this disease. Therefore, gene therapy/editing through inducing Hb F can be an ideal option to treat thalassemia patients, even in NTDT patients. Genome therapy is a novel strategy for improving hemoglobinopathies, especially B-TI by using (CRISPR)/Cas9 procedure.

Moreover, gene therapy was approved by FDA in 2022 by using ZYNTEGLO (betibeglogene autotemcel), the first cell-based gene therapy for the treatment of adult and pediatric patients with β-thalassemia who require regular red blood cell (RBC) transfusions **(24)**.

However, long term safety and efficacy is needed to follow this new therapeutic approach.

- Luspatercept for B-TI patients

The drug Luspatercept has been introduced for the treatment of sickle cell disease and B-TI patients. Luspatercept is a novel recombinant fusion protein that is composed of the modified extracellular domain of the human activin IIB receptor joined to a constant region of human IgG1. Luspatercept selectively traps GDF-11, GDF-8, and activin b; consequently, TGF-b signaling is considerably suppressed through Smad2/3 blockade. In addition, it can adjust erythropoiesis by increasing and improving the quality of mature RBCs. The FDA and European Medicine Agency (EMA) approved Luspatercept in 2020, and the recommended starting dose of the drug as 1 mg/kg once every 3 weeks by subcutaneous injection for patients with B-thalassemia **(6, 25)**.

-Other novel treatments

Janus-associated kinase 2 (JAK2) inhibitors such as Ruxolitinib and Iron metabolism regulation such as mini Hepcidin have been investigated in thalassemia patients, but they are not approved yet in B-TI **(26, 27)**.

CONCLUSION

The therapeutic approach to B-TI is still challenging. Genome therapy technologies are among the top therapeutic strategies for hemoglobinopathies, particularly B-TI, that gene correction and regulation mechanisms lead to increasing Hb expression levels. Although genome editing strategies are promising for ameliorating B-TI, their challenges could not be ignored. Thus, genome editing technologies need to promote their safety and efficacy in future.

Although the clinical manifestations of TI are usually milder than TM, the prognosis and the rate of complications are often worse. Right-sided heart failure due to long-standing PHT, thrombosis and brain ischemia, EMH in some vital regions like spinal cord, and iron overload are major and life-threatening complications of B-TI. Close follow-up and monitoring of these patients for prevention of such complications and improved health-related quality of life (HRQoL) are recommended.

REFERENCES

1. Olivieri NF. The β-thalassemias. N Engl J Med. 1999; 341:99-109.
2. Rund D, Rachmilewitz E. β-Thalassemia. N Engl J Med. 2005; 353:1135–1146.
3. Haddad A, Tyan P, Radwan A, Mallat N, Taher A. β-Thalassemia intermedia: A bird's-eye view. Turk J Haematol. 2014;31(1):5–16.
4. Taher AT, Musallam KM, Karimi M, et al. Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a re-

gion of endemicity: the OPTIMAL CARE study. Blood. 2010;115(10):1886–1892.
5. Karimi M, Cohan N, De Sanctis V, Mallat NS,3 and Taher A. Guidelines for Diagnosis and Management of Beta-Thalassemia Intermedia. Pediatric Hematology and Oncology, 31:583–596, 2014.
6. Saeidniaa M, Fazelib P, Arghavan Farzid A, Nezhade A, Shabani-Borujenif M, Erfanig M, Tamaddonh G and Karimi M. An Expert Q4 Overview on Therapies in Non-Transfusion-Dependent Thalassemia: Classical to Cutting Edge in Treatment. HEMOGLOBIN https://doi.org/10.1080/03630269.2022.2158099.
7. Taher A, Isma'eel H, Cappellini MD. Thalassemia intermedia: Revisited. Blood Cells Mol Dis. 2006; 37:12–20.
8. El Rassi F, Cappellini MD, Inati A, Taher A. Beta-thalassemia intermedia: An overview. Pediatric Annals. 2008;37(5):322–328.
9. Weatherall DJ. Thalassemia intermedia: cellular and molecular aspects. J Hematol. 2001;86(Suppl 1):186–188.
10. Weatherall DJ. Phenotype-genotype relationships in monogenic disease: lessons from the thalassaemias. Nature Reviews Genetics. 2001; 12:245–255.
11. Moorchung N, Arya V, Agarwal S. Modifier genes and beta thalasaemia intermedia. Haema. 2006; 9:505–510.
12. Aessopos A, Kati M, Meletis J.Thalassemia intermedia today: should patients regularly receive transfusions? Transfusion. 2007; 47:792–800.
13. Karimi M, Nikrooz P, Kashef S, et al. RBC alloimmunization in blood transfusion-dependent beta thalassemia patients in southern Iran. Int J Lab Hematol. 2007;29(5):321–326.
14. Spanos T, KarageorgaM, Ladis V, et al. Red cell alloantibodies in patients with thalassemia.Vox Sang. 1990; 58:50–55.
15. Taher AT, Porter JB, Viprakasit V, et al. Deferasirox effectively reduces iron overload in non-transfusion-dependent thalassemia (NTDT) patients: 1-year extension results from the THALASSA study. Ann Hematol. 2013;92(11):1485–1493.
16. Musallam KM, Cappellini MD, Wood JC, et al. Elevated liver iron concentration is a marker of increased morbidity in patients with beta thalassemia intermedia. Haematologica 2011; 96(11): 1605–1612.
17. Karimi M, Darzi H, Yavarian M. Hematologic and clinical responses of thalassemia intermedia patients to hydroxyurea during 6 years of therapy in Iran. J Ped Hematol Oncol. 2005; 27:380–385.
18. Dixit A, Chatterjee TC, Mishra P, et al. Hydroxyurea in thalassemia intermedia–a promising therapy. Ann Hematol. 2005; 84:441–446.
19. Karimi M, Cohan N, Moosavizadeh K, et al. Adverse effects of hydroxyurea in β-thalassemia intermedia patients: 10 years' experience. Ped Hematol Oncol. 2010; 27:205–211.
20. Karimi M, Haghpanah S, Farhadi A, Yavarian M. Genotype-phenotype relationship of patients with β-thalassemia taking hydroxyurea: a 13-year experience in Iran. Int J Hematol. 2012;95(1):51–56.
21. Karimi M, Zekavat OR, Haghpanah S, et al. Comparative study of hypogonadism in beta-thalassemia intermedia patients with and without hydroxyurea. Hematology. 2012;17(2):122–124.
22. Taher A, Vichinsky E, Musallam K, et al. editors. Guidelines for the management of Non-Transfusion Dependent Thalassaemia (NTDT). Nicosia (Cyprus): Thalassaemia International Federation; 2013.
23. Cai L, Bai H, Mahairaki V, et al. A universal approach to correct various HBB gene mutations in human stem cells for gene therapy of beta-thalassemia and sickle cell disease. Stem Cells Transl Med. 2018;7(1):87–97.
24. ZYNTEGLO [prescribing information], Somerville, MA; bluebird bio, Inc.; August 2022.
25. Taher AT, Cappellini MD. Luspatercept for b-thalassemia: beyond red blood cell transfusions. Expert Opin Biol Ther. 2021;21(11):1363–1371.
26. Taher AT, Karakas Z, Cassinerio E, et al. Efficacy and safety of ruxolitinib in regularly transfused patients with thalassemia: results from a phase 2a study. Blood. 2018;131(2):263–265.
27. Casu C, Chessa R, Liu A, et al. Minihepcidins improve ineffective erythropoiesis and splenomegaly in a new mouse model of adult b-thalassemia major. Haematologica. 2020;105(7): 1835–1844.

Correspondence Address:
Prof. Dr. Mehran Karimi MD
Professor of pediatric Hematologist-Oncologist, American Hospital Dubai, Dubai, UAE
E-mail: mkarimi820@gmail.com

THE COMPLICATIONS OF THALASSEMIA INTERMEDIA

Prof. Dr. Zeynep Karakas
Istanbul University, Istanbul Medical Faculty, Department of Pediatrics, Divisiion of Hematology/Oncology/İstanbul/Türkiye

ABSTRACT

Thalassemia Intermedia (TI) is known as Non-transfusion-dependent thalassemia (NTDT) for patients who do not require regular lifelong transfusions to survive. TI may require intermittent, frequent or even regular transfusion in certain clinical situations and periods such as pregnancy, surgery, infection. On the other hand, in childhood, cases of TI are usually more likely to require regular blood transfusions due to children need more Hb for optimal growth.

Despite high hemoglobin levels and fewer transfusions, most thalassemia-related complications occur more frequently in patients with NTDT than in patients with TDT, because the rarity of blood transfusion stimulates compensatory mechanisms to overcome chronic anemia, causing various complications in patients with NTDT. Although, the chronic anemia, ineffective erythropoiesis and iron load are the same responsible factors, clinical course differ in patients with TDT and NDTD because of regular transfusion decrease most of complications. Patients with NTDT develop specific complications that are rare in TDT such as leg ulcers, gall stones, thrombosis and pulmonary hypertension and usually occur at an older age. Recent studies indicate that patients with NTDT have high rate of complications such as thrombosis, pulmonary hypertension, nonfocal brain infarction, iron overload, spinal cord compression, and decreasing quality of life with age.

Diagnosis, treatment, and follow-up of complications require a multidisciplinary approach involving many subspecialists of pediatric and adult medicine, including pediatric child and adult cardiologists, endocrinologists, and radiologists, under the direction of a hematologist.

Keywords: Thalassemia Intermedia, NTDT, complications

BACKGROUND

Beta-Thalassemia (β-thal) is became a serious worldwide public health problem due to migration. Based on clinical presentations and genotypes, are classified as thalassemia minör, intermedia, or major phenotype. Thalassemia Minor is the heterozygous form of the disease and recogntion of carriers is important for preventing the severe disease. Patients with β-thal Intermedia show a heterogeneous wide spectrum of clinical manifestations, ranging from nearly asymptomatic to severe transfusion required anemia and disease and therapy related complications **(1, 2)**.

In clinical practice, the homozygous form of thalassemias is currently defined related to transfusion requirements (**3)**. Thalassemia Major now defined as Transfusion-dependent thalassemia (TDT) is a condition that patients cannot produce adequate hemoglobin to survive without blood transfusion. Thalassemia Intermedia is also known as Non-transfusion-dependent thalassemia (NTDT) for patients who do not require regular lifelong transfusions to survive. TI may require intermittent, frequent or even regular transfusion in certain clinical situations and periods such as pregnancy, surgery, infection **(3, 4)**. On the other hand, in childhood, cases of TI are usually more likely to require regular blood transfusions due to children need more Hb for optimal growth **(5)**.

NTDT includes clinically three forms: 1. β-thalassemia intermedia (β-TI), 2. hemoglobin E/β-thalassemia (mild and moderate forms), and 3. α-thalassemia intermedia (hemoglobin H disease) **(2-4)**. β-TI is the most common subtype of NTDT. A

large clinical heterogeneity has been shown in Hb E/β-thal syndrome that the interaction of β-thal with Hb E results in milder clinical symptoms known clinically TI **(6)**.

Although patients with NTDT have less severe diseases than those with TDT, it is remains unclear whether these patients have a significant disease burden that affects their quality of life **(7, 8)**. The most important reason for this uncertainty is that the frequency and treatability of disease- and treatment-related complications are very heterogeneous among patients **(9, 10)**.

A multidisciplinary approach is very important in Thalassemia Intermedia, which progresses with many complications throughout life, and requires great patience, patient-doctor cooperation, medical and paramedical information and skills. Diagnosis, treatment and follow-up of complications require subspecialists from many pediatric and adult medicine, including pediatric child and adult cardiologists, endocrinologists, and radiologists, under the direction of a hematologist.

COMPLICATIONS

Ineffective erythropoiesis, which is responsible for skeletal disorders, osteopenia and hepatosplenomegaly, chronic anemia, which is responsible for splenomegaly and growth retardation, and iron overload, which creates a non-transfusion-related iron load in the liver as a result of increased gastrointestinal iron absorption, are the main factors responsible for the complications that develop in NDTD **(11)**.

Despite high hemoglobin levels and fewer transfusions, most thalassemia-related complications occur more frequently in patients with NTDT than in patients with TDT, because the rarity of blood transfusion stimulates compensatory mechanisms to overcome chronic anemia, causing various complications in patients with NTDT **(3, 4, 12)**. Although, the chronic anemia, ineffective erythropoiesis and iron load are the same responsible factors, clinical course differ in patients with TDT and NDTD because of regular transfusion decrease most of complications **(13, 14) (Figure 1)**. With the advances in the understanding of pathophysiology and therapeutic modalities of beta thalassemia Intermedia, there has been a significant improvement in the management and the life expectancy of the patients. Today, there is a different pattern of complications than previously observed in thalassemias because patients are surviving longer. Patients with NTDT develop specific complications that are rare in TDT such as leg ulcers, gall stones, thrombosis and pulmonary hypertension and usually occur at an older age **(13, 15) (Figure 2)**. This has translated into new complications being identified that are associated with increasing age **(16)**.

Recent studies indicate that patients with NTDT have high rate of complications such as thrombosis, pulmonary hypertension, nonfocal brain infarction, iron overload, spinal cord compression, and decreasing quality of life with age **(8, 12, 17, 18)**.

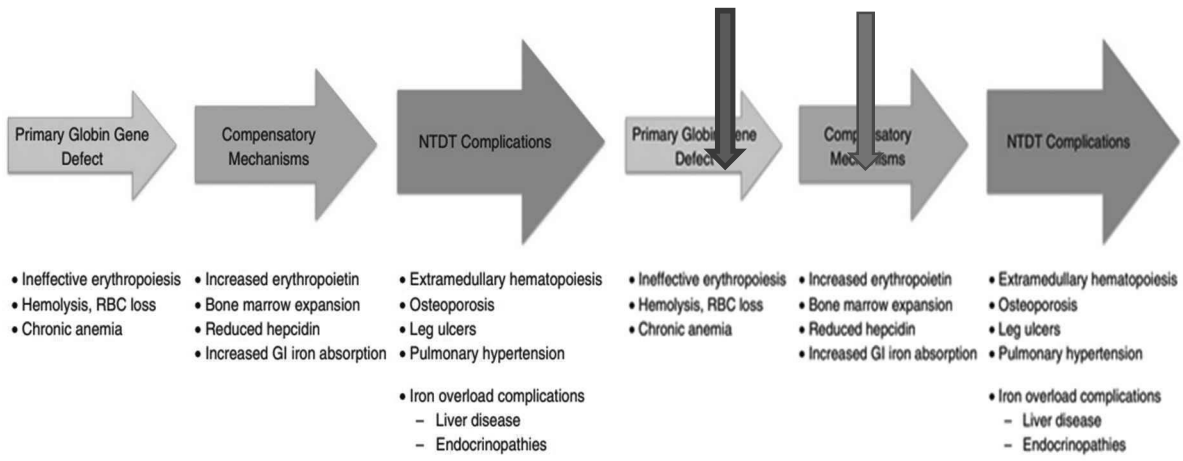


Figure 1: *Pathophysiology of NTDT* [13, 14]

GI, gastrointestinal; NTDT, non-transfusion-dependent thalassemia; RBC, red blood cell
: Transfusion

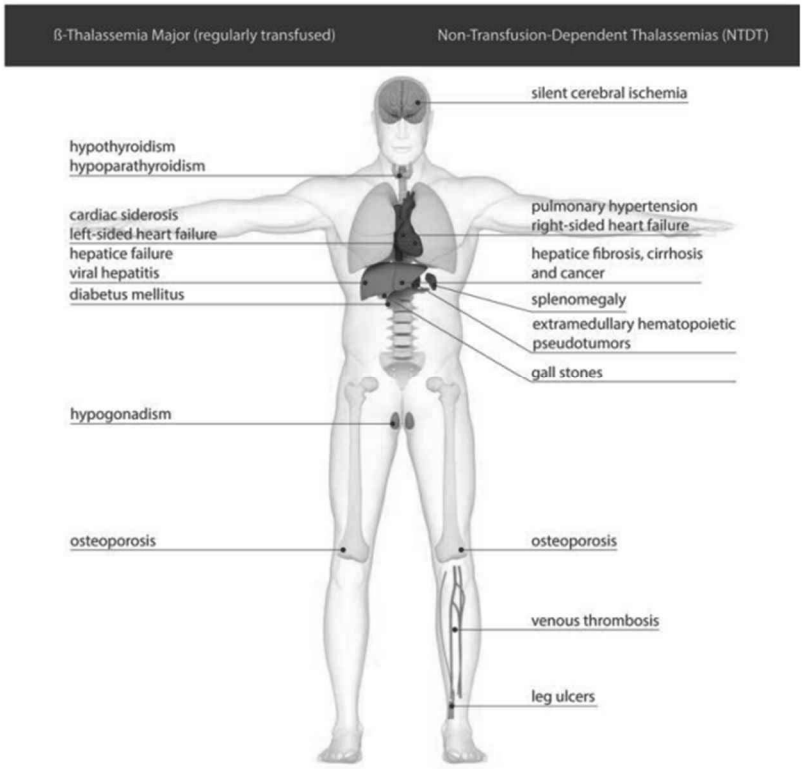


Figure 2: *Common clinical complications profile seen in patients with nontransfusion-dependent thalassemias compared regularly transfused β-thalassemia*

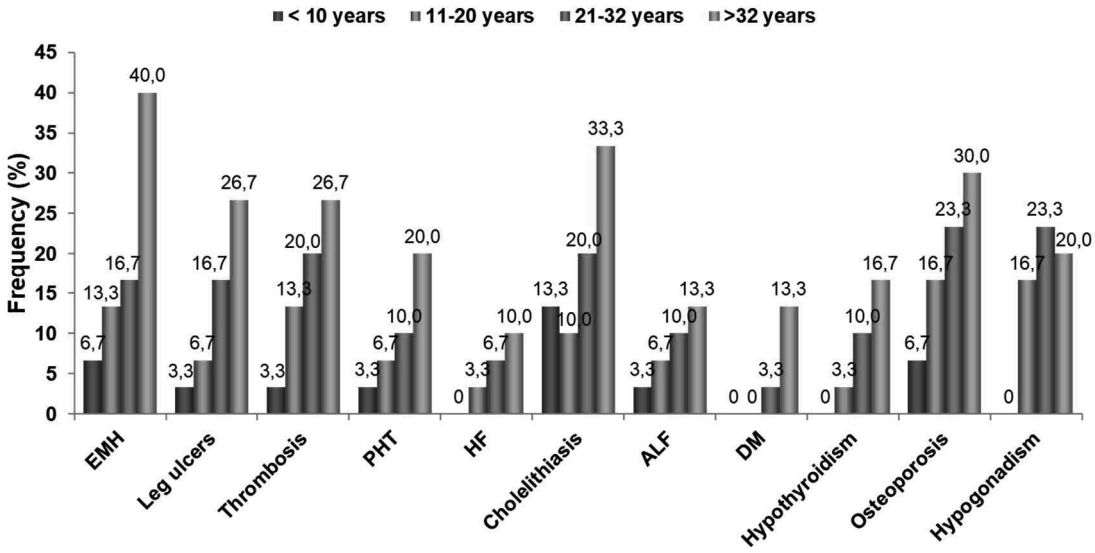


Figure 3: *Age related complications in patients with treatment naive Thalassemia Intermedia*[15]

IRON LOAD

Hemosiderosis occur secondary to increased iron absorption and irregular transfusion. The patients who do not receive transfusions have an iron load rate of approximately 2–5 g/year compared to transfused patients have an iron load of 7.5–15.1 g/year **(10)**. After the cutoff of 5 mg Fe/g dry weight liver iron concentration (LIC), an increase by 1 mg Fe/g dry weight is independently correlate with an in-

creased risk of thrombosis, osteoporosis, pulmonary hypertension (PHT), leg ulcers, endocrinopathies, and other organ injuries **(19, 20)**.

The morbidities associated with iron overload can manifest as early as 10 years of age, even in the absence of blood transfusions; however, they are rare below the age of 10 years **(18)**.

Patients with NTDT are at a specific risk for hepatic fibrosis, cirrhosis, liver failure, and possibly hepatocellular carcinoma due to more hepatic iron deposition **(19, 20)**. Cardiac iron accumulation occurs in NTDT, but at a considerably slower rate than in TDT **(13)**. In contrast, cardiac disease in NTDT is associated with chronic right heart failure secondary to PHT **(21)**. Patients with postsplenectomy have a higher incidence of iron overload-related complications than those who have not undergone splenectomy, suggesting that the spleen may be a reservoir and scavenger of excess iron, including nontransferrin-bound-iron **(11, 12)**.

COAGULOPATHY

The hypercoagulable state in NTDT is associated with a prevalence of thromboembolisms (TE), ranging from 3.9% to 14%. TEs are mostly venous, such as deep vein, pulmonary, portal vein, and cerebral thromboses, but recurrent arterial thrombosis may also occur **(10, 11)**. In the largest epidemiological study, Taher et al. described on 8,860 patients with thalassemia, of whom 2190 had β-TI **(22)**. They reported that TE occurred 4.38 times more frequently in β-TI than in β-TM, with more venous TE in β-TI compared with arterial TE in β-TM. They were more prevalent in patient's post-splenectomy than in patients who had not undergone splenectomy (22.5% vs. 3.5% in patient's post-splenectomy vs. patients not splenectomized). This is likely due to the scavenger effect of the spleen of abnormal RBCs and procoagulant platelets; therefore, its removal can increase the risk of thrombotic events **(11, 21, 22)**. Other risk factors include older age, total hemoglobin level less than 9 gm/dL, platelet count >500 × 109/L, nucleated RBCs >300 × 106/L, low HbF levels, and history of thrombotic events **(11)**.

EXTRAMEDULLARY HEMATOPOEISIS

EMH is almost exclusive to NTDT compared with TDT. EMH is a physiological compensatory phenomenon in which the reactivation of hematopoietic sites from fetal life occurs in the presence of chronic IE **(7, 8, 11)**. This may lead to the formation of erythropoietic tissue masses (hematopoietic pseudotumors) in potentially any site of the body **(10, 11)**. One of the most serious locations is the paraspinal region, accounting for 11–15% of EMH cases, which may cause various neurological symptoms due to spinal cord compression **(11, 23)**. However, it has been hypothesized that paraspinal EMH is asymptomatic in more than 80% of the cases and diagnosed incidentally by radiological imaging. The risk is increased in older age, lower HbF levels, and patients without transfusions **(23)**.

PULMONARY HYPERTENSION

The prevalence of PHT ranged from 4.8% to 59% in patients with NTDT, depending on the cohort studied and the method of diagnosis. Hypercoagulability and hemolysis with subsequent NO and arginine deficiency are factors that may result in vasculopathy and microthrombi development. PHT, the leading cause of right-sided heart failure in NTDT, presents with nonspecific symptoms, such as dyspnea, weakness, and fatigue, which may be difficult to distinguish from those of anemia. Echocardiogram is generally used for diagnosis, which likely overestimates the prevalence, and right heart catheterization is required for definitive diagnosis. The risk is increased with age, post-splenectomy, nucleated RBCs >300 × 106/L, and history of TE. PHT was found less frequent in patients who had previously received transfusions, iron chelation, or hydroxyurea **(11, 21, 24)**.

BONE ABNORMALITIES

Bone abnormalities in NTDT are multifactorial, including sustained IE, subsequent bone marrow expansion, iron overload, and genetic factors **(11)**. The classic skeletal abnormalities, such as facial bone deformities and upper jaw protrusion associat-

ed with thalassemia, are more evident in NTDT compared with those in TDT **(7, 8, 11)**.

High prevalence of low bone marrow density, bone pain, and fractures reported in patients with TDT and NTDT, which increase with age **(25)**. Significantly higher rate of osteoporosis in β-TI showed compared with that in β-TM, whereas the prevalence of osteopenia was lower in β-TI than in β-TM **(26)**. The pooled prevalence of fracture among patients with thalassemia was 16%, with subgroup analysis describing prevalences of 18% and 7% in patients with TDT and NTDT, respectively. The risk of osteoporosis in NTDT increases with female sex, iron overload, splenectomy, and low HbF levels and decreases with hydroxyurea and iron chelation **(11)**.

LEG ULCERS

Leg ulcers were reported to occur in 7.9% of patients with β-TI and up to 22% of patients with hemoglobin E/β-thalassemia **(6, 11)**. There are multiple hypotheses regarding the etiology of leg ulcers, including chronic hypoxia due to anemia, increased oxygen affinity of HbF, hypercoagulable state, and iron overload. Leg ulcers usually manifest in the second decade of life after minor trauma, most commonly on the medial or lateral malleoli. They heal slowly and tend to recur or become chronic, causing pain and disability **(27)**. Increasing age, iron overload, hypercoagulability, and splenectomy are risk factors for the development of leg ulcers **(11, 13, 27)**.

ALLOIMMUNISATION

Alloimmunization is significantly influenced by age since it tends to be more prevalent in older patients than in those who received transfusions when younger **(28)**. A Greek study showed that alloimmunization developed less frequently in patients transfused before the age of 3 years compared with after (20.9% vs. 47.5%, respectively) **(29)**. The rate of alloimmunization in patients with thalassemia differs according to population, which is likely related to the homogeneity of donor and recipient populations. Alloimmunization also increases with the number of units transfused, unfiltered blood, units transfused without extended crossmatch, splenectomy, and during pregnancy **(10, 11)**. It was found that at the same number of transfusions, patients with NTDT had a higher incidence of alloimmunization than patients with TDT **(28)**.

Other complications in NTDT include growth retardation and delayed puberty, severe splenomegaly, gallstones, and decreased quality of life **(4, 11, 30, 31)**. Important issues in the follow-up of NTDT patients who are ≥10 years summarized in Table 1.

Table 1: *Management of specific complications in NTDT* [4, 9]

Complication	Risk, recommendations	Management

Thrombotic disease (including overt/ silent strokes	Higher risk in Adult NTDT: Pregnancy, family history of thrombosis, post-splenectomy, age >35 y, minimal blood transfusion, hemoglobin <10 g/dL, platelets ≥500 × 109/L, nucleated RBC ≥300 × 106 /L, PH, immobility, surgery Transfusion therapy for the primary or secondary prevention of thrombotic or cerebrovascular disease in high-risk patients should be considered.	Consultation with cardiology, neurology, and vascular surgery Antiplatelet therapy: after splenectomy, especially with platelets ≥500 × 109/L Primary and secondary prophylaxis with anticoagulant/ antiplatelet therapy in high-risk patients with NTDT patients Patients who develop thrombosis should be treated per local or international guidelines. Antiplatelet therapy in patients who underwent splenectomy at any platelet count, especially with platelets ≥500 × 10^9/L
Pulmonary hypertension	Lower prevalence among patients with NTDT receiving HU, transfusions, or iron chelation therapy Preventive anticoagulant therapy in at high-risk patients	NTDT patients: annual routine echocardiographic assessment in adults to assess TRV, especially in β-thalassemia intermedia patients with elevated platelet counts (≥500 × 109/L), and history of thrombosis and with post-splenectomy, Referral to a multidisciplinary or specialized PH clinic for treatment Patients with pulmonary hypertension may benefit: # Blood transfusion targeting a pretransfusion hemoglobin of >10 g/dL) # Hydroxyurea # Sildenafil citrate #Adequate control of iron overload status # Anticoagulant therapy

Extramedullary hematopoiesis	NTDT pts with symptoms and signs of spinal cord compression should be promptly evaluated for paraspinal extramedullary hematopoietic pseudotumors, preferably with MRI of the spine. MRI is currently the standard of care for diagnosis of EMH with active pseudotumors showing intermediate signal intensity in both T1- and T2-weighted images Biopsy is not routinely recommended due to the risk of hemorrhage • There is no sufficient evidence to recommend blood transfusion or hydroxyurea therapy to prevent extramedullary hematopoietic pseudotumors in NTDT patients. However, a beneficial effect may be observed when used for different indications.	NTDT pts with paraspinal extramedullary hematopoietic pseudotumors should be promptly managed and followed by a team including a neurologist, neurosurgeon, and radiation specialist Management strategy includes blood transfusion therapy, hydroxyurea, radiotherapy, and/or surgery depending on severity and acuity of symptoms Hypertransfusion for a pretransfusion target hemoglobin >10 g/dL as the cornerstone of management for extramedullary hematopoietic pseudotumors Surgical decompression and radiotherapy offer faster control of paraspinal lesions causing sensory and/or motor deficits; Use of dexamethasone before and after surgery can minimize the risk of exacerbation of tissue edema and worsening of symptoms[4]
Leg ulcers	The skin of NTDT patients should always be inspected on routine physical examination. The following treatment measures may also be considered in patients who have persistent leg ulcers, although no clinical trials to supporting their use exist: # Hydroxyurea # Dialzep (vasodilators) # Oxygen chamber # Skin grafts # Platelet-derived wound healing factors and granulocyte macrophage # Anticoagulation • There is no sufficient evidence to recommend blood transfusion, iron chelation, or hydroxyurea therapy for the prevention of leg ulcers in NTDT patients, although when used for different indications, a beneficial effect may be observed	Referral to dermatology or vascular medicine/surgery for treatment per local standards Keep legs and feet raised above the level of the heart Apply topical antibiotics and occlusive dressing Consider applying topical sodium nitrite cream[4] Consider pentoxifylline, hydroxyurea, and transfusion[4]

Endocrine and bone disease #	Evaluation for growth by standing and sitting height every 6 months, and bone age when needed • In patients who fall off the growth curve (>5%), have decreased height velocity or delayed bone age, perform evaluation of growth hormone stimulation, insulin-like growth factor (IGF)-1 level, IGF-BP3 level, deferoxamine toxicity, and other hormonal tests, Standards for prevention of osteoporosis (behavioral, hormonal, vitamins, and supplements) in patients with NTDT should follow guidelines and recommendations in transfusion-dependent β-thalassemia major patients. nutritional imbalances • Tanner staging annually • In patients with evidence of pubertal delay, perform evaluation of gonadotropin-releasing hormone, luteinizing hormone, follicle-stimulating hormone, testosterone, estradiol, pelvic ultrasound, zinc deficiency, growth retardation, and hypothyroidism Annual screening with # Free thyroxine and thyroid-stimulating hormone # Calcium, phosphate, vitamin D: Annually # Parathyroid hormone: if indicated # Fasting blood sugar: Annually # Oral glucose tolerance test: if indicated # Adrenocorticotropic hormone stimulation test # Bone mineral density spine, hips, radius, ulna (dual-energy X-ray absorptiometry): Annually # Other hormonal and nutritional imbalances • Spine imaging: for back pain or neurological findings	Referral to a pediatric or adult endocrinology department for treatment per local or international guidelines Positive experience with bisphosphonates in thalassemia but lack of supporting data specific to NTDT
Hepatosplenomegaly	Otherwise, avoid splenectomy and treat patients initially with transfusion therapy to avoid the increased risk of thrombosis and infection[4]	Splenectomy is indicated in cases of hypersplenism or symptomatic splenomegaly The use of agents such as ruxolitinib to decrease spleen size is experimental [30,31]
Gallstones		Cholecystectomy if recurrent painful attacks[4]
Liver fibrosis, cirrhosis, and hepatocellular carcinoma	Immunization against hepatitis A and hepatitis B Adequate management of concomitant viral hepatitis, if present (direct-acting antiviral therapy, such as glecaprevir-pibrentasvir, ledipasvir-sofosbuvir, or sofosbuvir-velpatasvir for patients with active hepatitis C);	Referral to hepatology department for treatment per local Standards

Pregnancy	Assess iron overload: Optimize iron overload management before conception Hold oral iron chelation for conception and pregnancy; may switch to deferoxamine if needed during the second and third trimesters especially if worsening iron overload resulting in cardiac dysfunction Hold deferasirox and deferiprone during breastfeeding Monitor serum ferritin levels monthly Evaluate cardiac status: Transthoracic echocardiogram before conception and every trimester Cardiology assessment for PH and risk management Evaluate liver function every trimester Evaluate thyroid function every trimester[4]	Screen for gestational diabetes at 16 and 28 wks. Check calcium and vitamin D; replete as necessary Screen for RBC antibodies before conception Establish viral status (hepatitis B, hepatitis C, and HIV) Provide appropriate immunizations (hepatitis B, pneumococcal vaccine, and influenza) Start folic acid before conception Assess hemoglobin concentration: if <10 g/dL, consider transfusion therapy Consider prophylactic anticoagulation with aspirin or LMWH for women considered at high risk (pre- and/or post-partum) Monitor fetal health: monthly fetal ultrasound[4]

NTDT: non-transfusion-dependent thalassemia; TRV: tricuspid-valve regurgitant jet velocity; HU: hydroxyurea. # LMWH, low molecular- weight heparin; RBC, red blood cell.

CONCLUSION

With NTDT being a disease mostly diagnosed in childhood, the transition from pediatric to adult clinics has many difficulties. Therefore, for optimum follow-up of NTDT patients, the patient should be followed up with a multidisciplinary team including the nurse, the social worker, the pediatric hematologist, the adult hematologist, and many subspecialists (at least pediatric and adult endocrinologist and cardiologist).

REFERENCES

1. Musallam K.M, Cappellini M.D, Viprakasit V, Kattamis A, Rivella S, Taher A.T. Revisiting the non-transfusion-dependent (NTDT) vs. transfusion-dependent (TDT) thalassemia classification 10 years later. Am. J. Hematol. 2021, 96, E54–E56.
2. Kattamis A, Kwiatkowski JL, Aydinok Y. Thalassaemia. Lancet. 2022 Jun 18;399 (10343):2310-2324.
3. Taher AT, Musallam K, Capellini MM (Editors). Guidelines fort he Management of Non-Transfusion Dependent β-Thalassemia. Published by Thalassemia International Federation. Nicosia, Cyprus, 3rd Edition, 2023, 1-129.
4. Saliba AN, Musallam KM, Taher A. How I treat non-transfusion-dependent β-thalassemia, 142:11, Blood. 2023 Sep 14;142(11):949-960.
5. Hokland P, Daar S, Khair W, S S, Taher A, Torti L, Hantaweepant C, Rund D. Thalassaemia—A global view. Br J Haematol. 2023;201:199–214.
6. Khainga AA, Myinta PP, Paiboonsukwongb K, Winc N, Fucharoenb S and Sripichaib O. Clinical Severity of β-Thalassemia Pediatric Patients in Myanmar. Hemoglobin. 46:1, 66–70.
7. Vichinsky E. Non-transfusion-dependent thalassemia and thalassemia intermedia: epidemiology, complications, and management. Current Medical Research & Opinion 32:1, 2016, 191–204.
8. Sleiman J, Tarhini A, Bou-Fakhredin R, Saliba AN,Cappellini MD and Taher AT. Non-Transfusion-Dependent Thalassemia: An Update on Complications and Management. Int. J. Mol. Sci. 2018, 19, 182;
9. Shash H Non-Transfusion-Dependent Thalassemia: A Panoramic Review. Medicina 2022, 58, 1496-1516
10. Haddad, A.; Tyan, P.; Radwan, A.; Mallat, N.; Taher, A. Beta-Thalassemia Intermedia: A Bird's-Eye View. Turk. J. Haematol. 2014,31, 5–16. F
11. Taher, A.T.; Musallam, K.M.; Karimi, M.; El-Beshlawy, A.; Belhoul, K.; Daar, S.; Saned, M.S.; El-Chafic, A.H.; Fasulo, M.R.; Cappellini, M.D. Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a

region of endemicity: The OPTIMAL CARE study. Blood 2010, 115, 1886–1892.
12. Taher, A.T.; Cappellini, M.D. How I manage medical complications of β-thalassemia in adults. Blood 2018, 132, 1781–1791.
13. Musallam KM, Rivella S, Vichinsky E, Rachmilewitz EA. Non-transfusion dependent thalassemias. Haematologica 2013;98:833-44
14. Viprakasit V, Tyan P, Rodmai S, Taher AT. Identification and key management of non-transfusion-dependent thalassaemia patients: not a rare but potentially under-recognised condition. Orphanet J Rare Dis 2014;9:131.
15. Taher, A.T.; Musallam, K.M.; El-Beshlawy, A.; Karimi, M.; Daar, S.; Belhoul, K.; Saned, M.S.; Graziadei, G.; Cappellini, M.D. Age-related complications in treatment-naive patients with thalassaemia intermedia. Br. J. Haematol. 2010, 150, 486–489.
16. Gupta V, Aggarwal P. Complications in Transfusion-Dependent Thalassemia. Indian Pediatrics. 59:15, 2022, 911-912
17. Borgna-Pignatti, C.; Marsella, M.; Zanforlin, N.: The natural history of thalassemia intermedia. Ann. N. Y. Acad. Sci. 2010, 1202, 214–220.
18. Taher, A.T.; Musallam, K.M.; and Cappellini, M.D.: β-Thalassemias. N Engl J Med 2021;384:727-43.
19. Musallam, K.M.; Cappellini, M.D.; Taher, A.T. Evaluation of the 5 mg/g liver iron concentration threshold and its association with morbidity in patients with beta-thalassemia intermedia. Blood Cells Mol. Dis. 2013, 51, 35–38.
20. Musallam, K.M.; Cappellini, M.D.; Wood, J.C.; Motta, I.; Graziadei, G.; Tamim, H.; Taher, A.T. Elevated liver iron concentration is a marker of increased morbidity in patients with beta thalassemia intermedia. Haematologica 2011, 96, 1605–1612.
21. Derchi, G.; Galanello, R.; Bina, P.; Cappellini, M.D.; Piga, A.; Lai, M.E.; Quarta, A.; Casu, G.; Perrotta, S.; Pinto, V.; et al. Prevalence and risk factors for pulmonary arterial hypertension in a large group of beta-thalassemia patients using right heart catheterization: A Webthal study. Circulation 2014, 129, 338–345.
22. Taher, A, Isma'eel, H, Mehio, G, Bignamini, D, Kattamis, A, Rachmilewitz, E.A, Cappellini, M.D. Prevalence of thromboembolic events among 8,860 patients with thalassaemia major and intermedia in the Mediterranean area and Iran. Thromb. Haemost. 2006, 96, 488–491
23. Haidar, R.; Mhaidli, H.; Taher, A.T. Paraspinal extramedullary hematopoiesis in patients with thalassemia intermedia. Eur. Spine J. 2010, 19, 871–878.
24. Fraidenburg, D.R.; Machado, R.F. Pulmonary hypertension associated with thalassemia syndromes. Ann. N. Y. Acad. Sci. 2016,1368, 127–139.
25. Vogiatzi, M.G.; Macklin, E.A.; Fung, E.B.; Cheung, A.M.; Vichinsky, E.; Olivieri, N.; Kirby, M.; Kwiatkowski, J.L.; Cunningham, M.; Holm, I.A.; et al. Bone disease in thalassemia: A frequent and still unresolved problem. J. Bone Miner. Res. 2009, 24, 543–557.
26. Charoenngam, N.; Rittiphairoj, T.; Ponvilawan, B. Fracture prevalence in thalassemia: A systematic review and meta-analysis. Arch. Osteoporos. 2021, 16, 171.
27. Mehta, V.; Kirubarajan, A.; Sabouhanian, A.; Jayawardena, S.M.; Chandrakumaran, P.; Thangavelu, N.; Cader, R.; Mettananda, S.; Bandara, D.; Khan, S.; et al. Leg Ulcers: A Report in Patients with Hemoglobin E Beta Thalassemia and Review of the Literature in Severe Beta Thalassemia. Acta Haematol. 2022, 145, 334–343.
28. Ang, A.L.; Lim, C.Y.; Ng, W.Y.; Lam, J.C.M. Non-transfusion dependent thalassemia is independently associated with higher alloimmunization risk than transfusion dependent thalassemia and would benefit the most from extended red cell antigen matching. Transfusion 2021, 61, 2566–2577.
29. Spanos, T.; Karageorga, M.; Ladis, V.; Peristeri, J.; Hatziliami, A.; Kattamis, C. Red cell alloantibodies in patients with thalassemia. Vox Sang. 1990, 58, 50–55.
30. Taher, A.T.; Karakas, Z.; Cassinerio, E.; Siritanaratkul, N.; Kattamis, A.; Maggio, A.; Rivella, S.; Hollaender, N.; Mahuzier, B.; Gadbaw, B.; et al. Efficacy and safety of ruxolitinib in regularly transfused patients with thalassemia: Results from a phase 2a study. Blood 2018, 131, 263–265.
31. Taneja, K.; Verma, C.; Mahajan, A. Can ruxolitinib avert splenectomy in patients with thalassaemia: A short term case series. Br. J. Haematol. 2022, 196, 1111–1113.

Correspondence Address:
Prof. Dr. Zeynep Karakas
Istanbul University, Istanbul Medical Faculty, Department of Pediatrics, Divisiion of Hematology/Oncology/İstanbul/Türkiye
E-mail:zeynepkar@hotmail.com

CHAPTER 8
SICKLE CELL SYNROMES

SICKLE CELL SYNDROMES

Yurdanur Kilinç, Yusuf Ünal Sarıkabadayı, Pelin Kan Özkaya
SANKO University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hamatology, Gaziantep-Türkiye

ABSTRACT

All the hemoglobin disorders which carries HbS and the other structura Hb disorders are cumulated under the title "sickle cell syndromes". Basic physiopathological event is thrombosis tendency of sickled red blood cells and results with vasculopathy which leads to ischemia, infarct and necrosis in tissues. As a result, the complications of sickle cell disease are dependent to vasculopathy.

Keywords: Sickle cell anemia, sickling, vasculopathy

INTRODUCTION

As a result of exchange od valine with glutamine at 6.th position of B gene, HbS may be formed in red blood cell (RBC)s. Mutation is genetic disorder is seen with single amino aicid substitution in beta gene and shows otosomal recessive inheritance. The patients who carries HbS in homozygous status is called sickle anemia or homozygous sickle cell disease. The coinheritance of HbS and the other emoglobin disorders is called sickle cell syndromes. The patients with sickle cell anemia with homozygous HbSS state or double heterozygous with HbS which is together with severe mutations which result severe clinical courses may lead to vaso-occlusive crisis initially and may cause tissue and organ damages as a result of physiopathological changes and complications ensue. Sickle cell anemia patients may be followed at outpatient clinics periodically beginning in the first 3 months of life for the prevention of complications and maintain normal life span **(6)**.

Table 1: *Preventive Strategies in Sickle Cell Disease* [16]

- Prevention of İnvasive Pneumococci infections
- Immunization
- Screening of renal disease
- Screening of pulmonary hypertension
- Screening of EKG and ECHO
- Screening of hypertension
- Screening of retinopathy
- Screening the risk of the stroke by neuroimaging technics
- Screening of pulmonary disease
- Counselling of heathy productivity and fertility

In case of coinheritance of HbS together with thalassemic mutations, clinical outcome is more severe with severe thalassemic mutations, then treatment is the same asin transfusion dependent thalassemia patients **(7)**.

In homozygote HbSS or double heterozygote status with severe thalassemic mutations, the outcome of patients is dependent to the, percentages physiıpathological changes causes vazo-occlusion, pain, tissue and organ failure **(8-12)**. In Türkiye, the clinical course of sickle cell anemia patients with mild and severe clinical presentations **(9-13)**. The patients with Benin type or haplotype 19 is seen in our country about 80% of the patients which is characteristic for the patients who live in Northwestern part of Africa **(8)**. It is necessary to canalize the severity, the understanding of the epigenetic modulators and management of the patients carefully. Plenty of agents are given to patients fort eh investigational searches or therapeutically for decreasing sickling or increasing HbF 5. As is seen in our country, double eterozygosity of HbS with severe thalassemic mutations, clinical outcome and treatment is the same with thalassemia major.

Preventive strategies (Table 1) has changed the clinical courses for long follow-up period **(6, 13-14)**. The complications could be prevented by preventive strategies in SCD (sickle cell disease) **(15-18)**.

Chorion villus sampling in 8-12th weeks of embrio or cordosynthesis at 14-22th weeks of fetal life prenatally may leads to prevention of birth. From 22th weeks of prenatal life, rights of fetuses are began in accordans with las. Prenatal genetic diagnosis (PGD) is applied fort of the embrio or fetuses with special targets, for example donor selection for stem cell transplantation.

Basically, three treatment modalities are changes the courses of SCD. New agents for example usage of hydroxy carbamide, red blood cell transfusion and stem cell transplantation. If HLA compatible sibling donor is available, stem cell transplantation must be performed as soon as possible if suitable criteria present (3); indications are still limited for the alternative donor usage. In patients with HbSS or S-beta thalassemia with severe courses, stem cell transplantation must be performed **(17)**.

If medical stuff is educated for the complications of SCD, it can be prevented (Table 2). The prevention of the life-threatening infections, destruction of central nervous system and stroke, may leads to decrease death in childhood in SCD.

HbF levels are decrees fastly in first 6 months of life, instead HbS levels are increased steadily 6 % more and the patient is asymptomatic. The increase in HbS levels are about 32% to 1 year and 61% to 2 years and 96 % to 8 years. When HbS concentration is more than 30% in red blood cells, the risks of crises are increased as predisposing factors ensue. After 1 years, infections, dehydratations presents vaso-occlusive crises begin, hand-foot syndrome may be seen 2-4 years. Etc Especially Daily food and fluid intake must be enough. Normal vaccines, pneumococcus and meningococcus vaccines must begin in 2 months to 3 years period. The available pneumococci vaccines are Prevnar 13 and Pneumo 23 vaccines **(19)**.

For the prevention of 13 different serotypes od Streptococcus pneumoniae, Prevnar 13 is applied to children from 6th week to 5 years post natally (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23). The vaccination is done by i.m. route as 0.5ml single dose., must repeat at 2, 4, 6th months and as 4 doses at 12-15th months. If the child failed to complete the vaccination, 1 more completion-catch up dose must be given at 24-59th months. The patients at 5 years are assumed as high risk patients and vaccination scheme of pneumocci must be completed **(20)**. PSV23 (Pneumovax) first dose must be given form the age of 2 and after the 8 weeks after completion of Prevnar 13 series, booster is given after 5 years after first dose. The children in playing age or school ages 2 doses are enough between 3 months **(23-28)**.

REFERENCES

1- Kilinc Y, The physipathology in sickle cell anemia. I. Tthalassemia and Hemoglobinopatyhies Education Book , pp 20-23,107-109, 2014.

2- Kilinc Y, Gurgey A KUmi M, Altay Ç, -:Alpha-thalassemia, Glucose-6-phosphate dehydrogenase deficiency and HbS frequency in the cord bood samples form Adana Region. DOĞA ScienceBulletin, 10:2, 162-167, 1986.

3- Kilinc Y, Sickle Cell anmeia:Diagnosis, follow-up, treatment and transfusion. The Book of Diagnosis and Treatment in Thalassemia and Hemoglobinopathies, Canatan D, Aydınok Y(Editörs), Retma Matbaa Ltd Şt, , pp 255-262, 2007.

4- Kilinc Y, Abnormal hemoglobins. The Bulletin of Turkish Pedatric Hematology Society.2(3):1;6-19, 2008.

5- - Kilinc Y, New trends in the treatment of sickle cell anemia. Kılınç Y, editör, Sickle Cell Anemia, 1. edition, Ankara, Türkiye Clinics, p 73-76, 2021, .

6- Alouch JR, Kilinc Y, Aksoy M, Yüreğir GT, Bakioğlu I, Kutlar A, Kutlar F, Huisman THJ. Sickle cell anemia among Eti-Türks:hematological, clinical and genetic observations. British J Haematol 64:1, 45-49, 1986.

7- Gonzales-Redondo JM, Kutlar F, Kutlar A, Stoming TA, Pablos J Ma, KILINÇ Y, Huisman THJ:HbS (C)B+ thalassemia:different mutations are associated with different levels of normal HbA. Brit J Haematol 70:85-89, 1988.

8- Kilinc Y, Kümi M, Yılmaz B, Tanyeli A. Zinc and copper values in serum and erythrocytes in sickle cell homozygous and trait children. Acta Paediatrica Scandinavica 80:873- 874, 1991

9- Kilinc Y, Acartürk E, Kümi M. Echocardiographic findings in mild and severe forms of sickle cell anemia. Acta Paediatrica Japonica 1993; 35: 243-246.
10- Karabay-Bayazıt A, Noyan A, Aldudak B, Özel A, Anarat A, Kilinc Y, Şaşmaz İ, Gali E, Anarat R, and Dikmen N: Renal functions in children with sickle cell anemia. Clinical Nephrology 57:2, 127-130,
11- Kilinc Y, New Trends in the treatment of sickle cll anemia. 6. International Thalassemia Congressand Summer Schoolsayfa 99-102, 2013, .
12- KilincÇ Y, Şaşmaz İ, Antmen B, Kozanoğlu H, Soyupak S, Altunbaşak Ş:Stroke in sickle cell anemia. In "Focus on Sickle Cell Research" Plasmar RL(editor),New Biomedical Books, Nova Publishers, New York,2004, chapter 3, pp 59-68.
13- Kilinc Y, Stroke in sickle cell anemia. 5th Inyernational Thalassemia Summer School Book., The publications of National Thalassemia Federation, 167-180, 2009.
14- Antmen B, Pediatric stem cell transplantation in sickle cell anemia, . Kılınç Y, editör, Sickle cell anemia. 1 st edition, Ankara. Türkiye Clinics, 2021. . Kılınç Y, p 20-26, 2021,
15- Kilinc Y, Almacioğlu M: Follow-up algorithm in sickle cell anemia. . Kılınç Y, editor, Sickle Cell Anemia, 1st edition, Ankara. Türkiye Clinis, p 20-26, 2021.
16- Yawn BP, John-Sowah J. Management of Sickle Cell Disease: Recommendations from the 2014 Expert Panel Report. Am Fam Physician. 2015 Dec 15;92(12):1069-76. PMID: 26760593.
17- Kassim, A. A., and DeBaun, M. R. The case for and against initiating either hydroxyurea therapy, blood transfusion therapy or hematopoietic stem cell transplant in asymptomatic children with sickle cell disease. Expert Opin. Pharmacother. 15, 325–336 (2014).
18- Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, Smith WR, Panepinto JA, Weatherall DJ, Costa FF, Vichinsky EP. Sickle cell disease. Nat Rev Dis Primers. 2018..
19- Engel ER, Howard AL, Ankus EJ, Rico JF: Advances in sickle cell disesase management. Advances in Pediatrics, 67(2020), 57-71.
20- Kibar F, Köksal F, Serin MS, Kilinc Y, Akan E: Immunugenecity of pneumococcal capsular polysaccharide vaccine in patients with sickle cell anemia. Microbiol Bulletin 1996;31, 29-37.
21- American Academy of Pediatrics. Active and passive immunization. Immunization in special clinical circumstances:asplenia and functional asplenia. In:Kimberlin DW, Brady MT, Jackson MA, et al , editorsRed Book 2018 report of the committee of infectious diseases. 31th edition. Itasca (IL): American Academy of Pediatri ,2018, pp 88-90.
22- Robinson CL, Bernstein H, Romero JR, Szilagy P: Advisory Committee on Immunization Practices recommended immunization schedules for children and adolescents aged 18 years or younger-United States, 2019. MMVR Morb Mortal Wkly Rep 2019; 68(5):112-114.
23- Chou ST, Alsawas M, Fasano RM, Field JJ, Hendrickson JE, Howard J, et al. American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. Blood Adv. 2020 Jan 28. 4 (2):327-355.
24- Yawn BP, John-Sowah J. Management of Sickle Cell Disease: Recommendations from the 2014 Expert Panel Report. Am Fam Physician. 2015 Dec 15;92(12):1069-76. PMID: 26760593. 18- Lanzkowsky P, Lipton J, Fish J. Lanzkowsky's Manual of Pediatric Hematology and Oncology 6th Edition, chapter 11, 166-196, 2016.
25- Kilinç Y, Şaşmaz İ, Antmen B, Kozanoğlu H, Soyupak S, Altunbaşak Ş:Stroke in sickle cell anemia. In "Focus on Sickle Cell Research" Plasmar RL(editor),New Biomedical Books, Nova Publishers, New York,2004, chapter 3, pp 59-68. 26-Brandow AM, Carroll CP, Creary S, Edwards-Elliott R, Glassberg J, Hurley RW, et al. American Society of Hematology 2020 guidelines for sickle cell disease: management of acute and chronic pain. Blood Adv. 2020 Jun 23. 4 (12):2656-2701.
26- Houwing ME, de Pagter PJ, van Beers EJ, Biemond BJ, Rettenbacher E, Rijneveld AW, Schols EM, Philipsen JNJ, Tamminga RYJ, van Draat KF, Nur E, Cnossen MH; SCORE Consortium. Sickle cell disease: Clinical presentation and management of a global health challenge. Blood Rev. 2019 Sep;37:100580. doi: 10.1016/j.blre.2019.05.004.
27- DeBaun MR, Jordan LC, King AA, Schatz J, Vichinsky E, Fox CK, et al. American Society of Hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults. Blood Adv. 2020 Apr 28. 4 (8):1554-1588
28- Lanzkowsky P, Lipton J, Fish J. Lanzkowsky's Manual of Pediatric Hematology and Oncology 6th Edition, chapter 11, 166-196, 2016.

Correspondence Address;
Prof. Dr. Yurdanur Kılınç
SANKO University, Faculty of Medicine, Department of Pediatrics, Dividion of Pediatric Hamatology, Gaziantep-Türkiye
E-mail: kyurdnur@cu.edu.tr

Table 2: *Possible complications in Sickle Cell Disease 18*

Acute Complications	Chronic Complications
	Developmental-mental delay
Acute chest syndrome	Neurocognitive dysfunction
Stroke	Indirect hyperbilirubinemia
Acute anemia;	Albuminuria
- Splenic sequestration crisis	Isosthenuria
-Aplastic crisis	Critical kidney damage
- Hyperhemolytic crisis	Delayed puberty
Cholelithiasis	Erectile dysfunction
Renal papillary necrosis	Avascular necrosis
Priapism	Retinopathy
Bone marrow infarction	Pulmonary hypertension
Osteomyelitis	Cardiomegaly
Glaucoma after hyphema	Diastolic heart failure
Retinal infarction	Anemia
Meningitis	Leukocytosis
Septicemia	Functional asplenia
Splenic infarction	Pregnancy complications
Spleen sequestration	Skin (leg) ulcers
	Chronic pain
	Psychosocial complications

Table 3. *Routine Health Maintenance in Patients with Sickle Cell Disease 28*

Laboratory studies	Starting age	Frequency
Complete blood count/reticulocyte count	At diagnosis	Quarterly to yearly with differential monthly if receiving HU
Hemoglobin quantitation	At diagnosis	Yearly
Red cell antigen typing	At diagnosis	
Liver and renal functions	At diagnosis	Yearly
Urinalysis	1 year	Yearly
HIV, hepatitis B, C		Yearly if receiving transfusions
Pulse oximetry	At diagnosis	Quarterly to yearly
Pulmonary function	5 years	Every 3 years
Sleep study		If symptoms present
Eye examinations	5 years for SCD-SC	Yearly
	8 years for SCD-SS	Yearly
Transcranial Doppler	2 years	Based on prior results
Brain MRI/A		If school difficulties, abnormal or repeatedly conditional TCD, neurological symptoms
Abdominal ultrasound		If symptoms of cholelithiasis
Hip radiograph/MRI		If symptoms of AVN
Echocardiogram	10 years	Every 3 years or more frequent if abnormal

AVN, avascular necrosis; TCD, transcranial Doppler; HU, hydroxyurea.

TRANSFUSION IN SICKLE CELL SYNDROMES

Prof. Dr. İlgen Şaşmaz
Çukurova University Faculty of Medicine, Department of Pediatric Hematology, Adana-Türkiye

ABSTRACT

Used correctly, transfusions can prevent organ damage and save the lives of patients with sickle cell disease. Used unwisely, transfusion therapy can result in serious complications. Transfusions are indicated for either episodic events triggered by an acute complication or a necessary medical intervention. In contrast to these episodic indications, some clinical problems require long-term suppression of circulating sickle cells. Chronic transfusion therapy may be warranted for the primary prevention of stroke and prevention of stroke recurrence. It may also be used to treat chronic debilitating pain, pulmonary hypertension, chronic heart failure, and anemia associated with chronic renal failure.

The choice of several methods, such as simple transfusion, exchange transfusion, and erythrocytapheresis, depends on the specific requirements of the patient and available resources. Erythrocytapheresis is a method of automated red blood cell exchange recently reported to be effective in stabilizing or reducing iron overload for chronically transfused sickle cell patients. Because the body has no physiological mechanism to actively excrete excess iron, repeated blood transfusions lead to increased body iron burden, including iron deposition into the liver, heart and endocrine organs.

Red blood cell transfusion is a critical part of SCD management. It is vital to carefully weigh the indications for transfusion before transfusing any patient with SCD. Various guidelines are available to assist in decision-making in cases of stroke, acute chest syndrome, pregnancy, and in the perioperative setting.

Keywords: Sickle cell anemia, erythrocyte transfusion, HbS

INTRODUCTION

Used correctly, transfusions can prevent organ damage and save the lives of patients with sickle cell disease. With erythrocyte transfusion therapy, the burden of sickled cells is reduced and oxygen carrying capacity and perfusion are improved. However, it should not be forgotten that unnecessary transfusions will lead to various complications, from mild to severe **(1-6)**.

Complications from sickling are related to the proportion of red cells containing HbS. These risks may be minimised by reducing the %HbS through transfusion, but no single %HbS target covers all indications. Randomised controlled studies have shown a transfusion target of HbS ≤30% (compared with no transfusion) is effective in reducing incidence rates of stroke, vaso-occlusive crises, acute chest syndrome, priapism and new symptomatic avascular necrosis. However, other randomised trials have shown higher targets of <35% and<50% reduced pain rates in prophylactically transfused pregnant women and perioperative complications in surgical patients transfused preoperatively, respectively. Some observational studies used %HbS targets of 25-40% in acute chest syndrome (ACS) **(2, 7, 8)**.

Acutely ill SCD patients may deteriorate rapidly so transfusion issues should be considered early, including any recent transfusions, previous haemolytic transfusion reactions, and alloantibody formation. Baseline investigations should include: full blood count, reticulocyte count, and blood group with antibody screen. The transfusion request form must clearly state that the patient has SCD so that special transfusion requirements are met. For patients presenting to a different hospital from usual, their primary hospital should be contacted for their baseline Hb, reticulocyte count, transfusion history, red cell

phenotype/genotype and history of alloantibodies. The patient may have a card bearing details of their phenotype and/or alloantibodies. Red cell units usually have to be ordered from the National Blood Service and this may introduce delay, especially for individuals with alloantibodies. Meticulous attention should be paid to all aspects of SCD management, particularly adequate analgesia and hydration, to help prevent the development of critical organ complications for which transfusion may be required **(6-9)**.

Transfusions are divided into two categories: acute episodic transfusions, which provide treatment or stabilization of complications, and chronic prophylactic transfusions, which provide protection from future complications. Indications for transfusion are shown in Table 1. In patients with SCD, transfusion can be performed as simple transfusion and blood exchange. The transfusion option to be applied is selected according to the patient's clinical condition and facilities **(2, 3, 6, 9)**.

The viscosity of sickle red cells is much higher than that of normal red cells, and the risk of hyperviscosity at a given Hb is dependent on the %HbS and the haematocrit. Increased viscosity compromises oxygen delivery and exacerbates the sickling process.

The viscosity effect of sickle red cells is reduced but not eliminated by the presence of normal red cells. Simple transfusion leads to a rise in haematocrit, and any increment in oxygen carrying capacity is offset by increased blood viscosity. Exchange transfusion removes HbScontaining cells and decreases blood viscosity. When the pre-transfusion Hb is close to steady state or is high for other reasons exchange transfusion is preferred. Normal red cells support maximum oxygen transport at Hb 14-16 g/dl, but in untransfused sickle cell anemia patients, it is lower at 10-11 g/dl because of the higher viscosity of sickle red cells. In such patients, it is unwise to exceed a post-transfusion Hb target of 10-–11 g/dl, without an accompanying reduction in %.

HbS to less than 30% **(6-9)**.

If transfusion is needed, patients with SCD must be given ABO-compatible, extended Rh- and Kell-matched units. If there are clinically significant red cell antibodies (current or historical) then the red cells selected should be negative for the corresponding antigens **(6-9)**. Patients with SCD must also have extended RBC antigen typing performed, which may assist with further serological testing and selection of red cell units if haemolytic reactions occur or there are complex transfusion requirements. Blood provided for SCD patients should be HbS negative and, where possible, be <10 days old for simple transfusion and <7 days old for exchange transfusion, but older blood may be given if the presence of red cell antibodies makes the provision of blood difficult **(6-9)**.

Erythrocytes should be leukocyte filtrated and irradiated. With transfusion, the oxygen carrying capacity of the blood increases, the rate of sickled erythrocytes decreases, and clinically, microvascular perfusion in the tissues increases **(1, 3, 6-9)**.

Patients with multiple red cell alloantibodies or antibodies to rare antigens need a clear agreed plan because blood may be difficult to source in the elective or emergency setting. Close liaison between all clinical teams, the hospital transfusion laboratory and the National Blood Service is essential to ensure appropriate provision of blood **(6-9)**.

All clinicians managing patients with SCD must be aware of the risk of haemolytic transfusion reactions to ensure prompt recognition and management. Close liaison is needed with haemoglobinopathy specialists and blood services for investigation and management. Any adverse events or reactions related to transfusion should be appropriately investigated and reported to local risk management systems and to National Haemovigilance **(6-9)**.

With the increasing availability and application of molecular Rh typing, it is becoming clear that many supposed autoantibodies are in fact Rh alloantibodies due to variant RH alleles that are capable of causing haemolytic reactions **(7-10)**.

A transfusion history should be obtained in all SCD patients requiring transfusion, whether elective or emergency. This includes details of the patient’s red cell phenotype and any red cell antibodies (current and historical). Hospitals should have robust systems in place to enable transfusion laboratories to clearly identify samples for sickle cell patients. Close communication is essential between clinical and laboratory teams so that appropriate blood is given **(7-10)**.

Simple transfusions increase blood viscosity. Exchange transfusion corrects the symptoms of anemia without causing increased viscosity and volume overload. Exchange transfusion also reduces the risk of transfusion related iron overload. It can be done manually or automatically by erythocytapheresis with an apheresis device. However, its disadvantages are that it has a higher cost, requires a larger number of donors, and requires the use of intravenous catheters. The differences between simple transfusion and exchange transfusion are shown in Table 2 **(7-11)**.

Transfusion in Acute Complications

In SCD patients, blood transfusion may be needed in emergency situations to increase oxygen carrying capacity and reduce vaso-occlusive complications. Simple transfusion is preferred when the primary reason for transfusion is to prevent or reverse the effects of severe anemia (such as aplastic crisis). Exchange transfusion is the preferred option when an urgent reduction in HbS% is required without an undesirable increase in blood viscosity (such as severe ACS) **(6-9, 11)**.

Transfusion in Sudden Severe Complications

Patients with SCD can present as acutely unwell with multiorgan failure, which may be related directly to their SCD or may be caused by another factor such overwhelming sepsis. Improvement has been seen after exchange transfusion but this has usually been provided in conjunction with organ support in the Intensive Care Unit so it is difficult to elucidate the impact of the Exchange transfusion alone. In patients with multiorgan failure, exchange transfusion should be performed aiming for S% ,30% and Hb of 10 g/dl. Acute intrahepatic cholestasis is another life-threatening complication of SCD characterized by rapid deterioration in liver function tests and hepatomegaly, where observational data suggests that exchange transfusion may be of benefit.

The causes of death in SCD are ACS, stroke, sepsis and multiple organ failure, and these conditions are often accompanied by a decrease in hemoglobin. Transfusions improve tissue oxygenation and perfusion and reduce vaso-occlusion. Although there are no controlled clinical studies conducted in such life-threatening situations, the medical approach is based on clinical observations. However, some studies have shown that aggressive transfusion regimens improve organ functions and life expectancy in patients with multiple organ failure. In the above cases, the aim is to keep the Hb S level below 30% **(1-6)**.

Acute chest syndrome

ACS is defined as an acute illness characterized by fever and/or respiratory symptoms in association with a new pulmonary infiltrate on chest radiograph. Severity can vary from a mild self-limiting pneumonic illness to respiratory distress syndrome and multiorgan failure and it can be rapidly progressive. Prospective studies show that between 65% to 72% of patients with ACS are transfused and that both simple transfusion and RCE are associated with improvements in clinical, radiologic, and oxygenation outcomes. Simple transfusion to a Hb level of 10 to 11g/dL should be considered in symptomatic ACS with Hb.1.0 g/dL below baseline or if oxygen saturations cannot be maintained. 92% on room air or if oxygen requirements increase. RCE should be considered in patients with features of severe disease (e.g., worsening hypoxia, increasing respiratory rate, decreasing platelet count, decreasing Hb concentration, multilobar disease on chest radiograph, and neurologic complications) or if patients deteriorate despite initial simple transfusion.

In adult patients, an arterial blood gas performed on room air may be useful in guiding treatment decisions and a partial pressure of oxygen ,9.0 kPa (65 mm Hg) has been recommended as an indication for transfusion, but transfusion should not be delayed in rapidly deteriorating patients with a lesser degree of hypoxia **(6, 11, 25-28)**.

International guidelines recommend that patients with an acute ischemic stroke should be treated with exchange transfusion aiming for Hb of 10 g/dl and HbS 30%. If the patient presents with an ischemic stroke and markedly reduced Hb (6 g/dl), an initial simple transfusion may be required, followed by an exchange transfusion to reduce HbS to 30%. Alt-

hough there is little evidence on the efficacy of transfusion in hemorrhagic stroke, exchange transfusion would usually be recommended in this situation in part because of the possibility of neurosurgery **(6, 9-11)**.

Preoperative blood transfusion

In a multicenter study conducted by the SCD Preoperative Transfusion Group, no difference was found in terms of postoperative complications between simple transfusion to keep Hb at 10 g/dl and blood exchange to keep Hb S below 30%. However, transfusion-related complications were more common in the blood exchange group. For minor surgical procedures that do not require general anesthesia, patients do not need transfusion before the procedure. Simple transfusion is recommended for pediatric patients with uncomplicated Hb SS to keep their hemoglobin level at 10 g/dl before low-moderate risk surgery. Blood exchange is recommended to keep the Hb S level below 30% in patients who require long-term anesthesia, high-risk operations such as cholecystectomy, open heart surgery and joint replacement, and patients with high initial hemoglobin values and impaired pulmonary functions **(6, 16)**.

In the case of emergency surgery, the decision to transfuse will require careful discussion between the surgeon, anesthesiologist and haematologist. In practice, clinicians should consider simple preoperative transfusion in patients with Hb<9 g/dL. If the Hb value is >9 g/dL, the surgery should be continued with blood available in case of postoperative complications **(6, 16, 17)**.

Acute priapism

Initial management of acute priapism will include pain relief, alfa-adrenergic agents, and penile aspiration or irrigation. There is no randomized trial evidence of either simple or exchange transfusion in acute priapism, and observational data show variable responses to transfusion. Patients who do not respond to aspiration and irrigation will need shunt procedures, and these are performed under general anesthesia, so transfusion may be indicated for these patients. Exchange transfusion aiming for Hb of 10 g/dL and HbS,0%could be considered if shunt procedures are not effective in relieving the priapism. Recurrent attacks of priapism can lead to impotence. Chronic transfusion can be administered prophylactically to these patients to keep the Hb S level below 30%. The duration of these programs is limited and the patient should be evaluated after 6-12 **months (6, 18)**.

Pregnancy

Pregnancy in women with SCD is associated with increased risks of sickle-related complications such as pain crises, pulmonary complications and infection; maternal and perinatal mortality, pregnancy-related complications; and fetal complications **(18-20)**.

Prophylactic transfusion is not routinely required in pregnant women with SCD, but should be considered for pregnant women in the following situations:

Previous or current medical, obstetric, or fetal problems related to SCD

Women who have previously used hydroxyurea due to severe SCD

Multiple pregnancy

Women receiving long-term transfusions to prevent stroke or treat severe complications of SCD should continue regular transfusions throughout pregnancy.

Transfusion should be considered in pregnant women whose anemia worsens or who develop acute SCD complications (acute chest syndrome, stroke, etc.).

It is recommended that the target Hb level be at least 7 g/dL and the HbS level be <50% **(6-9, 18)**.

Chronic Transfusion Treatment

The aim of chronic transfusion treatment is to keep the Hb S level between 30-50% according to the following indications that develop or may develop in SCD. Transfusions are repeated every 3-4 weeks. Although simple transfusion can be performed, iron overload can also be reduced by performing erythrocytapheresis or blood exchange. These indications;

a. Prevention of stroke (Primary, recurrent or prophylactic)
b. Pulmonary hypertension and chronic lung disease
c. Vital organ failure
d. Chronic, refractory pain that does not respond to treatment
e. Anemia associated with chronic renal failure
f. Recurrent priapism
g. After liver or kidney transplantation

Transfusion is not recommended in cases of chronic anemia, uncomplicated painful crisis, infections, and minor surgery that does not require general anesthesia **(1-9)**.

Erythrocyte Transfusion Complications

Erythrocyte transfusions can cause serious problems, morbidity and even mortality. Transfusion has risks of alloimmunization, iron overload, and transfusion-transmitted infection **(6-9, 18)**.

Patients with SCD may be at a greater risk for transfusion reactions due to the higher number of red cell units transfused at one time. Febrile non-hemolytic transfusion reactions and allergic transfusion reactions are the most common types of transfusion reactions experienced by any patient receiving red cell transfusions. They have a prevalence of 1,000-3,000 per 100,000 units transfused and 112.2 per 100,000 units transfused, respectively. The less common but more serious transfusion reactions that could lead to significant morbidity and mortality such as acute hemolytic transfusion reactions (prevalence: 2.5-7.9/100,000 units transfused), DHTR (prevalence: 40/100,000 units transfused), transfusion related acute lung injury (prevalence: 0.4-1/100,000 units transfused), transfusion associated circulatory overload (prevalence: 10.9/100,000 units transfused), and septic transfusion reactions (prevalence: 0.03–3.3/100,000 units transfused) may also occur in the frequently transfused SCD patient population **(18, 21, 22)**.

Alloantibody formation against eryhtrocyte blood group antigens is a major complication for patients with SCD. Our understanding of the pathophysiological mechanisms that contribute to this phenomenon is limited. One factor that increases alloantibody induction in patients with SCD is their exposure to red cell antigens present in the blood donor population but lacking on their own red cells. Others factors are immune dysregulation in white blood cell subsets and their response to heme, platelets, eryhtrocytes and complement. The inflammatory status of the recipient at the time of RBC exposure may also influence whether a patient becomes alloimmunized or not; red cell transfusion in the setting of vaso-occlusive crisis, ACS, a viral illness, or other inflammatory disorder may increase the risk for alloimmunization **(21-23)**.

In order to mitigate alloantibody formation, it is a common practice to match the eryhtrocyte antigens for Rh D, C, and E, and K in accordance with the National Institutes of Health (NIH) Expert Panel, American Society of Hematology, and British Society for Haematology guidelines **(30, 34, 43, 99)**. The RBC alloimmunization rate is reported to be as high as 50% without any prophylactic matching but decreases significantly with prophylactic matching. With limited prophylactic matching (i.e., C, E, K), the RBC alloimmunization rate ranges from 5–24% and with extended prophylactic matching that includes matching for Duffy, Kidd and S antigens, the RBC alloimmunization rate can be as low as 7% **(24, 25)**.

Patients with SCD may also develop autoantibodies. SCD disease-related inflammation and RBC membrane changes with neoantigen exposure may contribute to the predisposition for autoantibody formation **(18)**.

The ASH guideline panel suggests immunosuppressive therapy over no immunosuppressive therapy in patients with SCD (all genotypes) with an acute need for transfusion and at high risk for acute hemolytic transfusion reaction or with a history of multiple or life-threatening delayed hemolytic transfusion reactions (DHTR) differently than other guidelines (conditional recommendation based on very low certainty in the evidence about effects). These are rare clinical situations in which patients are experiencing life-threatening anemia that require immediate red cell transfusion and either compatible blood cannot be found (i.e., patients with alloantibodies for whom antigen negative blood is unavailable) and/or the patients have a history of repeated episodes of severe hemolytic transfusion reactions with or without an antibody specificity identified (even when compati-

ble blood is available). The hematologist and transfusion medicine specialist should have ongoing discussions to weigh the potential benefits and harms associated with transfusion vs the effect of ongoing life-threatening anemia and to consider the respective mechanisms of action for choice of therapy (IVIg, steroids, or rituximab) **(9)**.

The ASH guideline panel suggests immunosuppressive therapy (IVIg, steroids, rituximab, and/or eculizumab) over no immunosuppressive therapy in patients with SCD (all genotypes) with a delayed hemolytic transfusion reaction and ongoing hyperhemolysis (conditional recommendation based on very low certainty in the evidence about effects). A DHTR was defined as a significant drop in hemoglobin within 21 days posttransfusion associated with 1 or more of the following: new red cell alloantibody, hemoglobinuria, accelerated HbS% increase with a concomitant fall in HbA posttransfusion, relative reticulocytopenia or reticulocytosis from baseline, significant LDH rise from baseline, and exclusion of an alternative cause.

Hyperhemolysis is defined as a rapid hemoglobin decline to below the pretransfusion level and rapid decline of the posttransfusion HbA level. Immunosuppressive therapy should be initiated promptly in patients with life-threatening hemolysis. The hematologist and transfusion medicine specialist should discuss potential benefits and harms associated with specific immunosuppressive therapies.

First-line immunosuppressive agents include IVIg and high-dose steroids; the second-line agent is eculizumab. Rituximab is primarily indicated for potential prevention of additional alloantibody formation in patients who may require further transfusion. Depending on the length of steroid therapy, weaning to avoid precipitation of a vaso-occlusive episode should be considered. Avoidance of further transfusion is recommended unless patients are experiencing life-threatening anemia with ongoing hemolysis. Supportive care should be initiated in all patients, including erythropoietin with or without IV iron **(9)**.

For individuals receiving chronic transfusion, iron overload is a common complication. It is often seen in individuals receiving chronic simple transfusion. The approximate net iron load for patients with SCD receiving 10 to 15 mL/kg of RBC every 4 weeks is 0.25 to 0.42 mg/kg/d. To reduce the iron load, partial manual exchange, or phlebotomy of whole blood before transfusion, may be performed. Partial manual exchange reduces the net iron load by 15% to 20% when compared to simple transfusion. Exchange transfusion can reduce the net iron by approximately 85% when compared to simple transfusion **(26)**.

It is essential that both intermittently and regularly transfused patients are monitored for iron overload and treated accordingly. Ferritin is an unreliable marker of iron overload as it remains elevated for weeks after a painful crisis **(27)**. Furthermore, changes in ferritin with chelation therapy may be absent even when changes in hepatic iron levels are significant. Therefore, assessment of liver iron concentration using validated non-invasive magnetic resonance imaging techniques is recommended for patients with suspected or documented transfusional iron overload; a testing frequency of every 1-2 years has been suggested **(9, 11)**.

Despite exchange transfusion significantly reducing the net iron load, monitoring for iron overload with serum ferritin and/or liver iron content (LIC) remains crucial. A retrospective study by Fasano and colleagues observed that SCD patients receiving exchange transfusion had elevated serum ferritin levels and LIC **(26)**.

Virology testing [hepatitis B, hepatitis C and human immunodeficiency virus (HIV)] should be undertaken at presentation and hepatitis B vaccination should be given to all patients with SCD irrespective of previous or prospective planned transfusions. SCD patients on regular transfusions should be screened annually for hepatitis B, hepatitis C and HIV **(6-9)**.

In conclusion, red blood cell transfusion is a critical part of SCD management. It is vital to carefully weigh the indications for transfusion before transfusing any patient with SCD. Various guidelines are available to assist in decision-making in cases of stroke, ACS, pregnancy, and in the perioperative setting. However, these guidelines also state that the evidence remains limited and additional studies are needed to determine best practices.

***Table 1:** Indications for transfusion in SCD [3]*

Indication	Type of transfusion
Acute transfusion indication	
Symptomatic anemia: aplastic crisis, acute splenic sequestration	Simple transfusion
Acute clinical stroke or TIA	Exchange transfusion
Acute hepatic sequestration/ intrahepatic cholestasis	Simple or exchange transfusion
ACS	Simple or exchange transfusion
Acute multiorgan failure	Simple or exchange transfusion
Pre operative (surgeries Lasting >1 hour and require general anesthesia)	Simple or exchange transfusion
Pregnancy*	Simple or exchange transfusion
Chronic transfusion indication	
Primary stroke prevention	Simple or exchange transfusion
Secondary stroke prevention	Simple or exchange transfusion
Recurrent VOC	Simple or exchange transfusion

*Pregnant women with severe or frequent SCD-related complications or high-risk pregnancy.

Table 2: *Comparison between simple and exchange transfusion [3]*

	Simple transfusion	Exchange transfusion
Description	• Infuse donor blood without removing patient's blood	Remove patient's blood during or shortly before infusing donor blood • May be partial or total exchange • May be performed manually or via apheresis
Indication /recommendation	• Acute anemia	
	• Acute complications	• Severe ACS failing to respond to simple transfusion • Acute complications with Hb ≥9 g/dL • Conditions requiring reduced HbS percentage (i.e., stroke/stroke prevention)
Potential risks and/or limitations	• Hyperviscosity • Iron	• Iron overload (less frequent) • Requires specialized equipment and trained staff • Costly (compared to simple transfusion)

REFERENCES

1. National Institutes of Health; National Heart, Lung, and Blood Institute. The management of sickle cell disease. 4th ed. NIH Publication No.02-2117. Bethesda, MD: National Heart, Lung, and Blood Institute, 2002.
2. Heeney MM, Ware RE. Sickle cell disease In: Orkin SH, Nathan DG, Ginsburg D, Look TA eds. Hematology of infancy and childhood. 8th ed. Philadelphia, WB Saunders Company, 2014;675-714.
3. Han H , Hensch L, Tubman N. Indications for transfusion in the management of sickle cell disease Hematology Am Soc Hematol Educ Program. 2021 Dec 10;2021(1):696-703. doi: 10.1182/hematology.2021000307.PMID: 34889416

4. Inati A, Mansour AG, Sabbouh T, et al.Transfusion therapy in children with sickle cell disease. J Pediatr Hematol Oncol. 2017;39(2):126-132.
5. Rees DC, Robinson S, Howard J. How I manage red cell transfusions in patients with sickle cell disease. Br J Haematol. 2018;180(4):607-617.
6. Howard J. Hematology Am Soc Hematol Educ Program. 2016 Dec 2;2016(1):625-631. doi: 10.1182/asheducation-2016.1.625.
7. Davis BA, Allard S, Qureshi A, et al. Guidelines on red cell transfusion in sickle cell disease. Part I: principles and laboratory aspects. Br J Haematol 2017;176:179-191.
8. Davis BA, Allard S, Qureshi A, et al. Guidelines on red cell transfusion in sickle cell disease Part II: indications for transfusion. Br J Haematol 2017;176:192-209.
9. Chou ST, Alsawas M, Fasano RM, et al. American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. Blood Adv 2020;4:327-55.
10. Sippert E, Fujita, CR, Machado D et al. (2015) Variant RH alleles and Rh immunisation in patients with sickle cell disease. Blood Transfusion, 13, 72–77.
11. Management of Sickle Cell Disease: Recommendations from the 2014 Expert Panel Report Yawn BP, Buchanan GR, Afenyi-Annan AN, et al. J.JAMA. 2014 Sep 10;312(10):1033-48. doi: 10.1001/jama.2014.10517.PMID: 25203083.
12. Howard J, Hart N, Roberts-Harewood M, et al. BCSH Committee. Guideline on the management of acute chest syndrome in sickle cell disease. Br J Haematol. 2015;169(4):492-505.
13. Emre U, Miller ST, Gutierez M, et al. Effect of transfusion in acute chest syndrome of sickle cell disease. J Pediatr. 1995;127(6):901-904.
14. Vichinsky EP, Neumayr LD, Earles AN, et al; National Acute Chest Syndrome Study Group. Causes and outcomes of the acute chest syndrome in sickle cell disease. N Engl J Med. 2000;342(25):1855-1865.
15. Velasquez MP, Mariscalco MM, Goldstein SL,et al. Erythrocytapheresis in children with sickle cell disease and acute chest syndrome. Pediatr Blood Cancer. 2009;53(6):1060-1063.
16. Vichinsky EP, Luban NLC, Wright E, et al. A comparison of conservative and aggressive transfusion regimens in the perioperative management of sickle cell disease. The Preoperative Transfusion in Sickle Cell Disease Study Group. N Engl J Med 1995 27;333(4):251-253.
17. Howard J, Malfroy M, Llewelyn C, et al. The Transfusion Alternatives Preoperatively in Sickle Cell Disease (TAPS) study: a randomized controlled multicenter clinical trial. Lancet. 2013;381:930-938.
18. Tanhehco YC, Shi PA, SchwartzJ. Guidelines on red cell transfusion in sickle cell disease. Ann Blood. 2022;7:9 1-13.
19. Oteng-Ntim E, Ayensah B, Knight M, et al. Pregnancy outcome in patients with sickle cell disease in the UK--a national cohort study comparing sickle cell anaemia (HbSS) with HbSC disease. Br J Haematol 2015;169:129-37.
20. Oteng-Ntim E, Meeks D, Seed PT, et al. Adverse maternal and perinatal outcomes in pregnant women with sickle cell disease: systematic review and meta-analysis. Blood 2015;125:3316-3325.
21. Delaney M, Wendel S, Bercovitz RS, et al. Transfusion reactions: prevention, diagnosis, and treatment. Lancet 2016;388:2825-2836.
22. Zhang D, Xu C, Manwani D, et al. Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. Blood 2016;127:801-809.
23. Yazdanbakhsh K, Shaz BH, Hillyer CD. Immune Regulation of sickle Cell Alloimmunization. ISBT Sci Ser 2017;12:248-253.
24. O'Suoji C, Liem RI, Mack AK, et al. Alloimmunization in sickle cell anemia in the era of extended red cell typing. Pediatr Blood Cancer 2013;60:1487-1491.
25. Lasalle-Williams M, Nuss R, Le T, et al. Extended red blood cell antigen matching for transfusions in sickle cell disease: a review of a 14-year experience from a single center (CME). Transfusion 2011;51:1732-1739.
26. Fasano RM, Leong T, Kaushal M, et al. Effectiveness of red blood cell exchange, partial manual exchange, and simple transfusion concurrently with iron chelation therapy in reducing iron overload in chronically transfused sickle cell anemia patients. Transfusion. 2016;56(7):1707-1715
27. Porter J. Concepts and goals in the management of transfusional iron overload Am. J. Hematol.2007;82:1136–1139.
28. Vichinsky, E., Onyekwere, O., Porter, J., et al. Deferasirox in Sickle Cell Investigators. A randomised comparison of deferasirox versus deferoxamine for the treatment of transfusional iron overload in sickle cell disease. British Journal of Haematology, 2007;136, 501-508

Correspondence Address:
Prof. Dr. H. İlgen Şaşmaz
Çukurova University, Medical Faculty
Department of Pediatric Hematology, Adana, Türkiye
E-mail:ilgen@cu.edu.tr

COMPLICATIONS OF SICKLE-CELL SYNDROME

Joan-Lluis Vives Corrons
Emeritus Prof. University of Barcelona
Red Blood Cell and Hematopoietic Disorders Research
Institute for Leukaemia Research Josep Carreras
Rare Anaemias & Ektacytometry Unit
Clinical Centre for Ambulatory Medicine
Còrsega 209.08036-Barcelona
E-mail: jlvives@clinic.cat

ABSTRACT

Sickle-cell disease SCD is a complex genetic disorder caused by an abnormal hemoglobin called hemoglobin S (HbS). HbS leads to the formation of rigid, misshapen red blood cells, which can block blood vessels and damage various organs and tissues, resulting in a wide range of complications that require a multifaceted and personalized approach to management. Genetic counseling and psychosocial support are important throughout a patient's life, and routine clinical visits are essential for monitoring and intervention. The management of SCD complications involves a comprehensive approach, including pain management, blood transfusions, and organ-specific interventions with ongoing research and therapeutic advances offering hope for improved patient outcomes. Multidisciplinary teams are essential for providing comprehensive care, but such teams may not be available in all regions. Patients must be educated about infection prevention, pain management, and early detection of complications. Preventive measures such as folic acid intake and avoiding specific triggers are also crucial. This paper provides an overview of the 13 major complications associated with sickle-cell disease (SCD): 1. Painful vaso-occlusive crises (VOC) 2. Hemolysis and anemia, 3. Infections, 4. Acute chest syndrome (ACS), 5. Stroke, 6. Splenic sequestration crisis,7. Aplastic crisis due to parvovirus B-19 infection, 8. Organ damage with multiorgan failure, 9. Leg ulcers, 10. Priapism, 11. Retinopathy with vision problems and even blindness.12. Pulmonary hypertension and 13. Delayed Growth and Development due to chronic anemia.

INTRODUCTION

Sickle-cell syndrome, also known as sickle-cell disease (SCD), is a complex and multifaceted disease that can result in a wide range of complications affecting various organs and systems in the body. These complications can significantly impact the quality of life of individuals with the condition. However, since clinical manifestations depend not only on the genetic condition, managing SCD requires a comprehensive approach, including regular medical care, pain management, infection prevention, and supportive therapies to address the specific complications associated with this disorder. Research and medical advancements continue to improve the prognosis and quality of life for individuals with sickle-cell syndrome, offering hope for the future **(1)**.

SCD is due to the presence of an abnormal hemoglobin (Hb) called HbS, which results from a mutation in the HBB gene located in chromosome 11. The gene defect is a single nucleotide mutation of the β-globin gene, which results in glutamate (Glu) being substituted by valine (Val) at position 6. Hemoglobin (Hb) normally transports oxygen from the lungs to all the body tissues and red blood cells (RBCs) with normal Hb (HbA) are discoid-shaped and with a deformability that allows them to glide through blood vessels for about 120 days. In patients with SCD, the abnormal HbS

stick to one another and form long, rod-like structures. These structures cause RBCs to become stiff, assuming a sickle shape (sickle-cells) that become rigid and misshapen, causing blockages and damaging vital organs and tissues that are the origin of complications. In this chapter, we will explore the numerous complications associated with sickle-cell syndrome**.**

In Europe, the mortality rates of SCD during early childhood have decreased up to 95% due to the implementation of newborn screening, improvements in vaccination, especially with the pneumococcal vaccine, and the use of prophylactic and therapeutic antibiotics **(2).**

Although increased survival to adulthood is certainly progress, people with SCD die at a much younger age than race-matched peers and the overall median survival is 58 years. Adolescents and young adults in the second and third decades of life suffer significant morbidity with higher rates of SCD-related complications and higher health care costs **(3).** This is in part due to observations showing that these young adult patients receive fewer transfusions and are less likely to be on hydroxyurea and/or chelation therapy when eligible for such treatments as they transition from pediatric to adult care **(4)**.

The most common cause of death is cardiac, respiratory, renal, infectious, neurologic, gastrointestinal, and hepatobiliary disease in descending order, and leucocytosis remains a key predictor of poor outcome along with renal insufficiency recurrent episodes of acute chest syndrome, low Hb F concentration, severe anemia higher rates of hemolysis, and dactylitis before 1 year of age **(5)**. The first symptoms of SCD may be expected a few months after birth when the HbS level rises. While in less severe sickle cell disorders, clinical problems may develop later in life, SCD is a chronic disease characterized by anemia and multiorgan damage, but punctuated by acute painful episodes. These random crises are of variable severity and are triggered by different factors such as cold weather, infection, or dehydration. Chronic organ damage, as well as acute, random painful crises, can be life-threatening. They also can have a profound effect on all aspects of life; as a consequence, psychological and social problems are very common in these patients and their families. Genetic counselling and psychosocial support are pivotal at all stages of development and into adulthood **(6).**

CLINICAL COMPLICATIONS OF SCD

As mentioned before, SCD is a chronic disease, characterized by chronic hemolytic anemia associated with painful vaso-occlusive crises, progressive organic injuries due to vascular disease, infections, and severe complications affecting the chest, spleen, and kidney **(7)**. The most important complications of SCD are summarized in **Table 1**.

1. Vaso-occlusive crisis

Vaso-occlusive crises (VOC) are a hallmark of SCD and are painful episodes that occur when sickle cells, travelling through small blood vessels get stuck and block capillaries leading to tissue damage and pain **(8)**.

Pain is the most common complication of SCD, and the top reason that people with SCD go to the emergency department or hospital. It can occur in any part of the body but commonly occurs in the hands, feet, chest, and back. Infants are protected from these crises during the first months of life due to the high Hb F levels.

A pain crisis can start suddenly, be mild to severe, and can last for any length of time. Pain that comes suddenly and lasts for a short time is referred to as acute pain. Chronic pain is daily, ongoing pain lasting more than 6 months. People with SCD can experience acute pain, chronic pain, and/or both. The first episode of pain is usually dactylitis in the small bones of the hands and feet, and about 50% of the children present this manifestation at the age of 2-years-old. Pain frequency and intensity are variable; 1/3 of the patients do not suffer pain crises, while 1% present more than 6 episodes per year. Pain crises represent 50% to 60% of consultations and 60% to 80% of hospitalizations. Opioids are a class of drugs sometimes used to reduce pain. People with SCD should talk with their SCD provider to help make a pain management plan.

2. Hemolysis

SCD can cause the destruction of RBCs, leading to anemia and jaundice. Hemolysis is a prominent feature of the disease **(9)**. The existence of hemolysis in SCD has been documented by both indirect and direct methods. The existence of bone-marrow erythroid hyperplasia, reticulocytosis, indirect hyperbilirubinemia, and elevations of plasma hemoglobin and serum lactic acid dehydrogenase (LDH) values show hemolytic disease.

The first consequence of hemolysis is anemia, another common complication, which happens when sickle cells die faster than your body can replace them (hemolysis). That makes harder for your body organs to get oxygen, leading to tiredness, weakness and dizziness. You can also have a fast heartbeat and trouble breathing. Anemia ay also makes it harder for children to grow.

If anemia is severe, blood transfusions can help. But if you get them often, you may end up with too much iron in your blood, which can cause iron overload with concomitant heart liver and pancreas damage. Children are especially prone to splenic sequestration as a cause of hemolysis and anemia. This happens when sickle cells block blood flow through the spleen leading to painful and enlarged spleen. Some children won't show many symptoms, but others will feel weak, have pale skin or lips, feel sleepy or sluggish, have pain in the left side of the belly, and have a fast heartbeat.

3. Infections

Patients with SCD, especially infants and children, are more likely to experience harmful infections, particularly those caused by encapsulated bacteria pneumococcus (pneumonia), haemophilus (flu) and meningococcus (meningitis). Pneumonia is a leading cause of death in infants and young children with SCD and patients who require regular transfusions as part of their treatment are also at increased risk for viral hepatitis.

This increased infection risk is due to functional asplenia and impaired immune function (presence of plasma complement and/or opsonization disorders). 80% of patients with HbSS and HbSβ0 have functional asplenia before 1 year of life, and a complete loss of spleen function (auto splenectomy) at 5 years. The risk of fatal invasive pneumococcal disease is very high during the first 5 years of life as well as the increased risk of staph aureus infections, viridian's streptococcus, E. Coli, and Salmonella.

Symptoms may vary by the type of infection, but fever may be the first sign. An infection can be life-threatening for people with SCD, and they should go to the emergency department or hospital immediately for treatment if they think they might have an infection. Children and adults with SCD should get all recommended vaccinations, including a flu vaccination. As mentioned before, patients with SCD are considered "high risk" for certain infections and should follow a special vaccination schedule for the following vaccines. Additionally, for children under 5 years of age, daily penicillin (or another antibiotic prescribed by a doctor) is recommended.

4. Acute chest syndrome

Acute chest syndrome (ACS) is a frequent, and life-threatening complication, of patients with SCD that can result in lung injury, breathing difficulty, and low oxygen for the organs and tissues of the body. This severe pulmonary complication is a leading cause of mortality in SCD **(10)** and it is characterized by fever, respiratory distress, pain, hypoxemia, and lung infiltrates, easily identified on chest X-ray. ACS may occur when sickled cells block blood and oxygen from reaching the lungs or may be caused by a viral or atypical bacterial infection (mycoplasma and chlamydia), viruses and pulmonary fat embolisms coming from long bone infarction. In children, ACS is usually caused by an infection. The highest incidence occurs in the first decade, between 1 and 7 years. Moreover, more than 30% of patients suffer at least 1 episode, and it is the second most important cause of children's death. ACS is a medical emergency with **s**igns and symptoms similar to pneumonia (fever, chest pain, cough and difficulty breathing) and its treatment requires a multidisciplinary team.

5. Stroke

Stroke or cerebral vascular accident (CVA) is a central nervous system injury and a severe SCD

complication considered as an important cause of morbidity **(8, 11)**. In SCD, the stroke is 300 times more frequent than in the normal population, and the peaks of maximum incidence are between 2 and 8 years and over 50 years. 10% of children between 2 and 10 years old have clinical infarcts and 17% have silent infarcts associated with occlusion of the internal carotid and middle cerebral arteries. A stroke can happen if sickled cells get stuck in a blood vessel and block blood flow to the brain, making it harder for the brain to get the oxygen it needs to function properly. About 10% of children with SCD will have a symptomatic stroke. It is recommended that children with sickle cell anemia get a special type of exam called a transcranial Eco Doppler ultrasound (TCED) every year starting at 2 years old until they are 16 years old. A TCD can identify children who are at high risk of a stroke. This decreases the risk of stroke to 0.5% to 1%. However, if the blood speed in the middle cerebral artery is higher than 200 cm/sec, the risk increases to 10 to 13%. Cerebral infarction can be prevented with periodic transfusions every 3-4 weeks to maintain HbS <30%. Once a patient has presented a heart attack the risk of recurrence is 50%.

Some signs or symptoms of a stroke include: Sudden numbness or weakness, especially on one side of the body, sudden confusion or difficulty understanding speech, sudden trouble seeing in one or both eyes, sudden trouble walking, dizziness, loss of balance, or lack of coordination and sudden severe headaches with no known cause. A silent stroke is a stroke that occurs without any signs or symptoms. A person who experiences a silent stroke may not be aware of their stroke and it can only be detected using an imaging test known as magnetic resonance imaging (MRI). A silent stroke can lead to brain injury.

6. Splenic sequestration crisis:

They start during the 2nd or 3rd month of life and exhibit high mortality. Their recurrence is estimated at 50% after the first episode and can occur in 30% of children before 6 years. They create a life emergency episode when there is a sudden increase of the spleen (splenomegaly) associated with a hypovolemic shock. For this reason, it is of vital importance to educate the patient's family to aid in the prevention of these episodes. As mentioned before, 80% of patients with HbSS and HbSβ0 have functional asplenia before 1 year of age, and complete auto splenectomy at 5 years, but the patients with HbSC and HbS β+ are at risk of splenic sequestration throughout their life.

7. Aplastic crisis

Aplastic crisis (AC) is due to the infection by parvovirus B-19 that blocks the erythropoiesis and therefore the production of RBCs in the bone marrow. This leads to severe non-regenerative anemia with very low values of hemoglobin concentration and reticulocytes.

8. Organ damage

Since in SCD there is a decrease of local blood flow, problems related to the heart, lung, kidney, and tissues may appear because not enough oxygen is reaching these organs. Accordingly, this can lead to a life-threatening multiorgan failure and to long-term health issues. Symptoms can be different depending on the organ(s) affected but some symptoms can include: Difficulty breathing, irregular heartbeat, nausea, swelling in the hands and feet and jaundice **(12)**.

9. Leg Ulcers

Chronic leg ulcers can develop in individuals with SCD due to a poor circulation of blood in the lower part of the leg. These ulcers are often painful and challenging to heal, leading to reduced quality of life. Leg ulcers happen more frequently in males than in females. The combination of several factors, such as trauma, infection, inflammation (swelling) may facilitate the apparition of ulcers in a local poor blood flow in the smallest blood vessels of the legs.

10. Priapism

Sickle RBCs in the penis can cause a persistent and often painful erection known as priapism. Repeated episodes that happen for a short time, also known as stuttering priapism, can last for a few minutes and up to three hours. A prolonged priapism is an epi-

sode that lasts more than 2 hours. It's important to seek medical treatment when priapism lasts more than 2 hours. Repeated episodes over time and delayed treatment can cause permanent damage and erectile dysfunction **(13)**.

11. Retinopathy

Sickle-cell retinopathy is an eye condition that can lead to partial vision loss, and, in severe cases, blindness. This is due to the block of blood flow in the fine vessels of the eye retina and the patient suddenly experiences vision problems, leading to permanent blindness. The blockage can occur in any part of the eye, but the most common is a blockage in the blood vessels of the retina (retinopathy).

12. Pulmonary Hypertension

Patients with SCD are at greater risk than the general population for high blood pressure in the lungs, which can be life-threatening Pulmonary hypertension is a rare SCD complication that occurs when the blood vessels in the lungs become narrow and stiff, leading to increased pressure in the pulmonary arteries. This can strain the right side of the heart, leading to heart failure.

Some signs and symptoms of high blood pressure in the lungs can include difficulty breathing, fatigue, chest discomfort or pain, swelling of the ankles, legs, or abdomen (belly) and light-headedness or dizziness.

13. Delayed Growth and Development

Children with the sickle-cell syndrome may experience delayed growth and development due to chronic anemia and frequent illness. Proper nutritional support and regular medical care are essential to help manage these challenges.

CLINICAL MANAGEMENT OF COMPLICATIONS

All these complications are complex, and their prevalence is highly variable. For this reason, it is unlikely that all the services necessary for the best diagnosis, follow-up, and treatment of SCD patients can be offered by only one health care provider (HCP), and a multi-disciplinary team of health and social services with local centers networking together with are the most convenient to offer a full range of services, including specialist access and supervision when required. In reality, however, such healthcare organization HCP is rarely available, even in developed countries, and the majority of care has to be delivered close to the patient's home by a local team or clinicians, with expertise in SCD and available for an in-person consultation or by telephone/internet communication. Regardless, it is of utmost importance that patients are educated on infection prevention, pain management, and early detection of complications starting with general measures that are beneficial to maintain health and avoid acute disease events. These measures include avoiding overexertion, excessive temperatures, hypoxia, and maintain an adequate water intake. It is always convenient to prevent megaloblastic erythropoiesis with the folic acid intake **(6, 12).**

The management of SCD complications involves a multifaceted approach, including pain management, blood transfusions, and potential organ-specific interventions. Advances in research have led to emerging therapeutics and improved clinical outcomes for SCD patients **(14)**. Understanding the complications of Sickle-Cell Disease is essential for healthcare providers and researchers working to improve the lives of those affected by this genetic disorder **(15, 16)**. Accordingly, the management of SCD patients must include 7 key issues including prevention programs, curative procedures and symptomatic and psychosocial interventions, from childhood to adult life **(Table 2)**. Genetic counselling and psychosocial support are pivotal at all stages of development and into adulthood transition. At the same time, routine clinical visits allow for the acquisition of baseline laboratories that can help differentiate crisis events. This can be achieved with the following key points for the management of patients with SCD are summarized in **Table 3.**

REFERENCES

1. Brandow AM, Liem RI. Advances in the diagnosis and treatment of sickle cell disease. J Hematol Oncol 2022; 15: 20

2.European Network for Rare and Congenital Anemias (ENERCA) Recommendations for Centres. of Expertise in Rare Anemias. A White Book. Prodrug Multimedia, S.L. 2014. ISBN: 978-84-939018-8-2.

3. Azar S, Wong TE. Sickle Cell Disease. A brief update. Med Clin N Am. 2017; 101;375-393

4. Blinder MA, Vekeman F, Sasane M, et al. Age-related treatment patterns in sickle cell disease patients and the associated sickle cell complications and healthcare costs. Pediatr Blood Cancer. 2013; 60:828-835

5. Russo G, De Franceschi L, Colombatti R, Rigano P, Perrotta S, Voi V, Palazzi G, Fidone C, Quota A, Graziadei G, Pietrangelo A, Pinto V, Ruffo GB, Sorrentino F, Venturelli D, Casale M, Ferrara F, Sainati L, Cappellini MD, Piga A, Maggio A, Forni GL. Current challenges in the management of patients with sickle cell disease - A report of the Italian experience. Orphanet J Rare Dis. 2019 May 30;14(1):120. doi: 10.1186/s13023-019-1099-0. PMID: 31146777; PMCID: PMC6543611.

6. De Baun MR, Jordan LC, King AA, et al. American Society of Hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults. Blood Clinical Advances. 2020; 4:1554-1588.

7. Pinto VM, Balocco M, Quintino S, Forni GL. Sickle cell disease: a review for the internist. Intern Emerg Med 2019; 14: 1051–64.

8. Amanda M. Brandow, C. Patrick Carroll, Susan Creary, Ronisha Edwards-Elliott, Jeffrey Glassberg, Robert W. Hurley, Abdullah Kutlar, Mohamed Seisa, Jennifer Stinson, John J. Strouse, Fouza Yusuf, William Zempsky, Eddy Lang; American Society of Hematology 2020 guidelines for sickle cell disease: management of acute and chronic pain. *Blood Adv* 2020; 4 (12): 2656–2701. doi: https://doi.org/10.1182/bloodadvances.2020001851

9. Kato, G., & Gladwin, M. (2009). Mechanisms and Clinical Complications of Hemolysis in Sickle Cell Disease and Thalassemia. In M. Steinberg, B. Forget, D. Higgs, & D. Weatherall (Authors), *Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management* (pp. 201-224). Cambridge: Cambridge University Press. doi:10.1017/CBO9780511596582.016

10. Wynn, R., Bhat, R., & Monagle, P. (2017). Sickle Cell Disease. In *Pediatric Hematology: A Practical Guide* (pp. 95-102). Cambridge: Cambridge University Press. doi:10.1017/CBO9781139942430.018

11. J. Strouse, Chapter 18 - Sickle cell disease,Editor(s): Michael J. Aminoff, François Boller, Dick F. Swaab, Handbook of Clinical Neurology, Elsevier,Volume 138,2016, pp:311-324. https://doi.org/10.1016/B978-0-12-802973-2.00018-5

12. Montalembert M, Fester A, Colombatti R, et al. On behalf of the European Network for Rare and Congenital Anaemias. ENERCA clinical recommendations for disease management and prevention of complications of sickle cell disease in children. Am J Hematol. Letter to Editor. 30 August 2010

13. Birnbaum BF, Pinzone JJ. Sickle cell trait and priapism: a case report and review of the literature. Cases J. 2008 Dec 30;1:429. doi: 10.1186/1757-1626-1-429. PMID: 19116025; PMCID: PMC2628646

14. Mañú Pereira MDM, Colombatti R, Alvarez F, Bartolucci P, Bento C, Brunetta AL, Cela E, Christou S, Collado A, de Montalembert M, Dedeken L, Fenaux P, Galacteros F, Glenthøj A, Gutiérrez Valle V, Kattamis A, Kunz J, Lobitz S, McMahon C, Pellegrini M, Reidel S, Russo G, Santos Freire M, van Beers E, Kountouris P, Gulbis B. Sickle cell disease landscape and challenges in the EU: the ERN-EuroBloodNet perspective. Lancet Haematol. 2023 Aug;10(8):e687-e694.

15. Mark T. Gladwin, Gregory J. Kato, Enrico M. Novelli. Sickle-Cell Disease MacGraw Hill Medical. 2021. ISBN 978-1-260-45859-6

16. Sociedad Española de Hematologia Pediatrica (SEOP). Enfermedad de células falciformes. Guia de práctica clínica. SEHOP-2019

Table 1. Most important complications of SCD

1. Vaso-occlusive crises
2. Hemolysis
3. Infections
4. Acute Chest Syndrome
5. Stroke
6. Splenic sequestration syndrome
7. Organ damage
8. Leg ulcers
9. Priapism
10.Retinopathy
11. Pulmonary hypertension
12. Delayed growth and development
13. Aplastic crises

Table 2. The 7 key points for the general management of SCD

1. Education, information, and advice regarding sickle cell disease (given to health workers, parents, and/or patients);
2. Prevention of infections by extended vaccinations (pneumococcal) and prophylaxis (penicillin)
3. Follow up with patients to identify those at risk for certain adverse outcomes
   - Stroke by monitoring them by transcranial Doppler scanning
   - Severe disease by monitoring the number of painful events per year
4. Treatment of chronic conditions by blood transfusion or hydroxycarbamide (Hydrea)
5. Treatment of acute crisis
   - Blood transfusion for acute stroke or acute chest syndrome,
   - Antibiotics for the infection,
   - Tailored analgesia for a painful crisis
   - Other
6. Monitoring and treatment of iron overload;
7. Curative therapy by hematopoietic stem cell transplantation (in severe cases, when possible)

Table 3. General clinical approach of patients with SCD

1. Universal neonatal screening.
2. Early prophylaxis with penicillin
3. Vaccination
4. Hydroxyurea administration in both children and adolescents
5. Blood transfusions
6. Iron Chelation
7. Hematopoietic Stem Cell Transplantation (HSCT)
8. Treatments in development

CHAPTER 9
COMPLICATIONS IN THALASSEMIA

UPDATE ON ENDOCRINE COMPLICATIONS IN β-TRANSFUSION DEPENDENT THALASSEMIA

Vincenzo De Sanctis[1], Ashraf T Soliman[2], Shahina Daar[3], Ploutarchos Tzouli[4], Maria Concetta Galati[5], Giuseppe Raiola[6], Mohamed A. Yassin[7]

[1] Coordinator of ICET-A Network (International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine) and Pediatric and Adolescent Outpatient Clinic, Quisisana Hospital, Ferrara, Italy

[2] Department of Pediatrics, Division of Endocrinology, Hamad General Hospital, Doha, Qatar

[3] Department of Haematology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Sultanate of Oman & Stellenbosch Institute for Advanced Study, Wallenberg Research Centre at Stellenbosch University, Stellenbosch 7600, South Africa

[4] Department of Diabetes and Endocrinology, Whittington Hospital, University College London, London, UK

[5] Department of Oncohematology, Pugliese-Ciaccio Hospital of Catanzaro, Italy,

[6] Department of Paediatrics, Pugliese-Ciaccio Hospital, Catanzaro, Italy,

[7] Hematology-Oncology Department, National Centre for Cancer Care and Research, Doha, Qatar

ABSTRACT

With the optimization of frequent transfusions and chelation regimens, and the availability of new imaging techniques that allow assessment of iron overload in several organs,

β-transfusion-dependent thalassemia (TDT) has changed from a pediatric disease with poor life expectancy to a chronic disease with open ended prognosis. Nevertheless, hepatic, cardiovascular and, mainly, endocrine complications still occur. The etiology of endocrine complications is multifactorial and partially unknown, although in thalassemia, iron overload is the major factor. Many diagnostic and therapeutic approaches of endocrine complications have been adopted from those in the general population to patients with TDT; however, some crucial aspects of the diagnosis and treatment of growth disorders and endocrine complications in TDT patients remain to be elucidated. The aim of the present update is to report the recent advancements in selected areas of endocrine complications in patients with TDT.

Keywords: Transfusion-dependent thalassemia, short stature, endocrine complications

INTRODUCTION

Clinically, β-thalassemias can be classified as β-transfusion-dependent thalassemia (TDT) or thalassemia major and non–transfusion-dependent thalassemia (NTDT) or thalassemia intermedia (TI) according to the severity of clinical and hematological phenotypes. These are related to genotype which consists of the combination of two β- thalassemia gene variants of the wide spectrum of variants prevailing in the respective population, in homozygous or compound heterozygous state. Co-inheritance of one or more α-globin gene deletions ($-\alpha^{3.7}$) and the presence of the *Xmn*I polymorphism and other polymorphisms are associated with lesser severity of the disease. Current treatment of TDT patients consists of regular lifelong blood transfusions (starting before the age of 2-3 years) and efficient iron chelation regimes to prevent and treat the severe iron load and organ toxicity. The only curative therapy is bone marrow transplantation, with gene therapy still in the experimental stage **(1)**.

Over the last half of the 20th century, the deleterious effects of complications due to iron overload in TDT patients have been well recognized; at present endocrine complications constitute a major health issue for a large proportion of patients with TDT particularly those of advanced age **(2, 3)**.

The aim of the present update is to report the recent advances in selected areas of endocrine complications in patients with TDT.

Short stature and growth hormone deficiency/insufficiency

Short stature is defined as height which is two standard deviations below the mean height for age and sex or below the 3rd percentile or more than two standard deviations below the mid parental height. Of note, independently of growth percentile, an annual growth velocity (GV) ≤ 3rd percentile must be considered a red flag that requires a prompt clinical and laboratory investigations.

In the past, short stature has been attributed to several factors, such as: chronic anemia, iron overload, associated endocrine complications [hypogonadism, disorders of growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis, diabetes], and non-endocrine conditions [chronic liver diseases, nutritional deficiencies, body disproportion with flattening of the vertebral bodies (platyspondilosis) at the dorsal-lumbar region, a complication of intensive iron chelation therapy with desferrioxamine] **(4-7)**. Nowadays, adherence to modern transfusion and iron chelation protocols and avoidance of iron chelator overdosage has clearly reduced the risk for short stature, and may have potentially reduced endocrine complications in children with TDT. However, longer-term studies are required to confirm whether these favorable results will continue to be observed in late adolescents and young adults.

Recently, a systematic review and meta-analysis including 74 studies from five continents [70.2% (Asia), 16.2% (Europe), 6.7% (Africa), 2.7% (America), 1.3% (Oceania), and 2.7% (Multicenter)] on the growth status of patients with TDT was reported by Arab-Zozani et al. **(8)**.

The overall mean age of the participants was about 14 years. SS (≤ 3rd centile) was identified when the patient's height was more than two standard deviations below the mean for age, gender, and ethnicity; GR when the height of the subject was lower than the Mid Parental Height (MPH) and GHD deficiency was defined when the peak of GH, during a provocative test was ≤ 5 ng/mL.

The prevalence of SS, growth retardation (GR), and growth hormone deficiency (GHD) was 48.9%, 41.1%, and 26.6%, respectively. The prevalence of both SS and GR was higher in males compared to females: 61.9% vs. 50.9% and 51.6% vs. 33.1%, respectively. No significant difference was found in patients among various populations.

Similar percentages of GHD (34%) were reported by Campo et al. **(9)** in 31 TDT (27 adults and 4 children) and by Gagliardi et al. **(10)** in 18 out of 81 (22.2%) adult TDT patients.

A low prevalence of GHD (4.5%) was reported in a survey promoted by ICET-A and published in 2019, in 202 children and adolescents (< 18 years) followed in Malaysia, Egypt and Oman **(11)**. All reported GHD diagnoses were made in Malaysian thalassemic children with short stature. The cut-off limit for the diagnosis of GHD was <10 ng/mL, using an ultrasensitive chemiluminescence immunometric assay. The wide variability could be due to small sample sizes, different diagnostic protocols, tests and cut-off used for the assessment of GH secretion and criteria used for the diagnosis of GHD.

In another study, 10 out of 28 TDT (35%) had GHD, including 5 cases with partial GHD. Only one patient with complete GHD had normal insulin growth factor -1 (IGF-1) level **(12)**.

A variable response to GH replacement therapy has been reported in children and adolescents and it is unclear whether its use in adults provides any consistent or clear benefits **(13)**.

Essentially, no well-defined signs or symptoms to suspect GHD in adult TDT patients have been reported. Therefore, the clinical evaluation of GHD is difficult because signs and symptoms may be subtle and nonspecific, and universal provocative testing in all patients is difficult because the approach is cumbersome and expensive **(9)**. The ICET-A proposed and revised some recommendations based on current evidence from literature **(14, 15)**. In particular, it recommended the evaluation of GH secretion in adult TDT patients in the presence of:

- Severe and/or prolonged history of iron overload.

- Serum IGF-1 level <-2 standard deviations for age and sex.
- Significant pituitary iron deposition and/or gland shrinkage.
- Documented dilated cardiomyopathy.
- Severe osteoporosis.

Hypothyroidism, hypocortisolism and hypoparathyroidism

Hypothyroidism in TDT patients is the result of primary gland failure or secondary to hypothalamus or pituitary gland damage due to iron overload. The severity of clinical manifestations generally reflects the degree of thyroid dysfunction. Annual screening of thyroid stimulating hormone (TSH) and free T4 (FT4) level is recommended, starting from the age of 10 years **(16)**. Subclinical hypothyroidism (SCH) is an asymptomatic state diagnosed when peripheral thyroid hormone levels are within normal reference laboratory range, but serum TSH levels are mildly elevated. This condition occurs in 3% to 8% of the general population. It is more common in women than men, and its prevalence increases with age. Patients with secondary or central hypothyroidism (CH) have low FT4 and low or inappropriately normal TSH levels **(17)**. Most researchers have reported that subclinical forms are the more prevalent **(17, 18)**.

The various degrees of thyroid dysfunction are more common in patients who are anemic (poorly transfused) and/or poorly chelated (17%), either because of ineffective dose of chelator or non-compliance to iron chelation therapy (ICT) versus efficiently treated patients **(17)**. Non-compliance to ICT increased the risk of thyroid disorders by 6.38-fold (95% CI: 2.4–16.95) versus compliant subjects, suggesting that an adequate chelation regimen might minimize the burden of iron overload in the thyroid gland **(17)**.

All patients with primary hypothyroidism with TSH >10 mIU/L should be treated with L-T4 **(16, 17)**; there is insufficient evidence available for patients with SCH. Some authors observed reversibility of SCH after intensive ICT **(17)**.

Hypocortisolism

Chronic transfusion induces iron overload in several organs, including impairment of the pituitary gland and/or adrenal glands. The diagnosis of central adrenal insufficiency is relatively simple when glucocorticoid secretion is profoundly depressed. However, basal cortisol level may be normal in partial central adrenal insufficiency (CAI) and stimulation tests are then necessary to investigate the integrity of the hypothalamic-pituitary-adrenal (HPA) axis and establish the diagnosis.

Adrenal insufficiency/hypocortisolism is considered a rare/uncommon endocrine complication **(19-21)** while others have reported a prevalence between 13% and 46%, up to 61% of TDT patients, with an increased frequency in males versus females **(22)**. The extreme variability could be attributed to the heterogeneity of populations and the methods used for diagnosis (insulin tolerance test, ACTH test with 1 μg or 250 μg, salivary cortisol).

In general, the conventional normal cortisol response 30 or 60 minutes after cosyntropin ($ACTH_{[1–24]}$) stimulation test (SST) is considered to be ≥18 μg/dL (≥ 500 nmol/L) or lower (14 to 15 μg/dL; 386-413 nmol/L), depending on the assay used **(21)**.

In summary, it is important for clinicians, when evaluating patients for AI, to be aware of the assay-specific cut-off for the method used in their institution. Given the risks associated with failure to diagnose AI, in situations of high clinical probability and inconclusive hypothalamic-pituitary-adrenal (HPA) testing, a clinical empirical trial of treatment with glucocorticoids may be required **(23)**.

Hypoparathyroidism (HPT)

HPT is a relatively uncommon endocrine complication in TDT patients with the leading symptoms of hypocalcemia, associated with high serum phosphorus levels and very low or inappropriately normal-low parathyroid hormone (PTH) levels in relation to hypocalcemia. Concurrent magnesium deficiency may aggravate clinical signs of HPT **(24)**. The clinical manifestations of hypocalcemia are related to the degree of severity of the hypocalcemia itself, the age at onset, and duration of the disease. Although HPT has been reported mainly after the sec-

ond decade of life in TDT patients, a recent Italian study has reported a rather high prevalence (6.6%) in prepubertal subjects (mean age 11.3 years, range: 2–12 years) with severe iron overload (3,631 ± 1,636 ng/mL) **(25)**.

The cause of HPT is probably due to iron deposition and toxicity of parathyroid gland tissue. Moreover, in TDT patients with HPT, serum ferritin might act as a stimulator of Fibroblast Growth Factor 23 which controls the serum levels of 1,25-dihydroxyvitamin D3 values **(25, 26)**.

Calcium and vitamin D metabolites are currently the cornerstone of therapy. Overtreatment can lead to nephrolithiasis, thus monitoring of renal calcium excretion is necessary **(27)**.

In summary, screening for calcium metabolism should be considered in TDT patients with severe iron overload for early detection and proper treatment. Majid et al. **(28)** have proposed that in developing countries screening for parathyroid dysfunction should start from 9 years onwards. Further studies are required to assess the effects of iron overload on the synthesis, secretion and metabolism of FGF-23 **(29)**.

Glucose dysregulation

Glucose dysregulation (GD) is a common finding that develops insidiously and may aggravate the patients' quality of life and prognosis. The pathogenesis of glycemic abnormalities in β-TM is complex and multifactorial. It has been predominantly attributed to a combination of reduced insulin secretory capacity and insulin resistance (IR). These patients are a very heterogenous group with some individuals exhibiting mainly insulin deficiency and others predominantly IR.

However, it has been shown that a defect in β-cell insulin secretion can be present early before the development of glucose intolerance, resulting from toxic effects of iron deposition in the pancreas **(30, 31)**.

The wide variation in prevalence of GD in TDT patients has been attributed to a number of factors, such as the patient's age, the total and annual blood consumption, degree of iron load, the efficacy of chelation therapy based on the type of chelators and compliance to therapy. Others major identified risk factors in addition to iron overload are: active hepatitis C, nutrient deficiencies, genetic factors, and possibly splenectomy **(32)**.

Like the anterior pituitary, pancreatic cells are also very susceptible to oxidative damage.

A recent study by Pepe et al. **(32)** using MRI T2* to evaluate pancreatic iron content, found that 88.8% of a cohort of 1,079 TDT patients had detectable pancreatic iron at baseline. Pancreatic iron loading was present even at an early age, and 82.9% patients with normal glucose metabolism had abnormal pancreatic MRI.

In addition, Meloni et al. **(33)** found that only 5.2% of chelated patients had no pancreatic iron after 18 months follow up from baseline MRI study. Their results also showed that removal by chelation of hepatic iron precedes removal of stored pancreatic iron.

A retrospective study by Noetzli et al. **(34)** strongly suggests that pancreatic iron loading precedes cardiac iron loading by about 10 years and is an early marker of inadequate chelation.

The current international guidelines recommend that all patients with TDT should be screened with oral glucose tolerance test (OGTT) annually from puberty or from the age of 10 years if there is a family history of diabetes mellitus (DM). Other screening parameters, like hemoglobin A1c (HbA1c) should not be used because of low sensitivity in the TDT population **(35)**.

Routine OGTTs should consider at least 4 time points (0, 30, 60 and 120 minutes after OGTT), because in a retrospective study we found that an isolated high 1-h post-load glucose level (≥155 mg/dL; H-NGT) during the OGTT served as a simple biomarker to detect TDT patients at risk for GD **(36)**. In addition, the use of Real-Time continuous Glucose monitoring for few days may emerge as an early diagnostic tool of glycemic abnormalities in these patients **(37)**.

ADA and WHO recommend different cut-off points for the diagnosis of impaired fasting glucose (IFG). However, according to our experience, we could

miss more than half of impaired glucose tolerance (IGT) cases when using the WHO classification alone. Such findings suggest that the lower FPG cut-off point in the ADA recommendations may act as a better diagnostic index **(38)**. The combination IFG/IGT in β-TM patients who are severely iron overloaded constitutes a high-risk state for developing diabetes **(39)**. Management of these patients should include intensive chelation therapy as well as lifestyle modification and oral antidiabetic agents.

The recognition of GD in β-TM patients is an important aspect of care in these patients because early diagnosis and treatment with intensive chelation regimen (monotherapy or combined) can improve insulin secretion and glucose metabolism **(40, 41)**. In less severely iron overloaded patients, prediabetes may be reversible through the implementation of regular iron chelation therapy, associated with lifestyle modification programmes based on the adoption of healthier diet and increased levels of physical activity.

Hypogonadism and potential benefits and risks of sex-steroid therapy

Overall, acquired disturbances at any level of the hypothalamic–pituitary–gonadal (HPG) axis can lead to a clinical syndrome of hypogonadism and impairment of reproductive function. Secondary hypogonadism (also known as central hypogonadism or HH) is the most common endocrine complication in patients with TDT. It is mainly due to damage of the pituitary gland through iron toxicity. This condition is biochemically characterized by low or inappropriately normal gonadotropins levels along with low sex steroid levels **(42)**.

The anterior pituitary is particularly sensitive to iron overload, and toxicity to pituitary cells results in deficiency of hormones secretion, resulting in HH, short stature, secondary hypothyroidism and hypoparathyroidism. Noetzli et al. **(43)**, using magnetic resonance imaging (MRI) showed that 37of 57 (66%) chronically transfused patients had detectable pituitary iron, even in children as young as 4 years. In particular, the study found that patients with HH had significantly higher pituitary iron load and a smaller pituitary volume than those without HH. The volume loss of pituitary was also more specific to HH. In brief, the study found that pituitary iron started to deposit in the 1st decade of life and accelerated in adolescence, possibly related to increased transfusion demands due to acceleration of growth in adolescents.

In a recent review of 22 studies between 2017 and 2022 the prevalence of hypogonadism ranged between 22.2% and 82% and in another review from 14 Mediterranean and Middle East countries (n = 4,477, mean age: 16.5 years) the pooled prevalence of hypogonadism/delayed puberty was 45.6% (44). The high variability in the proportion of patients affected by hypogonadism could be explained by ethnic factors, different accessibility and compliance to therapy, economic status and genetic susceptibility. Susceptibility to HH development is associated with severity of genotype, as patients with severe underlying molecular defects have a higher annual blood consumption and a higher rate of iron loading and possibly a different vulnerability to free radical damage **(45)**.

Hypogonadism elicits different clinical consequences according to the type of hypogonadism (primary, secondary or mixed) and to the period of life of clinical manifestation (adolescence or young adult). Substantially, the overall goals of hormone replacement therapy (HRT) in hypogonadal patients are **(39)**:

1. To stimulate the development and maintenance of secondary sexual characteristics and normal sexual function.
2. To support pubertal growth spurt.
3. To build and sustain normal bone accretion and muscle mass.
4. To relieve the symptoms of hypogonadism.
5. To assist in the proper psychosocial adjustment of adolescents with hypogonadism.
6. To improve the quality-of-life.

To meet these objectives, a gradual increase over a period of 2–3 years is recommended until an adult dose of testosterone or estrogen is reached. This regimen will also prevent premature fusion of epiphyses. Progesterone is added after puberty induction or during sex hormone replacement to sus-

tain puberty in girls after breakthrough bleeding, after at least 2 years of treatment. Puberty induction in late-diagnosed patients must be individualised **(39, 43-45)**. A faster than normal increase in estrogen doses can be considered in such cases. Treatment is monitored by patient satisfaction and growth and development measures. Induction of fertility requires treatment with exogenous gonadotropins **(46, 47)**.

Researchers used different regimens, depending on their experiences, preferable administration route, patient's age, hormones pharmacodynamics /pharmacokinetics and local availability. Moreover, the available sex steroids formulations have variable potency, cost, tolerability ad adverse events, and formal guidelines regarding the use of sex steroid preparations have been delineated mainly on the experience in non-thalassemic patients **(46, 47)**.

HRT in TDT patients is extremely complex because of associated co-morbidities, such as iron overload, thrombophilic status (especially after splenectomy), chronic liver disease, gallbladder disease, impaired glucose tolerance or diabetes, and cardiomyopathy **(46, 47)**.

Testosterone replacement therapy is the mainstay of treatment in male hypogonadal β-TM patients.

Patients receive mainly intramuscular (i.m.) depot testosterone. There is limited experience with transdermal and oral formulations, or with i.m. testosterone undecanoate **(46)**.

The majority of TDT female patients with hypogonadism usually receive either oral conjugated estrogens (CE), transdermal estrogen (TE) or oral contraceptive pills (CO) and a very few use the vaginal ring (47, 48). Their use is complicated by the lack of estrogen formulations dedicated to younger patients. Compared with oral E2, theoretical benefits of TD use include the more physiologic route of delivery, avoiding first-pass effects in the liver that include the accumulation of non-physiologic estrogens observed after the oral route, and avoiding effects associated with a procoagulation state **(47, 48)**.

Thus, the initiation and the choice of a regimen should be carefully weighed and be dictated by tolerance in the attempt to ensure compliance. The optimal duration of HRT remains an unsolved issue and must be decided after considering the initial indication and the benefit-risk balance, which is specific for each patient.

Despite the evidence of a positive effect on the induction of a full pubertal maturation, the beneficial effects of sex steroid therapy on final height, bone mineral density and quality of life in subjects with TDT are very limited, while the relative information reported in the literature are from studies published several years ago.

The side effects and disadvantages of HRT in males include: pain and swelling at injection site, accelerated bone maturation, gynecomastia, erythema, inflammatory reaction, skin irritation, acne, nervousness and or irritability **(46)**. Moreover, in patients using testosterone gel, close skin contact with other person should be avoided after applying to prevent transfer of medication.

Out of 424 males with TDT, one patient (19-year-old), with DM developed atrial thrombosis during testosterone therapy. Other side effects of treatment reported in 95 young TDT patients included persistent pain in the injection site and mild to moderate gynecomastia (43.1%). Local reactions to transdermal testosterone patch occurred in 1/3 of patients and priapism in 2 patients on treatment with depot testosterone. One case report described the occurrence of heart failure in a TDT male that occurred concomitantly with increasing the dosage of testosterone **(49)**.

In 42 splenectomized TDT female patients on long-term HRT, 2/42 had severe adverse events (a stroke with right hemiparesis and an episode of transient monocular visual loss). Three patients presented with a deterioration of glucose homeostasis from normal OGTT to IGT and 1 from IGT to diabetes. Mild elevation of liver enzymes and bilirubin occurred in 3/42 (7.1%) female patients on HRT **(47)**.

In brief, hypogonadism requires appropriate estrogen or testosterone replacement for puberty induction and progression. Treatment should be individualized with the use of the appropriate regime for dose, duration, and route of administration in order

to meet patient's expectations and treatment goals. Several clinical questions pertaining to the impact of benefits and risks of sex-steroids therapy in TDT still remain unanswered. Nevertheless, our clinical experience provides evidence that starting HRT at physiological age may improve growth, bone mineral density, and patients' well-being. Table 1 summarize the consequences, observed in TDT patients with hypogonadism and the potential benefits and side effects of using sex steroid replacement in Qatar.

Table 1: *Consequences of hypogonadism in TDT patients and potential benefits of hormone replacement therapy (HRT)*

	Findings in TDT patients due to hypogonadism	**Findings in TDT patients during HRT**
Puberty	Delayed/absent puberty	Development of secondary sexual characteristics
Growth	Attenuated or absent pubertal growth spurt	Improved growth and pubertal growth spurt
Menstrual cycle	Primary or secondary amenorrhea	Increased uterine growth and induce menstruation
Bone mineral Density	Osteopenia and osteoporosis	Increased bone mineral density
Muscle mass/ strength	Decreased Lean Body mass	Increased LBM
Sense of wellbeing **Improved Mood** **QoL**	± Decreased sense of wellbeing ± Decreased mood. Decreased QoL	Improved sense of wellbeing Improve mood. Improved QoL
Libido	Decreased Libido	Improved Libido

Abbreviation = QoL: Quality of Life

CONCLUSION

With the optimization of transfusional and chelation regimens, β- TDT has changed from a pediatric disease with poor life expectancy into a chronic disease. In parallel with the increased lifespan, the comorbidities associated with the disease have become more prevalent. Despite the role of chelation therapy in the management of iron overload, the risk of secondary endocrine and metabolic complications remains considerable **(50, 51)**. Moreover, the data on reversibility of endocrine complications remains sparse and requires more clarity from larger multicentre studies.

Many diagnostic and therapeutic approaches of endocrine complications have been adopted from those of general populations to patients with TDT; however, some crucial aspects of the diagnosis and treatment of growth disorders and endocrine complications in TDT patients remain to be elucidated. Therefore, all patients with endocrinopathies should be managed by multidisciplinary teams including the key role of an endocrinologist who has expertise in the diagnosis and management of endocrine complications of subjects with hemoglobinopathies.

REFERENCES

1. Kattamis A, Kwiatkowski JL, Aydinok Y. Thalassemia.Lancet.2022;399(10343):2310-24.
2. Carsote M, Vasiliu C, Trandafir AI, et al. New Entity-Thalassemic Endocrine Disease: Major Beta-Thalassemia and Endocrine Involvement. Diagnostics (Basel). 2022;12(8):1921.
3. Farmakis D, Porter J, Taher A, Domenica Cappellini M, Angastiniotis M, Eleftheriou A. 2021 Thalassaemia International Federation Guidelines for the Management of Transfusion-dependent Thalassemia. Hemasphere. 2022; 6(8):e732.
4. Multicentre study on prevalence of endocrine complications in thalassaemia major. Italian working group on endocrine complications in non-endocrine diseases. Clin Endocrinol (Oxf). 1995;42(6):581-6.

5. Soliman AT, De Sanctis V, Kalra S. Anemia and growth. Indian J Endocrinol Metab. 2014;18 (Suppl 1):S1-5.
6. De Sanctis V, Soliman AT, Elsedfy H, et al. Growth and endocrine disorders in thalassemia: The international network on endocrine complications in thalassemia (I-CET) position statement and guidelines. Indian J Endocrinol Metab. 2013;17(1):8-18.
7. Kyriakou A, Skordis N. Thalassaemia and aberrations of growth and puberty. Mediterr J Hematol Infect Dis. 2009;1(1):e2009003.
8. Arab-Zozani M, Kheyrandish S, Rastgar A, Miri-Moghaddam E. A Systematic Review and Meta-Analysis of Stature Growth Complications in β-thalassemia Major Patients. Ann Glob Health. 2021;87(1):48.
9. Campo MR, Farese A, Correale M, et al. Retrospective Analysis of Endocrine Dysfunctions in a Population of Adult Polytransfused Patients: Correlation of GH-IGF1 Axis Alteration with Cardiac Performance. Biomed Res Int. 2018;2018:6047801.
10. Gagliardi I, Mungari R, Gamberini MR, et al. GH/IGF-1 axis in a large cohort of ß-thalassemia major adult patients: a cross-sectional study. J Endocrinol Invest.2022; 45:1439–45.
11. De Sanctis V, Soliman AT, Canatan D, et al. An ICET-A survey on occult and emerging endocrine complications in patients with β-thalassemia major: Conclusions and recommendations. Acta Biomed. 2019; 89(4):481-9.
12. Dhouib NG, Khaled MB, Ouederni M, et al. Growth and endocrine function in Tunisian thalassemia major patients. Mediterr J Hematol Infect Dis.2018;10(1):e2018031.
13. Ngim CF, Lai NM, Hong JY, et al. Growth hormone therapy for people with thalassaemia. Cochrane Database Syst Rev.2020; 5(5):CD012284.
14. Soliman A, De Sanctis V, Elsedfy H, et al. Growth hormone deficiency in adults with thalassemia: an overview and the I-CET recommendations.Georgian Med News. 2013;222:79–88.
15. De Sanctis V, Soliman AT, Candini G, et al. Insulin-like Growth Factor-1 (IGF-1): Demographic, Clinical and Laboratory Data in 120 Consecutive Adult Patients with Thalassaemia Major. Mediterr J Hematol Infect Dis. 2014;6(1):e2014074.
16. De Sanctis V, Soliman AT, Duran Canatan D, et al. How to screen, evaluate and treat hypothyroidism in homozygous β-thalassemia (β-thal) patients.Endo.Thal. Riv It Med Adolesc.2019;17(1);104-10.
17. De Sanctis V, Soliman AT, Canatan D, et al. Thyroid Disorders in Homozygous β-Thalassemia: Current Knowledge, Emerging Issues and Open Problems. Mediterr J Hematol Infect Dis. 2019;11(1):e2019029.
18. Yassouf MY, Alquobaili F, Kabalan Y, Mukhalalaty Y. Compliance with Deferoxamine Therapy and Thyroid Dysfunction of Patients with β-Thalassemia Major in Syria. Hemoglobin. 2019; 43:218–21.
19. Miri-Aliabad G, Moghadam MN, Naderi M, et al. Adrenal Insufficiency in Patients with Beta Thalassemia Major in the Southeast of Iran. IJHOSCR.2022; 16 (3): 173-6.
20. Ambrogio AG, Danesi L, Baldini M, et al. Low-dose Synachten test with measurement of salivary cortisol in adult patients with β-thalassemia major. Endocrine. 2018;60(2):348-54.
21. Poggi M, Samperi I, Mattia L, et al. New Insights and Methods in the Approach to Thalassemia Major: The Lesson From the Case of Adrenal Insufficiency. Front Mol Biosci. 2020;6:162.
22. Karen E. Huang KE, Mittelman SD, Coates TD, Geffner ME, Wood JC. A significant proportion of thalassemia major patients have adrenal insufficiency detectable on provocative testing. J Pediatr Hematol Oncol. 2015; 37(1): 54–9.
23. Javorsky BR, Raff H, Carroll TB, et al. New Cutoffs for the Biochemical Diagnosis of Adrenal Insufficiency after ACTH Stimulation using Specific Cortisol Assays. J Endocr Soc.2021;5(4):bvab022.
24. Majumder A, Basu S. Hypoparathyroidism in a Case of Transfusion Dependent Thalassemia. ASEAN. 2020,35(1):129-32.
25. Mahmoud RA, Khodeary A, Farhan MS. Detection of endocrine disorders in young children with multi-transfused thalassemia major. Ital J Pediatr. 2021;47(1):165.

26. Majumder A, Basu S. Hypoparathyroidism in a Case of Transfusion Dependent Thalassemia. ASEAN.2020,35(1):129-32..
27. De Sanctis V, AT, Canatan D, et al. An ICET- A survey on hypoparathyroidism in patients with thalassaemia major and intermedia: a preliminary report. Acta Biomed. 2017; 88(4): 435–44.
28. Majid H, Jafri L, Ahmed S,Talati J, Moiz B, Khan AH. Unique classification of parathyroid dysfunction in patients with transfusion dependent thalassemia major using Nomogram-A cross sectional study.Ann Med Surg.2019;45:22-6.
29. Tangngam H, Mahachoklertwattana P, Poomthavorn P, et al. Under-recognized Hypoparathyroidism in Thalassemia. J Clin Res Pediatr Endocrinol. 2018;10(4):324-30..
30. Cunningham MJ, Macklin EA, Neufeld EJ, et al. Complications of β -thalassemia major in North America. Blood 2004;104:34-9.
31. Angelopoulos NG, Zervas A, Livadas S, et al. Reduced insulin secretion in normoglycaemic patients with β -thalassaemia major. Diab Med. 2006;23:1327-31.
32. Pepe A, Pistoia L,Gamberini MR, et al. The Close Link of Pancreatic Iron with Glucose Metabolism and With Cardiac Complications in Thalassemia Major: A Large, Multicenter Observational Study. Diabetes Care. 2020;43(11):2830-9.
33. Meloni A, Pistoia L, Ricchi P, et al. Prospective changes of pancreatic iron in patients with thalassemia major and association with chelation therapy. Blood Adv.2022:bloodadvances. 2022008805.
34. Noetzli LJ, Papudesi J, Coates TD, Wood JC. Pancreatic iron loading predicts cardiac iron loading in thalassemia major. Blood. 2009;114(19):4021-6.
35. De Sanctis V, Soliman A, Tzoulis P, et al. The prevalence of glucose dysregulations (GDs) in patients with β-thalassemias in different countries: A preliminary ICET-A survey. Acta Biomed. 2021; 92(3): e2021240.
36. De Sanctis V, Soliman A, Tzoulis P, Daar S, Pozzobon GC, Kattamis C. A study of isolated hyperglycemia (blood glucose ≥155 mg/dL) at 1-hour of oral glucose tolerance test (OGTT) in patients with β -transfusion dependent thalassemia (β-TDT) followed for 12 years. Acta Biomed. 2021; 92(4): e2021322.
37. Soliman AT, Yassin M, El-Awwa A, De Sanctis V. Detection of glycemic abnormalities in adolescents with beta thalassemia using continuous glucose monitoring and oral glucose tolerance in adolescents and young adults with β-thalassemia major: Pilot study. Indian J Endocrinol Metab. 2013;17:490-495.
38. De Sanctis V, Daar S, Soliman AT, et al. Screening for glucose dysregulation in β-thalassemia major (β-TM): An update of current evidences and personal experience. Acta Biomed 2022; 93(1): e2022158.
39. De Sanctis V, Daar S, Soliman AT, Tzoulis P, Karimi M, Kattamis C. Evolution of glucose-insulin homeostasis in children with β-thalassemia major (β-TM): A twenty-year retrospective ICET- A observational analysis from early childhood to young adulthood. Acta Biomed. 2022; 93(3): e2022243.
40. Platis O, Anagnostopoulos G, Farmaki K, Posantzis M, Gotsis E, Tolis G. Glucose metabolism disorders improvement in patients with thalassaemia major after 24-36 months of intensive chelation therapy. Pediatr Endocrinol Rev 2004;2 (Suppl 2):279-81.
41. Farmaki K, Angelopoulos N, Anagnostopoulos G, Gotsis E, Rombopoulos G, Tolis G. Effect of enhanced iron chelation therapy on glucose metabolism in patients with beta-thalassaemia major. Br J Haematol 2006;134:438-44.
42. De Sanctis V, Soliman AT, Candini G, Elsedfy H. The recommendation of the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine for the assessment of growth hormone secretion in thalassemia. Indian J Endocrinol Metab. 2015;19(2): 306–7.
43. Noetzli LJ, Panigrahy A, Mittelman SD, et al. Pituitary iron and volume predict hypogonadism in transfusional iron overload. Am J Hematol. 2012;87(2):167-71.doi: 10.1002/ajh.22247.
44. Soliman AT, Elalaily R, Soliman N. Prevalence of Short Stature and Endocrinopathy in β-thalassemia Major Patients on Conventional Therapy in the Mediterranean and the Middle East countries. 60th Annual Meeting of the ES-

PE. Horm Res Paediatr. 2022;95 (suppl 2):1–616.
45. Yassin MA, Soliman AT, De Sanctis V, Yassin KS, Abdulla MAJ. Final Height and Endocrine Complications in Patients with β-Thalassemia Intermedia: Our Experience in Non-Transfused Versus Infrequently Transfused Patients and Correlations with Liver Iron Content. Mediterr J Hematol Infect Dis. 2019; 11(1): e2019026.
46. De Sanctis V, Soliman AT, Yassin MA, et al. Hypogonadism in male thalassemia major patients: pathophysiology, diagnosis and treatment. Acta Biomed. 2018; 89 (Suppl 2): 6–15.
47. De Sanctis V, Soliman AT, Elsedfy H, et al. Review and Recommendations on Management of Adult Female Thalassemia Patients with Hypogonadism based on Literature Review and Experience of ICET-A Network Specialists Mediterr J Hematol Infect Dis.2017;9(1): e2017001.
48. De Sanctis V, Soliman AT, Daar S, et al. The experience of a tertiary unit on the clinical phenotype and management of hypogonadism in female adolescents and young adults with transfusion dependent thalassemia. Acta Biomed. 2019;90 (1):158-67.
49. De Sanctis V, Daar S, Soliman AT, Elsedfy H, Khater D, Di Maio S. Does Testosterone Replacement Therapy Promote an Augmented Risk of Thrombotic Events in Thalassemia Major Male Patients with Hypogonadism? Indian J Endocrinol Metab. 2017; 21(4): 636–7.
50. Shah FT. Endocrine complications in thalassaemia syndromes. Haematologica 2017; 102(s1):35-8.
51. Habbash F, Al-Bati W, Al-HashimH. Endocrine Complications and the Effect of Compliance with Chelation Therapy in Patients with Beta Thalassemia Major in Eastern Province of Saudi Arabia . J Blood Med. 2022:13: 763-73.

Conflict of interest statement: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Correspondence Address:
Vincenzo De Sanctis, MD
Coordinator of the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescence Medicine (ICET-A) and Adolescent Outpatient Clinic, Quisisana Hospital
Ferrara, Italy
44121 Ferrara, Italy
Tel: +39 0532 770243
E-mail: vdesanctis@libero.it

CARDIAC PROBLEMS AND MANAGEMENT IN THALASSEMIA

Specialist Dr. Zehra Diyar Tamburacı Uslu
University of Health Sciences, Antalya Education and Research Hospital,
Department of Pediatric Cardiology Antalya-Türkiye

ABSTRACT

Life quality and expectancy of transfusion dependent thalassemia major and thalassemia intermedia patients are gradually increasing in recent years. This remarkable improvement mainly depends on decrease in mortality due to cardiac complications. Nevertheless, cardiac complications related to iron overload are still the main reason of mortality and morbidity. In terms of cardiac problems, the early evaluation of thalassemic patients with chronic transfusion and iron overload who, at the same time, have a hyperdynamic circulation due to chronic anemia is very important in order to decrease mortality and morbidity rate among these patients. Cardiac iron buildup and cardiac functions are not merely dependant on ferritin level. Iron does not accumulate homogeneously in the heart. Cardiac structure and functions are heterogeneous in thalassemia major patients. Therefore, in order to identify disritmic tendency, an increased QT dispersion, which manifests ventricular repolarization in electrocardiogram (ECG) is an important indicator for cardiac arrhythmias and iron cardiomyopathy. At the conventional echocardiography examines; there is not a decrease in the global left ventricul functions and exercise capacities until the very last stage of the illness. Thus; patients with risk of heart failure, chronic transfusion patients and iron overload patients are uneasy to be determined in the early period. Tissue Doppler is a quite common technique and can be applied in many centers. This is why, if T2* MR is unable to evaluate some cases, they can be assessable with tissue Doppler in terms of systolic and diastolic dysfunctions in the early stage. In the early period of non-transferrin-dependent iron accumulation after multiple transfusions, other diagnostic tests other than cardiac T2* MRI may be completely normal. With the drop in T2* signal value; chance in treatment and arrangement of chelation therapy can decrease iron accumulation and prevent progress. Accordingly, thalassemia patients should definitely have cardiac examinations (physical examination, ECG, holter ECG, (echocardiography) periodically, if possible, T2* MR should be performed on these patients.

Keywords: Thalassemia major, cardiac iron overload, ferritin, electrocardiography, echocardiography, tissue Doppler echocardiography, T2* MR

INTRODUCTION

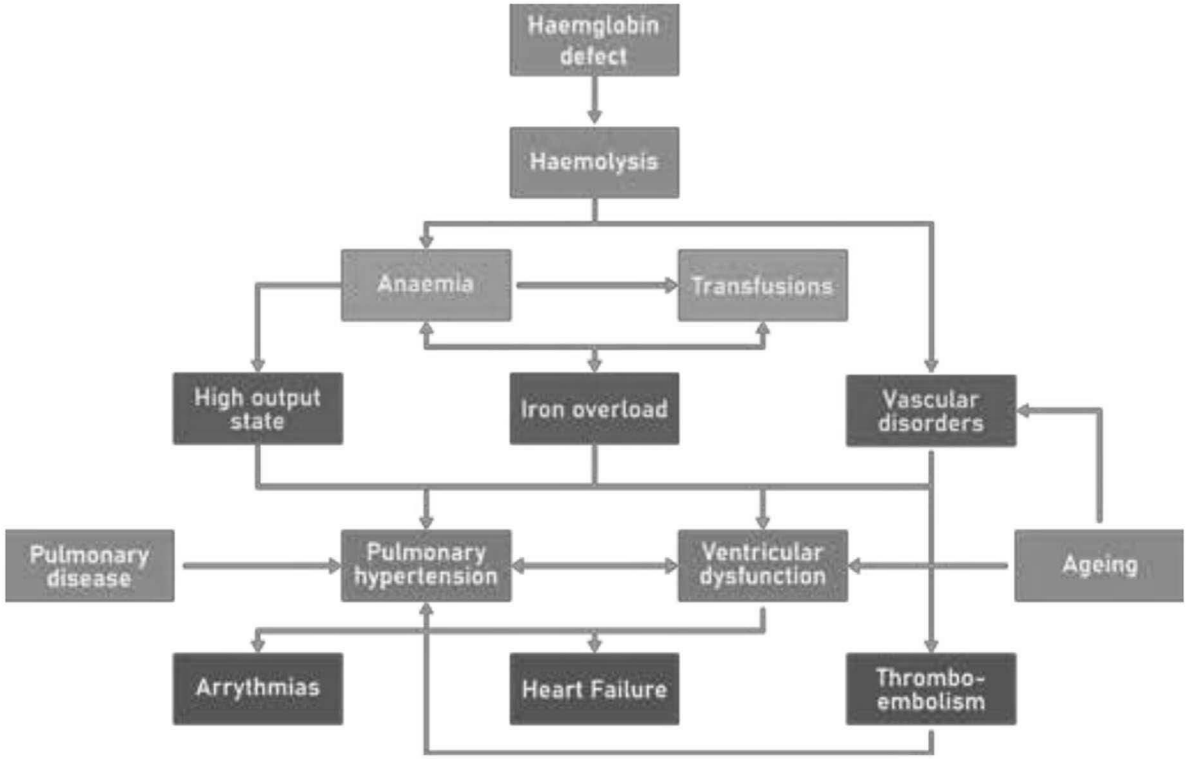
Cardiovascular (CV) complications are the leading cause of mortality in patients with thalassaemia, including thalassaemia major (TM) and thalassaemia intermedia (TI). Today, cardiovascular mortality has decreased significantly. This improvement has resulted from the effective implementation of modern diagnostic and therapeutic modalities. Among other advances, magnetic resonance imaging (MRI)-guided chelation therapy with the use of the T2* technique has been estimated to account for 71% reduction of mortality due to iron overload and 62% reduction of all-cause mortality since 2000 **(1)**.

PATHOPHYSIOLOGY

Pathophysiology is determined by two main factors; the severity of the genetically transmitted haematological defect and the applied therapy including

blood transfusions and iron chelation, which is determined by physicians' therapeutic choices as well as patients' access to and compliance with the prescribed regimens. A third factor with increasing influence on the pathophysiology of cardiovascular disease in thalassemia is aging and is reflected in the clinical course **(2)**.

Figure 1. Pathophysiology of cardiovascular disease in thalassaemia (modified from Farmakis et al, Eur J Heart Fail 2017;19: 479-489).



CARDICA SIDE EFFECTS

The underlying pathological mechanism is common in both forms of transfusion dependent (TDT) and non transfusion dependent (NTDT) thalassemia disease causing cardiac dysfunction due to increased workload on the heart. Heart failure due to iron storage in the heart due to multiple blood transfusions in TDT is the major cause of death. Cardiac iron deposition occurs primarily in the ventricle and most commonly in the epicardium. Moreover, free iron interacts with calcium channels and leads to disruption of myocardial contractility. In NTDT, on the other hand, has a lesser effect on the heart since intestinal iron absorption primarily leads to iron storage in the liver. In NTDTs, cardiac involvement may be due to other cardiac causes such as pulmonary hypertension and thrombosis rather than excessive iron deposition. Chronic anemia, increased cardiac output in NTDT is one of the main pathophysiological mechanisms in cardiac involvement. Even if TDT is well transfused, increased cardiac output may have an effect to some degree.

The spectrum of cardiac disease includes left and/or right ventricular dysfunction with or without heart failure, pulmonary hypertension, tachyarrhythmias such as atrial fibrillation, bradyarrhythmias such as atrioventricular block, valvular disease, pericarditis and myocarditis. Further CV disorders include, thromboembolic events resulting from either venous or arterial thrombosis, cerebrovascular disease manifested as either ischaemic or haemorrhagic stroke and vascular abnormalities including endothelial dysfunction and increased arterial stiffness.

Thalassemic Cardiomyopathy (Iron Load Cardiomyopathy).

Iron overload cardiomyopathy constitutes the main form of heart disease in thalassemia patients who receive regular blood transfusions but cannot be adequately chelated due to lack of compliance or access to iron chelation regimens **(3)**.

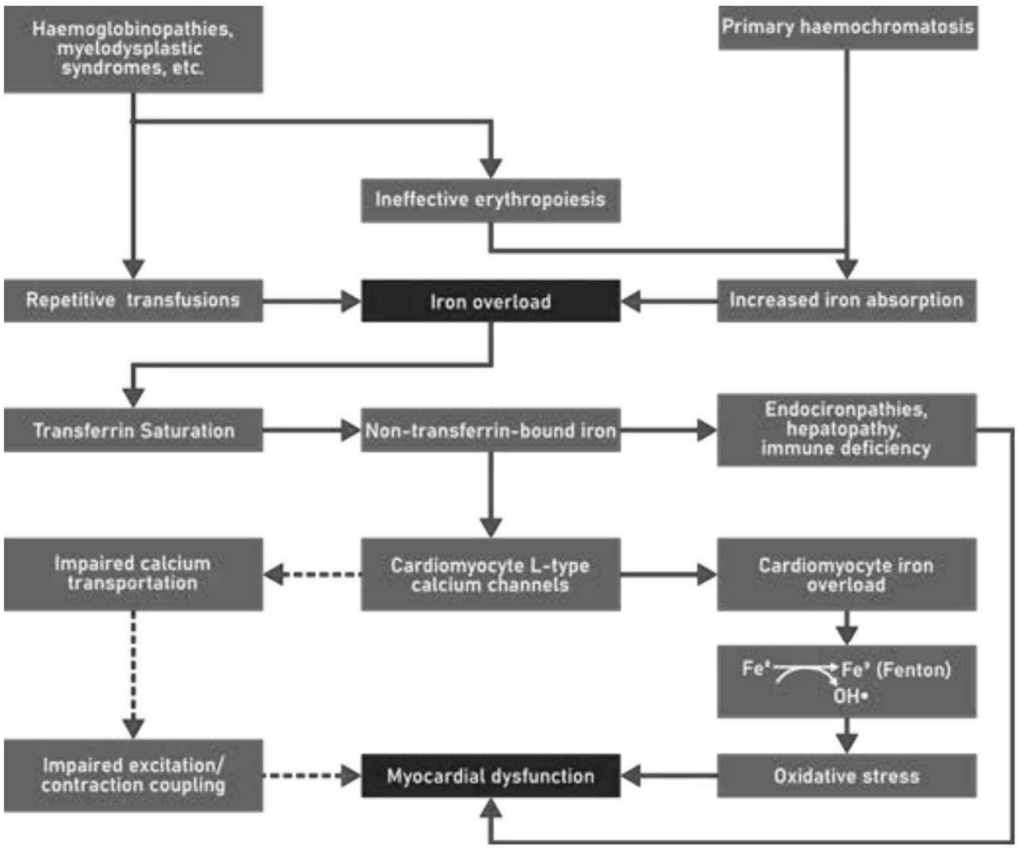
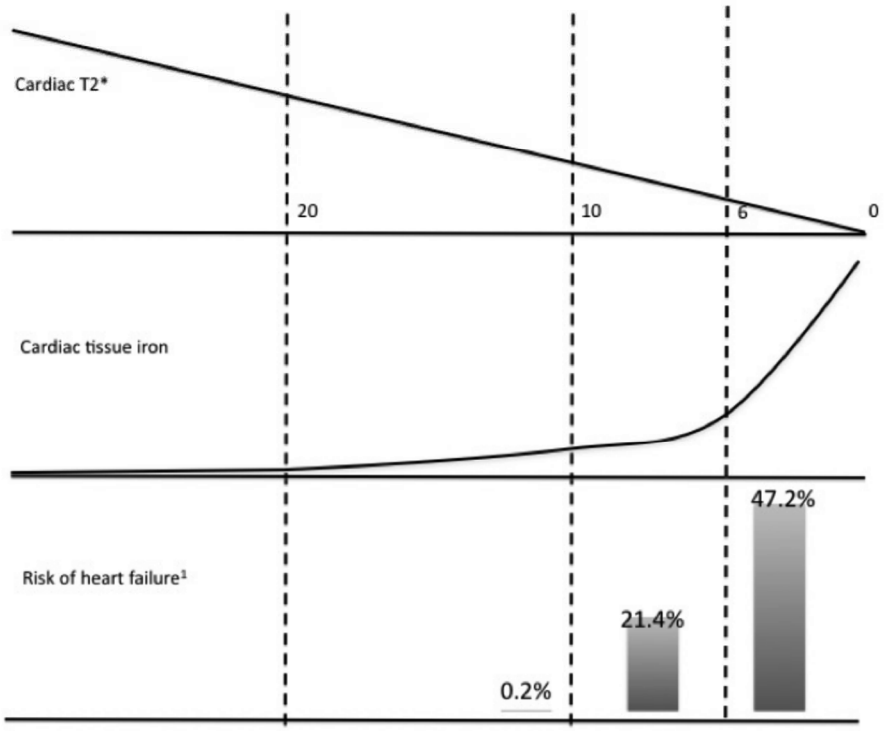


Figure 2. Pathophysiology of iron overload cardiomyopathy (from Kremastinos et al., Circulation 2011;124: 2253-2263).

Left ventricular failure is more common than right ventricular failure in more than 80% of cases. This form of cardiomyopathy may manifest as two different phenotypes; whether or not with LV dilatation, it either leads to heart failure with hypokinetic cardiomyopathy with decreased left ventricular (LV) contractility, or leads to heart failure as restrictive cardiomyopathy with severely impaired LV diastolic function and preserved contractility. The deterioration that starts with diastolic dysfunction over time becomes evident with the deterioration of systolic functions. Many patients with severe iron overload continue with normal or near-normal systolic left ventricular function sometimes for long periods of time, but are at risk of acute decompensation, often due to intercurrent diseases. Patients often consult with symptoms of increased pulmonary venous pressure (dyspnea, orthopnea, and exercise dyspnea). In the presence of significant myocardial iron overload (T2* 60%), rhythm disturbances including decreased systolic function, heart failure, conduction abnormalities, and ventricular arrhythmias, are seen and predict poor prognosis **(3)**.

Genetic factors may predispose to heart failure in thalassemia patients. Again, it has been reported in studies that left ventricular failure may be seen more in people carrying the apolipoprotein e4 allele **(4)**. Kremastinos at al. study of the HLA-DRB1*1401 and HLA-DQA1*0501 allele frequencies were found to be higher in patients with left heart failure than in healthy patients without heart failure **(5)**.

Figure 3. Schematic representation of the relationship among cardiac iron overload, as estimated by magnetic resonance imaging T2*, left ventricular ejection fraction and the risk of heart failure in patients with thalassaemia (from Kremastinos et al., Circulation 2011;124:2253-2263).



High-output failure

In the absence of regular transfusions able to maintain an adequate pretransfusional haemoglobin concentration, chronic anaemia leads to a compensatory increase in cardiac output. In addition, high-output state also contributes to heart disease within contemporary thalassaemia populations including TI or sub-optimally transfused TM patients, to an extent that is directly related to the severity of residual chronic anaemia.

Valvular heart disease

Valvular heart disease concerns an increasing prevalence of mainly mild to moderate disorders including mitral valve prolapse, mitral and aortic valve regurgitation and some sporadic cases of severe aortic stenosis. These lesions have been partly related to cardiac remodelling in the context of a high output state and, interestingly, to a coexisting disorder of elastic tissue resembling hereditary pseudoxanthoma elasticum (PXE) **(6)**. This disorder is primarily seen in middle-aged or elderly patients, usually with non-regularly treated TI, and is followed by cutaneous, ocular, vascular and valvular lesions.

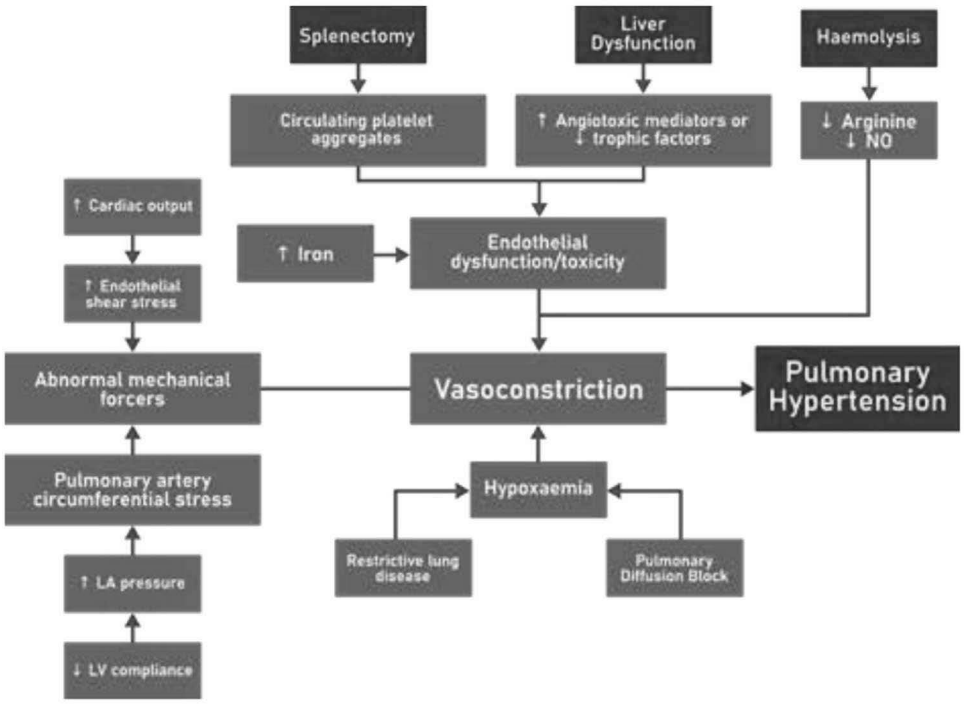
Myocarditis/pericarditis

Although congestive heart failure is the main cause of death, almost half of the patients with thalassemia major experience an episode of acute pericarditis/myocarditis throughout their lives. The most common ECG anomalies are T wave negativity, ST segment elevation, pathological Q wave and decreased R wave amplitude. While acute pericarditis was quite common before regular chelation therapy, today this complication has decreased significantly with effective thalassemia management. Like acute cases of pericarditis, cases of acute myocarditis have been reduced by better management of iron overload and anemia, and by improving the quality of blood to be transfused.

Pulmonary hypertension

Many studies have reported that chronic hypoxia and damage caused by iron storage may lead to pulmonary hypertension in these patients. Another factor thought to be related is thrombotic lesions caused by hypercoagulability. In addition, it has been shown to be associated with splenectomy, age, and chronic hemolysis **(7)**.

Figure 4. Pathophysiology of pulmonary hypertension in thalassaemia. LA, left atrium.



In non-transfused TI patients, particularly those with previous splenectomy, a high occurrence of thromboembolic complications is also observed, including deep vein thrombosis, pulmonary embolism, stroke, portal vein thrombosis and others. The prevalence of thromboembolic disease is reported to be considerably lower in regularly treated TM patients (5%) compared to TI patients with a history of splenectomy (29%) **(8)**.

Conduction disorders and dysrhythmias

Iron does not show a homogeneous accumulation in the heart. Therefore, if the QT dispersion, which is evaluated to determine the susceptibility to dysrhythmia and indicates ventricular repolarization, is increased; it is an important indicator for cardiac arrhythmias and iron cardiomyopathy. While the rhythm may be completely normal, ST depression and T wave negativity, especially occuring with exercise, may be seen as infrequent atrial and ventricular extrasystoles, and frequent atrial and ventricular extracytoles.

Major arrhythmias are more common in the group that receives transfusion (over 200 units) and does not receive good chelation. Progressive cardiac damage should be considered in the presence of cardiac arrhythmia (atrial fibrillation, atrial flutter, recurrent ventricular arrhythmias) and early cardiac dysfunction. Cardiac iron accumulation in thalassemia patients may affect the cardiac conduction system, therefore conduction delays and heart blocks may be seen. With 24-hour Holter ECG monitoring, it was determined that atrial and especially ventricular extrasystoles (early beat) increased significantly and there were intermittent ventricular tachycardia attacks. Outcomes are worse for those who start chelation therapy at adolescence. If there is a decrease in the ejection fraction with resistant arrhythmias, this is accepted as a sign that cardiac failure will increase significantly within a year. AF is the most common arrhythmia, and increased iron overload and left atrial enlargement increase the likelihood of arrhythmias. Atrial or multifocal atrial tachycardias may also develop. If AF has occurred in the presence of iron overload, chelation therapy should be intensified. Although MRI is a good tool for predicting T2* arrhythmias, arrhythmias can also be seen in normal T2* values.

Impaired neurohumoral activation

Autonomic imbalance is held responsible for the pathophysiology of arrhythmogenesis, especially ventricular arrhythmias and sudden cardiac death, due to increased sympathetic activity and decreased

vagal tone. Cardiac autonomic changes can be seen without heart failure, peripheral and autonomic neuropathy.

Cerebrovascular diseases

Cerebrovascular diseases in the form of either ischaemic or haemorrhagic stroke has been reported in patients with thalassaemia. Ischaemic strokes have been associated with underlying atrial fibrillation in patients with TM, while haemorrhagic strokes with the aforementioned PXE-like elastic tissue disorder in patients with TI, with PXE-related vascular lesions comprising calcification and increased risk of rupture and bleeding **(9)**.

EVALUATION AND MONITORING OF CARDIAC SIDE EFFECTS

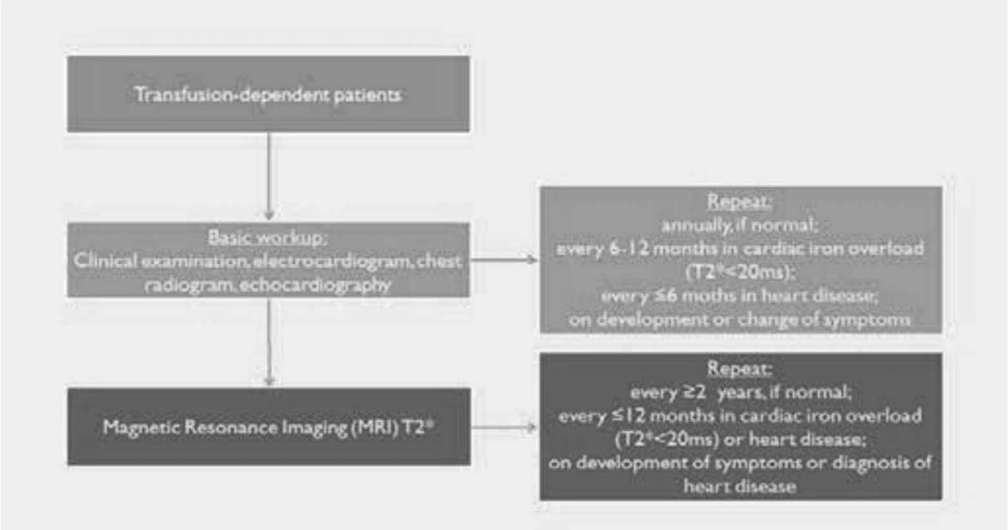
Regular evaluation of cardiac status in patients with thalassemia is achieved by regular evaluation of ECG, holter, echocardiography, and, if possible, cardiac MRI. Iron accumulation may cause delayed conduction and vague repolarization anomalies on the ECG. Echocardiography, on the other hand, is sensitive for systolic and diastolic dysfunction metrically, but is relatively specific for iron accumulation. Tricuspid regurgitation jet flow is significant for the recognition of pulmonary hypertension. Cardiac MRI is the gold standard for monitoring cardiac status because it provides both predictive estimation of cardiac iron load and assessment of right and left ventricular volume. In the early period of non-transferrin-dependent iron accumulation after multiple transfusions, other diagnostic tests other than cardiac T2* MRI may be completely normal.

All patients with thalassemia should have regular annual cardiac monitoring. In the presence of cardiovascular disease, the frequency and content of cardiac assessment should be tailored to meet the needs of each patient and administered at shorter intervals (e.g., 3 or 6 months) according to disease severity. In addition, the development of new symptoms potentially suggestive of cardiovascular disease such as dyspnea, chest discomfort, frequent palpitations, syncope or fainting, lower extremity edema, fatigue or exercise intolerance should prompt immediate action and referral to a thalassemia center.

How to assess?

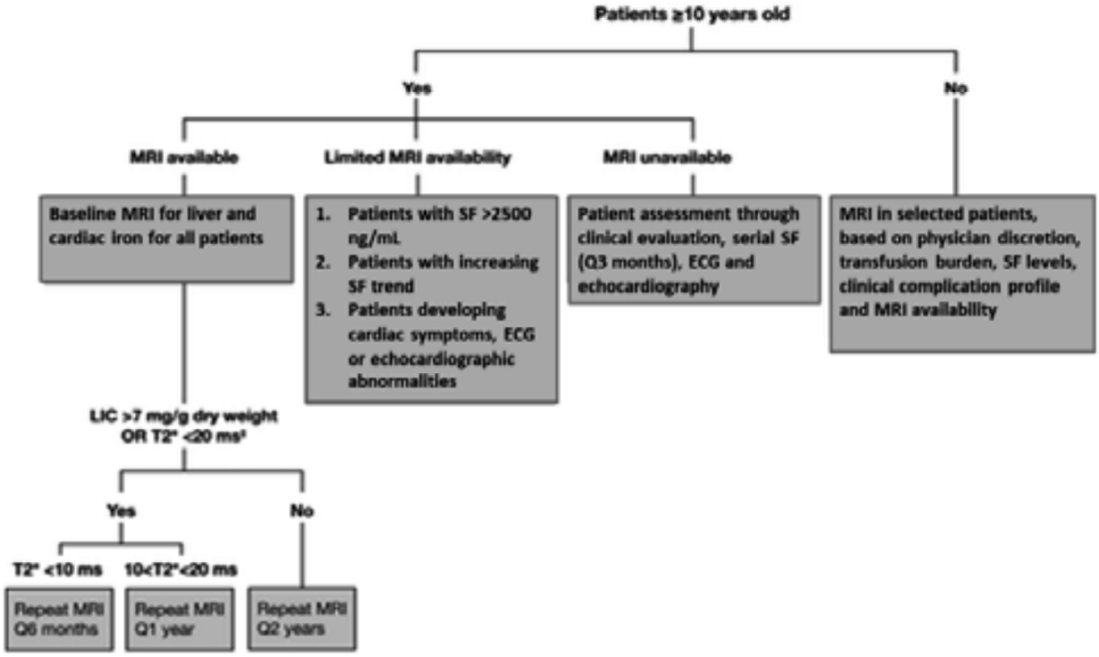
The regular annual basic CV assessment consists of; history taking, physical examination; electrocardiogram (ECG), transthoracic echocardiography (TTE) **(2)**. **(Figure 5)**:

Figure 5. Basic algorithm for the cardiac evaluation of patients with thalassaemia (modified from Farmakis et al, Eur J Heart Fail 2017;19:479-489). DFO, deferoxamine; DFP, deferiprone; HfrEF, heart failure with reduced left ventricular ejection fraction; MRA, mineralocorticoid receptor inhibitor; RAASi, renin-angiotensin-aldosterone system inhibitor.



A typical echocardiographic examination should report heart cavity dimensions, left ventricular wall thickness, left ventricular systolic and diastolic function indices, right ventricular systolic function indices, tricuspid regurgitation flow rate, heart valve morphology and function, and the presence of pericardial fluid or other abnormalities such as shunts or intracavitary masses. It should be kept in mind that increased Doppler velocities may be high due to chronic anemia, not true heart disease. Evaluation of cardiac iron content by cardiac MRI T2* should be performed simultaneously with hepatic MRI T2* according to patient's iron load and local protocols. Typically, the first MRI T2* scan is done 7-10 years after the start of blood transfusions and is repeated every 2 years. In the absence of access to MRI T2*, left ventricular diastolic or systolic worsening detected by serial echocardiography is a stimulus for cardiac iron overload.

Figure 6. A proposed algorithm to guide magnetic resonance imaging (MRI) T2* use according to local availability (LIC, liver iron concentration; MRI, magnetic resonance imaging; SF, serum ferritin; modified from Viprakasit et al., Am J Hematol 2018;93: E135-E137)



- ambulatory ECG monitoring for the evaluation of frequent palpitations or known arrhythmias or to assess the arrhythmogenic risk of patients with systolic LV dysfunction or heart failure;
- cardiac biomarkers, including cardiac troponins (e.g., in suspected myocarditis) or natriuretic peptides (e.g., for the evaluation of patients with known or suspected heart failure);
- cardiac magnetic resonance imaging for the more accurate assessment of cardiac cavities, systolic LV function and myocardial tissue characterization;
- exercise testing such as exercise ECG or ergospirometry for the assessment of functional capacity or arrhythmias;
- right cardiac catheterization for the evaluation of pulmonary artery pressure in patients with elevated TRV (e.g., >3 m/s, despite optimal transfusion therapy and a pre-transfusional haemoglobin level close to 100 g/l).
- lung function tests, high-resolution chest computed tomography (CT), CT pulmonary angiography or lung scanning along with careful LV ventricular function evaluation are required for the comprehensive diagnostic assessment of confirmed pulmonary hypertension.
- assessment should always take into consideration the parameters of the main disease, such as blood transfusion and iron chelation programme, pre-transfusional haemoglobin level and serum ferritin concentration, as well as parameters rela-

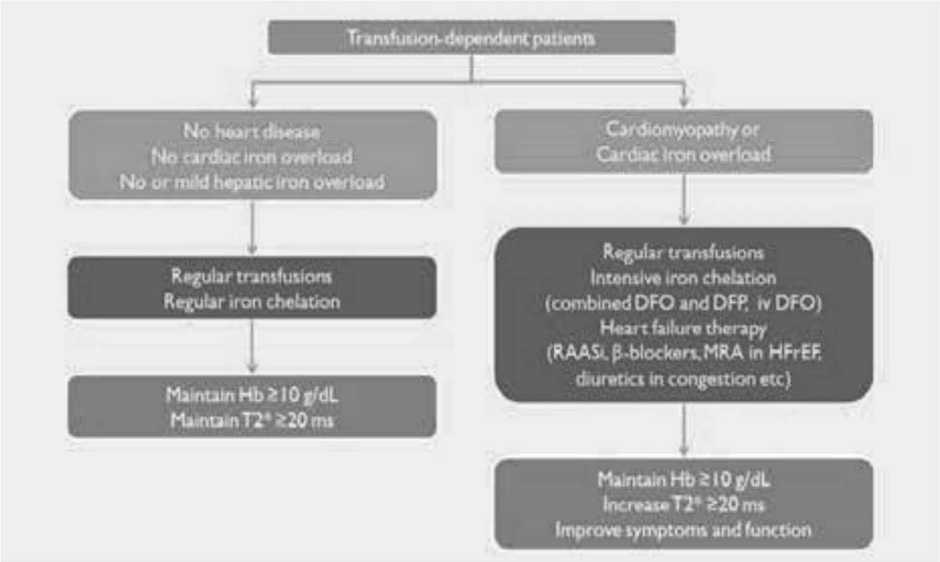
ted to other systems such as liver or endocrine disease. In addition, CV assessment should always be performed in close collaboration and communication with the thalassaemia physician who oversees the patient's whole monitoring and treatment.

Disease specific therapy

The management of CV abnormalities in thalassaemia patients should take the pathophysiology and characteristics of the main underlying disease into consideration. As a result, disease-specific therapy, including regular blood transfusions aiming at a pre-transfusional haemoglobin level of 100 g/l and iron chelation regimens aiming at a cardiac T2* value greater than 20 ms, hold the key role for the prevention and management of CV disease (Figure 7). Patients with high levels or serum ferritin or hepatic iron overload with or without cardiac iron overload should be treated with combined chelation therapy (e.g., deferoxamine and deferiprone), while those with acute or advanced iron overload-induced heart failure may require continuous intravenous infusion of deferoxamine.

It should further be stressed that cardiac dysfunction generally lags cardiac iron deposition by several years, while cardiac iron clearance is also a very slow process requiring several years to complete. In the presence of significant cardiac iron overload (T2*<20 ms) patients are at risk of rapid deterioration, even in the presence of normal or near normal systolic LV function, while a drop in LV EF may often carry a dire prognosis. As a result, chelation regimes must be adjusted to ensure a rapid fall in cardiac iron content.

Figure 7. A basic algorithm for the management of thalassaemia patients on regular blood transfusions (DFO: deferoxamine; DFP: deferiprone; DFX: deferasirox; Hb: haemoglobin concentration; ACEi: angiotensin converting enzyme inhibitors; ARB: angiotensin II receptor blockers; AFib: atrial fibrillation;modified from Farmakis et al, Eur J Heart Fail 2017;19:479-489).



Cardioactive therapy

Undertaking a healthy lifestyle, in terms of diet, regular exercise, body weight control and smoking abstinence, in accordance with general guidelines on CV prevention is crucial for the prevention of CV disease in combination with proper disease-specific therapy. In addition, the management of CV risk factors and of complications arising from other systems or organs such as diabetes, thyroid disease, or liver disease, is also of key importance. CV prevention is becoming even more important today in view of the increasing risk of age-related complications in ageing thalassaemia patients.

CV TREATMENT

The CV management of patients should take under consideration the following tips:

- CV disease should primarily prompt optimisation of disease-specific therapy, including blood transfusions and iron chelation as well as investigation and treatment of comorbid conditions such as endocrine or metabolic disease.
- Cardiac dysfunction and heart failure due to iron overload may be reversed with intensified iron chelation therapy and this possibility should be considered in decision making regarding more permanent CV interventions such as implantable cardioverter defibrillator (ICD) implantation (using MRI conditional devices), catheter ablation of arrhythmias, permanent ventricular assist device implantation or cardiac transplantation.
- A pacemaker may be need for the management of atrioventricular block, often related to iron overload; in this case leads should be MRI conditional to allow periodic evaluation of iron overload by the T2* technique.
- Thalassaemia patients generally have low blood pressure levels, and therefore use of blood pressure-lowering medications such as renin-angiotensin-aldosterone system inhibitors should be cautious.

  In addition, the use of vasopressors and other blood support therapies in patients with hypotensive heart failure should rather be targeted to renal perfusion and other surrogates instead of blood pressure values. Thalassaemia patients often have restrictive cardiac physiology, usually due to iron overload, and increased vascular stiffness, that may render them sensitive to hypovolaemia during diuresis. Anticoagulation in patients with atrial fibrillation may be challenging; nonregularly treated patients with previous splenectomy carry an increased risk of thromboembolism, while patients with pseudoxanthoma elasticum-like lesions may carry an increased risk of bleeding (Aessopos, Farmakis & Loukopoulos, 2002). The use of general risk prediction scores such as the CHA2DS2-VASc may be inappropriate for thalassaemia patients, as it may underestimate their potential risk.

REFERENCES

1. Modell, B., Khan, M., Darlison, M., Westwood, M.A., et al. (2008) Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. Journal of Cardiovascular Magnetic Resonance: Official Journal of the Society for Cardiovascular Magnetic Resonance. [Online] 10, 42. Available from: doi:10.1186/1532-429X-10-42
2. Farmakis, D., Triposkiadis, F., Lekakis, J. & Parissis, J. (2017) Heart failure in haemoglobinopathies: pathophysiology, clinical phenotypes, and management. European Journal of Heart Failure. [Online] 19 (4), 479–489. Available from: doi:10.1002/ejhf.708.
3. Kremastinos, D.T. & Farmakis, D. (2011) Iron overload cardiomyopathy in clinical practice. Circulation. [Online] 124 (20), 2253–2263. Available from: doi:10.1161/ CIRCULATIONAHA.111.050773.
4. Bazrgar M, Karimi M, Peiravian F, Fathzadeh M, Fattah MJ. Apolipo protein E4 allele and the risk of left ventricular dysfunction in thalassemia major. Iranian Cardiovascular Research Journal 2007; 1(1): 13-9
5. Kremastinos DT, Flevari P, Spyropoulou M, Vrettou H. Asso ciation of heart failure in homozygous beta thalassemia with the major histocompatibility complex. Circulation 1999; 100: 2074-8
6. Aessopos, A., Farmakis, D. & Loukopoulos, D. (2002) Elastic tissue abnormalities resembling pseudoxanthoma elasticum in beta thalassemia and the sickling syndromes. Blood. [Online] 99 (1), 30–35. Available from: doi:10.1182/blood.v99.1.30
7. 2021 Guidelines For The Management of Transfusion Dependent Thalassaemia(TDT) Publishers Thalassaemia International Federation Editors: Cappellini, M.D. Farmakis, D. Porter, J. Taher, A
8. Cappellini, M.D., Robbiolo, L., Bottasso, B.M., Coppola, R., et al. (2000) Venous thromboembolism and hypercoagulability in splenectomized patients with thalassaemia intermedia. British Journal of Haematology. [Online] 111 (2), 467–473. Available from: doi:10.1046/j.1365-2141.2000.02376.x.
9. Aessopos, A., Farmakis, D. & Loukopoulos, D. (2002) Elastic tissue abnormalities resembling pseudoxanthoma elasticum in beta thalassemia and the sickling syndromes. Blood. [Online] 99 (1), 30–35. Available from: doi:10.1182/blood.v99.1.30.

Correspondence Address:

Specialist Dr. Zehra Diyar Tamburacı

University of Health Sciences, Antalya Education and Research Hospital, Department of Pediatric Cardiology Antalya -Türkiye

E-mail: tamburacizehra@gmail.com

LIVER COMPLICATIONS IN THALASSEMIA

Specialist Dr. Vedat Aslan MD
University of Health Sciences, Antalya Education and Research Hospital, Department of Hematology Antalya -Türkiye

ABSTRACT

The liver is the most important organ in iron metabolism. It has mechanisms for controlling the amount of iron obtained from food, the safe reutilization of free iron released after the breakdown of aged red blood cells by macrophages, and its safe storage. It synthesizes important proteins such as hepcidin, ferritin, and transferrin. Therefore, in diseases characterized by excessive iron accumulation, it can be damaged at an early stage. Moreover, many diseases requiring frequent transfusions in thalassemia patients are associated with viruses that can cause liver damage, whether they are transfusion-dependent or not. All thalassemia major and intermedia patients should be closely monitored for liver iron accumulation and viral hepatitis.

Keywords: Liver, complication, thalassemia

INTRODUCTION

Physical examination: In the early stages of iron accumulation in the liver, the physical examination findings are indicative of thalassemia-related symptoms; therefore, hepatomegaly may be observed in the early stages. In advanced stages, depending on the degree of cirrhosis, physical examination findings such as palmar erythema, ascites, spider nevi, and signs of hepatic encephalopathy may be observed. The liver can shrink in advanced-stage cirrhosis.

Laboratory: Values and imaging method vary depending on the severity of liver damage. While mild elevations of AST, ALT, ALP and GGT can be observed in the early stages, prolonged prothrombin time, increased direct bilirubin and low albumin are prominent in the advanced stages.

Ferritin: Ferritin is an indirect indicator of the amount of stored iron. Since it is a positive acute phase reactant, it may increase more than expected in case of inflammation. This increase may also be due to the release of excess amounts from damaged cells when liver damage develops. Since it is very easy to reapply and accessible, it can be very helpful in iron accumulation when used well. The targeted ferritin level in thalassemia patients is below 500 ng/mL.

Pathophysiology: The liver is the most important organ in iron metabolism. Hepatocytes produce not only transferrin and ferritin, which are involved in the transport of iron, but also hepcidin, which determines the use of iron in other organs. It regulates ferroportin, which removes excess free iron from the cell. Due to frequent transfusions in thalassemia patients, iron is primarily taken up by macrophages and accumulated in hepatocytes in the liver. Since erythrocyte production in thalassemia patients is defective, hypoxia increases erythropoietin and, in this case, hepcidin decreases. Depletion of hepcidin causes an increase in free iron (non-transferrin-bound iron). As the iron released after tranferrin is filled, toxic iron species begin to increase. In this case, DMT1 (divalent metal transporter 1) increases ferritin production by taking this iron. Ferritin carries much more iron than transferrin, but as the amount of free iron increases, it may exceed the carrying capacity even though ferritin increases. In this case, iron accumulates primarily in hepatocytes and cell damage occurs with oxygen radicals.

While hepcidin reduces the absorption of excess iron in transfusion-dependent patients, it increases iron absorption in non-transfusion-dependent thalassemia intermedia patients.

Transferrin reaches saturation when the total amount of iron in the body exceeds the amount of iron required for erythrocyte production, myoglobin and enzymes in the muscles. When transferrin increases from 35% to 70% of its binding capacity, free iron binds to other proteins and molecules (such as albumin, citrate, acetate). Iron bound to substances other than transferrin is called non-transferrin-bound iron (NTBI).

NTBI is transported into the cell via L-type calcium channels. This excess iron entering the liver, heart, and endocrine parenchymal cells interacts with hydrogen peroxide and initiates the Haber-Weiss reaction as a Fenton agent. Free oxygen radicals (ROS) resulting from this reaction cause oxidation in the cell lipid membrane. Cell death occurs in a cell whose cell and mitochondrial membrane integrity is lost.

DIAGNOSIS AND STAGING

If advanced-stage cirrhosis has developed, ultrasound imaging may be sufficient, but it is not suitable for early-stage iron accumulation. The gold standard for determining early-stage liver iron accumulation is dry iron measurement from the sample obtained by liver biopsy. Biopsy can also distinguish liver fibrosis, if present, and other causes of liver damage. The amount of iron accumulated, and the degree of fibrosis can be measured by liver biopsy, but this is an invasive method and is difficult to repeat.

NON INVASIVE METHODS

1: Liver Iron Measurement: Liver iron measurement with MR R2 or R2* is preferred as the standard method in thalassemia patients. Using this method, the relaxation rates T2 and T2* are measured from the spin position created by the magnetic field. As iron accumulation increases, this time shortens. The 1/T2 and 1/T2* values provide R2 and R2* results. The measurement results are calculated and provided as Femg/g dry weight **(1)**. Hepatic liver iron concentration (LIC) is directly related to ferritin **(2)**. To monitor the effectiveness of chelation therapy, it is recommended to be performed annually. Treatment is recommended to be initiated for patients with levels exceeding 5 mg/g dry weight who are transfusion-independent. When the level falls below 3 mg/g dry weight, discontinuation of treatment is recommended **(3)**. In transfusion-dependent thalassemia patients, levels up to 7 mg/kg of dry weight are considered as moderate iron loading, while those up to 15 mg/g dry weight are considered as severe iron loading **(4)**. Values above 16 mg/g dry weight are associated with a significantly increased risk of fibrosis.

2: Liver Fibrosis Assessment: Transient elastography (TE), a newly developed technique, can replace the need for liver biopsies in fibrosis evaluation. This method, which utilizes pulse-echo technology, allows for the non-invasive measurement of liver fibrosis **(5)**. As mechanical vibrations from the ultrasound probe pass through the liver tissue, they generate varying amounts of different ultrasonic echoes, depending on the degree of fibrosis. In patients with thalassemia, a correlation has been observed between the liver stiffness measured using TE and the liver iron concentration (LIC) and serum ferritin levels **(6)**. However, in some studies, this correlation has not been established, necessitating further research for standardization **(7)**. The patient's body mass index and concomitant liver steatosis can affect the results, so the use of larger probes is recommended **(8)**.

LIVER IRON OVERLOAD TREATMENT

Three chelators used in the treatment of thalassemia iron overload are also successfully employed in reducing liver iron levels. Desferrioxamine (DFO), Deferiprone (DFP), and Deferasirox (DFX) treatments can be used as single or combination agents depending on the amount of iron accumulation. In cases of excessive iron buildup, DFO is reported to be more successful in preventing fibrosis development **(9)**.

In intermediate thalassemia, the level of ferritin begins to increase in the liver before it becomes very high. Iron accumulation occurs without transfusion due to a decrease in hepcidin and an increase in iron absorption. The iron absorbed from the intestine accumulates in the liver rather than in macrophages. Therefore, it is necessary to start iron

chelation therapy when ferritin is above 800 ng/mL, even without regular transfusions **(3)**.

HEPATITIS C

The prevalence of hepatitis C in thalassemia patients varies in every country and region. The most frequently detected HCV genotype in thalassemia patients is GT 1b. Over the past 20 years, the frequency of donor screenings has increased, and with the development of molecular techniques, the number of new cases has significantly decreased. Unfortunately, this is often not achieved in developing countries.

Diagnosis and Treatment of Hepatitis C

All thalassemia patients should be investigated for HCV infection.

1: Anti-HCV antibodies
2: HCV-RNA from those who test positive for anti-HCV antibodies
3: Transient elastography (TE) for liver fibrosis assessment in patients with positive results
4: Determination of HCV genotype
5: Treatment planning based on genotype (if new generation treatments are not available, consider other options).

In the treatment of HCV, direct-acting antiviral agents have taken the place of interferon and have been very successful. They have achieved a sustained viral response rate of over 95% and can be considered a complete cure. The treatment duration is 8-12 weeks. Chelation therapies are not contraindicated during the treatment, but some medications, such as antiarrhythmics, may interact.

In patients with Hepatitis C infection, autoimmune hemolytic anemia and autoimmune hepatitis can be observed. In recent publications, some cases have been reported where liver function abnormalities due to autoimmune hepatitis have been detected in patients who have received treatment for Hepatitis C and have tested negative for HCV RNA. Therefore, monitoring for autoimmune pathologies in patients is also necessary. Additionally, patients receiving antiviral treatment without interferon and based on genotype may also develop autoimmune hepatitis during treatment **(10)**.

HEPATITIS B

The prevalence of Hepatitis B has increased in some geographical areas. As donor testing and Hepatitis B vaccination rates increase, the prevalence decreases.

1: HBsAg (Hepatitis B surface antigen)
2: Measurement with HBV DNA PCR method, HBeAg
3: Monitoring the fibrosis stage and liver function tests

Treatment involves antiviral agents and interferon therapy. Interferons carry a risk of myelosuppression. The primary goal should be to protect all thalassemia patients through vaccination. The ongoing effectiveness of the vaccine requires regular measurement of antiHBs levels.

The Hepatitis E virus can be transmitted from blood donors who consume contaminated products. It can manifest itself with exacerbations in patients with acute hepatitis or in those previously diagnosed with chronic hepatitis. It is recommended to test for IgM anti-HEV and, if necessary, check the viral load. It can be treated with Ribavirin and interferon.

HEPATOCELLULER CARCINOMA (HCC)

As patients' lifespans increase and cardiac early mortality decreases, the frequency of HCC in older age groups has risen. HCC can also occur at a young age in patients with hepatitis B or C infections and detectable liver iron accumulation. In patients with HCV infection, a decrease in hepcidin synthesis increases iron accumulation and accelerates HCC development. In chronic hepatitis B patients, the risk of HCC development exists even before cirrhosis develops. Additionally, inflammation-induced free oxygen radicals increase carcinogenesis.

1: Abdominal ultrasound (at least every 6 months in cirrhotic patients)
2: AFP (not a reliable marker).

In cirrhotic patients with HCV and Hepatitis B infections:

In those not dependent on transfusions, LIC levels above 5 mgFe/g dw

In those dependent on transfusions, LIC levels above 7 mgFe/g dw

Screening should be performed every 6 months for individuals with serum ferritin levels above 1000.

Annual MRI with R2 or R2* should be used to monitor liver iron accumulation.

CHOLESTASIS

Due to increased ineffective erythropoiesis in thalassemia patients, gallstones are frequently observed. Therefore, the risk of developing cholestatic hepatitis is high. In most patients, cholecystectomy is performed due to the risk of cholangitis, cholestasis, and pancreatitis. In sickle cell anemia patients, intravascular cholestasis can be observed due to occlusive crises.

REFERENECES

1. Garbowski, M.W., et al., *Biopsy-based calibration of T2* magnetic resonance for estimation of liver iron concentration and comparison with R2 Ferriscan.* Journal of Cardiovascular Magnetic Resonance, 2014. **16**(1): p. 40.
2. Shehata, S.M., M.I. Amin, and E.S.H. Zidan, *MRI evaluation of hepatic and cardiac iron burden in pediatric thalassemia major patients: spectrum of findings by T2*.* Egyptian Journal of Radiology and Nuclear Medicine, 2019. **50**(1): p. 68.
3. Taher, A.T. and A.N. Saliba, *Iron overload in thalassemia: different organs at different rates.* Hematology Am Soc Hematol Educ Program, 2017. **2017**(1): p. 265-271.
4. Allali, S., et al., *Management of iron overload in hemoglobinopathies.* Transfus Clin Biol, 2017. **24**(3): p. 223-226.
5. Di Marco, V., et al., *Noninvasive assessment of liver fibrosis in thalassaemia major patients by transient elastography (TE) - lack of interference by iron deposition.* Br J Haematol, 2010. **148**(3): p. 476-9.
6. Pipaliya, N., et al., *Comparison of Tissue Elastography With Magnetic Resonance Imaging T2* and Serum Ferritin Quantification in Detecting Liver Iron Overload in Patients With Thalassemia Major.* Clin Gastroenterol Hepatol, 2017. **15**(2): p. 292-298.e1.
7. Ou, G., et al., *Utility of Transient Elastography in Estimating Hepatic Iron Concentration in Comparison to Magnetic Resonance Imaging in Patients Who are Transfusion-Dependent: A Canadian Center Experience.* Hemoglobin, 2017. **41**(1): p. 21-25.
8. Xia, B., et al., *Feasibility and Efficacy of Transient Elastography using the XL probe to diagnose liver fibrosis and cirrhosis: A meta-analysis.* Medicine (Baltimore), 2018. **97**(39): p. e11816.
9. Brittenham, G.M., *Iron-chelating therapy for transfusional iron overload.* N Engl J Med, 2011. **364**(2): p. 146-56.
10. Fleischer-Stępniewska, K., et al., *Risk of autoimmune hepatitis reactivation in patients with chronic hepatitis C and autoimmune hepatitis treated with direct-acting antivirals.* Pol Arch Intern Med, 2019. **129**(3): p. 215-218.

Correspondence Address:
Specialist Dr. Vedat Aslan MD
University of Health Sciences, Antalya Education and Research Hospital, Department of Hematology Antalya, Türkiye
E-mail: vedataslan21@yahoo.com

PREGNANCY, FERTILITY AND REPRODUCTIVE PROBLEMS IN THALASSEMIA

Assoc. Prof. Dr. Ziynet Alphan Üç[1]/Prof. Dr. Süheyla Görar[2]
[1]University of Uşak, Usak Training and Research Hospital
Department of Endocrinology and Metabolism Uşak-Türkiye
[2]University of Health Sciences, Antalya Training and Research Hospital,
Department of Endocrinology and Metabolism Antalya-Türkiye

ABSTRACT

Thalassemia is the most common hereditary hemoglobin disorder in the world. Transfusion-dependent thalassemia (TDT) refers to a severe clinical genotype associated with severe anemia and need for regular blood transfusions in the clinical course, and therefore management and follow-up of patients with TDT is require a multidisciplinary approach considering the systemic comorbidities due to chronic anemia and transfusion-related iron overload. Hypogonadotropic hypogonadism and fertilization problems arising from the involvement of reproductive organs are the most common endocrine comorbidities.

Controlling chronic anemia with transfusions and keeping iron overload at an optimum level with appropriate and adequate chelation treatments are the basic approaches to preserving reproductive functions in TDT patients. In this way, a healthy pubertal process, protection of gonadal functions, minimization of fertility problems, prevention of long-term complications of hypogonadism, and physical and social well-being can be ensured. In patients with thalassemia, follow-up of puberty, administration of appropriate hormone replacement therapies, genetic counseling for couples who desire pregnancy, assessment of eligibility for pregnancy, examine and management of systemic comorbidities are required. If pregnancy achieved the peripartum/postpartum periods should be followed up by a multidisciplinary team along with an individualized management approach.

Keywords: Thalassemia, fertility, pregnancy, reproductive problems

BACKGROUND

Thalassemia is the most common hereditary hemoglobin disorder in the world. Although it has a higher prevalence in Mediterranean countries, the Arabian Peninsula and Asia, it spreads all over the world with international migration movements. Thalassemia manifests with different clinical and hematological findings depending on the inheritance pattern. Transfusion Dependent Thalassemia (TDT), which is severe anemia and requires regular blood transfusions due to the severe clinical genotypic structure, requires multidisciplinary follow-up and management. The control of iron overload due to repeated blood transfusions depends on use of appropriate and adequate chelation treatments. Additionally, TDT patients are also at increased risk of multisystemic problems due to chronic anemia and transfusion-related iron overload **(1)**.

In patients with TDT, the follow-up of endocrine system along with potential dysfunctions is considered to be of critical importance. The most common endocrine disorders are hypogonadotropic hypogonadism (HH) and fertilization problems due to the involvement of reproductive organs. Fertility of both male and female genders in adults is possible with the functioning of the hormonal control mechanisms of the hypothalamic–pituitary–gonadal (HPG) axis **(1) (Figure 1)**.

Gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus stimulates the pituitary gonadotropic hormones (follicle stimulating hormone-FSH, luteinizing hormone-LH). FSH enables the synthesis of estrogen by the ovarian granulosa cells. FSH and estrogen stimulate the LH receptors and lead to androgen production by increasing the sensitivity of cells to LH. Increased estrogen takes part in the oocyte maturation and ovulation. Fertilization of mature oocyte with male reproductive cell (sperm) results in pregnancy. In the absence of pregnancy, estrogen level decreases, progesterone level increases, increased endometrial hyperplasia and resulting in a thickened endometrial wall. Menstruation occurs with the tissue breakdown and shedding of the endometrium following the vasomotor changes associated with estrogen-progesterone withdrawal. Any factor (i.e., genetic, anatomic, chronic diseases, etc.) disrupting the continuity of this process leads to loss/deficiency of reproductive functions **(2)**.

Male reproductive organs consist of testes, epididymis, vas deferens, ejaculatory ducts and urethra. Testes consist of seminiferous tubules (sertoli and germ cells) and leydig cells. Similar to the mechanisms in the female sex, hypothalamic GnRH stimulation results in release of FSH and LH hormones. FSH stimulates the sperm production by seminiferous tubules while LH stimulates the release of testosterone by leydig cells. LH secretion is regulated by testosterone levels and FSH secretion is regulated though inhibin hormone levels secreted by sertoli cells. The amount of sperm produced by seminiferous tubules regulates the rate of inhibin secretion **(3)**.

HPG axis remains restrained until puberty. With the onset of puberty, pulsatile release of GnRH becomes active, reproductive axis resumes activity and starts the puberty process. Delayed puberty is defined as the no signs of puberty onset by age 13 in girls and age 14 in boys **(4)**. The arrested puberty, which is defined as the absence of further pubertal progression for more than 1 year after puberty has started, often occurs in thalassemia major (TM) cases with moderate-to-severe iron overload. In these patients, annual growth velocity may also slow down or stop **(1)**. In individuals with thalassemia, the iron overload leads to primary hypogonadism by causing damage to gonads and leads to secondary hypogonadism by damaging the cells that release pituitary gonadotrophins. Hypogonadism is defined as the absence of testicular growth (smaller than 4 ml) in boys by the age of 16, and the absence of breast development in girls. Delayed puberty and hypogonadism are the most important clinical markers of iron overload. Primary hypogonadism is rarely reversible and HH is considered to be the most prevalent endocrinopathy in patients with thalassemia **(5)**. In primary hypogonadism, gonadotrophin levels increase due to gonadal deficiency. While in secondary hypogonadism, due to involvement of either hypothalamus or hypophysis, the gonadotrophin hormone and testosterone/estrogen levels are both low. In both sexes, albeit differs across countries and centers, hypogonadism affects 40-90% of TDT patients **(6)**. Rate of adult-onset hypogonadism in TDT patients ranges between 8.3-12% **(7)**.

PATHOPHYSIOLOGY IN FERTILITY PROBLEMS

Spontaneous fertility can occur in patients with spontaneous puberty and maintained menstrual functions who receive regular blood transfusions and appropriate chelation treatment. However, most of these patients are subfertile because of HH secondary to transfusional hemosiderosis **(6-7)**. Iron overload in the cells that produce pituitary gonadotropic hormones (LH and FSH) may lead to impairment of synthesis and release of these hormones. Similarly, in most of the patients with optimal follow-up and treatment, gonadal (ovaries and testes) functions are normal, whereas rarely gonadal dysfunction may also occur as a result of iron overload in gonads **(5)**.

Gonadotroph cells in adenohypophysis are especially sensitive to increasing iron concentration in the circulation. The impaired pulsatile secretion of LH and FSH by the affected cells decrease or eliminate the stimulation of gonads and thus reduces the synthesis of sex hormones **(8)**. The increase in transferrin receptor expression in the adenohypophysis cells and increase in the pituitary gland blood supply during puberty may expedite the accumulation of

high circulatory levels of iron in these cells **(9)**. Although standard measurements of iron load and chelation intensity are used to identify the gonadal dysfunction, they are inadequate for a reliable assessment of pituitary hormone releasing capacity and the reproductive potential **(10)**.

In detecting the pituitary iron load, magnetic resonance imaging (MRI) is an important diagnostic tool. Although MRI enables visualization of pituitary iron stores in TDT patients under age 10, volume loss in the gland and significant clinical symptoms can only be identified during the second decade of life. The degree of pituitary iron load and pituitary volume loss are the independent predictors of hypogonadism **(11, 12)**.

In women with TDT, oxidative stress is considered the main causative factor underlying the ovarian follicle ageing and physiological fertility decline. Pathogenesis is multifactorial with contribution of the increase in production of reactive oxygen radicals, decrease in enzymatic antioxidant defense mechanisms, functional errors in mitochondrial mechanisms, destroyed micro-environment and decreased estradiol production by the granulosa cells **(13)**. There is also evidence that ovarian iron overload affects the oocyte count and impairs the oocyte functions. In female TM patients with iron overload, increase in follicular fluid redox activity and accumulation of hemosiderin in endometrial epithelium have been demonstrated in some studies **(14, 15)**. Iron overload is considered likely to be responsible not only for the oocyte dysfunction but also the premature ovarian failure and the decrease in clinical pregnancy rate of assisted reproductive techniques (ART) **(1, 15, 16)**. Moreover, in women with TM the ovarian volume is known to decline to postmenapousal levels by the age of 30, which is considered to be related to the deficient stimulation of gonadotropin and the possible iron overload in the ovary tissue. The ovarian reserve, which can be evaluated with antimüllerian hormone (AMH) and antral follicle count (AFC), was found to be lower in women with TM compared to control subjects **(17, 18)**. AMH level is correlated with non-transferrin-bound iron (NTBI), which suggests the potential role of labile iron in lowering the oocyte quality **(19)**. Despite these concerns, the likelihood of pregnancy to be achieved via hormonal stimulation in women with TDT who have primary or secondary amenorrhea, indicates that ovarian functions can be maintained at least partially. Also, studies with female TM patients who underwent in vitro fertilization (IVF) reported that, despite having significant decline in ovarian reserve in AMH and AFC measurements, oocyte quality was not affected in these women along with achievement of high fertility rates **(6, 15, 19, 20)**.

In women with thalassemia, problems with pubertal period and impaired gonadal functions lead to the menstrual problems, the failure in development of secondary sex characteristics, decrease in fertilization and the osteopenia-osteoporosis via long term effects on the bone metabolism, in addition to adverse outcomes in psychosocial aspects. Accordingly, these patients are considered to benefit from the hormone replacement therapy (HRT) and treatment monitoring in terms of a healthier sexual development and better quality of life.

HORMONE REPLACEMENT THERAPY

In adult female patients with hypogonadism, HRT aims to ameliorate the estrogen deficiency symptoms, preserve secondary sex characteristics, prevent bone loss and to improve physical and social well-being. However, HRT requires a careful approach with consideration of certain treatment-related risks during follow up, such as thromboembolic events, stroke, hypertension, cardiovascular events, gallbladder diseases, breast cancer, endometrial hyperplasia/cancer, cirrhosis and hepatocellular adenoma/carcinoma. In this regard, appropriateness of each patient should be considered before the initiation of HRT, along with the implementation of an individualized treatment. Due to limited number of studies on the utility of HRT in thalassemia patients, there are no standardized treatment protocols for this group of patients. Treatment recommendations are based on the studies addressing the contraceptive treatments in healthy women, and those investigating the effects and complications of HRTs in women with hypogonadism for other reasons or postmenopausal women **(6, 7)**.

The replacement of estrogen-progesterone hormones by cyclic treatments or combined oral contraceptives (COC), to sustain the continuity of menstrual cycle, constitutes the basis of treatment in women with hypogonadism.

Cyclic hormone replacement is based on concomitant use of estrogen and progesterone preparations or use of progesterone preparate as add-on to continuing estrogen replacement for a period of 12-14 days. Three types of estrogen are used for the estrogen replacement, including estradiol, ethinyl estradiol (synthetic form) and conjugated equine estrogens. In cyclic replacement, the preferred regimens are oral micronized estradiol (1-2 mg/day), oral conjugated equine estrogen (0,625-1,25 mg) or transdermal estradiol (100 mcg/day). The transdermal estrogen compared to oral preparations was shown to yield more physiological estrogen concentrations. For progesterone replacement, medroxyprogesterone acetate 10 mg (12-14 days/month) or micronized progesterone 200 mg/day (continuous) are the recommended regimens. Cyclic estrogen-progesterone hormone replacement includes more physiological replacement doses and does not inhibit ovulation and pregnancy **(21)**.

In female patients with hypogonadism, there is no consensus on the ideal hormone replacement protocol. However, several centers prefer COCs (estrogen and progesterone in the same preparation) as the first-line HRT **(22)**. COCs are more potent and contain supraphysiological doses. COCs often contain ethinyl estradiol or estradiol valerate. Over the years, the estrogen doses of COCs are decreased to reduce the risk of thromboembolic events. Medroxyprogesterone acetate and norethisterone enanthate are the first developed progesterone regimens. Later on, synthetic progestins such as norgestrel, levonorgestrel (second generation), gestodene, desogestrel (third generation), drospirenone, dienogest (fourth generation) were added to the group. Third generation progestins have minimum effect on blood glucose, plasma insulin concentrations and lipid profile. For this reason, they are suitable for use in patients with lipid disorders or diabetes **(22, 23)**.

Unlike healthy menopausal women, HRT treatment in TM patients is complicated by comorbidities, and more prone to potential risks (i.e., iron overload, thrombophilic status, chronic liver disease, impaired glucose tolerance/diabetes and cardiovascular disease). Thalassemia intermedia (TI) was reported to be associated with 4.38-fold higher risk of thromboembolic events compared to TM **(24)**. In a study with 735 thalassemia patients (683 TM and 52 TI) at 9 Italian centers, venous thromboembolism was noted in 32 patients overall, including 3.95% of TM patients and 9.61% of TI patients, as associated with increased incidence of organ dysfunction **(25)**. Due to first-pass elimination effect of oral estrogen (ethinyl estradiol), production of various hormone-binding globulins, coagulation factors and lipoproteins increase, which increases the VTE risk. Increase in VTE risk is highest in the first year of treatment. Moreover, the risk varies according to properties as different molecules and doses of estrogen and type of progestin in the COCs **(22, 23, 26)**.

HRT should be avoided in patients with previous history of VTE. Use of transdermal estrogen is suggested to be associated with lower risk of VTE. Currently, transdermal estrogen and micronized progesterone seem to be the most reliable and physiological treatment alternatives. Although COCs are effective in endometrial preservation, natural progesterone preparations may be more convenient for reducing the cardiovascular event risk and possible risk of breast cancer **(27)**.

A panel of international experts have gathered under a program, namely “The International Network on Endocrine Complications in Thalassemia (ICET)” program, to guide the management and follow up of endocrine dysfunctions and growth and development disorders in clinical practice and reported their consensus recommendations in 2017 **(22)**. The recommendations proposed for the use and follow up of HRT in thalassemia patients with hypogonadism in the ICET-A report, are as follows **(22)**:

- In the pretreatment period, assessment of hepatic and cardiac iron load along with use of intensive chelation therapy for those with iron overload is recommended. In these patients, liver dysfunction is basically associated with liver siderosis and chronic hepati-

tis (hepatitis C). Besides, chronic hemolysis in TM may cause bilirubin gallstone formation. In case of acute liver disease, HRT is contraindicated.

- Elevated liver enzymes (3-6 times higher the normal limit) are amongst contraindications. In patients receiving chronic hepatitis C treatment with antiviral agents and those with serious liver siderosis (Fe/g dry weight >7 mg/g), intense chelation treatment is recommended before HRT.
- One month after the initiation of HRT, if the liver enzymes increase by more than 100% or basal bilirubin levels are high, liver function test should be performed once monthly for at least three months, to decide whether or not the treatment will be continued. The likelihood of chronic use of third generation contraceptives or HRTs to cause formation of gallstones by influencing serum lipid profile should not be disregarded.
- Before the start of COC, the assessment of risk/benefit status and identification of increased thrombophilia risk is recommended for each patient, while in the presence of known thrombophilic etiologies, consulting with an expert specialized in this field is recommended.
- In patients with risk factors for VTE, transdermal estrogen and micronized progesterone are the most reliable and physiological treatment alternatives. Transdermal estrogens may cause local irritation, and thus administration by rotating the application site is recommended.
- In patients with splenectomy, if HRT treatment will be applied, low-dose aspirin or anticoagulant treatment should be added to the treatment.
- Clinical follow-up should be performed in women with diabetes (insulin dependent/nondependent). COCs have no significant impact on daily insulin requirement, diabetes control or progression of retinopathy. In case of cardiovascular disease or serious microvascular complications (nephropathy or active proliferative retinopathy), COC use should be avoided.
- Juvenile or adult women with hypogonadism should be recommended to abstain from alcohol and tobacco and to exercise daily in order to prevent obesity.

In conclusion, in a female TM patient with hypogonadism, general status of the patient, ongoing chelation treatment and presence of complications should be considered in the treatment plans regarding HRT. In order to minimize the possible risks, high doses of sex hormones should be avoided. The goal is to reach the average serum estradiol levels of 100 pg/ml (400 pmol/l) in women with normal menstrual cycle. Progesterone is generally used for 12-14 days each month in order to start menstrual bleeding. Micronized progesterone is recommended due to more favorable safety profile in terms of cardiovascular and breast conditions. However, the strongest evidence for endometrial preservation is provided for COC **(22)**.

Considering the medical eligibility criteria for contraceptive use (MEC), 'US-MEC: The United States Medical Eligibility Criteria' report was published in 2016 **(28)**. Accordingly, Category 1. No restriction for the use of the contraceptive method., Category 2. Advantages of contraceptive method generally outweigh the theoretical or proven risks, Category 3. Theoretical or proven risks of contraceptive method generally outweigh the advantages, Category 4. Contraceptive use carries an unacceptable health risk.

Conditions for which there is no restriction for the use of the contraceptive method in women with thalassemia: There is no restriction for the use of estrogen/progesterone tablet, patch, vaginal ring (Category 1). In chronic hepatitis, COC use does not increase hepatocellular cancer risk, severity and progression of liver fibrosis (Category 1). In women with insulin dependent/nondependent diabetes, COC has limited effect on the increase in daily insulin requirement and has no influence on long term diabetes control or retinopathy progression (Category 1).

Conditions that represent an unacceptable health risk if the contraceptive method is used in women with thalassemia: Family history for venous thrombosis in the first-degree relatives (Category 2). In asymptomatic gallbladder diseases there is minimal increase of risk (Category 2). A higher risk is evident in those with gallbladder disease receiving treatment (Category 3). In migraine without or with aura, the risk is high (Category 2-4, respectively). In atherosclerotic cardiovascular diseases, the risk is high (Category 3/4). Known thrombogenic mutation and previous history of thromboembolism (Category 4). In presence of nephropathy, neuropathy and retinopathy, the risk is high (Category 4).

Follow-up of women initiating HRT is important and necessary. Before starting the treatment, systemic physical examination should be performed and repeated at each visit. In terms of puberty development, Tanner staging should be evaluated by a pediatric endocrinologist every 3-6 months after the onset treatment. In sexually active adult individuals, pelvic evaluation should be performed every 3-6 months. Besides, hormonal assessment (thyroid hormones, gonadotropins and estradiol) serum lipids, and kidney and liver functions should be examined once yearly. Glucose metabolism should be evaluated in every 3-6 months along with the glucose tolerance test in selected cases for diabetes screening. For the evaluation of bone health, lumbar and femoral neck bone densitometry can be conducted once in 1-2 years depending on the risk status. Also, iron load of the patient should be measured every 3-6 months and appropriate chelation treatment should be planned by a hematologist. For thromboembolism, family and personal history should be questioned and screening for thrombophilia should be performed once yearly **(22)**.

PRENATAL EVALUATION AND COUNSELLING IN WOMEN WHO DESIRE PREGNANCY

In thalassemia, while the FSH/LH and estradiol levels from the onset of puberty are used to identify the hypogonadism, monitoring of these hormones may be inadequate in evaluating the potential for fertility. AMH measurement and follow-up of AFC are the tools recommended for the evaluation of fertilization. AMH is produced by granulosa cells of preantral, and antral follicles and it has low variability, representing an ideal marker in the thalassemia population. It is important in determining the ovarian reserve among patients who were planned to receive hormone stimulation treatment. AFC decreases with the advancing age. Low ovarian reserve is considered to foresee the decreased chance of spontaneous pregnancy and non-response to hormonal stimulation **(18)**. In women with fertility planning, age at pregnancy desire is also important besides the severity and duration of iron overload. Advanced age is a negative factor. In this respect, receiving timely counselling in case of pregnancy expectation is considered to enable a proper management planning and implementation of necessary procedures **(6)**.

Fertility management requires a careful planning and preparation which also includes the provision of counselling to partners before pregnancy. Ideally, management of the process is conducted by a multidisciplinary team of hematologist, fertility specialist, obstetrician/gynecologist, cardiologist, endocrinologist, and psychologist **(6)**. Potential partner should be tested for thalassemia gene mutations, and provision of the appropriate genetic counselling in accordance with the test results is mandatory. If both partners are homozygous for thalassemia, the use of donor gametes, preferably donor sperm, is the ideal option given that sperm can be more easily available from sperm banks. Use of donor eggs is technically more complicated with an unpredictable success rate. If the partner is heterozygous, mutation can be detected prior to conception via the pre-implantation genetic diagnosis (PGD). This may be the most acceptable opting to eliminate the risk of affected pregnancy. Finally, in patients with severe organ damage or where both partners have TM, an alternative option may be adoption **(29)**.

Pregnancy may occur spontaneously in some women and via ART in others. In the planned pregnancy, induction of ovulation may be indicated when both partners are thalassemic, and in women with primary or secondary amenorrhea, or

failure to conceive despite normal menstrual function. The success in induction is considered to be associated with the degree of the functional ovarian reserve protected from the iron overload injury. There are various recommended ovarian induction regimens, however there is no specialized protocol for this group of patients, so the treatment should be individualized. Dose and frequency of gonadotropin injections are determined based on follicle count/size and estradiol levels **(6, 29, 30)**. Ovarian induction regimens are associated with multiple pregnancy and increase in the risk of ovarian hyperstimulation syndrome. Therefore, ovulation-induction procedures should be performed by an experienced team. There is no evidence regarding the hazardous effect of iron chelation treatment during ovulation induction. However, in patients receiving oral chelators (deferasirox or deferiprone), treatment switch to deferoxamine (DFO) is recommended in case of pregnancy desire and/or before the ovulation/spermatogenesis induction **(29)**.

Before fertility treatment, eligibility for pregnancy, concomitant medications, systemic disorders, and complications of partners should be assessed within pre-pregnancy counselling. Partners should be acknowledged about the treatment and risks of pregnancy. Recommendations for pre-pregnancy evaluation of patients with thalassemia are presented in Table 1.

Cardiac complications continue to be the primary cause of death in TDT, necessitating close cardiac monitoring throughout the pregnancy. During pregnancy, cardiac load is increased by at least 25-30% due to increased heart rate and stroke volume. Benign arrythmias may occur. Chronic anemia and increased systemic vascular resistance may result in left ventricular dysfunction. Additionally, in patients with TDT, cardiac reserve may be decreased due to myocyte damage related with iron overload, particularly the NTBI **(31)**. This situation, along with iron overload, poses a considerable risk for premature mortality due to heart failure. As chelation treatments are discontinued during pregnancy, cardiac functions may worsen, especially in patients with borderline myocardial function or siderosis. All women with TDT should be evaluated in terms of cardiac functions before pregnancy. Resting and exercise echocardiography and electrocardiogram (ECG) should be performed in addition to 24-hour holter monitoring to control cardiac arrhythmias. Quantify iron levels can be measured and can correlated these to left ventricular dimensions by modified MRI technique using gradient T2* measurements. Cardiac MRI should be used to identify patients at risk (T2*<20ms). If cardiac iron load and the complications in particular are detected, iron chelation treatment should be intensified to enable cardiac T2*>20ms before the pregnancy **(6, 29)**.

In female patients, pre-pregnancy liver iron load should be evaluated. Liver iron concentration (LIC) is measured by MRI. If iron load is high, intensified chelation should be applied before conception. In the pre-pregnancy period, liver iron should be <7mg/g (dry weight). Ultrasound imaging should be performed particularly in patients TI, due to increased risk of gallstone formation. In those with positive findings regarding the gallstone or biliary sludge, cholecystectomy may be considered before pregnancy. Hepatitis C virus (HCV) or hepatitis B virus (HBV) may further accelerate the liver fibrosis in thalassemia patients. The sufficient blood donor control, widespread HBV vaccination and antiviral treatments are important measures to minimize this risk. In Hepatitis C positive women, antiviral agents should be used to achieve Hepatitis C RNA negativity. Currently, next generation treatments have replaced the interferon-based protocols and are commonly used in thalassemia patients with chronic HCV **(7, 32)**.

Before pregnancy, bone mineral density should be measured via vertebral imaging and dual-energy X-ray absorptiometry (DEXA) screening and if osteoporosis/osteopenia is present appropriate treatment should be planned. In order to optimize bone health, before and during pregnancy, vitamin D and calcium replacement should be performed regularly. Vitamin D is recommended not only for bone health, but also to minimize the risk of gestational diabetes **(6, 29, 33)**.

In pregnant women with thalassemia, an immunosuppressed status is likely due to high estrogen levels, excessive iron load and splenectomy (if

performed). The antibodies formed against transfusion related viral infections should be controlled due to their potentially adverse effects on pregnancy. All patients should be screened for human immunodeficiency virus (HIV), HBV, HCV, and measles. In HIV positive patients who demand fertility, convenient antiviral agents should be used to reduce the risk of vertical transmission. Besides, cesarean delivery and breastfeeding should be avoided **(34)**.

Patients should be screened for diabetes, hypothyroidism and acquired red blood cell antibodies. Instead of HbA1c, fructosamine levels should be used for glycemic control assessment with targeted fructosamine levels of <300 nmol/l for at least 3 months before conception **(34, 35)**. Symptoms of mild adrenal hypofunction (i.e., asthenia, muscle weakness, arthralgia, and weight loss) may be masked by conventional thalassemia symptoms, in suspected cases, an endocrinology consultation is necessary. For the evaluation of adrenal function, basal cortisol levels (between 8:00-9:00 a.m.) should be measured along with cortisol response to ACTH or insulin stimulation, when necessary, and the results should be interpreted by an endocrinologist **(36)**.

Before pregnancy, patient's medications should be reviewed, and dietary habits, smoking and alcohol consumption should be evaluated, and folic acid, calcium and vitamin D supplementation should be started. Bone deformities, especially cephalopelvic disproportion may influence the pregnancy and management of delivery. Despite appropriate transfusion management, osteoporosis and scoliosis are frequent in TM. Therefore, in the presence of osteoporosis, estrogen hormone replacement should be started in the prenatal period, in addition to bisphosphonates, when necessary. In order that spinal anesthesia becomes possible, enhancement of bone density is important. Bisphosphonates are contraindicated during pregnancy and breastfeeding period, and ideally be discontinued at least 6 months before the conception due to long half-life. Other medications such as interferon, ribavirin and hydroxyurea should be discontinued at least 6 months before the onset of fertility treatment. In levothyroxine (LT4)-treated patients with hypothyroidism, LT4 dosage should be increased during the pregnancy period for maintaining the euthyroid status. Hyperthyroidism is rare in thalassemia patients. In patients with history of hyperthyroidism or use of another antithyroid medication, the treatment should be switched to propylthiouracil **(29)**.

Pregnancy does not alter the natural course of the disease. Risk of pregnancy-related complications is similar to general population. There is no increase in fetal malformation risk. Fetal growth retardation and premature delivery risks are doubled. Thrombotic risk may increase. In a meta-analysis of 17 studies conducted between 2000 and 2017 with 417 pregnant women, maternal mortality was found to be 0.48%. The rate of intrauterine growth retardation was similar to the normal population in singleton pregnancies (%8,9), whereas a higher rate was noted in multiple pregnancies. Pregnancy loss, low birth weight, prematurity, and ART-associated multiple pregnancy (1.6-18.9%) were the other complications **(6, 37)**.

In pregnancy, the main goal is to maintain the pretransfusion hemoglobin (Hb) concentrations above 100 g/L. In pregnant women with TI, due to the dilutional decrease in hemoglobin, transfusion requirement may arise during pregnancy. The risk of potential alloimmunization and hemolysis that leads to deepening of fetal anemia should be considered, and if transfusion is considered necessary it should be based on concordant phenotypic matching. On the other hand, in patients without significant iron overload but with sufficient cardiac function, DFO was shown not to be needed during pregnancy. Patient should be informed about the association of pregnancy, albeit carrying certain risks, often with a favorable outcome **(6, 29)**.

MANAGEMENT OF PREGNANCY

In each trimester, evaluation of cardiac function via echocardiography as well as assessment of liver and thyroid functions is recommended. In pregnancies started with provision of optimum iron load, no serious cardiac complications were reported. All patients should be screened for gestational diabetes at week 16 and if the results are normal, screening should be repeated at weeks 24 and 28. The follow

up of patients diagnosed with diabetes should be performed at diabetes-pregnancy clinics with monthly measurement of serum fructosamine levels. The fetal growth should be monitored via regular ultrasonography screenings starting from the 24^{th}-26^{th} weeks, preferably by a perinatologist. As chronic anemia would influence fetal growth, pre-transfusion Hb levels should be at least 100 g/L. This value is considered particularly relevant for non-transfusion-dependent thalassemia patients, whereas is also recommended for transfusion-dependent patients **(38)**.

In pregnancy, increased coagulability occurs as a result of increase in fibrin and coagulation factors and decrease in fibrinolytic activity. Besides, venous blood flow velocity decreases during pregnancy. Splenectomized patients without previous history of transfusion are particularly at risk of this condition. For this reason, in transfusion-naïve splenectomized TI patients with history of repeated miscarriage, thromboprophylaxis is recommended during pregnancy and in the postpartum period. In these patients, thromboprophylaxis is performed with low-molecular-weight heparin by the second trimester. If platelet count >600.000/cm^3, several women with splenectomy may already be on low-dose aspirin (75 mg) and should be administered also with low-molecular-weight heparin **(32, 39)**. For mothers with TM, regular folic acid supplementation is recommended prevent megaloblastic anemia alongside the control of chronic anemia. As in normal pregnancies, in order to reduce to incidence of spina bifida, folic acid should be started before conception. Folic acid supplementation is applied as 5 mg/day (6, 7).

Regular vitamin D and calcium supplementation is recommended to optimize bone health not only before pregnancy but also during pregnancy. In hypothyroid patients receiving LT4 treatment, for maintaining euthyroidism during pregnancy, pre-pregnancy serum TSH levels should be assessed along with adjusting the LT4-dose to enable TSH levels between the lower reference limit and 2.5 mU/L. When pregnancy is confirmed, LT4 doses should be increased by 20-30% **(7, 40)**. Use of chelation treatment during pregnancy as well as the choice of the best chelation agent is controversial. Currently, chelation treatment is discontinued with the onset of pregnancy and is not given throughout the pregnancy. If cardiac function is impaired during pregnancy, DFO can be used carefully after the first trimester. The teratogenic potential this agent is inconclusive **(7)**. For this reason, DFO has been used in some high-risk pregnancies particularly in the third trimester. The reason for DFO to be preferred during pregnancy is the inability of placental transfer due to large molecular size. In case new onset cardiac dysfunction during pregnancy, DFO can be given in earlier stages based on the risk/benefit assessment **(6, 7, 34)**. Data on fetotoxicity potential of newer oral chelator agents are not sufficient **(41)**.

MANAGEMENT OF DELIVERY

There is no consensus on the method and timing of delivery. If pregnancy is not complicated, spontaneous induction of delivery can be considered, after a careful individual-based evaluation by a multidisciplinary team, considering the personal preferences of the patient. However, in many women with thalassemia, cesarean delivery is preferred due to short stature and skeletal deformities accompanying normal fetal growth and more commonly due to cephalopelvic disproportion. In some TM patients, epidural anesthesia is preferred due challenging intubation and trauma risk during general anesthesia caused by severe maxillofacial deformity **(6, 29)**.

In pregnant women with known significant cardiac iron overload or dysfunction, in order to minimize the risk of cardiac decompensation or arrythmia during delivery, DFO infusion (2 g intravenous for 24-hour) may be considered **(34)**.

POSTPARTUM CARE

Deferoxamine can be re-started after delivery, due to very low concentrations in human milk and lack of oral absorption. Nonetheless, breastfeeding experience in patients taking DFO is inadequate. Breastfeeding should be encouraged in all cases, except for those with HIV and/or hepatitis C RNA positivity and/or HBV surface antigen (HbsAg) positivity due to risk of vertical transmission via human milk. Especially in patients with splenectomy, due to

higher risk of infection, antibiotic treatment is recommended after delivery **(6)**.

There is a limited number of clinical studies regarding the postpartum thromboprophylaxis However, guidelines recommend low-molecular-weight heparin prophylaxis during hospitalization, for 7 days following the vaginal delivery and for 6 weeks following the cesarean delivery **(7, 34)**. In cases of abortion or termination of pregnancy, risk thromboembolism continues, and low-molecular-weight heparin prophylaxis should be supplied during the abortion and for at least 7 days afterwards **(34)**.

All patients should be provided with counselling on contraception. As a male thalassemia HH patient is not expected to have spontaneous fertility, the female partner does not need contraception. Due to risk of infection, intra uterine devices should be avoided. In the breastfeeding period, calcium and vitamin D supplementation should be continued, however bisphosphonate treatment for osteoporosis can only be given after stopping the breastfeeding **(6, 29)**.

FERTILITY AND SPERMATOGENESIS INDUCTION IN MALES

In males with thalassemia, as in females, the same pathogenetic mechanism affecting the HPG axis may lead to infertility due to delayed/arrested puberty, absence of incomplete development of secondary sex characteristics and gonadal insufficiency. In males with thalassemia, iron overload in gonads leads to primary hypogonadism, and iron overload in pituitary gonadotropin hormone releasing cells leads to HH. Primary hypogonadism is rarely reversible, while HH is the most prevalent endocrine disorder in patients with thalassemia **(5, 42)**.

In these patients, for the evaluation of hypogonadism; height-weight measurement, development of secondary sex characteristics, penile length, body hair distribution, axillary and pubic hair development, presence of gynecomastia and size of testes should be determined on physical examination. If any, puberty follow-up cards should be examined; previous history of injury, trauma or mumps with likely influence on gonad functions, concomitant medications, erection and libido status, known genetic diseases should be questioned; thyroid functions, prolactin levels, liver function tests, gonadotropin and testosterone levels should be measured; and pituitary imaging and sperm analysis should be performed. Sperm analysis is recommended to be performed at least 3 times with 6–8-week intervals and after 2-3 days of sexual abstinence **(42)**. Measurement serum testosterone levels should be performed at its peak hours in the morning between 7:00 and 10:00 hours. In healthy non-obese males, lower limit of morning total testosterone level is reported as 264 ng/dL **(43)**.

In male thalassemia patients with hypogonadism, 2 main goals of hormonal treatment are; androgenization and stimulation of fertility. Treatment plan should be individualized. In the adulthood, in patients with hypogonadism; for virilization and its maintenance, preservation of bone health in the long term, reduction of cardiovascular risks, sexual and psychosocial well-being, testosterone hormone replacement should be given. For testosterone replacement, injectable, transdermal gel or patch, oral tablet or pellet forms of preparations can be used **(42, 43)**.

The most important period in the development of hypogonadism is puberty in thalassemia. Close follow-up of puberty development, appropriate chelation and management of testosterone hormone replacement therapy will prevent the development of hypogonadism and enable preservation of fertility. In male thalassemia patient with HH onset before completion of pubertal development, testes are generally smaller than 5 ml. In these patients, treatment with both Human Chorionic Gonadotropin (HCG) and Human Menopause Gonadotrophin (HMG) or recombinant FSH is usually required to induce spermatogenesis.

The initial regimen of hCG is usually 2,000–3,000 IU administered intramuscularly twice a week. Testosterone levels are measured every 2-3 months and clinic response is followed-up. If 8-12 months of HCG treatment has not resulted in sperm production and the patient is completely virilized, FSH treatment should be added. Even in individuals with

decreased sperm count and mobility, sperm banking procedures are recommended. When pregnancy occurs, FSH treatment may be stopped and spermatogenesis may be maintained with HCG, only. This treatment regimen for sperm production is continued for a maximum of 2 years. If it is not achieved, there is no indication to continue treatment **(29, 44)**.

Development of techniques like intracytoplasmic sperm injection (ICSI) enhanced the rate of conception, even in oligo-asthenospermic patients. For this reason, unless they have azoospermia, in all patients with desire to have a child in the future, sperm cryopreservation should be considered to preserve fertility **(44)**.

REFERENCES

1. Castaldi MA, Cobellis L. Thalassemia and infertility. Hum Fertil (Camb). 2016 Jun;19(2):90-6. doi: 10.1080/14647273.2016.1190869. Epub 2016 Jun 23. PMID: 27.335.221.
2. Corrine K Welt, MD. Normal menstrual cycle. Post TW, ed. UpToDate. Waltham, MA: UpToDate Inc. http://www.uptodate.com.Accessed on Apr 17, 2023.
3. Gardner D, Shoback D. Testes. Greenspan's Basic and Clinical Endocrinology 9th (eds) / 2011. 395-400.
4. Pozo J, Argente J. Delayed puberty in chronic illness. Best Pract Res Clin Endocrinol Metab. 2002 Mar;16(1):73-90. doi: 10,1053/beem.2002.0182. PMID: 11987900.
5. De Sanctis V, Soliman AT, Elsedfy H, et al. Growth and endocrine disorders in thalassemia: The international network on endocrine complications in thalassemia (I-CET) position statement and guidelines. Indian J Endocrinol Metab. 2013 Jan;17(1):8-18. doi: 10.4103/2230-8210.107808
6. Sayani FA, Singer ST, Carlberg KT, et al. Fertility and Pregnancy in Women with Transfusion-Dependent Thalassemia. Hematol Oncol Clin North Am. 2023 Apr;37(2):393-411. doi: 10.1016/j.hoc.2022.12.008.
7. Farmakis D, Porter J, Taher A, et al. 2021 Thalassaemia International Federation Guidelines for the Management of Transfusion-dependent Thalassemia. Hemasphere. 2022 Jul 29;6(8):e732. doi: 10.1097/HS9.0000000000000732.
8. Toumba M, Sergis A, Kanaris C, et al. Endocrine complications in patients with Thalassaemia Major. Pediatric Endocrinology Reviews: PER. 2007 Dec;5(2):642-648. PMID: 18084158. 2007.
9. Çetinçakmak MG, Hattapoğlu S, Menzilcioğlu S, et al. MRI-based evaluation of the factors leading to pituitary iron overload in patients with thalassemia major. J Neuroradiol. 2016 Jul;43(4):297-302. doi: 10.1016/j.neurad.2016.03.001.
10. Farmaki K, Tzoumari I, Pappa C, et al. Normalisation of total body ironload with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. Br J Haematol. 2010 Feb;148(3):466-75. doi: 10.1111/j.1365-2141.2009.07970. x.
11. Lam WW, Au WY, Chu WC, et al. One-stop measurement of iron deposition in the anterior pituitary, liver, and heart in thalassemia patients. J Magn Reson Imaging. 2008 Jul;28(1):29-33. doi: 10.1002/jmri.21433.
12. Noetzli LJ, Panigrahy A, Mittelman SD, et al. Pituitary iron and volume predict hypogonadism in transfusional iron overload. Am J Hematol. 2012 Feb;87(2):167-71. doi: 10.1002/ajh.22247. Epub 2011 Dec 27.
13. Pafumi C, Leanza V, Coco L, et al. The reproduction in women affected by cooley disease. Hematol Rep. 2011 Jan 13;3(1):e4. doi: 10.4081/hr.2011.e4. Epub 2011 Mar 23.
14. Birkenfeld A, Goldfarb AW, Rachmilewitz EA, et al. Endometrial glandular haemosiderosis in homozygous beta-thalassaemia. Eur J Obstet Gynecol Reprod Biol. 1989 May;31(2):173-8. doi: 10.1016/0028-2243(89)90178-0.
15. Roussou P, Tsagarakis NJ, Kountouras D, et al. Beta-thalassemia major and female fertility: the role of iron and iron-induced oxidative stress. Anemia. 2013; 2013:617204. doi: 10.1155/2013/617204. Epub 2013 Dec 16. PMID: 24396593.
16. Reubinoff BE, Simon A, Friedler S, et al. Defective oocytes as a possible cause of infertility in a beta-thalassaemia major patient. Hum Reprod. 1994 Jun;9(6):1143-5. doi: 10.1093/oxfordjournals.humrep.a138647.
17. Uysal A, Alkan G, Kurtoğlu A, et al. Diminished ovarian reserve in women with transfusion-dependent beta-thalassemia major: Is iron gonadotoxic? Eur J Obstet Gynecol Reprod Biol. 2017 Sep; 216:69-73. doi: 10.1016/j.ejogrb.2017.06.038.
18. Chang HH, Chen MJ, Lu MY, et al. Iron overload is associated with low anti-müllerian hormone in women with transfusion-dependent β-thalassaemia. BJOG. 2011 Jun;118(7):825-31. doi: 10.1111/j.1471-0528.2011.02927. x.
19. Singer ST, Vichinsky EP, Gildengorin G, et al. Reproductive capacity in iron overloaded women with thalassemia major. Blood. 2011 Sep 8;118(10):2878-

81. doi: 10.1182/blood-2011-06-360271. Epub 2011 Jul 14. PMID: 21757620; PMCID: PMC3172803.
20. Mensi L, Borroni R, Reschini M, et al. Oocyte quality in women with thalassaemia major: insights from IVF cycles. Eur J Obstet Gynecol Reprod Biol X. 2019 May 13; 3:100048. doi: 10.1016/j.eurox.2019.100048. PMID: 31404374
21. Chon SJ, Umair Z, Yoon MS. Premature Ovarian Insufficiency: Past, Present, and Future. Front Cell Dev Biol. 2021 May 10; 9:672890. doi: 10.3389/fcell.2021.672890.
22. De Sanctis V, Soliman AT, Elsedfy H, et al. Review and Recommendations on Management of Adult Female Thalassemia Patients with Hypogonadism based on Literature Review and Experience of ICET-A Network Specialists. Mediterr J Hematol Infect Dis. 2017 Jan 1;9(1):e2017001. doi: 10.4084/MJHID.2017.001.
23. Rossouw JE, Anderson GL, Prentice RL, et al; Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002 Jul 17; 288 (3):321-33. doi: 10.1001/jama. 288.3.321.
24. Taher A, Isma'eel H, Mehio G, et al. Prevalence of thromboembolic events among 8,860 patients with thalassaemia major and intermedia in the Mediterranean area and Iran. Thromb Haemost. 2006 Oct;96(4):488-91.
25. Borgna Pignatti C, Carnelli V, Caruso V, et al. Thromboembolic events in beta thalassemia major: an Italian multicenter study. Acta Haematol. 1998;99(2):76-9. doi: 10.1159/000040814.
26. Hugon-Rodin J, Gompel A, Plu-Bureau G. Epidemiology of hormonal contraceptives-related venous thromboembolism. Eur J Endocrinol. 2014;171: R221-230
27. Stuenkel CA, Davis SR, Gompel A, et al. Treatment of Symptoms of the Menopause: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2015 Nov;100(11):3975-4011. doi: 10.1210/jc.2015-2236.
28. CDC. U.S. medical eligibility criteria for contraceptive use, 2016. MMWR Recomm Rep 2016; 65 (No. RR-3):55-80.
29. Cappellini MD, Farmakis D, Porter J, et al. Guidelines for the management of transfusion dependent thalassemia (TDT). 4th edition. n.d: Thalassaemia International Federation; 2021.
30. Cassinerio E, Baldini IM, Alameddine RS, et al. Pregnancy in patients with thalassemia major: a cohort study and conclusions for an adequate care management approach. Ann Hematol. 2017 Jun;96(6):1015-1021. doi: 10.1007/s00277-017-2979-9.
31. Tsironi M, Karagiorga M, Aessopos A. Iron overload, cardiac and other factors affecting pregnancy in thalassemia major. Hemoglobin. 2010 Jun;34(3):240-50. doi: 10.3109/03630269.2010.485004.
32. Ambroggio S, Peris C, Picardo E, et al. β-thalassemia patients and gynecological approach: review and clinical experience. Gynecol Endocrinol. 2016;32(3):171-6. doi: 10.3109/09513590.2015.1109617.
33. Triunfo S, Lanzone A, Lindqvist PG. Low maternal circulating levels of vitamin D as potential determinant in the development of gestational diabetes mellitus. J Endocrinol Invest. 2017 Oct;40(10):1049-1059. doi: 10.1007/s40618-017-0696-9.
34. Petrakos G, Andriopoulos P, Tsironi M. Pregnancy in women with thalassemia: challenges and solutions. Int J Womens Health. 2016 Sep8; 8:441-51. doi: 10.2147/IJWH.S89308.
35. Spencer DH, Grossman BJ, Scott MG. Redcelltransfusiondecreases hemoglobin A1c in patientswithdiabetes. ClinChem. 2011 Feb;57(2):344-6. doi: 10.1373/clinchem.2010.157321.
36. De Sanctis, V, Soliman A.T, Elsedfy H, et al. (2016b) The ICET-A Survey on Current Criteria Used by Clinicians for the Assessment of Central Adrenal Insufficiency in Thalassemia: Analysis of Results and Recommendations. Mediterranean Journal of Hematology and Infectious Diseases. [Online] 8 (1). Available from: doi:10.4084/MJHID.2016.034 [Accessed: 12February 2021].
37. Origa R, Piga A, Quarta G, et al. Pregnancy and beta-thalassemia: an Italian multicenter experience. Haematologica. 2010 Mar;95(3):376-81. doi: 10.3324 /haematol.2009. 012393.
38. Bajoria, R. & Chatterjee, R. (2009) Current perspectives of fertility and pregnancy in thalassemia. Hemoglobin. [Online] 33 Suppl 1, S131-135. Available from: doi:10.3109/03630260903365023.
39. Leung TY, Lao TT. Thalassaemia in pregnancy. Best Pract Res Clin Obstet Gynaecol. 2012 Feb;26(1):37-51. doi: 10.1016/j.bpobgyn.2011.10.009.
40. Alexander EK, Pearce EN, Brent GA, et al. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid. 2017 Mar;27(3):315-389. doi: 10.1089/thy.2016.0457.
41. Diamantidis MD, Neokleous N, Agapidou A, et al. Iron chelation therapy of transfusion-dependent β-thalassemia during pregnancy in the era of novel drugs: is deferasirox toxic? Int J Hematol. 2016 May;103(5):537-44. doi: 10.1007/s12185-016-1945-y.

42. De Sanctis V, Soliman AT, Yassin MA, et al. Hypogonadism in male thalassemia majör patients: pathophysiology, diagnosis and treatment. Acta Biomed. 2018 Feb 16;89(2-S):6-15. doi: 10.23750/abm.v89i2-S.7082.
43. Bhasin S, Brito JP, Cunningham GR, et al. Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2018 May 1;103(5):1715-1744. doi: 10.1210/jc.2018-00229.
44. De Sanctis V, Soliman A, Mohamed Y. Reproductive health in young male adults with chronic diseases in childhood. Pediatr Endocrinol Rev. 2013 Mar-Apr;10(3):284-96.

Correspondance Address:
Assoc. Prof. Dr. Ziynet Alphan ÜÇ
Department of Internal Medicine
Division of Endocrinology and Metabolism
Usak University School of Medicine
64200, Usak, Türkiye
Phone +90 (274) 2212121
Mobile +90 (0505) 502 52 13
E-mail: ziynetalp@yahoo.com, ziynet.uc@usak.edu.tr

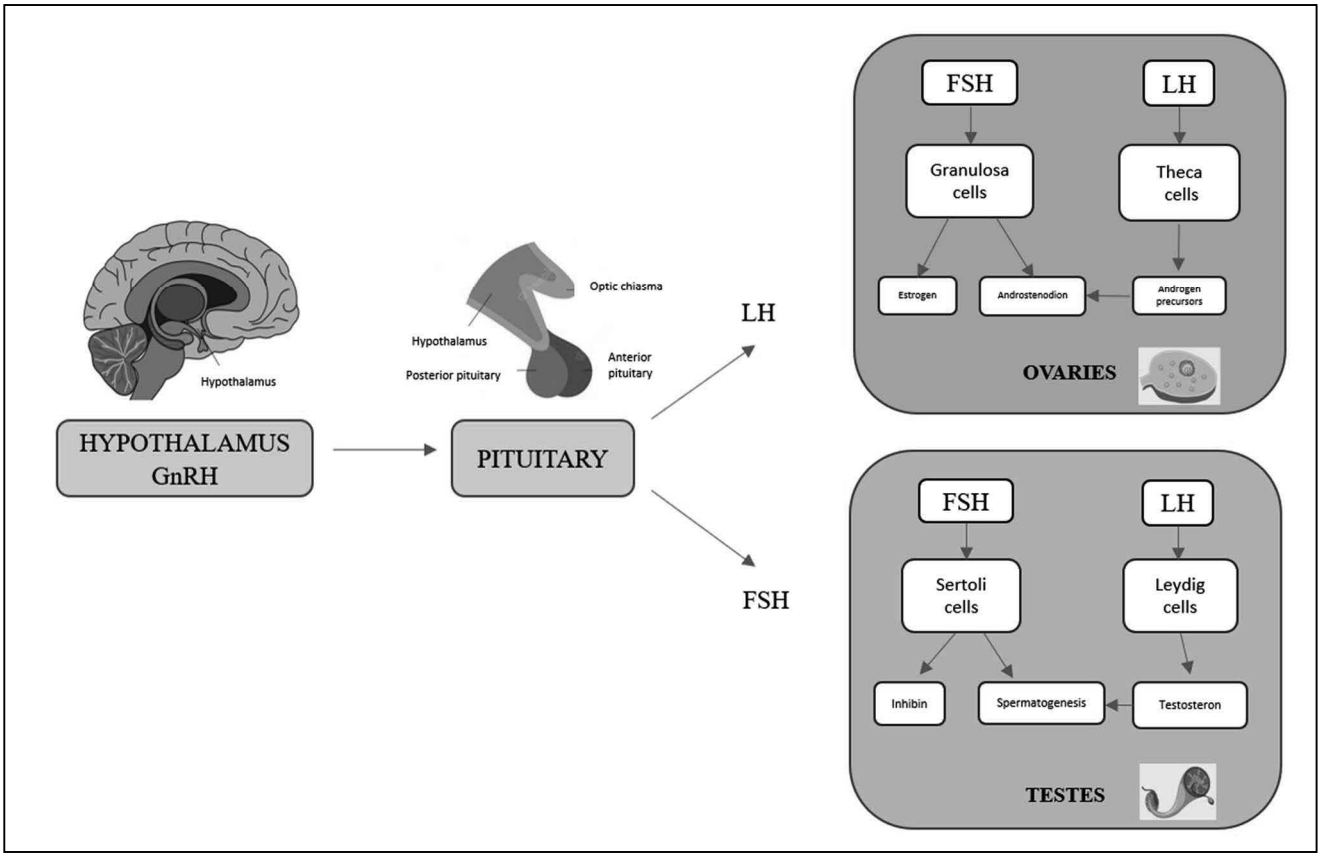


Figure 1: *Hormonal regulation of gonadal functions in males and females*

Table 1: *Before pregnancy evaluation*

Partner evaluation	Application of genetic tests for thalassemia and hemoglobinopathies, interpretation of results and provision of appropriate genetic counseling
Fertility evaluation	Evaluation of hypothalamic–pituitary–gonadal axis Menstrual history (primary or secondary amenorrhea) Ultrasonographic evaluation of uterus and ovaries AMH level, antral follicle count
Evaluation of iron overload	Proper chelation treatment to keep ferritin level <l.000 ng/ml Cardiac: ECHO, ECG, 24-hour holter monitoring, T2-MRI, target $T2^*$ >20 ms Liver: Liver function tests, MRI. Liver iron concentration should be <7 mg/g (dry weight) before pregnancy
Endocrinological evaluation	Thyroid function tests (TSH, free T4) Glucose tolerance test /fructosamine Vitamin D levels DEXA screening Adrenal gland evaluation
Infection screening	HIV, HCV, HBV, CMV Measles, toxoplasmosis, syphilis
Medical treatment evaluation	Medications to be discontinued: Chelators, bisphosphonates, ACE inhibitor, interferon, ribavirin, hydroxyurea, vitamin C, oral hypoglycemic agents Medications to be initiated: Folic acid, calcium, vitamin D, insulin treatment for those on oral antidiabetic
Thrombotic risk evaluation	Previous history for splenectomy Previous history for venous thromboembolic event Family history for thrombophilia If any, history for abortus in previous pregnancies

AMH: Antimüllerian hormone, ECHO: Echocardiography, ECG: Electrocardiography, MRI: Magnetic resonance imaging, HIV: Human immunodeficiency virus, HCV: Hepatitis C virus, HBV: Hepatitis B virus, CMV: Cytomegalovirus, DEXA: Dual-energy X-ray absorptiometry, TSH: Thyroid stimulating hormone, ACE: Angiotensin converting enzyme.

SKELETAL AND BONE COMPLICATIONS IN THALASSEMIA

Doga Turkkahraman[1], Merve Gullu[1]
[1]MD, University of Health Sciences, Antalya Training and Research Hospital, Department of Pediatric Endocrinology, Antalya, Türkiye

ABSTRACT

Bone abnormalities in thalassemia are quite frequent and range from a decrease in bone mineral density and consequent osteoporosis to spinal cord compression, back pain, and increased risk of the development of fractures. Its pathogenesis is multifactorial and mainly includes bone marrow expansion, ineffective erythropoiesis, hypogonadism, vitamin D deficiency, genetic factors, endocrine dysfunction, iron overload, iron chelation and reduced physical activity. Early start of regular transfusion and iron chelation is the priority of treatment in thalassemia. Annual pediatric endocrinology visits should be recommended to all thalassemia patients after the age of 10.

Keywords: Thalassemia, children, skeletal and bone complications

BACKGROUND

Bone abnormalities in beta-thalassemia major (β-TM) and beta-thalassemia intermedia (β-TI) are quite frequent and range from a decrease in bone mineral density (BMD) and consequent osteoporosis to spinal cord compression, back pain, and increased risk of the development of fractures. Its pathogenesis is multi-factorial and mainly includes bone marrow expansion, ineffective erythropoiesis, hypogonadism, vitamin D deficiency, genetic factors, endocrine dysfunction, iron overload, iron chelation and reduced physical activity **(1, 2, 3)**. The mechanisms leading to bone loss have not been completely clarified **(4)**. In previous reports up to 70% of adults with β-TM have low bone mass by using dual-energy X-ray (DEXA) **(5)**. The major skeletal changes in thalassemic patients are; osteoporosis, bone age delay and spondylometaphyseal abnormalities **(6)**.

OSTEOPOROSIS

Osteoporosis is defined as a decrease in BMD and disruption of the bone architecture leading to an increased risk of fractures. DEXA results were affected by the skeletal maturation and child's body size, so the diagnosis of osteoporosis in children should not be made on the basis of densitometric criteria alone. According to International Society of Clinical Densitometry (ISCD) guideline 2019, pediatric osteoporosis is defined by the occurrence of one or more vertebral compression fractures in the absence of local disease or high-energy trauma, or the presence of both low bone density for age (≤ -2 SDS) and a significant fracture history (≥ 2 long bone fractures by age 10, or ≥ 3 long bone fractures by age 19 years). Additionally, BMD Z-score of > −2, in this context, "does not preclude the possibility of skeletal fragility and increased fracture risk" **(7)**.

A decrease in bone mass can occur due to increased bone resorption or decreased bone formation. Etiological factors of osteoporosis in thalassemia are ineffective hematopoiesis, direct toxicity of iron to osteoblasts and iron chelation therapies. Endocrinological factors including hypogonadism, hypothyroidism, deficiency of growth hormone (GH) or insulin like growth factors, hypoparathyroidism and diabetes may cause osteopenia and osteoporosis via their effects on cartilage tissue, **Figure 1 (8, 9, 10)**. Hypogonadism and GH deficiency contribute to a failure to achieve peak bone mass in thalassemia patients **(11)**. The majority of the patients with β -

TI have decreased levels of IGF-1 that usually plays an essential role in bone remodeling cycle that stimulates osteoclasts and differentiation and proliferation of the osteoblasts. The receptor activator of the nuclear factor-kappa B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) pathway has been recognized as the final, dominant mediator of osteoclast proliferation and activation **(9)**. An increased level of RANKL leads to decreased bone thickness followed by bony deformities, osteopenia, and ultimately fractures, **Figure 2 (1, 11)**.

Additionally, Bsml Vitamin-D receptor (VDR) gene polymorphism has been shown to be linked with the osteopenia that develops in thalassemia **(12)**. The urinary excretion of urinary NTX has been shown to be a sensitive and reliable index of the hip BMD Z-score in patients with thalassemia **(12)**. In the pathogenesis of osteoporosis in β-TM, the WNT pathway has been proposed to participate and negative modulators of this signaling system, such as DKK-1 and sclerostin, have been also associated with BMD **(13)**.

BMD reduction was present in the spine, femoral neck, and distal radius in more than 2/3 of the patients with both β -TM and β -TI **(15)**. In a study of 18 children (aged 5.8 ± 1.5 years) with β -TM who received hypertransfusion and chelation therapy, Z-scores were noted to be <-2.5 in 22.2% of children, between -1 and -2.5 in 38.8%, and >1 in 38.8% **(16)**. In adolescent transfusion dependent thalassemia patients with suboptimal BMD, 61.3% of patients demonstrating a Z-score of < -2 and 22.6% having a Z-score between -1 and -2 **(17)**. In contrast, in a study of β -TM children and adolescents (aged 5–20 years) who received regular transfusion and chelation therapy, Z-scores were within the normal range for all subjects, with a mean Z-score of 0.42 for females and -0.41 for males **(18)**. DEXA is used to estimate BMD and the peripheral Quantitative Computer Tomography (pQCT) is used to assess the regional changes of BMD **(19)**. In adolescents, the mostly used site is lumbar spine for measurement of BMD by DEXA. Bone loss in β-TM largely involves the trabecular bone, maybe it is due to the close interaction between bone marrow and bone remodeling. Therefore, the most affected site is the lumbar spine due to its constitution of mainly trabecular bone **(20)**. Additionally, the accuracy of DEXA is affected by the presence of multiple degenerative changes at the spine e.g., osteophytes, osteochondrosis, vascular calcification.

MANAGEMENT OF OSTEOPOROSSIS IN THALASSEMIA

Due to the deleterious effect of iron toxicity, reducing iron overload must be the priority of treatment. Iron chelation should start as early as possible to prevent hypogonadism and GH deficiency. Most guidelines recommend to start iron chelation after 10 to 20 transfusions or when serum ferritin is above 1000 ng/ml **(14)**. The Transfusion Dependent Thalassemia (TDT) guideline (2021) recommends that thalassemia patients ≥10 years should be screened for serum calcium, phosphate, alkaline phosphatase, vitamin D, parathyroid hormone (PTH), and urinary calcium and phosphate excretion annually, and for osteoporosis by DEXA every two years **(21)**. Despite transfusion normalized hemoglobin levels, iron chelation and adequate hormonal replacement therapy, the thalassemia patients may continue to have progressive bone disease and BMD loss over time **(22, 23)**.

Bone-related abnormalities including hypocalcemia, hyperphosphatemia and vitamin D deficiency were very common among thalassemic patients. The prevalence of low BMD might be high in thalassemic children and adolescents due to the progression of disease or inadequate nutrient intake. Bone densitometry test is suggested routinely in this vulnerable group of children. Oral vitamin D (1000-1500 IU/day) and calcium (200-1000 mg/day) supplements should be recommended to the patients with thalassemia but the efficacy and exact treatment regimen have not yet been defined **(11)**.

Low serum zinc levels associated with hemolysis and oxidative damage is frequently observed in thalassemia by the effect of iron chelators **(24)**. Benefits for growth velocity and osteoporosis are based on uncontrolled studies **(25)**.

In chronic iron toxicity, vitamin C deficiency was possible due to hyperconsumption. In thalassemic patients with high iron overload and poor or no chelation, ascorbate deficiency may impair chon-

drocyte and osteoblast function, long bones growth leading subperiosteal hemorrhage, and fractures **(26)**. Additionally, the vitamin K2 combined with calcitriol, improves lumbar spine BMD **(27)**. Smoking should be discouraged to prevent early bone loss.

Bisphosphonates are the potent osteoclast inhibitors which constitute the treatment of choice in thalassemia associated osteoporosis and these drugs modify the biochemical markers of bone formation and resorption. They have been shown to be safe and efficacious in improving BMD and reducing bone complications and pain in both β-TM and β-TI **(28)**. Although there is no enough data that bisphosphonates reduce the number of fractures **(29)**. The effect of pamidronate on osteoporosis have not been properly evaluated in pediatric β-thalassemia patients **(30)**. Mostly used bisphosphonates in thalassemia are pamidronate and zoledronate. According to TDT guideline (2021), pamidronate is recommended 30 mg/month iv, and zoledronate 4 mg/ 3 months iv. Additionally, it has been underlined that the bisphosphonates should not be used longer than 24-36 months **(21)**.

Denosumab, a human monoclonal antibody to RANKL which works by decreasing the development of osteoclasts, reduces bone resorption markers and pain scores, and causes significant increase in lumbar BMD compared to placebo, but does not modify femoral BMD **(31)**. On the other hand, there is limited evidence for teriparatide, a recombinant peptide fragment of parathyroid hormone, that has a positive effect on vertebral BMD **(32)**.

FRACTURES

Fractures are more frequently seen in β-TM than β-TI. A decreased BMD is a major risk factor for the development of fractures in thalassemia. Depending on the study population and method of data collection in β-TM patients, the prevalence of fractures differs. In iron-overloaded patients with β-TM, fracture prevalence was ranged between 38% and 41% in two large studies in US and the most frequently reported fracture site was the upper extremity **(33, 34)**. In another study of 62 patients between 10 and 32 years of age, one in five had multiple or recurrent fractures and one in three had sustained fractures. Moreover, deformities occurred due to premature fusion of the epiphyses of the long bones at the lower tibia and fibula, upper humeral, and lower femoral epiphyses **(35)**. Vertebral fractures are usually asymptomatic and their prevalence varies from 2.6 to 13% **(36)**. Risk factors for fracture include increasing age, male gender, a history of sex hormone replacement and lower BMD. Males were more likely to be hypogonadal so more prone to fractures than females **(34)**. Bone fractures are more common in their mid to late 30s.

PAIN

Bone- and joint-related pains are common in thalassemic patients. It was determined that 30% of the patients complained of arthralgia and 25% of them complained of low back pain **(37)**. Back pain is most likely due to osteoporosis, compression fractures, and intervertebral disc degeneration. Adults aged >35 years experienced greater pain. There was a relationship between pain and low vitamin D levels, and there was a trend towards increased pain with lower bone density. Physical activity, prolonged standing and lifting of heavy objects trigger the pain but the most frequent pain trigger is low hemoglobin level **(38)**. Transfusion is important to relief pain but chelators used to inhibit iron toxicity may also cause pain. Additionally, first orally active iron chelator deferiprone had arthropathy side-effect and symptoms range from mild non-progressive arthropathy controllable with non-steroidal anti-inflammatory drugs to severe erosive arthropathy rarely that may progress even after treatment is stopped **(39)**.

INTERVERTEBRAL DISC CHANGES

A significant difference in the severity of disc degeneration on MRI and radiographs has been demonstrated between controls and TM patients in a study. In β-TM patients, platyspondyly, endplate irregularity, findings of intranuclear gas and calcification within discs were more common. The chelating agent deferoxamine deleteriously affect the integrity and strength of the annulus fibrosus fibers by chelating other trace elements or minerals and thus interfering with enchondral ossification, or possibly, by direct toxicity **(38)** especially with

early start, high doses, and reduced iron stores. These changes have not been observed with the oral chelators on the contrary, deferasirox has been associated with an increase in BMD.

SCOLIOSIS

An increased incidence of scoliosis associated with β-TM has been described. The S-shaped (right thoracic, left lumbar) scoliosis curve pattern was mostly seen. The prevalence of scoliosis was not gender related, irrespective of age and curve magnitude. Progression was mainly attributed to anemia, hemosiderosis, iron chelation therapy, and associated hormonal disorders previously described in patients with β-TM **(40)**.

EXTRAMEDULLARY HEMATOPOIESIS

The development of erythropoietic tissue outside the marrow cavity, known as extramedullary hematopoiesis (EMH), is a phenomenon that compensates for the decreased efficiency of the bone marrow in providing RBCs for the circulation **(41)**. The spleen, lymph nodes, liver, breast, spinal canal, prostate, heart, thymus, kidney, and adrenal glands can be involved in EMH contributing to the development of osteoporosis and deformities of the facial bones, obliteration of maxillary sinuses, and protrusion of the upper jaw, along with increased risk for fractures of long bones and spinal compression. The incidence of EMH may reach up to 20% in patients with β -TI compared to poly-transfused β-TM patients where the incidence remains less than 1%. However, more than 80% of cases may remain asymptomatic and the lesions are usually discovered incidentally by radiologic techniques **(41)**.

Early diagnosis of β -TI and optimal management of the underlying anemia and associated symptoms are important steps to prevent EMH and its complications. MRI is preferred for the evaluation of spinal cord compression due to an EMH **(42)**.

SPINAL CORD COMPRESSION

The treatment of spinal cord compression is blood transfusions, surgical decompression, and radiotherapy either singly or in combination **(41)**. While surgical decompression is an effective method that allows improvement of symptoms, radiotherapy alone remains to be the treatment of choice which hinders the hematopoietic activity thereby triggering the decline in the size of the mass and improvement of the associated symptoms **(43)**.

In conclusion, hypogonadism, hypothyroidism, deficiency of GH, hypoparathyroidism and diabetes may cause osteopenia and osteoporosis in thalassemic people via their effects on cartilage tissue. Osteoporosis is a progressive disease; therefore, prevention and early diagnosis are very important. In the management of thalassemia, adequate hormonal replacement, effective blood transfusion and iron chelation, calcium and vitamin D administration, physical activity is currently the main precautions. Bisphosphonates are used in the management of osteoporosis in thalassemia. Pamidronate and zoledronate are effective in increasing the BMD.

REFERENCES

1. Voskaridou E, Terpos E. New insights into the pathophysiology and management of osteoporosis in patients with beta thalassaemia. Br J Haematol. 2004;127(2):127–39
2. Taher AT, Musallam KM, Karimi M, et al. Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the Optimal Care Study. Blood. 2010;115(10):1886-1892.
3. Perrotta S, Cappellini MD, Bertoldo F, et al. Osteoporosis in beta-thalassaemia major patients: analysis of the genetic background. Br J Haematol. 2000;111(2):461-466.
4. Fung EB, Vichinsky EP, Kwiatkowski JL et al. Characterization of low bone mass in young patients with thalassemia by DXA, pQCT and markers of bone turnover. Bone. 2011;48(6):1305–12.
5. Filosa A, Di Maio S, Vocca S et al. Longitudinal monitoring of bone mineral density in thalassaemic patients. Genetic structure and osteoporosis. Acta Pediatr. 1997; 86:342–6.
6. Skordis N, Toumba M. Bone disease in thalassaemia major: recent advances in pathogenesis and clinical aspects. Pediatr Endocrinol Rev. 2011; 8:300–6.
7. Shuhart CR, Yeap SS, Anderson PA, et al. Executive Summary of the 2019 ISCD Position Development Conference on Monitoring Treatment, DXA Cross-calibration and Least Significant Change, Spinal Cord Injury, Peri-prosthetic and Orthopedic Bone

Health, Transgender Medicine, and Pediatrics. J Clin Densitom. 2019;22(4):453-471.
8. Toumba M, Skordis N. Osteoporosis syndrome in thalassaemia major: an overview. J Osteoporos. 2010; 2010:537673.
9. Salah H, Atfy M, Fathy A, et al. The clinical signifcance of OPG/sRANKL ratio in thalassemia patients sufering from osteopenia or osteoporosis in Egyptian patients. Immunol Investig. 2010; 39(8):820–32.
10. Piga A. Impact of bone disease and pain in thalassemia. Hematology Am Soc Hematol Educ Program. 2017;2017(1):272-277.
11. Wong P, Fuller PJ, Gillespie MT et al. Bone Disease in Thalassemia: A Molecular and Clinical Overview. Endocr Rev. 2016;37(4):320-346.
12. Dresner Pollack R, Rachmilewitz E, Blumenfeld A, et al. Bone mineral metabolism in adults with beta-thalassaemia major and intermedia. Br J Haematol. 2000;111(3):902-907.
13. Voskaridou E, Christoulas D, Plata E.et al. High circulating sclerostin is present in patients with thalassemia-associated osteoporosis and correlates with bone mineral density. Horm. Metab. Res. 2012, 44, 909–913.
14. Angastiniotis M, Eleftheriou A. Thalassaemic bone disease: an overview. Pediatr Endocrinol Rev. 2008;6(suppl 1):73-80.
15. Voskaridou E, Kyrtsonis MC, Terpos E, et al. Bone resorption is increased in young adults with thalassaemia major. Br J Haematol. 2001;112(1):36-41.
16. Vogiatzi MG, Autio KA, Schneider R, Giardina PJ. Low bone mass in prepubertal children with thalassemia major: insights into the pathogenesis of low bone mass in thalassemia. J Pediatr Endocrinol Metab. 2004;17(10):1415-1421.
17. Vogiatzi MG, Autio KA, Mait JE, et al. Low bone mineral density in adolescents with β -thalassemia. Ann NY Acad Sci. 2005; 1054:462– 466.
18. Christoforidis A, Kazantzidou E, Tsatra I, et al. Normal lumbar bone mineral density in optimally treated children and young adolescents with beta-thalassaemia major. Hormones (Athens). 2007;6(4):334-340.
19. Angastiniotis M, Pavlides N, Aristidou K, et al. Bone pain in thalassaemia: assessment of DEXA and MRI findings. J Pediatr Endocrinol Metab. 1998;11 Suppl 3:779-784.
20. Gaudio A, Morabito N, Catalano A, Rapisarda R, Xourafa A, Lasco A. Pathogenesis of Thalassemia Major-associated Osteoporosis: A Review with Insights from Clinical Experience. J Clin Res Pediatr Endocrinol. 2019;11(2):110-117.
21. Farmakis D, Porter J, Taher A, et al. 2021 Thalassaemia International Federation Guidelines for the Management of Transfusion-dependent Thalassemia. Hemasphere. 2022;6(8): e732. Published 2022 Jul 29.
22. Ladis V, Raptou P, Rigatou E, et al. Study of bone density by pQCT analysis in healthy adults and patients with B-thalassemia major and intermedia. Pediatr Endocrinol Rev. 2008;6 Suppl 1:127-131.
23. Carmina E, Di Fede G, Napoli N, et al. Hypogonadism and hormone replacement therapy on bone mass of adult women with thalassemia major. Calcif Tissue Int. 2004;74(1):68-71.
24. Ozturk Z, Genc GE, Gumuslu S. Minerals in thalassaemia major patients: an overview. J Trace Elem Med Biol. 2017; 41:1-9
25. Swe KM, Abas AB, Bhardwaj A, Barua A, Nair NS. Zinc supplements for treating thalassaemia and sickle cell disease. Cochrane Database Syst Rev. 2013;(6):CD009415.
26. Aghajanian P, Hall S, Wongworawat MD, et al.The roles and mechanisms of actions of vitamin C in bone: new developments. J Bone Miner Res. 2015;30(11):1945-1955
27. Ozdemir MA, Yilmaz K, Abdulrezzak U et al. The efficacy of vitamin K2 and calcitriol combination on thalassemic osteopathy. J Pediatr Hematol Oncol. 2013;35(8):623-627.
28. Voskaridou E, Terpos E, Spina G, et al. Pamidronate is an effective treatment for osteoporosis in patients with beta-thalassaemia. Br J Haematol. 2003;123(4):730-737.
29. Tsartsalis AN, Lambrou GI, Tsartsalis D, et al. The role of biphosphonates in the management of thalassemia-induced osteoporosis: a systematic review and meta-analysis. Hormones (Athens). 2018;17(2):153-166.
30. Morabito N, Lasco A, Gaudio A, et al. Bisphosphonates in the treatment of thalassemia-induced osteoporosis. Osteoporos Int. 2002;13(8):644-649.
31. Voskaridou E, Ntanasis-Stathopoulos I, Papaefstathiou A, et al. Denosumab in transfusion-dependent thalassemia osteoporosis: a randomized, placebo-controlled, double-blind phase 2b clinical trial. Blood Adv. 2018;2(21):2837-2847.
32. Tournis S, Dede AD, Savvidis C, et al. Effects of teriparatide retreatment in a patient with β-thalassemia major. Transfusion. 2015;55(12):2905-2910.
33. Vogiatzi MG, Macklin EA, Fung EB, et al. Prevalence of fractures among the thalassemia syndromes in North America. Bone 2006; 38(4):571–5.
34. Fung EB, Harmatz PR, Milet M, et al. Fracture prevalence and relationship to endocrinopathy in iron

overloaded patients with sickle cell disease and thalassemia. Bone. 2008;43(1):162-168.
35. Exarchou E, Politou C, Vretou E, Pasparakis D, Madessis G, Caramerou A. Fractures and epiphyseal deformities in beta-thalassemia. Clin Orthop Relat Res. 1984;(189):229-233.
36. Engkakul P, Mahachoklertwattana P, Jaovisidha S, et al. Unrecognized vertebral fractures in adolescents and young adults with thalassemia syndromes. J Pediatr Hematol Oncol. 2013;35(3):212-217.
37. Onur O, Sivri A, Gumruk F, Altay C. Beta thalassaemia: a report of 20 children. Clin Rheumatol 1999; 18:42–4.
38. Desigan S, Hall-Craggs MA, Ho CP, et al. Degenerative disc disease as a cause of back pain in the thalassaemic population: a case–control study using MRI and plain radiographs. Skeletal Radiol 2006; 35(2):95–102
39. Haidar R, Musallam KM, Taher AT. Bone disease and skeletal complications in patients with β thalassemia major. Bone. 2011;48(3):425-432
40. Papanastasiou DA, Baikousis A, Sdougos G, et al. Correlative analysis of the sagittal profile of the spine in patients with beta-thalassemia and in healthy persons. J Spinal Disord 2000; 13(2):113–7.
41. Taher A, Isma'eel H, Cappellini MD. Thalassemia intermedia: revisited. Blood Cells Mol Dis. 2006;37(1):12-20.
42. Coşkun E, Keskin A, Süzer T, Sermez Y, Kildaci T, Tahta K. Spinal cord compression secondary to extramedullary hematopoiesis in thalassemia intermedia. Eur Spine J. 1998;7(6):501-504.
43. Khandelwal N, Malik N, Khosla VK, Suri S. Spinal cord compression due to epidural extramedullary haematopoiesis in thalassemia. Pediatr Radiol. 1992;22(1):70-71.

Correspondence Address:
Doga Turkkahraman MD
University of Health Sciences, Antalya Training and Research Hospital, Department of Pediatric Endocrinology, Antalya, Türkiye
Mail: drdoga@hotmail.com

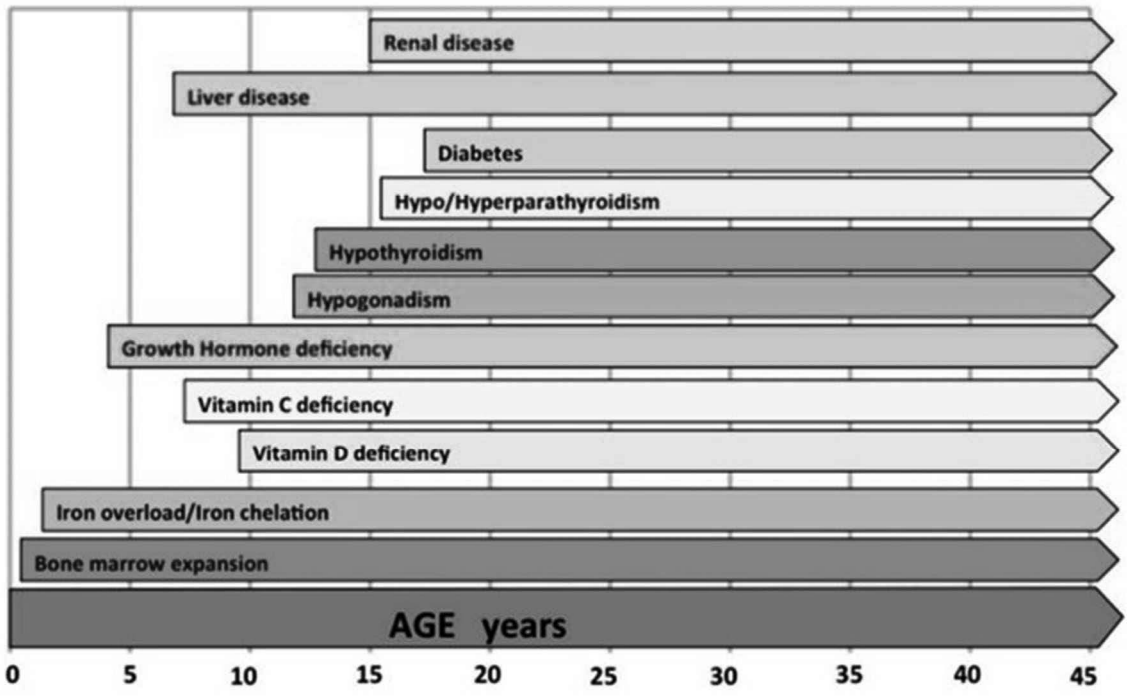


Figure 1: *The conditions relevant for bone disease and the approximate timeline of the occurrence of complications in suboptimal treatment* [10]

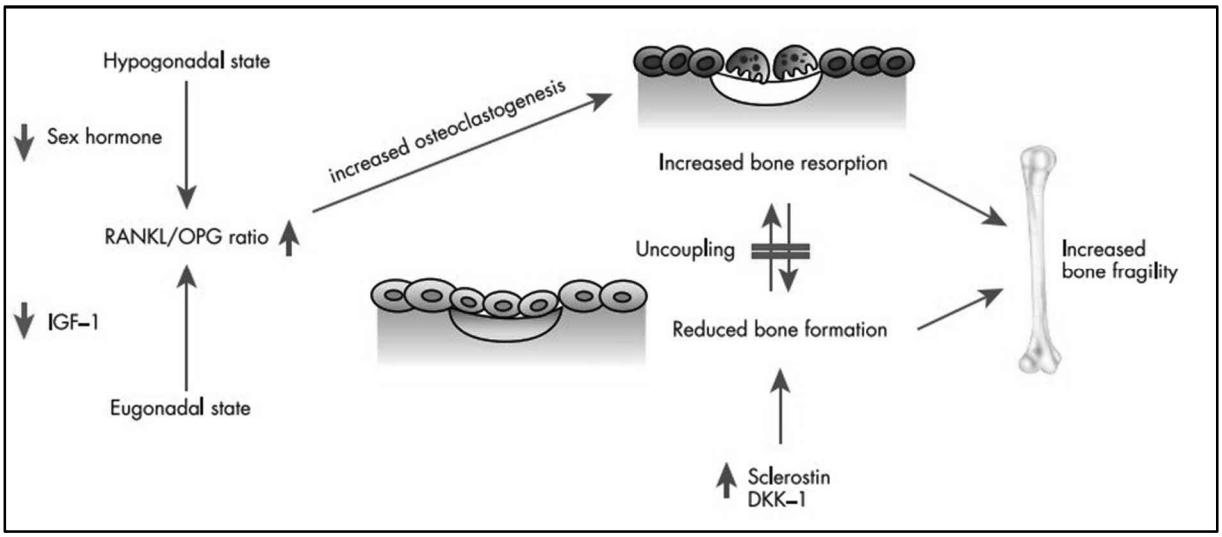


Figure 2: *The role of RANKL and WNT/-catenin signaling in thalassemia bone disease. Sex hormone and GH deficiency, leads to increased bone resorption through elevation of the RANKL/OPG ratio by mediating increased osteoclast activity. High levels of sclerostin and DKK-1 lead to reduced bone formation. The elevation of the RANKL/OPG ratio and enhanced sclerostin/DKK-1 results in low bone mass and increased bone fragility. DKK-1, Dickkopf-1.* [11]

ORAL AND DENTAL HEALTH PROBLEMS IN THALASSEMIA

Specialist Dr. Dt. Simge Canatan Keskin[1], Prof. Dr. Şükran Bolay[2]
[1]Bayındır Kavaklıdere Hospital, Oral and Dental Clinic- Ankara-Türkiye
[2]Hacettepe University Faculty of Dentistry, Department of Restorative Dentistry Ankara-Türkiye

ABSTRACT

Thalassaemia syndromes represent the most prevalent single-gene disorders worldwide. These disorders arise due to inadequate synthesis of either alpha or beta globin chains which, in turn, constitute the haemoglobin tetramer structure. To date, over 350 varieties of beta thalassaemia and over 200 forms of alpha thalassaemia have been identified at the molecular level.

The main pathophysiological mechanism of the disease is anaemia due to ineffective erythropoiesis and haemolytic anaemia, which is caused by anaemia. Consequently, patients might require regular blood transfusions throughout their lifetime. The clinical presentation varies depending on the severity of thalassaemia. The severity of clinical features may differ according to the degree of thalassaemia. Common academic sections will be included, author and institution formatting standards will be adhered to, citations will be consistent, and patients will be classified as having transfusion-dependent thalassaemia. Patients are categorized as transfusion-dependent thalassaemia (TDT) or major, transfusion-non-dependent thalassaemia (TNDT) or intermedia according to their transfusion status. There are four primary factors contributing to facial, oral, and dental issues in patients with TBT and TBOT. These factors include anaemia, ineffective erythropoiesis, iron overload, increased free radicals, and side effects that result from the use of chelation agents. As a consequence of these factors, all patients ultimately develop hyperplasia and maxillofacial enlargement, which can lead to maxillofacial deformities over time. Oral and dental health issues, such as dental caries and periodontal disease, are prevalent in thalassaemia patients as well as maxillofacial deformities. Notably, patients with thalassaemia experience tooth discolouration, gingival hyperpigmentation, and darkened skin colour due to iron overload.

Oral and dental health issues typically become critical among thalassaemia patients due to the delay in dental examinations and early diagnosis. Progression of dental caries and delay in restorative dental treatment can lead to abscess formation and the risk of infection spreading to the neck and facial tissues. With regular dental examinations in the diagnosis, treatment and follow-up of thalassaemia patients, oro-dento-facial problems can be prevented before they occur.

Keywords: Thalassaemia, oral and dental health

INTRODUCTION

Thalassaemia syndromes are prevalent single-gene disorders worldwide, which are correlated with a decrease in the synthesis of either alpha or beta globin chains making up the haemoglobin tetrameric structure. The designation of thalassaemias varies according to the name of the affected globin chain and the severity of the clinical condition. Currently, over three hundred and fifty variants of beta thalassaemia and more than two hundred types of Alpha Thalassaemia have been identified at the molecular level. Alpha/Beta thalassaemia has four clinically defined forms: silent carrier (ST), carrier (TT), intermedia (TI), and major (TM). Depending on transfusion status, it is classified as transfusion-dependent thalassemia (TDT) or major, transfusion non-dependent thalassemia (TNDT) or intermedia **(1-4)**. Patients with TDT or TNDT are particularly

susceptible to facial, oral, and dental problems due to the disease's physiopathology **(5)**.

REASONS FOR ORAL AND DENTAL HEALTH PROBLEMS

There are four underlying causes of facial, oral and dental problems in TBT and TBOT patients. These are anaemia, ineffective erythropoiesis, increased iron load and free radicals, and side effects of chelation agents.

The primary cause in the physiopathology of the disease is ineffective erythropoiesis and haemolytic anaemia. Due to inadequate beta chains, haemoglobin production is inhibited, leading to anaemia. The bone marrow signals to the bone marrow to work hard and cannot produce enough, resulting in ineffective erythropoiesis. Additionally, the short lifespan of the erythrocytes produced leads to haemolysis. The bone marrow signals the bone marrow to increase red blood cell production due to anaemia resulting from haemolysis and ineffective erythropoiesis. However, the production of red blood cells is abnormal. Over time, the bone marrow cavities such as those in the skull, facial bones, and ribs enlarge, leading to distinct facial features and radiographic findings known as “chipmunk or rodent face” **(1-4)**.

The second cause is increased iron load in the body due to transfusion and gastrointestinal absorption of iron. Following iron overload, accumulation in saliva and gingiva increases. In a study of 96 patients with iron overload (71 thalassaemia major, 10 thalassaemia intermedia and 15 thalassaemia carriers), 30 patients with iron deficiency anaemia and 35 healthy controls, salivary and serum iron, iron binding and ferritin levels were compared. Salivary levels of iron and ferritin were found to be higher in Thalassaemia patients than in the control group. However, salivary iron and ferritin levels were significantly lower in patients with iron deficiency **(6)**.

A study was performed to evaluate liver iron load, which is the gold standard for measuring iron load in patients, and gingival biopsy. The results showed a significantly high iron load in the gingiva of the patients, while no significant change was observed in the liver iron load. The study included 22 TBI patients and 20 healthy controls. The measurements included the plaque index, gingival index, periodontal pocket depth, and gingival biopsies were taken from all patients. Gingival and liver tissues were examined histopathologically for inflammation, iron deposition and fibrosis. Mild gingivitis and gingival iron deposition were observed in all patients. Nonetheless, there was no correlation found between gingival iron accumulation and hepatic iron deposition. Therefore, more studies are recommended to measure iron load in patients **(7)**.

The third reason for the increase in free radicals is iron overload. Free radicals impact the entirety of the body, leading to the deterioration of oral hygiene and the development of dental caries. Salivary oxidative stress indicators, protein, iron, and pH were observed and compared in healthy individuals with dental caries and in patients with transferrin-bound iron saturation. It was suggested that oxidative stress played a key role in the development of dental caries in patients with TBT and that natural antioxidants should be used accordingly **(8)**.

Desferrioxamine (DFX), deferiprone (DFP) and deferasirox (DFX) chelating agents are used alone or in various combinations, depending on the patient's condition. Although chelation agents have no direct impact on oral or saliva structure, research suggests that chelators negatively impact the immune system by inducing zinc deficiency and ultimately result in aphthous ulcers in the mouth **(9)**. Furthermore, it has been emphasised that DFP, one of the oral chelators, causes oral aphthous lesions by causing neutropenia and affecting the immune system **(5)**.

ORAL AND DENTAL HEALTH PROBLEMS

In patients with TBT, decreased haemoglobin production due to a deficiency in haemoglobin beta chain synthesis is a defect that leads to increased ferritin levels, excessive haemolysis and iron overload **(10, 11)**. As a consequence, hyperplasia and maxillofacial enlargement occur, which could result in maxillofacial deformities **(12, 13)**. The major facial features seen in thalassaemia patients include prominent cheekbones and widening of the upper jaw due to erythroid hyperplasia with collapse of

the nasal bridge. Such modifications result in a characteristic facial appearance known as the "chipmunk or rodent face" **(14)**. Other orofacial anomalies consist of maxilla protrusion and malocclusions of varied degrees **(15)**, maxillary incisors protrusion (overjet) and enlarged spacing (diastema), abnormal anterior open bite, occlusal anomalies and saddle nose deformity, and delayed pneumatisation of the maxillary antrum **(16)**. Secondary effects of thalassaemia include dental caries, pale-coloured gingiva, burning sensation of the tongue, painful swelling of the salivary glands and dry mouth, and decreased IgA levels resulting in reduced salivary protection **(17)**. Furthermore, thalassaemia patients may have teeth that are discoloured and have short crowns and roots **(18)**. Facial features and oral symptoms of thalassaemia patients are shown in Table 1 **(12)**.

Table 1: *Facial features and oral symptoms of Thalassaemia patients* **(12)**

	Features	Cause
Facial features	Class II malocclusion	Maxillary protrusion, mandibular atrophy
	Maxillary protrusion **(19, 20)**	Early fusion of occipital sutures, hyperplasia of anterior maxillofacial structures
	Lateral displacement of orbits **(21)**	Marrow overgrowth in maxillary bone
	Chipmunk facies **(22, 23)**	Malar prominence, saddle nose, frontal bossing
	Brodie syndrome **(24)**	Mandibular arch is telescoped within the maxillary arch
	Pneumatisation of paranasal sinuses	Hyperplasia of marrow in frontal, temporal and facial bones **(20, 21)**
	Malocclusion	Maxillary protrusion, increased overjet, anterior open bite
	High caries index **(20)**	Poor oral hygiene, less phosphorous and Ig A in saliva
Oral manifestations	Mucosal pallor, atrophic glossitis	Decreased haemoglobin levels
	Severe gingivitis	If splenectomy done **(22)**
	Inflammation of salivary glands	Iron deposits
	Dark coloured gingiva	High ferritin levels in blood
	Thin mandibular cortex (**20)**	Marrow proliferation, expansion of medulla
	Multiple diastemas	
	Roots-short and spike shaped, taurodontism **(20)**	

In radiographs of the jaw and skull in thalassaemia patients, a decrease in overall bone density resulting from bone marrow enlargement, absence of the inferior alveolar canal, small maxillary sinuses, thin lamina dura, decreased thickness of the inferior mandibular cortex, and a prominent antegonial notch can be seen **(25, 26)**.

Oral and dental health issues, such as dental caries and periodontal disease, are prevalent in thalassaemia patients as well as maxillofacial deformities. According to studies, the prevalence of dental caries and periodontal disease is higher in thalassemia patients than in the control group **(18, 27)**. The increased incidence of dental caries in thalassaemia patients may be due to the chronic nature of the disease, which leads patients to neglect primary and preventive dental care because they are overwhelmed by the life-threatening nature of the disease. Moreover, insufficient knowledge about oral health, inappropriate nutrition, and malocclusion may also contribute to the increase in dental caries **(28)**. Thalassaemia patients experience morphological changes in their teeth. These changes notably involve a reduction in tooth dimensions, such as the mesio-distal width, and an increase in the number of tooth pits and fissures. Furthermore, pits and fissures result in higher chances of dental caries due to endocrine dysfunction and inadequate oral hygiene **(29, 30)**.

Iron overload due to frequent blood transfusions causes oxidative stress, which is the most common problem in thalassaemia patients **(31)**. The increased total protein in saliva may be considered a risk factor for dental caries because of its effect on salivary flow rate and buffering capacity **(32)**. The main components of this system are various lactoperoxidase and myeloperoxidase compounds secreted by salivary glands and polymorphonuclear neutrophils, respectively. One of the primary functions of salivary peroxidases is to regulate the oral bacteria responsible for caries **(33)**. Additionally, enhancing the protein concentration of saliva has a direct effect on the viscosity of saliva, which may play a role in dental caries **(34)**.

Iron is a critical nutrient necessary for the growth of microorganisms and linked to biofilm development in bacterial species. Streptococcus mutans proliferation is increased by oxidative stress due to iron accumulation from repeated blood transfusions **(35)**. Additionally, the increased oxidative stress during growth and development in the oral cavity impacts tooth structure **(36)**. These factors increase the susceptibility to dental caries in patients with thalassaemia.

Some studies have indicated that low salivary IgA levels in thalassaemia patients may cause more caries than in healthy individuals **(37, 38)**. In their study, Siamopoulou et al. **(37)** showed that the decrease in salivary IgA in Thalassaemia patients and the associated increase in oral bacteria led to an increase in the tooth and surface level index (DMFT= total number of decayed (D), missing (M) or filled (F) teeth or surfaces in the permanent dentition). Other studies have indicated that endocrine dysfunction in thalassaemia patients is the main reason for the high prevalence of dental caries in these patients **(29)**. Luglie et al. **(39)** demonstrated in a study that decreased salivary urea was effective in increasing caries prevalence in thalassaemia patients. Kalman et al. **(38)** claimed that salivary sodium, potassium and calcium levels were significantly decreased in thalassaemia major patients. Figure 1 shows the reasons for the prevalence of dental caries in thalassaemia patients.

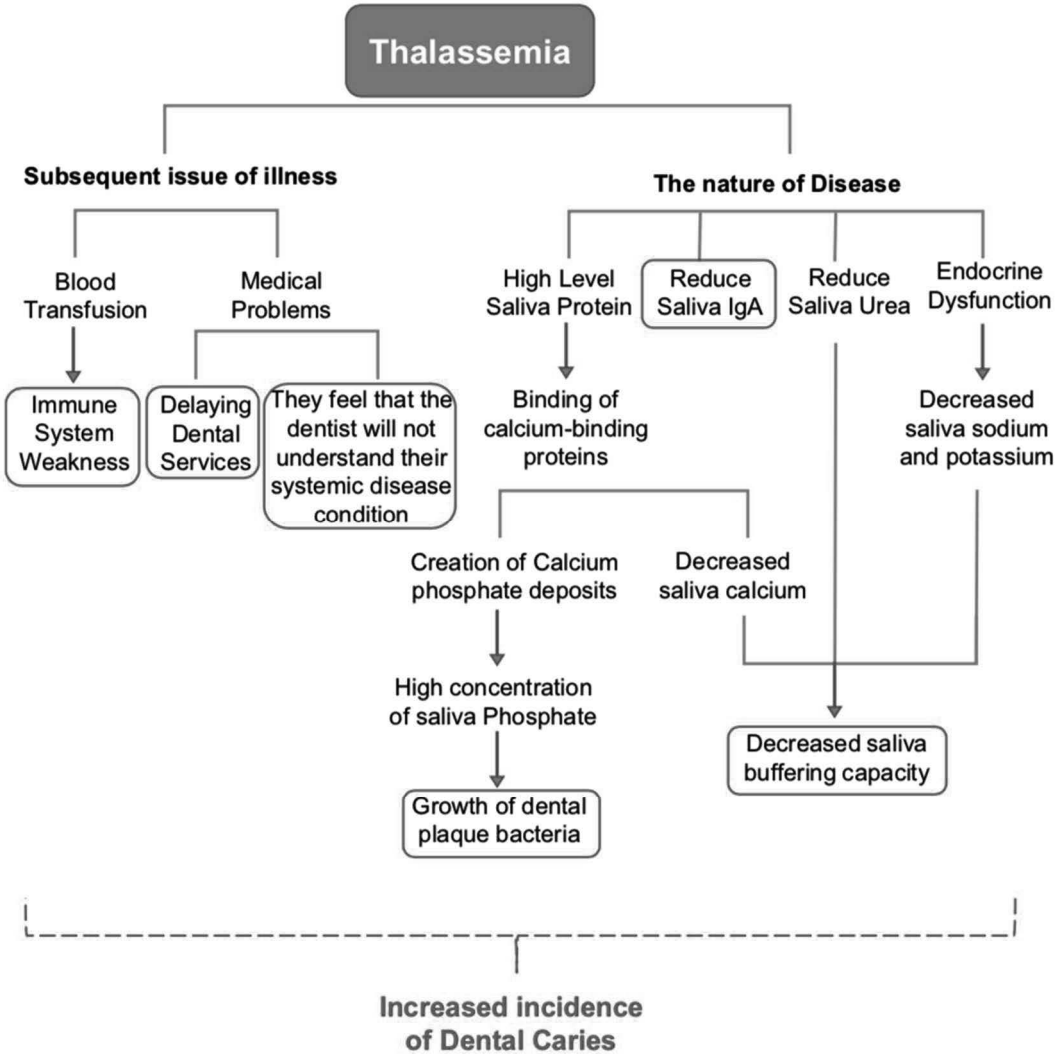


Figure 1: *The reasons for the prevalence of dental caries in Thalassaemia patients (40)*

Free radicals may impair the function of the salivary glands, leading to a reduction in saliva production. This, in itself, may alter the antimicrobial components of saliva, including lysozyme and IgA. Patients with thalassaemia major have lower levels of lysozyme than normal people. Salivary lysozyme plays a crucial role in degrading bacterial cells through its interaction with chaotropic ions (thiocyanate, perchlorate, iodide, bromide, nitrate, chloride, and fluoride) and bicarbonate **(41)**. Due to changes in salivary components, it affects the function of saliva in maintaining the balance of oral flora, including bacteria that automatically cause gingivitis. The increased virulence of Porphyromonas Gingivalis, one of the main subgingival bacteria, plays an important role in the progression of periodontal disease. Porphyromonas gingivalis requires iron, one of the essential nutrients, to survive in the periodontal tissues. The iron used by this pathogen plays a significant role in both its growth and virulence **(42)**.

Literature studies have emphasised that the incidence of gingivitis may be higher in patients with Thalassaemia major than in healthy individuals, and it has been suggested that this may be due to local factors or oral-maxillo-facial features of the disease **(27, 37, 39)**. Ay et al. **(43)** compared blood lipid levels and periodontal parameters (gingival index, plaque index, bleeding on probing, probing depth) of thalassaemia major patients with controls and found that all periodontal parameters were significantly higher in thalassaemia major patients.

In patients with thalassaemia, painful swelling of the salivary glands and dry mouth may be observed,

which leads to decreased salivary protection **(39)**. In a study involving sixty-five thalassaemia patients, dry mouth was found in 74%, burning in the oral mucosa in 79%, numbness in the oral mucosa in 34.6%, atrophic glossitis in 37%, recurrent aphthous ulcer in 18.5% and taste impairment in 24.7%. In addition, oral lichen planus and varices were found in the same study **(44)**.

Dermatological symptoms in thalassaemia patients include dark skin colour and gingival hyperpigmentation due to iron overload **(2)**. A direct correlation between skin colour and body iron levels in thalassaemia patients has been observed **(45)**. Hyperpigmentation of the gingiva is caused by the excessive accumulation of melanin in the basal and suprabasal layers of the epithelium **(46)**. Studies have demonstrated that elevated ferritin levels in the blood is the reason for the darkening of gingival colour **(47)**. A further study substantiated a significant correlation between serum ferritin level, gingival hyperpigmentation, and skin colour in individuals with thalassaemia **(48)**. Moreover, within thalassaemia patients, the gingiva and the inside of the mouth become pale due to anaemia, and pain and burning in the tongue may be observed due to folate deficiency **(39)**.

TREATMENT AND RECOMMENDATIONS

Oral and dental health issues typically become critical among thalassaemia patients due to the delay in dental examinations and early diagnosis **(12)**. Thalassaemia not only affects the child, but also creates a psychosocial situation for the family and requires support. This situation may lead to dental care and treatment procedures of thalassaemia patients being overlooked or ignored. Consequently, thalassaemia patients are more likely to lose teeth **(39)**.

The progression of dental caries and failure to carry out restorative dental treatment in time leads to abscess formation and the risk of infection spreading to the neck and facial tissues. At this stage, it is more likely that the tooth will be extracted rather than restored. In these patients, early detection of oral problems allows for easier and more cost-effective treatment. Moreover, the frequency of dental visits should be increased for these patients **(40)**.

A conservative treatment approach is generally preferred for patients with thalassaemia. Different orthodontic appliances (fixed, removable, functional, extraoral) are recommended for the correction of various orthodontic dentofacial deformities and malocclusions **(49)**. For these patients, a protective and preventive orthodontic approach is required to decrease the chance of trauma and improve stomatognathic function and facial appearance **(50)**. Effective orthodontic treatment interventions typically involve using high-pull headgear and functional appliances to restrict maxillary sagittal and vertical growth while increasing mandibular growth. Due to regular blood transfusions in these patients, it is advisable to avoid orthognathic surgeries as much as possible **(49)**. Therefore, early and preventive interventions, as well as growth modifications, are generally preferred treatment options **(49, 50)**.

Any invasive procedure in thalassaemia patients should be performed after antibiotic treatment and immediately after a blood transfusion. Before dental treatment, liver function and coagulation tests should be carried out and the patient's haematologist should be consulted. For less severe forms of thalassaemia, orofacial defects and malocclusions may be treated surgically, followed by orthodontic treatment to align the teeth. Mild types of malocclusions can be corrected through orthodontic treatment during childhood. It is advised to exercise caution when prescribing drugs that may cause liver damage. It is recommended to avoid prescribing tetracycline, metronidazole and erythromycin estolate. Paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin are safe alternatives. Sedation or anaesthesia can be utilised to enhance control and cooperation during dental treatment of thalassaemia patients. When general anaesthesia is necessary, it is essential to consult the anaesthesia and haematology departments. In thalassaemia patients who receive continuous blood transfusions, possible hepatitis B or C or HIV carriage should be considered. Caution should be taken in patients with thalassaemia due to potential complications relating to impaired immunity, hepatic function, splenectomy and cardiovascular problems. Those who have undergone splenectomy must receive prophylactic antibiotics. It is advisable to adopt a multidiscipli-

nary approach involving a dentist, haematologist and orthodontist **(12)**.

In conclusion, oro-dento-facial problems can be prevented before they occur if thalassaemia patients are under the control of a dentist from childhood for diagnosis, treatment and follow-up. As preventive measures are an integral part of the management of dental problems, it is important that dentists have the necessary knowledge and equipment about the disease, and that clinicians do not ignore the oral and dental health problems of thalassaemia patients. Dentists should provide information and education programmes to thalassaemia patients and their families about the problems to consider and the solutions available.

REFERENCES

1. Weatherall DJ. The Thalassemia syndromes (4th edition). BlackwellScientific Publications Oxford. 2001;597-629.
2. Cao A, Galanello R. Beta-thalassemia. Genet Med. 2010;12(2):61-76.
3. Origa R. Beta-Thalassemia. Genet Med. 2017;19(6):609-19.
4. Piel FB, Weatherall DJ. The Alpha-Thalassemias. N Engl J Med. 2014;371(20):1908-16.
5. Bozkurt Y. Talasemide ağız ve diş sağlığı. Talasemi ve hemoglobinopatiler Antalya Retma Matbaası. 2007:223-6.
6. Canatan D, Akdeniz SK. Iron and ferritin levels in saliva of patients with Thalassemia and iron deficiency anemia. Mediterr J Hematol Infect Dis. 2012;4(1):e2012051.
7. Caliskan U, Tonguc MO, Ciris M, et al. The investigation of gingival iron accumulation in Thalassemia major patients. J Pediatr Hematol Oncol. 2011;33(2):98-102.
8. Akbarnejad AA, Mahjoub S, Tamaddoni A, et al. Salivary Oxidative Stress, Total Protein, Iron and pH in Children with Beta-Thalassemia Major and their Correlation with Dental Caries. J Dent (Shiraz). 2022;23(3):266-71.
9. Erdogan E, Canatan D, Ormeci AR, et al. The effects of chelators on zinc levels in patients with Thalassemia major. J Trace Elem Med Biol. 2013;27(2):109-11.
10. Rechavi G, Rivella S. Regulation of iron absorption in hemoglobinopathies. Curr Mol Med. 2008;8(7):646-62.
11. Nienhuis AW, Nathan DG. Pathophysiology and Clinical Manifestations of the beta-Thalassemias. Cold Spring Harb Perspect Med. 2012;2(12):a011726.
12. Helmi N, Bashir M, Shireen A, et al. Thalassemia review: features, dental considerations and management. Electron Physician. 2017;9(3):4003-8.
13. Amirabadi F, Saravani S, Miri-Aliabad G. et al. The Association between Dental Health Status and Oral Health-Related Quality of Life of Children Diagnosed with Beta-Thalassemia Major in Zahedan City, Iran. . Int J Pediatr. 2019;7(2):8985-91.
14. Elangovan A, Mungara J, Joseph E, et al. Prevalence of dentofacial abnormalities in children and adolescents with Beta-Thalassaemia major. Indian J Dent Res. 2013;24(4):406-10.
15. Hattab FN. Patterns of physical growth and dental development in Jordanian children and adolescents with Thalassemia major. J Oral Sci. 2013;55(1):71-7.
16. Pirinccioglu AG, Akpolat V, Koksal O, et al. Bone mineral density in children with Beta-Thalassemia major in Diyarbakir. Bone. 2011;49(4):819-23.
17. Aldallal S, Alkathemi M. Orodental Considerations in Thalassemia Patients. J of Haematol and Blood Disord 2016;2(2):205.
18. Hattab FN. Mesiodistal crown diameters and tooth size discrepancy of permanent dentition in Thalassemic patients. J Clin Exp Dent. 2013;5(5):e239-44.
19. Cutando Soriano A, Gil Montoya JA, Lopez-Gonzalez Garrido Jde D. Thalassemias and their dental implications. Med Oral. 2002;7(1):36-40, 1-5.
20. Madhok S, Madhok S. Dental considerations in Thalassemic patients. IOSR-JDMS. 2014;13:57-62.
21. Trent RJ. Diagnosis of the haemoglobinopathies. Clin Biochem Rev. 2006;27(1):27-38.
22. Tunaci M, Tunaci A, Engin G, et al. Imaging features of Thalassemia. Eur Radiol. 1999;9(9):1804-9.
23. Kharsa MA. Orthodontic Characteristics of Thalassemia Patients. Orthod Cyber Journal. 2008.
24. Adeyemo TA, Adeyemo WL, Adediran A, et al. Orofacial manifestations of hematological disorders: anemia and hemostatic disorders. Indian J Dent Res. 2011;22(3):454-61.
25. Hazza'a AM, Al-Jamal G. Radiographic features of the jaws and teeth in Thalassaemia major. Dentomaxillofac Radiol. 2006;35(4):283-8.
26. Bayati S, Keikhaei B, Bahadoram M, et al. Radiographic Features of the Maxillofacial Anomalies in Beta-Thalassemia Major: With New View. World J Plast Surg. 2021;10(3):78-83.
27. Hattab FN, Hazza'a AM, Yassin OM, et al. Caries risk in patients with Thalassaemia major. Int Dent J. 2001;51(1):35-8.
28. Olivieri NF. The Beta-Thalassemias. N Engl J Med. 1999;341(2):99-109.

29. Al-Wahadni AM, Taani DQ, Al-Omari MO. Dental diseases in subjects with Beta-Thalassemia major. Community Dent Oral Epidemiol. 2002;30(6):418-22.
30. Hattab FN. Thalassemia major and related maxillofacial complications: clinical and radiographic overview with reference to dental care. Int J Exp Dent Sci. 2017;6:1-10.
31. Kohgo Y, Ikuta K, Ohtake T, et al. Body iron metabolism and pathophysiology of iron overload. Int J Hematol. 2008;88(1):7-15.
32. Dewi SR, Septhimoranie S, Muchzal S. Correlation of saliva characteristics and caries in Beta-Thalassemia major patients. . Majalah Kedokteran Gigi Indones. 2020;6:100-5.
33. Preethi BP, Reshma D, Anand P. Evaluation of Flow Rate, pH, Buffering Capacity, Calcium, Total Proteins and Total Antioxidant Capacity Levels of Saliva in Caries Free and Caries Active Children: An In Vivo Study. Indian J Clin Biochem. 2010;25(4):425-8.
34. Zussman E, Yarin AL, Nagler RM. Age- and flow-dependency of salivary viscoelasticity. J Dent Res. 2007;86(3):281-5.
35. Oh E, Andrews KJ, Jeon B. Enhanced Biofilm Formation by Ferrous and Ferric Iron Through Oxidative Stress in Campylobacter jejuni. Front Microbiol. 2018;9:1204.
36. Kasai S, Mimura J, Ozaki T, et al. Emerging Regulatory Role of Nrf2 in Iron, Heme, and Hemoglobin Metabolism in Physiology and Disease. Front Vet Sci. 2018;5:242.
37. Siamopoulou-Mavridou A, Mavridis A, Galanakis E, et al. Flow rate and chemistry of parotid saliva related to dental caries and gingivitis in patients with Thalassaemia major. Int J Paediatr Dent. 1992;2(2):93-7.
38. Kalman S, Atay AA, Sakallioglu O, et al. Renal tubular function in children with Beta-Thalassemia minor. Nephrology (Carlton). 2005;10(5):427-9.
39. Luglie PF, Campus G, Deiola C, et al. Oral condition, chemistry of saliva, and salivary levels of Streptococcus mutans in Thalassemic patients. Clin Oral Investig. 2002;6(4):223-6.
40. Moosazadeh M, Gorji NE, Nasiri P, et al. Comparison of decayed, missing, filled teeth index between Thalassemia major patients and control group in Iran: a systematic review and meta-analysis. BDJ Open. 2020;6(1):23.
41. Nuraini P, Wahluyo S, Pradopo S. et al. Effects of Iron Accumulation on Dental Caries, Gingivitis, and Candida albicans Infection in Children with Beta Thalassemia Major: A Narrative Review. Acta Med Philipp [Internet]. 2023;57(3).
42. Lewis JP. Metal uptake in host-pathogen interactions: role of iron in Porphyromonas gingivalis interactions with host organisms. Periodontol 2000. 2010;52(1):94-116.
43. Ay ZY, Orucoglu A, Kilinc G, et al. Does the periodontal health of Thalassemia major patients have an impact on the blood lipid profiles? A preliminary report. J Pediatr Hematol Oncol. 2007;29(10):694-9.
44. Hamid RN, Hamid S, jabber W. Oral Manifestations for Thalassemia Patients at Thalassemia Center Located at Ramadi Women and Children Hospital. Systematic Reviews in Pharmacy. 2020;11(3).
45. Bucak IH, Almis H, Benli S, et al. The Assessment of Skin Color and Iron Levels in Pediatric Patients with Beta-Thalassemia Major Using a Visual Skin Color Chart. Hemoglobin. 2017;41(2):120-3.
46. Patil KP, Joshi V, Waghmode V. et al. Gingival depigmentation: A split mouth comparative study between scalpel and cryosurgery. Contemp Clin Dent. 2015;6(Suppl 1):S97-S101.
47. Gimenez Garcia RM, Carrasco Molina S. Drug-Induced Hyperpigmentation: Review and Case Series. J Am Board Fam Med. 2019;32(4):628-38.
48. Gajjar S, Kaur H, Girdhar G, et al. Identifying Gingival Pigmentation Patterns and Skin Color and Its Co-relation With Serum Ferritin Levels in Thalassemic Patients. Cureus. 2022;14(8):e28015.
49. Einy S, Hazan-Molina H, Ben-Barak A, et al. Orthodontic Consideration in Patients with Beta-Thalassemia Major: Case Report and Literature Review. J Clin Pediatr Dent. 2016;40(3):241-6.
50. Cappellini MD, Cohen A, Porter J, et al. Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT). 3rd ed. Nicosia (CY)2014.

Correspondence Addreess:
Specialist Dr. Dt.Simge Canatan Keskin
Dental Clinic of Bayindir Hospital, Kavaklıdere, Ankara, Türkiye
E-mail: smgcntn@gmail.com
Tel: +905384232972

SPLENECTOMY IN THALASSAEMIA PATIENTS

Specialist Dr. Yiğithan Yasin Keskin[1], Assoc. Prof. Dr. Mehmet Ali Koç
1. Ministry of Health, Elbistan State Hospital, General Surgery Clinic, Kahramanmaraş-Türkiye
2. Ankara University Medical Faculty, General Surgery Clinic, Ankara-Türkiye

ABSTRACT

The main defect in β-thalassaemia is the diminished or lacking synthesis of β-globin chains and a surplus of α-globin chains, which results in an imbalanced globin chain ratio. Consequently, it leads to inefficient erythropoiesis and splenomegaly due to widespread, premature destruction of red cell precursors in the bone marrow and extramedullary regions, particularly in the spleen.

The primary indication for splenectomy in thalassemia patients dependent on transfusions is hypersplenism. The objective of splenectomy in these patients is to decrease iron overload by reducing transfusion requirements and blood consumption. Although splenectomy may decrease the necessity for transfusions in thalassemia patients, it may also result in severe negative consequences with minimal benefit.

Complications stemming from splenectomy include perioperative complications like haemorrhage, atelectasis, and subphrenic abscess/hematoma and pulmonary hypertension, as well as postoperative complications such as thromboembolism, sepsis and mortality. Splenectomy can impede immune function in the long term, heightening susceptibility to encapsulated microorganisms like Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, gram-negative bacilli (including Escherichia coli, Klebsiella, and Pseudomonas aeruginosa) and parasites such as malaria.

Current studies suggest that regular transfusion therapy combined with targeted iron chelation may help to reduce the need for splenectomy as well as the complications of thalassaemia. Due to the lack of sufficient good quality evidence from randomised controlled trials, splenectomy has no clear implications in terms of the benefit-harm balance in patients with thalassaemia. On the other hand, splenectomy may still be a good treatment option in selected patients.

Keywords: Thalassaemia, splenectomy, complication

INTRODUCTION

The primary abnormality in β-thalassaemia is a decreased or completely absent synthesis of β-globin chains and an excessive production of α-globin chains, causing an imbalance in globin chains. Consequently, there is inefficiency in erythropoiesis and splenomegaly due to the destruction of immature red blood cell precursors in both the bone marrow and other sites, especially the spleen **(1)**.

An enlarged spleen may reduce the quantity of red blood cells, platelets, and white blood cells in the bloodstream. Additionally, it can cause abdominal pain or lead to various disorders. Splenectomy may be especially beneficial in the treatment of patients with Transfusion Dependent Thalassaemia (TDT) **(2, 3)**.

The main indicator of the need for splenectomy in TDT patients is hypersplenism. Indications for hypersplenism include an increase in red blood cell transfusion requirement exceeding 200-220 mL/kg per year, symptomatic splenomegaly and severe pancytopenia **(1, 4)**. The primary objective of performing splenectomy on patients with TDT is to diminish iron overload, thereby reducing transfusion requirements and blood consumption **(5)**. By ensuring appropriate levels of haemoglobin are maintained before transfusion and adequate inter-

vals are given between transfusions, the chances of splenectomy within the first decade of life for individuals with thalassaemia has decreased significantly from 57% during the 1960s to merely 7% by the 1990s **(6)**. Splenectomy ought to be avoided, particularly in children under the age of 5, as there is a likelihood of post-splenectomy sepsis occurring **(7)**.

Splenectomy is a surgical procedure that generally requires the complete removal of the spleen **(8)**. In addition, certain centres have carried out partial splenectomy as a means to alleviate hypersplenism while maintaining the immune function of the spleen **(9-11)**. Long-term outcomes of partial splenectomy remain unknown. Like all abdominal procedures, laparoscopy is associated with less intraoperative blood loss, perioperative morbidity, and mortality compared to open surgery. Moreover, laparoscopic splenectomy offers shorter hospital stays and superior cosmetic results, which are additional benefits **(12-14)**.

INDICATIONS FOR SPLENECTOMY

Splenectomy is indispensable in thalassaemia patients when the ailment is not sufficiently relieved by blood transfusion **(15)**. The purpose of splenectomy is not solely to decrease the requirement for blood transfusions, but also to relieve symptomatic cytopenia or symptomatic splenomegaly (left upper quadrant pain or risk of rupture) and to prevent transfusion-induced iron overload **(1, 5)**. On the contrary, it is recognised that the spleen serves as a storage site for iron and has a diminishing impact on unbound iron in the bloodstream **(16)**.

Studies have shown that there is a notable decrease in the need for transfusion following splenectomy **(17)**, also showed a reduction in iron overload and serum ferritin levels after splenectomy **(18)**. However, after splenectomy, a significant increase in platelet count was observed from 24 hours postoperatively **(17)**.

The tendency to perform splenectomy in thalassaemia patients has decreased in recent years due to concerns about its efficacy and possible long-term side effects. Observational studies confirm that the risk of venous thromboembolism, pulmonary hypertension, leg ulcers and silent cerebral infarction is higher in splenectomised Non-Transfusion Dependent Thalassemia patients than in non-splenectomised patients **(19-22)**. It has been reported that the prevalence of pulmonary hypertension in patients with thalassaemia who have undergone splenectomy can be as high as 59-75% **(23, 24)**. Changes in some haematological parameters, such as thrombocytosis and an increase in nucleated red blood cells after splenectomy, may be related to these complications **(2, 3)**. A hypercoagulable state has been described in non-transfusion dependent thalassaemia patients due to the presence of activated platelets and abnormal red blood cells in the pre-splenectomy period **(25)**. After splenectomy, these abnormalities become more pronounced because of the loss of the spleen's beneficial role in removing these procoagulant platelets and red blood cells **(25)**.

Splenectomy is also a risk factor for serious post-splenectomy infections associated with encapsulated microorganisms such as Streptococcus pneumoniae, Haemophilus influenzae and Neisseria meningitidis, and other infectious diseases such as malaria **(15, 26)**.

In conclusion, splenectomy can reduce the need for transfusions in patients with thalassaemia, but it can also lead to serious adverse outcomes. There are no guidelines with strong evidence to support the relative effectiveness, safety and risks of splenectomy compared with other available treatment options in patients with thalassaemia **(18)**.

PRE-OPERATIVE ASSESSMENT AND FOLLOW-UP AFTER SPLEENECTOMY

Vaccination against the above encapsulated microorganisms should be given at least 2 weeks before splenectomy. If this could not be done earlier, vaccination can be given at least 2 weeks after surgery. Influenza vaccination is also recommended **(7)**.

Blood parameters (biochemistry, complete blood count, bleeding profile) are analysed prior to elective surgery and preoperative replacements may be performed if necessary. If thrombocytopenia is present, intravenous steroid or IVIG administration may also be required **(27)**.

The role of radiological imaging in preoperative preparation is questionable **(27)**. However, although ultrasound is the primary non-invasive examination, computed tomography is recommended, especially in patients with suspected accessory spleen or severe splenomegaly **(28)**. If cholelithiasis is present after these imaging studies, simultaneous cholecystectomy may be planned.

The operation may vary depending on the general condition of the patient, the size of the spleen and the preference of the surgical team. Spleen with a long axis >25 cm or spleen crossing the midline or entering the pelvis is not suitable for laparoscopic approach **(29)**. Open splenectomy is usually performed through a supraumbilical midline or left subcostal incision in the supine position. For laparoscopic splenectomy, the patient is placed in the right semi-lateral position, supported by a pillow on the back to form a 45-degree angle with the operating table **(28)**. Generally, one camera port and two working ports are used. Although open splenectomy is more advantageous in terms of operation time and ease of surgical technique, laparoscopic splenectomy is more advantageous in terms of hospital stay, postoperative pain, early onset of gastrointestinal motility and cosmetic appearance.

As thalassaemia patients are prone to thrombosis before and after splenectomy, venous thromboembolism prophylaxis should be given **(27)**.

In the postoperative period, appropriate pain palliation is performed in addition to close vital and physical examination and drain follow-up, if any. The patient's oral intake should be opened as early as possible postoperatively, but paralysis of the gastrointestinal system, which can occur after most open abdominal surgeries, especially after open splenectomy, should not be ignored.

Alternative approaches include partial surgical splenectomy **(30)** and splenectomy by embolisation **(31)**. Laparoscopic splenectomy appears to be associated with more complications due to the technical difficulty of the procedure. The surgeon's experience when using laparoscopic techniques and comorbidities such as cholecystitis may influence individual outcomes **(14, 32, 33)**.

COMPLICATIONS OF SPLENECTOMY

Perioperative complications such as haemorrhage, atelectasis, subphrenic abscess/hematoma and pulmonary hypertension, postoperative complications such as thromboembolism, sepsis and mortality can be seen in patients undergoing splenectomy.

As mentioned above, splenectomy can reduce immune function and increase the risk of infection, especially with encapsulated microorganisms such as Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, gram-negative bacilli such as Escherichia coli, Klebsiella and Pseudomonas aeruginosa, or parasites such as malaria. This requires immunoprophylaxis with vaccines and/or long-term chemoprophylaxis with antibiotics **(34)**. Pre-operative vaccination is known to reduce post-splenectomy infections **(35)**.

Thromboembolism, like infection, is a major complication after splenectomy and requires prompt diagnosis, effective treatment and good follow-up. One study investigated the effects of thrombocytosis and natural inhibitors on thrombosis after splenectomy and found that 7 (23.3%) of 30 patients undergoing splenectomy had thrombosis in the portal vein system detected by doppler ultrasound **(36)**. As in this study, portal vein thrombosis after splenectomy has been shown in several studies **(37, 38)**. In addition, another study that followed about 8000 patients for more than 25 years after splenectomy showed that the risk of deep vein thrombosis and pulmonary thromboembolism increased more than twofold **(39)**.

There is increasing evidence that thalassaemia-related complications such as pulmonary hypertension and cardiac dysfunction do not resolve after splenectomy **(30, 40)**.

The argument that regular transfusion therapy combined with an effective chelation regimen can modify the disease process and prevent the development of various Thalassaemia complications that previously required splenectomy is gaining strength. Over the years, increasing awareness and knowledge of the adverse effects of splenectomy has led to a gradual decrease in the tendency to

perform this procedure in Thalassaemia patients. This is supported by data from Italian registries. According to these data, the likelihood of undergoing splenectomy in the first decade of life has decreased significantly over the last 40 years **(6)**.

CONCLUSION

Current evidence based on observational studies suggests that regular transfusion therapy combined with targeted iron chelation may help reduce the need for splenectomy as well as the complications of thalassaemia. Individualisation and intensification of iron chelation therapy are alternative approaches to splenectomy to prevent iron overload and transfusion-related complications. Due to the lack of sufficient good quality evidence from randomised controlled trials, splenectomy has no clear implications in terms of the benefit-harm balance in patients with thalassaemia. On the other hand, it should be kept in mind that splenectomy may still be a good treatment option in selected patients.

REFERENCES

1. Cappellini MD, Cohen A, Porter J, et al. Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT). 3rd Edition. Nicosia, Cyprus. Thalassaemia International Federation. 2014.
2. Taher AT, Musallam, K, Karimi M, et al. Splenectomy and thrombosis: the case of thalassemia intermedia. J Thromb Haemost. 2010;8(10):2152-8.
3. Casale M, Cinque P, Ricchi P, et al. Effect of splenectomy on iron balance in patients with beta-thalassemia major: a long-term follow-up. Eur J Haematol. 2013;91(1):69-73.
4. Machado NO, Grant CS, Alkindi S, et al. Splenectomy for haematological disorders: a single center study in 150 patients from Oman. Int J Surg. 2009;7(5):476-81.
5. Rachmilewitz EA, Giardina PJ. How I treat thalassemia. Blood. 2011;118(13):3479-88.
6. Piga A, Serra M, Longo F, et al. Changing patterns of splenectomy in transfusion-dependent thalassemia patients. Am J Hematol. 2011;86(9):808-10.
7. Farmakis D, Porter J, Taher A, et al. 2021 Thalassaemia International Federation Guidelines for the Management of Transfusion-dependent Thalassemia. Hemasphere. 2022;6(8):e732.
8. Townsend JCM, Beauchamp RD, Evers BM, et al. Sabiston Textbook of Surgery New Delhi, India: Elsevier. 2004;2:17th Edition.
9. Sheikha AK, Salih ZT, Kasnazan KH, et al. Prevention of overwhelming postsplenectomy infection in thalassemia patients by partial rather than total splenectomy. Can J Surg. 2007;50(5):382-6.
10. Al-Salem AH. Splenectomy for Children With Thalassemia: Total or Partial Splenectomy, Open or Laparoscopic Splenectomy. J Pediatr Hematol Oncol. 2016;38(1):1-4.
11. De Montalembert M, Girot R, Revillon Y, et al. Partial splenectomy in homozygous beta thalassaemia. Arch Dis Child. 1990;65(3):304-7.
12. Canda AE, Sucullu I, Ozsoy Y, et al. Hospital experience, body image, and cosmesis after laparoscopic or open splenectomy. Surg Laparosc Endosc Percutan Tech. 2009;19(6):479-83.
13. Winslow ER, Brunt LM. Perioperative outcomes of laparoscopic versus open splenectomy: a meta-analysis with an emphasis on complications. Surgery. 2003;134(4):647-53; discussion 54-5.
14. Konstadoulakis MM, Lagoudianakis E, Antonakis PT, et al. Laparoscopic versus open splenectomy in patients with beta thalassemia major. J Laparoendosc Adv Surg Tech A. 2006;16(1):5-8.
15. Boone KE, Watters DA. The incidence of malaria after splenectomy in Papua New Guinea. BMJ. 1995;311(7015):1273.
16. Taher A, Musallam KM, El Rassi F, et al. Levels of non-transferrin-bound iron as an index of iron overload in patients with thalassaemia intermedia. Br J Haematol. 2009;146(5):569-72.
17. Merchant RH, Shah AR, Ahmad J, et al. Post Splenectomy Outcome in beta-Thalassemia. Indian J Pediatr. 2015;82(12):1097-100.
18. Taher AT, Musallam KM, Cappellini MD, et al. Optimal management of beta thalassaemia intermedia. Br J Haematol. 2011;152(5):512-23.
19. Vichinsky E. Advances in the treatment of alpha-thalassemia. Blood Rev. 2012;26 Suppl 1:S31-4.
20. Musallam KM, Taher AT. Thrombosis in thalassemia: why are we so concerned? Hemoglobin. 2011;35(5-6):503-10.
21. Crary SE, Buchanan GR. Vascular complications after splenectomy for hematologic disorders. Blood. 2009;114(14):2861-8.
22. Taher AT, Musallam KM, Nasreddine W, et al. Covert brain ischaemia in splenectomised adults with thalassemia intermedia: An emerging entity. Thromb Haemost. 2010;104(3):652-3.
23. Phrommintikul A, Sukonthasarn A, Kanjanavanit R, et al. Splenectomy: a strong risk factor for pulmonary hypertension in patients with thalassaemia. Heart. 2006;92(10):1467-72.

24. Du ZD, Roguin N, Milgram E, et al. Pulmonary hypertension in patients with thalassemia major. Am Heart J. 1997;134(3):532-7.
25. Taher AT, Viprakasit V, Musallam KM, et al. Treating iron overload in patients with non-transfusion-dependent thalassemia. Am J Hematol. 2013;88(5):409-15.
26. Cadili A, de Gara C. Complications of splenectomy. Am J Med. 2008;121(5):371-5.
27. Bona R. Elective (diagnostic or therapeutic) splenectomy. medi media. Sep 2022.
28. Fisichella PM, Wong YM, Pappas SG, et al. Laparoscopic splenectomy: perioperative management, surgical technique, and results. J Gastrointest Surg. 2014;18(2):404-10.
29. Rosen M, Brody F, Walsh RM, et al. Hand-assisted laparoscopic splenectomy vs conventional laparoscopic splenectomy in cases of splenomegaly. Arch Surg. 2002;137(12):1348-52.
30. Morsy MF, Alnajjar AA, Almuzainy IS, et al. Splenectomized versus non-splenectomized patients with thalassemia major. Echocardiographic comparison. Saudi Med J. 2008;29(9):1310-4.
31. Pringle KC, Spigos DG, Tan WS, et al. Partial splenic embolization in the management of thalassemia major. J Pediatr Surg. 1982;17(6):884-91.
32. Iolascon A, Andolfo I, Barcellini W, et al. Recommendations regarding splenectomy in hereditary hemolytic anemias. Haematologica. 2017;102(8):1304-13.
33. Laopodis V, Kritikos E, Rizzoti L, et al. Laparoscopic splenectomy in beta-thalassemia major patients. Advantages and disadvantages. Surg Endosc. 1998;12(7):944-7.
34. Cappellini MD, Cohen A, Eleftheriou A, et al. Chapter 10: Splenectomy in β-thalassaemia. In: Guidelines for the Clinical Management of Thalassaemia, 2nd Revised edition. Thalassaemia International Federation, (accessed 10 January 2019). 2008.
35. Akhtar IK, Ashraf M, Khalid IU, et al. Surgical outcome of spelenectomy in Thalassemia major in children. Pak J Med Sci. 2016;32(2):305-8.
36. Canatan D, Zorlu M, Bayir N, et al. Thrombosis After Splenectomy in Patients with Thalassemia. Turk J Haematol. 2001;18(4):259-63.
37. Wang M, Zhang M, Li J, et al. Risk factors of portal vein thrombosis in patients with beta thalassemia major after splenectomy: laparoscopic versus open procedure. Hepatogastroenterology. 2014;61(129):48-54.
38. Hassan MN, Tahereb GM, Ahmad T, et al. Correlation of splenectomy with portal vein thrombosis in beta-thalassemia major. J Pak Med Assoc. 2011;61(8):760-2.
39. Kristinsson SY, Gridley G, Hoover RN, et al. Long-term risks after splenectomy among 8,149 cancer-free American veterans: a cohort study with up to 27 years follow-up. Haematologica. 2014;99(2):392-8.
40. Aessopos A, Farmakis D, Deftereos S, et al. Cardiovascular effects of splenomegaly and splenectomy in beta-thalassemia. Ann Hematol. 2005;84(6):353-7.

Correspondance Address:
Yiğithan Yasin Keskin
Ministry of Health, Elbistan State Hospital, General Surgery Clinic Kahramanmaraş-Türkiye
E-Mail: yigithan_keskin@hotmail.com
Tel: +905367655787

THROMBOEMBOLIC PROBLEMS AND TREATMENT IN THALASSEMIA

Melike Sezgin Evim
Adalet Meral Güneş
Uludag University Medical Faculty Dept.of. Pediatric Hematology Bursa, Türkiye

ABSTRACT

As life expectancy increases in patients with thalassemia, complications such as thromboembolism have begun to occur. Its frequency is reported to be between 1.65-3.27% in different series. There are many reasons for its pathogenesis, such as abnormal erythrocytes due to the disease, splenectomy, endothelial damage, platelet activation, and congenital thrombophilia. Its frequency is higher in patients with thalassemia intermedia and splenectomy than in patients with thalassemia major. Venous thromboembolism is more common than arterial ones. Although thrombosis is seen everywhere, cerebrovascular thrombosis is of great importance in its sequelae and vital importance. In addition to stroke, transient ischemic attacks may occur. Asymptomatic or transient ischemic attacks are usually accompanied by impaired cognitive functions, and recurrent silent infarcts may cause permanent neurological damage. Although acetylsalicylic acid is given prophylactically to risk groups, thrombosis is also observed in patients taking acetylsalicylic. A regular transfusion program reduces the frequency of thromboembolism, and transfusions started after thromboembolism also reduce the risk of recurrence of thrombosis. Caution should be exercised in the use of direct oral anticoagulants due to liver toxicity.

Keywords: Thalassemia, thromboembolic problems, treatment

INTRODUCTION

Beta-thalassemia (β-thalassemia) is a congenital hemolytic anaemia and is characterised by a decrease in beta globin chain synthesis (β+) or absence (β0). The disease clinically presents in two forms: thalassemia major and thalassemia intermedia. In recent years, the life expectancy of patients with thalassemia has increased significantly with effective blood transfusion and effective chelation. As the patients get older, various complications occur. One of these complications is thromboembolic events **(1)**. Its incidence is higher, especially in cases with thalassemia intermedia and splenectomy **(2, 3, 4)**. Taher et al. **(5)**, in their study with 8860 thalassemia patients, the incidence was 1.65%. The frequency of venous thromboembolism (57%) was higher than that of arterial thromboembolism (40%). The frequency of both occurring together is 3%. Additionally, in the systemic screening of 11,791 patients, the incidence of cerebral thromboembolic events was 1.13% **(3)**. The Turkish Thalassemia Study Group reported the frequency of thromboembolic events as 3.27% in 519 β-thalassemia patients from 11 centres **(6)**.

PATHOPHSIOLOGY

There are many causes of hypercoagulability in patients with thalassemia, and these are given under the following headings **(7, 8)**.

a) Pathological changes in erythrocytes: Oxidation of alpha globulin chains forms hemichromes, and hemichromes precipitate by binding to erythrocyte membrane proteins. The heme molecule is separated from the precipitated hemichromes, and free iron that is not bound to transferrin is released and has a toxic effect. Free iron molecules oxidise erythrocyte membrane proteins by creating oxygen radicals, forming phosphatidylserine antigens. These antigens cause erythrocytes to become rigid

and deformed, causing them to aggregate. Oxidised erythrocytes also show a procoagulant effect by increasing thrombin generation **(9, 10)**.

In patients with β-thalassemia who receive regular erythrocyte transfusion, the level of deformed erythrocytes decreases, and the incidence of thromboembolism decreases. This explains why the incidence of thromboembolism is high in patients with thalassemia intermedia who are not regularly transfused **(11)**.

b) Platelet Activation: There is chronic platelet activation in patients with thalassemia; Increased platelet aggregation, shortening of platelet lifespan and increase in the number of platelets expressing CD62P (P-selectin) and CD63 cause continuous activation **(1, 12)**. Evidence of this increase is high levels of prostacyclin and thromboxane A2 in urine. The growth of these metabolites in urine was higher in both cases with thalassemia major and intermedia than in healthy subjects. But, no significant difference was found between the two clinical forms **(12)**.

In a study by Çelik Kurt et al. **(13)**, thrombin generation and platelet test tests (PFA-200) before and after transfusion in thalassemia were not different, and the results were known to be similar to the controls. It was concluded that these two tests were insignificant in detecting the risk of thrombosis. In addition, morphological changes in platelets observed in an electronic microscope due to chronic activation were seen in splenectomised patients and those with beta-thalassemia/haemoglobin E disease. This explains this population's high risk of pulmonary embolism **(14)**.

c) Activation of Endothelial Cells, monocytes and peripheral blood cells: In cell cultures, the adhesion of erythrocytes to endothelial cells increases in thalassemia and causes endothelial damage **(15)**. Therefore, markers indicating endothelial damage and activation; levels of endothelial adhesion proteins E-selectin-ELAM1 and intercellular adhesion molecule1-ICAM-1, vascular adhesion molecule1-VCAM-1 and von Willebrand factor were found high in patients with thalassemia **(16)**. Damage to endothelium activates tissue factors, leading to thrombosis in the areas of vascular inflammation. In addition, nitric oxide level decreases during hemolysis, causing vasoconstriction **(17)**.

As a result of widespread inflammation and apoptosis at the cellular level in cases with thalassemia, particles broken off from endothelial cells, monocytes, erythrocytes and platelets form new microparticles with a diameter of 01-2µm. Phosphatidylserine expression is high on the surface of these microparticles, and they cause vascular damage, increasing the incidence of thromboembolic events **(18, 19, 20)**. In a study conducted in Egypt, microparticle levels were even higher, especially in patients with splenectomy **(21)**. This is also evidence of the high frequency of thromboembolism in splenectomised beta-thalassemia patients. Additionally, in this study, thromboembolism risk factors were investigated, and the most significant factors were splenectomy, elevated total and direct bilirubin levels, and increased circulating microparticle levels.

d) Splenectomy: Splenectomy is performed in most patients with thalassemia. However, after splenectomy, thrombocytosis, circulating microparticles, and significantly an increase in the number of enlarged erythrocyte-microparticles expressing phosphatidylserine, cause thromboembolic events **(22)**. This frequency is higher, especially in thalassemia-intermediate patients with splenectomy **(2, 5)**. When splenectomised platelet content is investigated, plasma β2-thromboglobulin level was higher than the control group, resulting in hypercoagulability **(23)**. For all these reasons, the risk of thromboembolism, especially for those with splenectomy who are on regular erythrocyte transfusion, is reduced.

e) Other Causes: When acquired and hereditary thromboembolic factors are investigated, of the acquired factors, lupus anticoagulant positivity was determined in 16% of patients with thalassemia major. Additionally, anti-cardiolipin IgM and IgG antibodies are reported positive between 6-30%. Despite the high positivity rate of anti-cardiolipin antibodies found in cases with hepatitis C, thromboembolic events were not reported in any of them **(24, 25, 26)**.

Hereditary thromboembolic factors: factor V Leiden (FVL), prothrombin 20210 and methylene tet-

rahydrofolate reductase mutations did not significantly increase the risk of thromboembolism **(27, 28)**. However, Akar et al. reported intrahepatic thrombosis in a splenectomised patient with positive FVL and beta-thalassemia **(12)**. In addition, natural anticoagulants protein C and protein S and anti-thrombin III levels in plasma were found to be low **(29, 30)**. These low levels may have contributed to the development of thromboembolism.

CLINICAL FINDINGS

Deep vein thrombosis, pulmonary embolism, portal vein thrombosis or arterial thrombosis may develop in cases with thalassemia. However, cerebrovascular thrombosis is significant in terms of neurological sequelae and vital importance. Cerebrovascular events occur mainly in two different clinical situations: Stroke and silent cerebral infarcts.

Although clinical findings are evident in stroke cases, cerebral infarctions generally progress asymptomatic or as transient ischemic attacks. Impairment in cognitive functions is usually detected, especially recurrent silent infarctions that can cause permanent neurological damage. Both stroke and silent infarcts have been reported more frequently, especially in cases with thalassemia intermedia, than in patients with major **(3, 31, 32)**. Silent infarcts usually develop in the parietal and frontal regions, and subcortical white matter is affected. These lesions in the white matter may be small perivascular changes or maybe in the form of cavitation, fibrosis and sclerosis.

Diagnostic evaluation can be done with cranial magnetic resonance imaging (MRI). Particularly in cases with splenectomy and thrombocytosis, and in those with thalassemia intermedia who don't receive regular transfusion, silent infarcts may present, and these patients should be screened for thrombosis with cranial MRI at least once in 3 years to detect asymptomatic vascular damage (33). The best diagnostic approach to detecting vascular infarcts is diffusion-weighed cranial MRI. If, PET-CT and MRI are used together, functional neurologic deficits can be better evaluated **(34)**. In addition, prominent vessel diseases can be better detected by performing magnetic resonance angiography (MRA) and MRI together. Transcranial Doppler ultrasound can also show considerable vessel damage.

Serum neuron-specific enolase and S100 calcium-binding protein levels can be used as biomarkers showing brain damage **(35)**.

TREATMENT

Acetyl Salicylic Acid: Acetyl Salicylic Acid (ASA) has an anticoagulant effect by blocking thromboxane A2 synthesis and clearing platelet aggregate ision. It controversial to start routine aspirin in patients with splenectomy. Taher et al. **(5)** found the incidence of thromboembolism in thalassemia intermedia as 21.3%, and 52% of the cases were using ASA. However, it could be prophylactically given to children carrying high-risk factors for thromboembolism **(36)**.

These risk factors;

a. Splenectomy and thalassemia intermedia not on regular transfusion,
b. Thrombocytosis: There is no consensus on the cut-off value (>500x10^9)/L or >800-1000x10^9/L for starting aspirin.
c. Those with heavy iron load,
d. Those who are older (>20y)

Low dose 80mg/day aspirin can be used in these cases.

Regular Blood Transfusion: When the pathophysiology of thalassemic children is evaluated, the aggregation of erythrocytes and high thrombin generation cause thromboembolic. In addition, high free iron levels not bound to transferrin also cause endothelial damage with oxidative toxic effects. The risk of thromboembolic events will be reduced since the rate of damaged erythrocytes will decrease with regular transfusion **(37, 38)**. Chronic transfusions are especially recommended in symptomatic cerebrovascular thrombosis **(39)**.

Direct Oral Anticoagulants (DOAC): Direct oral anticoagulants show their effect by directly inhibiting Factor IIa or FXa. Compared to Warfarin, they have no interactions with drugs or foods and has a rapid impact. Although it is usually given to patients with atrial fibrillation and venous thrombo-

embolism, its use in thalassemia patients is limited **(40)**. The most crucial reason limiting its use is the hepatotoxic side effects of DOACs. For this reason, liver functional tests should be monitored in these patients **(41)**.

REFERENCES

1. Borgna Pignatti C, Carnelli V, Caruso V, et al. Thromboembolic events in beta-thalassemia major: an Italian multicenter study. Acta Haematol 1998;99:76–9.
2. Camaschella C, Cappellini MD. Thalassemia intermedia. Haematologica 1995;80:58–68.
3. Haghpanah S, Karimi M. Cerebral thrombosis in patients with beta-thalassemia: a systematic review. Blood Coagul Fibrinolysis, 2012;23:212-7
4. Cappellini MD, Poggiali E, Taher AT, et al.Hypercoagulability in β-thalassemia: a status quo. Expert Rev Hematol. 2012;5:505-11.
5. Taher A, Isma'eel H, Mehio G, et al. Prevalence of thromboembolic events among 8,860 patients with thalassaemia major and intermedia in the Mediterranean area and Iran. Thromb Haemost 2006;96:488–91
6. Akar N. Turkish Thalassemia Study Group: TurkishThalassemia Registry – 1997. Proceedings of the 25th National Hematology Congress, Istanbul, 1997, pp 123–126.
7. Taher AT, Otrock ZK, Uthman I,et al. Thalassemia and hypercoagulability. Blood Rev. 2008;22:283-92.
8. Sirachainan N. Thalassemia and the hypercoagulable state.Thromb Res. 2013;132:637-41.
9. Michaeli J, Mittelman M, Grisaru D, Rachmilewitz EA. Thromboembolic complications in beta-thalassemia major. Acta Haematol 1992;87:71–4.
10. Aessopos A, Farmakis D, Karagiorga M,et al. Pseudoxanthoma elasticum lesions and cardiac complications as contributing factors for strokes in beta-thalassemia patients. Stroke 1997;28:2421–4.
11. Moratelli S, De Sanctis V, Gemmati D, et al. Thrombotic risk in thalassemic patients. J Pediatr Endocrinol Metab 1998;11: 915–21.
12. Akar N, Kemahli S, Uysal Z, et al. Thromboembolism in beta-thalassemia major. Acta Haematol 1998;100:166.
13. Kurt ZDÇ, Sönmez Ç, Kaçar D, et al. Evaluation of coagulation parameters and impact of transfusion on coagulation in patients with beta-thalassemia major.Blood Coagul Fibrinolysis. 2022 ;33:266-271.
14. Senanayake MP, Lamabadusuriya SP. Cerebral thrombosis in beta-thalassaemia major. Indian J Pediatr 2001;68:1081–2.
15. Cappellini MD, Robbiolo L, Bottasso BM, et al.Venous thromboembolism and hypercoagulability in splenectomised patients with thalassaemia intermedia. Br J Haematol 2000;111:467–73.
16. Butthep P, Bunyaratvej A, Funahara Y, et al. Alterations in vascular endothelial cell-related plasma proteins in thalassaemic patients and their correlation with clinical symptoms. Thromb Haemost 1995;74:1045–9
17. Musallam KM, Taher AT. Thrombosis in Thalassemia: Why are we so Concerned? Hemoglobin,2011;35:503-10
18. Hugel B, Martinez MC, Kunzelmann C, Freyssinet JM. Membrane microparticles: two sides of the coin. Physiology (Bethesda) 2005;20:22–7.
19. Pattanapanyasat K, Noulsri E, Fucharoen S, et al. Flow cytometric quantitation of red blood cell vesicles in thalassemia. Cytometry B Clin Cytom 2004;57:23–31.
20. Habib A, Kunzelmann C, Shamseddeen W, et al. Circulating microparticles in lebanese patients with beta-thalassemia intermedia. In: ASH; 2006: Blood; 2006. p. 1589.
21. Youssry I, Soliman N, Ghamrawy M, et al. Circulating microparticles and the risk of thromboembolic events in Egyptian beta thalassemia patients. Ann Hematol. 2017;96:597-603.
22. Cappellini MD, Grespi E, Cassinerio E et al. Coagulation and splenectomy: an overview Ann N Y Acad Sci, 2005;1054:317-324.
23. Atichartakarn V, Angchaisuksiri P, Aryurachai K, et al.In vivo platelet activation and hyperaggregation in hemoglobin E/beta-thalassemia: a consequence of splenectomy. Int J Hematol 2003;77:299–303.
24. Giordano P, Galli M, Del Vecchio GC, et al. Lupus anticoagulant, anticardiolipin antibodies and hepatitis C virus infection in thalassaemia. Br J Haematol 1998;102:903–6.

25. Sharma S, Raina V, Chandra J, et al. Lupus anticoagulant and anticardiolipin antibodies in polytransfused beta thalassemia major. Hematology 2006;11:287–90.
26. Winichagoon P, Fucharoen S, Wasi P. Increased circulating platelet aggregates in thalassaemia. Southeast Asian J Trop Med Public Health 1981;12:556–60.
27. Zalloua PA, Shbaklo H, Mourad YA, et al. Incidence of thromboembolic events in Lebanese thalassemia intermedia patients. Thromb Haemost 2003;89:767–8
28. Iolascon A, Giordano P, Storelli S, et al. Thrombophilia in thalassemia major patients: analysis of genetic predisposing factors. Haematologica 2001;86:1112–3.
29. Mustafa NY, Marouf R, Al-Humood S, et al. Hypercoagulable state and methylenetetrahydrofolate reductase (MTHFR) C677T mutation in patients with beta-thalassemia major in Kuwait Acta Haematol, 2010;123:37-42.
30. Musumeci S, Leonardi S, Di Dio R, et al. Protein C and antithrombin III in polytransfused thalassemic patients. Acta Haematol 1987;77:30–3.
31. Nemtsas P, Arnaoutoglou M, Perifanis V, et al. Neurological complications of beta-thalassemia. Ann Hematol. 2015;94:1261-5.
32. Taher AT, Musallam KM, Nasreddine W, et al. Asymptomatic brain magnetic resonance imaging abnormalities in splenectomized adults with thalassemia intermedia. J Thromb Haemost. 2010;8:54-9.
33. Karimi M, Bagheri H, Rastgu F, et al. Magnetic resonance imaging to determine the incidence of brain ischaemia in patients with beta-thalassaemia intermedia. Thromb Haemost. 2010;103:989-93.
34. Musallam KM, Nasreddine W, Beydoun A, et al. Brain positron emission tomography in splenectomized adults with β-thalassemia intermedia: uncovering yet another covert abnormality. Ann Hematol. 2012;91:235-41.
35. Kanavaki A, Spengos K, Moraki M, et al. Serum Levels of S100b and NSE Proteins in Patients with Non-Transfusion-Dependent Thalassemia as Biomarkers of Brain Ischemia and Cerebral Vasculopathy. Int J Mol Sci. 2017; 18:2724.
36. Karimi M, Haghpanah S, Pishdad Pet al. Frequency of silent brain lesions and aspirin protection evaluation over 3 years' follow-up in beta-thalassemia patients. Ann Hematol. 2019; 98:2267-71.
37. Pazgal I, Inbar E, Cohen M, et al. C0431: High incidence of thrombotic cerebral lesions in adult patients with beta-thalassemia major. Thrombosis Research. 2014;133: S116.
38. Musallam KM, Taher AT, Karimi M, et al. Cerebral infarction in β-thalassemia intermedia: breaking the silence. Thromb Res. 2012; 130:695-702.
39. Karimi M, Cohan N, De Sanctis V, et al. Guidelines for diagnosis and management of Beta thalassemia intermedia. Pediatr Hematol Oncol. 2014;31:583- 96.
40. Apostolou C, Klonizakis P, Mainou M, et al. Rivaroxaban Use in Patients with Hemoglobinopathies. Hemoglobin. 2017; 41:223-4.
41. Bahrani S, Teimouri-Jervekani Z, Sadeghi M. Thrombotic Events and Anticoagulants in Beta-thalassemia Patients with Focus on Anticoagulants for Atrial Fibrillation: A Brief Review. Curr Probl Cardiol. 2022; 47:100912.

Correspondence Address:
Prof. Dr. Melike Sezgin Evim
Prof. Dr. Adalet Meral Güneş
Uludag University Medical Faculty
Dpt.of. Pediatric Hematology Bursa, Türkiye
E-mail:melikevim@yahoo.com
E-mail: adaletm@uludag.edu.tr

CHAPTER 10
LIFE IN THALASSEMIA

LIFE IN TRANSFUSION-DEPENDENT β-THALASSEMIA FROM PAST TO PRESENT

Prof. Leyla Ağaoğlu, MD
Acibadem Fulya and International Hospitals İstanbul-Türkiye

ABSTRACT

The β-thalassemias are one of the most common inherited monogenic diseases. Many patients present with severe anemia in early childhood. They can only survive with lifelong red blood cell (RBC) transfusions leadig to enormous iron overload and they are called transfusion-dependent thalassemia (TDT) patients. Before the introduction of regular transfusion and chelation therapy, TDT used to be a rapidly fatal disease. In the last few decades, the life expectancy of TDT patients has significantly increased, as reported by several groups in different countries. The improvement has been more marked in the wealthier parts of the world, and it can be attributed to several factors, including regular use of deferoxamine and, now, the availability of oral chelators. The evidence for improvement in survival with long-term deferoxamine therapy is beyond dispute. Before deferoxamine became available for the treatment of iron overload, prognosis was uniformly poor with most patients dying of cardiac complications in the second or third decade. But the iron chelation therapy with deferoxamine requires regular subcutaneous or intravenous infusions. This can lead to reduced quality of life and poor adherence, resulting in increased morbidity and mortality in thalassemic patients. The oral chelators, being more convenient to use and more powerful to chelate cardiac iron, are developped with the aim of solving this problem. Indeed studies showed that the oral chelators have a significant impact on quality of life and life expectancy of TDT patients. However now the serious complications due to ineffective erythropoiesis, anemia and iron overload are seen more and more in adults than in younger patients. Advances in the understanding of the pathology of the disease have led to the development of new treatment strategies. Soon with the use of this new options in clinical practice TDT, the hopeless patients of the past, will look forward to a long and productive life.

Keywords: Transfusion-dependent β-thalassemia, red blood cell transfusion, iron chelation, novel therapies, quality of life, survival

Thalassemia, an autosomal recessive disease caused by a single gene mutation, is named after the ancient Greek word “Talassa” meaning sea, since it was first described in patients living around the Mediterranean or originating from this region **(1)**. More than 300 β-thalassemia mutations have been studied in this disease and have been classified according to severity: β+ indicates mild mutations and β0 indicates severe mutations. In the former, there is a relatively slight decrease in β-globin chain synthesis, while in the latter there is no β-globin chain synthesis. This results in ineffective erythropoiesis and premature hemolysis of erythrocytes. Ineffective erythropoiesis leads to a decrease in hepcidin production in the liver, which leads to an increase in iron absorption from the intestine **(2)**. The diagnosis of these patients is made in the early years of life and they need to receive lifelong red blood cell (RBC) transfusions. Thus, transfusion-dependent β-thalassemia (TDT) emerges as the most severe form of the disease. In the last 40 years, the medical world has witnessed the transformation of TDT from a genetic disorder with an inevitably fatal course to a chronic disease that allows for a long life expectancy. This transformation has occurred with treatment.

The main steps of TDT therapy can be summarized in decades: 1960s—correction of hypoxia with

transfusion therapy; 1970s—reducing iron load with sc and/or IV chelation therapy; 1980s—complete healing with bone marrow transplantation; 2000s—development of oral iron chelators and taking their place in treatment. Among all these treatment methods, it is an indisputable fact that the dramatic prolongation in the life span of patients today is due to the regular use of deferoxamine. Additional factors for the effective use of deferoxamine are the availability of sufficient RBC suspensions from voluntary donors, their screening with reliable methods for the most common viral agents, the aggressive treatment of infections, the availability of deferoxamine and oral chelators, and the effective treatment of cardiac complications. For these reasons, the improvement in the quality of life and expected life expectancy of TDT patients is reported mainly from the developed countries of the world **(3)**.

For many years after Cooley first described the disease in 1925, treatment was limited to only minimal palliative applications: children whose hemoglobin levels went down to very low values such as 3-4 g/dL, were transfused and their levels were raised up to 6-7 g/dL **(4)**. As a result, all patients died in the first decade of life, mostly due to severe infections caused by chronic inactivity or complications of pathological fractures.

For the first time in 1964, Irving Wolman drew attention to the benefits of keeping hemoglobin values above 8g/dL **(5)**. As a result of these and other studies with similar findings, the routine "hypertransfusion" regimen has become the standard treatment of TDT **(6).** In a short time, it was understood that this method was not sufficient, growth and development were more normal in the first ten years of life, but thereafter, signs of iron overload such as stagnation, short stature, diabetes, delayed puberty, siderosis, liver failure, and cardiomyopathy appeared. Most of the patients died towards the end of their teens due to complications related to cardiac siderosis such as severe failure or fatal arrhythmia **(7)**.

In the early 1970s, the problem of siderosis was approached directly by Elizabeth Letsky et al. in London applying chelation therapy to remove excess iron from the body **(8, 9)**. British researchers used deferoxamine, a highly specific iron chelator, and showed that complications occur less frequently and later in a group of thalassemia patients who have migrated from Cyprus, if they are given 750mg deferoxamine (DFO) intramuscularly (IM) 5 days a week, compared to their peers **(10)**.

The first evidence of the beneficial effects of DFO treatment was obtained in the early 1980s, when Modell et al. showed that children who received more than 4 g of DFO per week were much less likely to be lost from excess iron overload than patients of the same age who received less or no DFO **(11)**. However, administration of DFO in this study varied from patient to patient, and the follow-up period was not sufficient to evaluate the effect of subcutaneous infusion, which was a new practice at the time, on survival.

Continuing studies over the next decade confirmed the beneficial effects of long-term subcutaneous DFO infusion on patients' survival **(12, 13)**. Nevertheless, it was proved in the mid-1990s that DFO treatment had a positive impact on patients' lives, without a doubt. Two separate studies with a follow-up period of more than 10 years have definitively shown that effective subcutaneous DFO administration in TDT provides a long life expectancy by reducing the complications of iron overload **(14, 15)**. Body iron load emerged as the most important factor affecting the clinical course in both studies. In the study of Olivieri et al. the efficacy of DFO treatment was monitored by serum ferritin values. TDT patients with less than 33% of their ferritin measurements above 2500 ng/mL had a probable cardiac disease-free survival rate of 100% after 10 years of DFO treatment and 91% after 15 years, while those with more than 67% of their ferritin measurements above 2500 ng/mL had a probable cardiac disease-free survival rate of 38% and 18% after 10 years and 15 years of DFO treatment respectively. Mortality was found to be 50% in patients who developed heart failure as a result of insufficient chelation **(15)**.

In the study of Brittenham et al., it was shown that body iron load, as assessed by magnetic measurement of liver iron stores, is closely related to the ratio of cumulative transfusional iron load (mmol/kg) and cumulative DFO use (g/kg). In pa-

tients who developed or died of cardiac disease, the ratio of transfusional iron load to DFO used was over 0,6 mmol/g, which was equivalent to liver iron concentration above 15mg/g dry weight. Patients with a ratio < 0,6 or a pre-chelation iron load < 14 mmol/kg had 100% survival, while those outside of these groups had only 25% survival at 25 years. Thus, this study revealed that body iron load before the onset of chelation therapy, total transfusional iron and total DFO amount used are factors affecting life, and early initiation of DFO reduces body iron load and prevents diabetes, heart disease and premature death in TDT **(14)**.

The findings of Brittenham et al. showing that compliance with DFO treatment is critical in prolonging life expectancy, has been supported by other studies. It has been shown that 95% of those who received 250 infusions/year (approximately 5 days/week) DFO treatment in 257 patients with TDT reached the age of 30, and only 12% of those who could not meet this target in treatment could reach the age of 30 **(16)**. In another study, Borgna-Pignatti et al. investigated 1146 thalassemia patients who were born between January 1, 1960 and December 31, 1987, followed up in seven centers in Italy, and received regular transfusions. By grouping them according to their birth years, they showed that the increase in survival was due to the reduction of cardiac mortality with early initiation of chelation therapy. The probability of survival in those born between 1970-74 was found to be 89% until the age 20 and 82% until the age 25. It was also shown that the risk of developing heart disease, diabetes and hypogonadism in those born in recent years was lower than those born in previous years **(17)**.

Borgna-Pignatti et al. reviewed 1073 patients born between 1960-1997, followed in the above-mentioned Italian centers over a longer period of time and published it again in 2005 **(18)**. However, it was noted that complications are still common and affect the quality of life of the patients. Heart disease, including heart failure, arrhythmias and myocardial infarction, is the first cause of death in patients (>50%), 7% of deaths are attributed to infection and 4% to liver disease. Complications are heart disease in 12% of patients born after 1970 and especially in males, diabetes in 6%, hypothyroidism in 11%, hypogonadism in 55%, osteoporosis and osteopenia in 52-96%. In addition, Borgna Pignatti et al. reported that hepatitis C virus antibodies were positive in 85% of Italian patients receiving chronic transfusion, while the same rate was 23, 35, 34 and 21 percent for patients from England, USA, France and India. They also stated that hepatotropic virus and excess iron may lead to the development of hepatocellular carcinoma, the probabiliy depending on the iron load; indeed they detected liver cancer in 23 patients. Morover, it was noted that ferritin levels have prognostic importance, low ferritin values go together with low heart failure and low risk of hypogonadism, and patients live longer. Thus, it was proven once again that iron overload is the main cause of death and complications in thalassemia. Compliance with chelation therapy was found to be the most effective factor in prolonging survival and it was concluded that alternative treatments such as oral chelators are needed for patients who do not comply with subcutaneous DFO infusions **(18)**.

In the study conducted by Modell et al. in which 796 TDT patients born after 1945 were investigated, the expected upward trend in the life curves was not observed and 50% of the patients were lost with iron overload complications before age 35. It was noted that there was no difference between those born in 1965-1974 and in 1975-1984. This high mortality in the UK has been attributed to the non-adherence of patients to subcutaneous DFO treatment, especially during adolescence. In addition, the causes of death were collected in three groups according to age and years: Death in those younger than 12 years was common before 1965 and occurred with anemia complicated by infection and/or hypersplenism. Premature deaths in recent years were due to bone marrow transplantation. The majority of deaths between the ages of 12-24 was the result of untreated or minimally treated iron overload. Long-term (>24 years) life was achieved with iron chelation therapy **(19)**.

Contrary to the study conducted by Modell et al. in England, the probability of survival was found to be much higher in TDT patients followed in a single center of the same country. In the study of Davis et

al. 103 patients born between 1957 and 1987 were followed in a center that applied a multidisciplinary approach, including clinical psychology, in order to ensure the highest level of compliance to DFO and chelation therapy. The probability of survival was found to be increased with the year of birth and no mortality was observed among those born after 1974. Thus, it was concluded that the difference with the results of the previous study stems from being treated in a "specialized center" **(20)**.

Ladis et al. investigated survival and causes of death in 647 thalassemia major patients born between 1958-2004 and followed in a single center in Greece. They obtained findings in parallel with other studies. The patients were gathered in two groups according to their birth before (group A) and after (group B) 1975 and the probability of survival at the age of 29 showed a significant difference between the two groups as 89.4% in group B and 73.5% in group A. This rate was 59% at age 46 for the entire group. In addition, patients were divided into three hemosiderosis groups according to their mean serum ferritin values in the last 5 years, as mild (<2000 µg/L), moderate (2000-4000 µg/L), and severe (>4000 µg/L). The probability of survival at 40 years of age was significantly different between these three groups (85.6%, 68.2% and 28.9%, respectively). There were 115 deaths in the series of Ladis et al. and the leading cause of mortality was hemosiderosis (76.5%) (cardiac disease in 71.3%), followed by sepsis (7.8%) and AIDS (6.1%). In the last 20 years, hematopoietic cell transplantation, which is the only curative method in the treatment of TDT, has also attracted attention among the causes of death (3.5%). Thus, it has been proven once again that the most important factor affecting life is compliance with chelation therapy and the effectiveness of this therapy **(21)**.

Continuation of cardiac disease as the leading cause of death in TDT has led researchers to seek alternative solutions to subcutaneous DFO treatment. The most important among these alternatives has been oral chelators. Deferiprone is the first oral chelator available to patients in Asia and Europe since the mid-1990s. Studies conducted in various countries such as England and Italy have shown that deferiprone is superior to subcutaneous DFO treatment in improving cardiac functions by removing cardiac iron **(22-24)**. Piga et al. retrospectively evaluated patients treated with deferiprone or DFO for at least 4 years and reported that cardiac dysfunction was significantly less in the deferiprone group than in the DFO group **(23)**. Borgna-Pignatti et al. investigated the effects of deferoxamine versus deferiprone in terms of cardiac events and probability of survival by examining data from TDT patients followed in seven centers in Italy. Cardiac events requiring treatment, including heart failure or arrhythmias, were observed in 14.5% of 359 patients in the DFO group and 4% of deaths were due to cardiac causes, while both conditions were 0% in the deferiprone group, which included 157 patients, and the difference was found to be highly significant **(24)**.

Modell et al.' Study published in 2008, revealed that there was a significant decrease in deaths due to excessive iron accumulation in TDT patients of all ages, at an average rate of 2.3/1000/year, since the early 2000s. It has been reported that it is the result of effective excretion of iron, deferiprone being the oral iron chelator used. In this context, the researchers state that the T2* cardiac magnetic resonance technique, which precisely measures the cardiac iron load, is of great importance. In this study, life expectancy was calculated as 17 years in 1970, 27 years in 1980, and 37 years in 1880, and since 2000, it has been reported that more than 80% of TDT patients have a life expectancy of 40 years or more **(25)**.

Deferasirox is the third chelator licensed in more than 70 countries in the world, especially in the USA and the European Union. Deferasirox is an effective once-daily oral iron chelator. As a result of a multinational, multicenter, randomized comparative phase III clinical study including Türkiye, it has been proven to be as effective and safe as DFO in reducing serum ferritin and liver iron concentration values at a dose of 30mg/kg/day **(26)**. Studies have shown that deferasirox has a good effect on cardiac iron **(27-29)**. In addition, phase III clinical studies have demonstrated a very important advantage of deferasirox: patients find deferasirox treatment much easier to administer than DFO treatment and are satisfied with this chelator. In the

study of Cappellini et al. 282 patients with beta-thalassemia who received DFO therapy and 289 patients with transfusion-induced hemosiderosis who were randomized to deferasirox therapy were evaluated for their satisfaction with the chelator they used and the suitability of using this chelator. Significantly more patients in the Deferasirox group than the DFO group reported being "very satisfied" or "satisfied" with treatment at weeks 4, 24 and at the end of the study (50.4% vs. 92%, 44% vs. 89.6% and 38.7% vs. 95.1% respectively; P<0.0001). At the same time given he majority of deferasirox patients found the treatment 'very useful' or 'useful' (95.5%, 91.7%, and 92.7%, respectively), while the majority of DFO patients found the treatment 'useless' (>60%). A total of 96.9% of the patients reported that they preferred deferasirox treatment to deferoxamine. At the end of the study, significantly more patients wanted to continue treatment with deferasirox **(30)**. According to these results, it is undoubted that TBT patients will adapt much better to deferasirox treatment and will not disrupt their iron chelation, this will positively affect the life expectancy and quality of life of the patients. Indeed, in recent years, studies examining the marriage, fertility and pregnancy status of TBT patients have attracted attention **(31, 32)**. Parenthood, which was once unthinkable for these patients, has now become ordinary.

However, despite all these developments in TDT treatment, studies show that the quality of life of patients is still lower than the general population, even in developed countries. Various measures have revealed the negative impact of time spent on transfusions, treatment and follow-up of complications on daily life. In the study of Tedone et al., in 105 TDT, the Psychological General-Well-Being Index (PGWBI) score was found to be significantly lower than the general population, especially in general health and vitality areas and in total PGWBI (p= 0.0001) **(33)**.

Another situation that requires a solution is the shift of complications due to ineffective erythropoiesis, anemia and iron overload, from childhood and adolescence to adult ages over time. While regular RBC transfusions and effective iron chelation prolonged life in TDT, exposure to adverse factors has also extended and complications that impair quality of life such as endocrinopathy, hepatitis, and thrombosis have became more noticeable in adult patients **(34)**.

In recent years, with a better understanding of the pathology of β-thalassemia, new drugs and methods that can improve the quality of life in TDT, by reducing the erythrocyte transfusion load, have been developed or are in development. They can be divided into three major groups according to their efforts to address different features of the underlying pathophysiology: those that correct the imbalance of α/β-globin chains (gene therapy, gene editing), those that target the late stage of ineffective erythropoiesis (luspatercept, JAK2 inhibitors) and those that reduce iron accumulation (hepsidin-like molecules, transmembrane protease serine 6 inhibitors) **(35, 36)**. Researches are continuing to determine the efficacy and safety of these new drugs which may open a new area in the treatment of TDT. In addition, cost-benefit analyzes should also be performed **(36)**.

Thalassemia is not fate, it is a disease that can be prevented by identifying carriers and prenatal diagnosis. Unprevented TDT cases are no longer helpless patients but individuals with a long and productive life expectancy. Today, effective DFO treatment, bone marrow transplantation and oral chelators which provide increased compliance with treatment and more effective removal of cardiac iron, have increased the quality of life of patients and significantly extended their life span. Also, with ongoing studies, the most appropriate treatment method and drug will undoubtedly be found for these patients in the near future.

REFERENCES

1. Greer JP, Arber DA, Glader B et al. Wintrobe's Clinical Hematology: thirteenth edition. Philadelphia, PA, USA. Wolters Kluwer Health Adis (ESP); 2013; 2312.
2. Taher AT, Musallam KM, Cappellini MD. β-Thalassemias. N. Engl. J. Med. 2021; 384: 273-282.
3. Kattamis A, Forni GL, Aydinok Y et al. Changing patterns in the epidemiology of β-thalassemia. Eur. J. Haematol. 2020; 105: 692-703.

4. Cooley T, Lee P. Series of cases of splenomegaly and peculiar changes in the bones. Trans Am Pediat Soc 1925; 37: 29.
5. Wolman LJ. Transfusion therapy in Cooley's anemia: Growth and health as related to long range hemoglobin levels. A progress report. Ann NY Acad Sci 1964; 119:736.
6. Schorr JB, Radel E. Transfusion therapy and its complications in patients with Cooley's anemia. Ann NY Acad Sci 1964; 119:703.
7. Engle MA, Erlandson M, Smith CH. Late cardiac complications of chronic, severe, refractory anemia with hemochromatosis. Circulation 1964; 30: 698-705.
8. Modell CB, Beck J. Long-term desferrioxamine therapy in thalassaemia. Ann NY Acad Sci 1974; 232:201-210.
9. Barry M, Flynn MD et al. Long-term chelation therapy in thalassaemia major: Effect on liver iron concentration, liver histology and clinical progress. BMJ 1974; 2:16.
10. Modell CB. Total management of thalassaemia major. Arch Dis Child 1977; 52: 489-500.
11. Modell B, Letsky E, Flynn D et al. Survival and desferrioxamine in thalassaemia major. BMJ 1982; 284:1081-1084.
12. Zurlo MG, Stefano P, Borgna-Pignatti C et al. Survival and causes of death in thalassaemia major. Lancet 1989; 2: 27-30.
13. Ehlers KH, Giardina PJ, Lesser M et al. Prolonged survival in patients with beta-thalassemia major treated with deferoxamine. J Pediatr 1991; 118:540-545.
14. Brittenham GM, Griffith PM, Nienhuis AW et al. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. N Engl J Med 1994; 331:567-573.
15. Olivieri NF, Nathan DG, MacMillan JH et al. Survival in medically treated patients with homozygous beta-thalassemia. N Engl J Med 1994; 331:574-578.
16. Gabutti V, Piga A. Results of long-term iron chelation therapy. Acta Haematol 1996; 95: 26-36.
17. Borgna-Pignatti C,Rugolotto S, De Stefano P et al. Survival and disease complications in thalassemia major. Ann NY Acad Sci 1998; 850:227-231.
18. Borgna-Pignatti C, Cappellini MD, De Stefano P et al. Survival and complications in thalassemia. Ann NY Acad Sci 2005; 1054: 40-47.
19. Modell B, Khan M, Darlison M. Survival in beta-thalassaemia major in the UK: data from the UK Thalassaemia Register. Lancet 2000; 355:2051-2052.
20. Davis BA, Porter JB. Results of long term iron chelation treatment with deferoxamine. In: Iron Chelation Therapy. Ed. Chaim Hershko, Kluwer Academic/Plenum Publishers, 91- 125, 2002.
21. Ladis V, Chouliaras G, Berdousi H et al. Longitudinal study of survival and causes of death in patients with talassemia major in Greece. Ann NY Acad Sci 2005; 1054:445-450.
22. Anderson LJ, Wonke B, Prescott E et al. Comparison of effects of oral deferiprone and subcutaneous desferrioxamine on myocardial iron concentrations and ventricular function in beta-thalassaemia. Lancet 2002; 360:516-520.
23. Piga A, Gaglioti C, Fogliacco E et al. Comparative effects of deferiprone and deferoxamine on survival and cardiac disease in patients with thalassemia major: a retrospective analysis. Haematologica 2003; 88:489-496.
24. Borgna-Pignatti C, Cappellini MD, De Stefano P et al. Cardiac morbidity and mortality in deferoxamine - or deferiprone- treated patients with thalassemia major. Blood 2006; 107:3733-3737.
25. Modell B, Khan M, Darlison M. Improved survival of thalassaemia majör in the UK and relation to T2* cardiovascular magnetic resonance. Journal of Cardiovascular Magnetic Resonance 2008; 10: 42.
26. Cappellini MD, Cohen A, Piga A et al. A phase 3 study of deferasirox (ICL670), a once-daily oral chelator, in patients with beta-thalassemia. Blood 2006; 107:3455-3462.
27. Cabantchik ZI, Link G, Glicktein H et al. Deferasirox (Exjade, ICL670): a journey into labile iron centers of living cardiomyocytes. Blood 2005; 106(11):abst 824.
28. Wood JC, Otto-Dues sel M, Gonzales I et al. ICL670 removes cardiac iron in a gerbil model of iron overload. Blood 2005; 106(11):abst 2695.
29. Porter JB, Taner MA, Pennell DJ et al. Improved myocardial T2* in transfusion dependent anemias receiving ICL670 (deferasirox). Blood 2005; 106(11):abst 3600.
30. Cappellini MD, Bejaoui M, Agaoglu L et al. Prospective evaluation of patient-reported outcomes during treatment with deferasirox or deferoxamine for iron overload in patients with □-thalassemia. Clinical Therapeutics 2007; 29:909-917.
31. De Sanctis V, Soliman AT, Hakim IE et al. Marital status et paternity in patients with Transfusion Dependent Thalassemia (TDT) and Non Transfusion Dependent Thalassemia (NTDT): an ICET – A survey in different countries. Acta Biomed. 2019; 3: 225-237.
32. Cassinerio E, Baldini IM, Alameddine RS et al. Pregnancy in patients with thalassemia major: a cohort study and conclusions for an adequate care management approach. Ann Hematol. 2017; 6: 1015-1021.

33. Tedone F, Lamendola P, Lopatriello S et al. Quality of life and burden of disease in Italian patients with transfusion- dependent beta-thalassemia. J. Clin. Med. 2022;11; 15.
34. Taher AT, Cappellini MA. How I manage medical complications of β-thalassemia in adults. Blood 2018; 132: 1781-1791.
35. Taher AT, Bou-Fakhredin R, Kattamis A et al. Improving outcomes and quality of life for patients with transfusion-dependent β-thalassemia: recommendations for best clinical practice and the use of novel treatment strategies. Expert Rev. Hematol. 2021; 14; 897-909.
36. Bou-Fakhredin R, Motta I, Cappellini MA. Advancing the care of β-thalassemia patients with novel therapies. Blood Transfus. 2022; 20: 78-88.

Correspondence Address:
Prof. Leyla Ağaoğlu, MD
Acibadem Fulya and International Hospitals İstanbul-Türkiye
E-mail: leyla.agaoglu@gmail.com

IMMUNITY AND VACCINES IN THALASSEMIA

Prof. Dr. Sefik S Alkan MD
Immunologist, Basel, Switzerland

ABSTRACT

In this short review, first I would like to deal with immunological changes observed in thalassemia patients. Secondly, I will discuss vaccination issues in these group of patients. Increased susceptibility to infections in thalassemia aroused a natural interest in the study of the immune status thalassemia. Immunological abnormalities in thalassemia are caused by either the illness, or therapy methods. The basis of the pathogenesis of the immunodeficiency in thalassemia is iron overload and allogenic stimulation caused by multiple blood transfusions. Immune deficiency in thalassemia includes abnormalities of the innate immune system such as decreased levels of complement, properdin, and lysozyme, reduced phagocytic ability of neutrophils, disturbed chemotaxis, and altered intracellular metabolism processes. Changes in adaptive immunity include increased levels of CD8 T cells, diminished activity of CD4 T cells and defective activity of Natural Killer (NK) cells, but increased levels of activated B lymphocytes. Elevated levels of most immunoglobulins also observed. Thalassemic patients are at increased risk of blood-borne infections due to multiple blood transfusions. The most important factors causing such alterations involve iron overload, oxidative stress, iron chelation therapy, splenectomy and chronic inflammation. Optimal vaccination is critical for all patients with thalassemia. Prior to splenectomy patients should receive the meningococcal conjugate vaccine and should be up to date for Hib and pneumococcal vaccines. Patients need to be immunized against hepatitis A and B. Annual monitoring of titers and booster immunizations, when indicated, will ensure patients are well protected.

INTRODUCTION

As said by Goethe in1800's "Blood is a very special juice". Red cells provide energy to the whole body (O2, glucose, ATP) and the white cells protect the whole body. From this, one can deduce that any defect in O2 carrying hemoglobin molecule in red cells must affect the whole body and that is what we see in thalassemia. These patients not only show enhanced susceptibility to infections because of coexistent immune deficiency but are also at risk of blood-borne infections resulting from multiple blood transfusions. Enhanced susceptibility to infections in β-thalassemia is associated with the interplay of several complex biological processes and was demonstrated to be also affected by external factors, such as blood transfusion, iron chelation therapy, splenectomy, as well as immune status **(1, 2)**.

The β-thalassemia is also associated with several abnormalities of the innate immune system observed from early childhood. These include decreased levels of complement, properdin and lysozyme, reduced phagocytic ability of neutrophils, disturbed chemotaxis, and altered intracellular metabolism processes that have been described in the literature **(1, 3)**. The disruption of chemotaxis and phagocytosis could be induced by multiple blood transfusions because of chronic immune stimulation by foreign proteins **(1)**. The first and most common cause of death in thalassemia patients is cardiac failure, while hepatic disease is the third most frequent cause **(4, 5)**. The standard treatment involves a lifelong blood transfusion therapy in order to preserve normal levels of hemoglobin **(1)**. Multiple blood transfusions result in the development of alloimmunization and the accumulation of iron in tissues leading to enhanced risk for transmitted

infections **(1, 6)**. The severe iron overload leads to progressive organ failure **(7)**.

IMMUNITY IN THALASSEMIA

A wide spectrum of immune defects that have been observed in thalassemic patients have been reviewed several times **(8-13)**.

Defects in Innate Immunity

In thalassemia, several components of the immune response show functional abnormalities. Altered cytokine as well as increased total leukocyte, neutrophil, and lymphocyte counts have been demonstrated in some groups of patients **(10)**. Defective chemotaxis and phagocytosis have also been described in thalassemic patients. Reduced opsonization and granulocyte phagocytosis as well as suppressed functioning of the complement system has also been reported **(4, 13, 14)**.

Mechanisms involved in the dysfunctional innate immune system comprise defects in neutrophil migration, phagocytosis, and the activation of pathogen-specific immune responses **(2)**. The authors suggested that decreased resistance to bacterial infections in β-thalassemia syndromes stemmed from chronic oxidative stress and the consequent diminished ability of PMN *(polymorphonuclear neutrophils)* to respond via respiratory burst to bacterial components **(15)**. Aberrant neutrophils may be considered a risk for disease-associated morbidity and mortality, perhaps neutrophil replenishment may prove to be beneficial in the diminishing of comorbidities and improvement of life quality in this group of patients **(13)**.

Defects in Adaptive Immunity

In thalassemic patients, increased levels of suppressor T cells, diminished activity of helper T cells (CD4) decreased T cell proliferative capacity and defective activity of Natural Killer (NK) cells, but increased levels of activated B lymphocytes have been observed **(4)**. Elevated levels of immunoglobulins IgG, IgM, and IgA indicate compromised immunoglobulin secretion. Thalassemic patients were found to have elevated levels of suppressor T cells, natural killer cells, and B cells, observed **(11, 16, 17)**. The most noticeable characteristic of altered lymphocytic subpopulations in thalassemia major patients seems to be increase in the ratio of T regulatory (CD4+/CD25+/Foxp3+) lymphocyte subset **(18)**.

Elevated lymphocyte counts in patients with thalassemia could be due to the repeated antigenic challenge from blood transfusions. A higher number of activated T cells was accompanied by an increased concentration of neopterin, could be due to the presence of chronic of the immune stimulation. However, decreased interferon gamma (IFN-γ) and IL-2 levels in subjects with elevated ferritin levels indicate, the immunosuppressive effect of iron overload in beta-thalassemia patients **(19)**.

B lymphocytes are responsible from the production of auto and alloantibodies against transfused red blood cells. The greater proportion of B cells, especially those with regulatory phenotype, expressing CD19, CD38, and CD24, but no difference in ratios of T cell subpopulation has been demonstrated in this group of patients **(20)**. Overexpression of CD38 and CD69 on the surface of lymphocytes in patients with thalassemia has been observed **(21)**. On the other hand, increased levels of IgA immunoglobin, but no difference in IgG, IgM, and IgE levels or complement components of C3 and C4 have been observed **(22)**. It is suggested that T-cell immune response was suppressed suggested caused by elevated levels of IL-17 and transforming growth factor β (TGF-β), but not IL-21 **(7)**. Also, decreased production of IFN-γ, IL-2, and IL-4 by activated lymphocytes from patients with β-thalassemia compared to the normal group has been observed **(19)**.

In thalassemia A state of an imunosenescence, a premature aging of lymphocytes been suggested **(19, 23)**. This may be due to the reduced expression of co-stimulatory molecule CD28 as this molecule plays a critical role in T-cell activation, proliferation, and survival **(25, 26)**. It has been found that CD8+ T cells lacking the expression of CD28 show some atypical features, including diminished T-cell receptor diversity, defective proliferation during antigen stimulation, and a suppressive effect on CD4 T-cell activation. The results of studies suggest that in β-thalassemia major patients, the aug-

mentation of senescence T cells, which impairs the functioning of the immune system, could be one of the reasons for greater susceptibility of infections **(25)**.

Disease-Related changes

In thalassemia reduced synthesis of β-globin chains and excess amount of α-chains which precipitate in the precursors of erythrocytes, leads to structural changes of the cell membrane. It has been demonstrated that the unbound α-globin continuously stimulates monocytes **(27)**. Multiple blood transfusions cause autoimmune hemolysis, alterations of T-and B-lymphocytes, as well as the effects of monocytes/macrophages functions **(1, 28)**.

Furthermore, the development of iron overload is associated with enhanced intestinal iron absorption related to ineffective erythropoiesis resulting from premature intramedullary death of erythrocytes as well as enhanced peripheral destruction of red cells; however, it could be also secondary to regular transfusions **(1, 29)**. Several studies have indicated that iron overload is the main factor of immune deficiency in β-thalassemia **(4)**. This may also stimulate the formation of anti-ferritin antibodies and subsequent enhanced production of circulating immune complexes **(30)**.

Chelation therapy which is used transfusions in order to prevent excessive iron load and have its complications. Application of chelating agent ferrioxamine provides direct evidence confirming the role of iron overload in immune system disturbances **(32)**.

External Causes

In addition to the disease-related factors, enhanced susceptibility to infections in β-thalassemia patients can be associated with external causes, such as blood transfusion, iron chelation therapy, and splenectomy and multiple blood transfusions have been associated with autoimmune hemolysis **(1)**. Heme exerts deleterious effects on the control of bacterial infections by hindering phagocytosis **(32)**. Chelation therapy (Desferrioxamine, Deferasirox, and Deferiprone) has its complications. Iron chelators may also contribute to Zn *deficiency* **(33)**.

Finally, splenectomy, which is a standard therapeutic intervention in β- thalassemia performed in order to remove the increased red blood cell consumption caused by hypersplenism, also causes a predisposition to infections and immune system modifications **(4, 33)**.

VACCINATION IN THALASSEMIA

Vaccines, as they prepare the immune system against pathogenic microorganism, are a great way to prevent many serious infectious diseases. However, as we have seen in the previous section, thalassemic patients represent an immunologically compromised patient group. Therefore, the protection against particularly childhood infections is of prime importance for thalassemic patients.

Immunization in Children and Adults with Thalassemia

The below recommendations are directly taken from: https://thalassemia.com/liing-with-thal-immunization.aspx#gsc.tab=0

(They are subject to change; parents should consult their own doctor for vaccinating their children)

Optimal immunization is critical for all patients with thalassemia, especially blood transfused patients and individuals who have been splenectomized. Prior to splenectomy patients should receive the meningococcal conjugate vaccine and should be up to date for Hib (*Haemophilus influenzae type B)* and pneumococcal vaccines.

Routine pediatric immunizations should be current and vaccination records should be checked annually. Beginning at two months of age, patients should be given a 7-valent conjugate pneumococcal vaccine as recommended. A booster with a 23-valent vaccine should be administered at 24 months. Pneumovax boosters should be considered every five to ten years. Check the pneumococcal titers following immunization. Severe local reactions can indicate high titer.

Patients need to be immunized against hepatitis A and B, especially patients on chronic blood transfusions. Annual monitoring of titers and booster immunizations, when indicated, will ensure patients are well protected. Individuals who are human immunodeficiency virus (HIV) positive or undergoing treatment for hepatitis C should not receive live virus vaccines. An annual influenza vaccination and annual PPD test for latent tuberculosis should also be administered. Particular attention should be given to the H1N1 virus, as this pathogen may cause more severe symptoms in patients with thalassemia.

HIV Infected Children

Children who are immune suppressed with HIV viral infection should not receive live virus vaccines: Measles, Mumps, Rubella; Oral Polio Vaccine; Varicella Vaccine. Nor should their siblings receive these vaccines without medical management to prevent infection of the immunosuppressed child.

Recommendations for children infected with the human immunodeficiency virus (HIV):

1. All routine inactivated vaccines (IPV, Hib, Hepatitis B, and DTaP) are recommended for all children.
2. Children who are six months or older receive the influenza vaccine (split dose with booster during the first season).
3. Children who are two years old or older receive pneumococcal vaccine.
4. The MMR vaccine is recommended only for children infected with HIV who are not severely immune compromised.
5. Live virus vaccines are contraindicated in all children who are infected with HIV with the above exception.

Children Receiving Intravenous Gamma Globulin: Children who are receiving intravenous gamma globulin (IVIG) have the possibility that live virus vaccines will be inactivated or that they will not develop immunity.

Children After Bone Marrow Transplantation: Centers performing bone marrow transplantation each have their own preferred schedule for reimmunization of their patients. These schedules should be followed. After immunocompetence is documented and all other immunizations are complete these children should receive the influenza vaccination annually.

Other Vaccines

New vaccines are being developed and will be available periodically. The Rotavirus Vaccine has recently been released and is not specifically indicated for children who have thalassemia. The meningococcal vaccine is available but is not generally recommended in most references. It would be indicated for splenectomized children and adults. This vaccine is not thought to be optimal and is not routinely administered at this center.

The Center for Disease Control (CDC) also recommends that all children and adults with thalassemia should get all recommended vaccinations, including a flu vaccination. People with thalassemia are considered “high risk” for certain infections, especially if they have had their spleen removed, and should follow a special vaccination schedule for the following vaccines:

Haemophilus influenzae type b (Hib)
Pneumococcal vaccines
Meningococcal vaccines

https://www.cdc.gov/vaccines/hcp/acip-recs/index.html (CDC). Pay special attention to the footnotes that provide special instructions for people with thalassemia.

SARS-CoV-2 and Thalassemia

Previously we have discussed several aspects of Covid-19 including immunology of the disease and several vaccine candidates **(34)**. Because thalassemia patients are immunocompromised, they are expected to be vulnerable patient population to the complications of COVID-19 infection. A new study aimed at evaluating the clinical and immunological response of patients with thalassemia to COVID-19 infection in comparison with controls **(35)**. Surprisingly this study concluded that most patients with transfusion-dependent thalassemia manifest with

mild or asymptomatic COVID-19. These patients were able to mount a statistically comparable IgG antibody response to COVID-19 akin to controls. However, the serological response cannot be sustained over three to six months as they have a more rapid fall in antibody titers when compared to the control group. This emphasizes the need for the protection of this vulnerable group by immunization.

Another recent study evaluated humoral response to Comirnaty vaccine in Thalassemia Major patients **[36].** They measured SARS-CoV-2–specific antibodies against Spike protein in 57 patients and 58 healthy blood donors. No statistically significant differences were observed between the two groups. Interestingly, splenectomy seems to correlate with a higher titer of antibodies to the Spike viral protein, although further studies are needed to confirm this finding. Further such studies are needed to evaluate cell-mediated immune responses.

Other studies suggest that the prevalence of SARSCoV-2 infection among β-thalassemia patients seems to be lower than in the general population; however, associated comorbidities confer the risk of more severe disease with a poorer prognosis. Regular transfusion therapy leads to a deficit in immune response and, thus, to higher susceptibility to infectious events **(37, 38)**.

REFERENCES

1. Asadov C. Prime Archives in Immunology. Vide Leaf; Santa Monica, CA, USA: 2020. Immunologic Abnormalities in β-Thalassemia; p. 224.8
2. Siwaponanan P., Siegers J.Y., Ghazali R., et al. Reduced PU.1 expression underlies aberrant neutrophil maturation and function in β-thalassemia mice and patients. Blood. 2017;129:3087–3099. doi: 10.1182/blood-2016-07-730135.
3. Ricerca B.M., Di Girolamo A., Rund D. Infections in thalassemia and hemoglobinopathies: Focus on therapy-related complications. Mediterr. J. Hematol. Infect. Dis. 2009;1:e2009028. doi: 10.4084/mjhid .2009.028.
4. Farmakis D., Giakoumis A., Polymeropoulos E et al. Pathogenetic aspects of immune deficiency associated with beta-thalassemia. Med. Sci. Monit. 2003;9:Ra19–Ra22 ;
5. Borgna-Pignatti C., Rugolotto S., De Stefano P., Piga A et al. Survival and disease complications in thalassemia major. Ann. N. Y. Acad. Sci. 1998;850:227–231. doi: 10.1111/j.1749-6632.1998.tb10479.x
6. Olivieri N.F., Brittenham G.M. Iron-chelating therapy and the treatment of thalassemia. Blood. 1997;89:739–761. doi: 10.1182/blood.V89.3.739].
7. Baharlou R., Davami M.H., Ahmadi Vasmehjani A., Ebrahimi M. Increased IL-17 and TGF-β serum levels in peripheral blood of patients with β-thalassemia major: Implication for continual transfusions role in T helper17-mediated proinflammatory responses. [Turk. J. Med. Sci. 2016;46:749–755. doi: 10.3906/sag-1409-57
8. Javad G, Saeid A and Mohammadmehdi N. Thalassemia and immune system dysfunction. International Journal of Current Research Vol. 3, Issue, 12, pp.105-108, December, 2011].
9. Asadov ChD Immunologic Abnormalities in β-Thalassemia. J Blood Disorders Transf 5: 224. 2014. doi: 10.4172/2155-9864.1000224;
10. Elsayh K.I., Mohammed W.S., Zahran A.M., Saad K. Leukocytes apoptosis and adipocytokines in children with beta thalassemia major. Clin. Exp. Med. 2016;16:345–350. doi: 10.1007/s10238-015-0361-].
11. Bazi A., Shahramian I., Yaghoobi H., Naderi M., Azizi H. The Role of Immune System in Thalassemia Major: A Narrative Review. J. Pediatr. Rev. 2017;6:29–36. doi: 10.5812/jpr.14508.
12. Anna Gluba-Brzózka, Beata Franczyk, Magdalena Rysz-Górzyńska et al. Pathomechanisms of Immunological Disturbances in β-Thalassemia. Int J Mol Sci. 2021 Sep; 22(18): 9677. doi: 10.3390/ijms22189677;
13. Ricerca B.M., Di Girolamo A., Rund D. Infections in thalassemia and hemoglobinopathies: Focus on therapy-related complications. Mediterr. J. Hematol. Infect. Dis. 2009;1:e2009028. doi: 10.4084/mjhid. 2009.028;
14. Vento S., Cainelli F., Cesario F. Infections and thalassaemia. Lancet Infect. Dis. 2006;6:226–233. doi: 10.1016/S1473-3099(06)70437-6.].
15. Amer J., Fibach E. Chronic oxidative stress reduces the respiratory burst response of neutrophils from beta-thalassaemia patients. Br. J. Haematol. 2005;129:435–441. doi: 10.1111/j.1365-2141. 2005.05463.x.]
16. Noulsri E., Lerdwana S., Fucharoen S., Pattanapanyasat K. Phenotypic characterization of circulating CD4/CD8 T-lymphocytes in β-thalassemia patients. Asian Pac. J. Allergy Immunol. 2014;32:261–269. doi: 10.12932/ap0426.32.3.2014;

17. Pourgheysari B., Karimi L., Beshkar P. Alteration of T Cell Subtypes in Beta-Thalassaemia Major: Impact of Ferritin Level. J. Clin. Diagn. Res. 2016;10:Dc14–Dc18.doi: 10.7860/JCDR/2016/16094.7272].
18. Bozdogan G., Erdem E., Demirel G.Y., Yildirmak Y. The role of Treg cells and FoxP3 expression in immunity of β-thalassemia major AND β-thalassemia trait patients. Pediatr. Hematol. Oncol. 2010;27:534–545. doi: 10.3109/08880018.2010.503334.].
19. Amirghofran Z. Double-faced cell-mediated immunity in beta-thalassemia major: Stimulated phenotype versus suppressed activity. Ann. Hematol. 2009;88:21–27. doi: 10.1007/s00277-008-0564-y].
20. Al-Awadhi A.M., Alfadhli S.M., Al-Khaldi D., Borhama M., Borusly M. Investigation of the distribution of lymphocyte subsets and zinc levels in multitransfused beta-thalassemia major patients. Int. J. Lab. Hematol. 2010;32:191–196. doi: 10.1111/j.1751-553X.2009.01161.x.].
21. Kyriakou D.S., Alexandrakis M.G., Kyriakou E.S., Liapi D., Kourelis T.V., Passam F., Papadakis A. Activated peripheral blood and endothelial cells in thalassemia patients. Ann. Hematol. 2001;80:577–583. doi: 10.1007/s002770100355.]
22. Ghaffari J., Vahidshahi K., Kosaryan M., Soltantooyeh Z., Mohamadi M. Humoral immune system state in ß thalassemia major. Med. Glas. 2011;8:192–196]
23. Amirghofran Z. Double-faced cell-mediated immunity in beta-thalassemia major: Stimulated phenotype versus suppressed activity. Ann. Hematol. 2009;88:21–27. doi: 10.1007/s00277-008-0564-y].
24. Gharagozloo M., Bagherpour B., Tahanian M., Oreizy F., Amirghofran Z., Sadeghi H.M., Hourfar H., Moayedi B. Premature senescence of T lymphocytes from patients with beta-thalassemia major. Immunol. Lett. 2009;122:84–88. doi: 10.1016/j.imlet.2008.12.003.]
25. Ghatreh-Samani M., Esmaeili N., Soleimani M., Asadi-Samani M., Ghatreh-Samani K., Shirzad H. Oxidative stress and age-related changes in T cells: Is thalassemia a model of accelerated immune system aging? Cent. Eur. J. Immunol. 2016;41:116–124. doi: 10.5114/ceji.2015.56973;
26. Saurwein-Teissi M., Lung T.L., Marx F., Gschösser C., Asch E., Blasko I., Parson W., Böck G., Schönitzer D., Trannoy E., et al. Lack of antibody production following immunization in old age: Association with CD8(+)CD28(-) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. J. Immunol. 2002;168:5893–5899. doi: 10.4049/jimmunol.168.11.5893.]
27. Wanachiwanawin W., Siripanyaphinyo U., Fucharoen S., Wasi P., Mawas F., Wiener E., Wickramasinghe S.N. Activation of monocytes for the immune clearance of red cells in beta zero-thalassaemia/HbE. Br. J. Haematol. 1993;85:773–777. doi: 10.1111/j.1365-2141.1993.tb03222.x.].
28. Singer S.T., Wu V., Mignacca R., Kuypers F.A., Morel P., Vichinsky E.P. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. Blood. 2000;96:3369–3373. doi: 10.1182/blood.V96.10.3369.
29. Taher A.T., Saliba A.N. Iron overload in thalassemia: Different organs at different rates. Hematology Am. Soc. Hematol. Educ. Program. 2017;2017:265–271. doi: 10.1182/asheducation-2017.1.265
30. Walker E.M., Jr., Walker S.M. Effects of iron overload on the immune system. Ann. Clin. Lab. Sci. 2000;30:354–365.
31. Cantinieaux B., Janssens A., Boelaert J.R., Lejeune M., Vermylen C., Kerrels V., Cornu G., Winand J., Fondu P. Ferritin-associated iron induces neutrophil dysfunction in hemosiderosis. J. Lab. Clin. Med. 1999;133:353–361. doi: 10.1016/S0022-2143(99)90066-5.
32. Martins R., Maier J., Gorki A.D., Huber K.V., Sharif O., Starkl P., Saluzzo S., Quattrone F., Gawish R., Lakovits K., et al. Heme drives hemolysis-induced susceptibility to infection via disruption of phagocyte functions. Nat. Immunol. 2016;17:1361–1372. doi: 10.1038/ni.3590]
33. Erdoğan E., Canatan D., Ormeci A.R., Vural H., Aylak F. The effects of chelators on zinc levels in patients with thalassemia major. J. Trace Elem. Med. Biol. 2013;27:109–111. doi: 10.1016/j.jtemb.2012.10.002.]
34. Canatan D, Alkan ŞŞ, editörler. Güncel COVID19 Bilgileri. 1. Baskı. Ankara: Türkiye Klinikleri; 2021. p.39-46
35. Kumari N, Gomber S, Dewan P, et al. COVID-19 Antibody Response in Patients with Thalassemia. Cureus 15(6): e40567. (June 17, 2023) DOI: 10.7759/cureus.40567)
36. Anastasi E, Marco Marziali M, Preziosi A, et al. Humoral immune response to Comirnaty (BNT162b2) SARS-Cov2 mRNA vaccine in Thalassemia Major patients Microbes Infect. 2022 Sep; 24(6): 104976. Published online 2022 Apr 4. doi: 10.1016/j.micinf.2022.104976)
37. De Sanctis V, Canatan D, Corrons JLV. et al. Preliminary data on COVID-19 in patients with hemoglobinopathies: a multicentre icet-a study. *Mediterr J Hematol Infect Dis.* 2020;12(1):e2020046. doi: 10.4084/mjhid.2020.046.
38. Torti L, Sorrentino F, Maffei L, et al. Epidemiological Surveillance of SARSCov2 in β-Thalassemia Patients

in the Last Two Years: Reinfection Rate, Insights and Future Challenges. Mediterr J Hematol Infect Dis. 2023; 15(1): e2023007. Published online 2023 Jan 1. doi: 10.4084/MJHID.2023.007

39. Canatan D, Vives Corrons JL, Piacentini G et al. Immigration and screening programs for hemoglobinopathies in Italy, Spain and Türkiye. Acta Biomed. 2021 Sep 2;92(4):e2021410. doi: 10.23750/abm.v92i4.11965.
DOI: 10.23750/abm.v92i4.11965

Acknowledgment

As the number of refugees from thalassemia endemic regions into Türkiye has increased dramatically ***[39]*** *the present imitative taken by Prof D Canatan, to put together recent findings on management of thalassemia is most appropriate. I congratulate Prof Canatan and thank him very much for including me for this effort as we celebrate 100th years of Turkish Republic. Many thanks to Drs. Mualla Alkan and Yağız Pat for critical reading the manuscript.*

Correspondence Address:
Prof. Dr. Şefik S Alkan
Immunologist, Basel, Switzerland
E-mail: sefik.alkan@gmail.com

CHAPTER 11
NEW METHODS IN DIAGNOSIS AND THERAPY

NEXT GENERATION SEQUENCING AND NEW METHODS IN THE DIAGNOSIS OF THALASSEMIA AND HEMOGLOBINOPTHIES

Prof. Dr Duran Canatan
Antalya Bilim University Faculty Member
President of the Mediterranean Blood Diseases Foundation Antalya-Türkiye
Antalya Genetic Diseases Evaluation Centre Responsible Manager

ABSTRACT

Thalassemia and haemoglobinopathies are the world's most common and public health single-gene diseases, ranging from none to very severe clinical picture, as a result of a significant decrease in the rate of inadequate synthesis of globin chains (α, β, γ, δ) that make up haemoglobins or structural defects in globin chains.

Alpha Thalassaemia syndromes consist of approximately 200 deletions, point mutations and rare large deletion mutations. In beta thalassaemia syndromes, more than 350 mutations have been identified, the majority of which are point mutations, but also rare large gene deletions, dominant β-thalassaemia mutations or unusual conditions of β-thalassaemia. There are currently 1866 known variants of abnormal haemoglobins.

Although conventional and molecular genetic diagnostic methods are used to identify such a wide range of molecular variations, targeted panel tests, whole exome analysis and whole genome analysis methods have been used in recent years, which allow rapid, multiple and high-throughput detection of genetic variants with next generation sequencing (NGS) method.

NGS technologies have achieved the capacity to sequence the entire human genome with ultra-high throughput, scalability and speed, a level not possible using Sanger sequencing technology. Although the Sanger Sequencing Method is still considered the "Gold Standard" in variant screening, NGS technologies have achieved the capacity to sequence the entire human genome with ultra-high efficiency, scalability and high speed.

The aim of this review is to review the new genetic methods used in the postnatal genetic diagnosis of thalassaemia and haemoglobinopathies, as well as the new genetic methods used in pre-implantation genetic diagnosis and non-invasive prenatal diagnosis.

Keywords: Thalassaemia, haemoglobinopathy, diagnosis, next generation sequencing, new methods

INTRODUCTION

Thalassaemia and haemoglobinopathies lead to ineffective erythropoiesis by damaging erythrocyte precursors or haemolytic anaemia by damaging mature erythrocytes as a result of a significant reduction in the rate of inadequate synthesis of the globin chains (α, β , γ, δ) that make up haemoglobins or structural defects in globin chains. Haemoglobinopathies lead to severe vasoclusive problems and organ destruction, as in Sickle Cell Anaemia (SCA), or polycythemia, or cyanosis, or unstable abnormal haemoglobins without causing any problem **(1)**. Alpha Thalassaemia syndromes are caused by three groups of mutation types including approximately 200 deletion and point mutations and rare large deletion mutations **(2)**. In beta thalassaemia syndromes, more than 350 mutations have been identified, the majority of which are point mutations, as well as rare large gene deletions, dominant

β-thalassaemia mutations or unusual conditions of β-thalassaemia **(3)**.

Gene modifiers affecting β-thalassaemia are important for the patient's phenotype. Primary modifiers determine the phenotypic severity associated with the location of mutations in different gene regions affecting HBB (β0, β+, β++). Secondary modifiers are gene expression affecting the amount and stability of α-globin chains that disrupt the α/β globin balance, and the effect of genes involved in γ-globin gene expression on HBS1L-MYB, BCL11A, KLF1 and C1orf77. Tertiary modifiers are gene variations such as VDR and HFE which affect the complications of patients with β-thalassaemia **(4)**. There are 1866 variants of abnormal haemoglobins known today. Although the most common and problematic ones are Hb S, E, D, C and O-Arab, new variants are defined as abnormal haemoglobins which cause polycythemia, cyanosis, haemolytic anaemia or are unstable without causing any problem. In order to identify such a wide range of molecular variations, besides conventional and molecular genetic diagnostic methods, next generation sequencing (NGS) has been used in recent years as target gen panel tests (GPT) or whole exome analysis (WES) and whole genome analysis (WGS), which allow rapid, multiple and high throughput detection of genetic variants **(5, 6)**. The aim of this review is to review new genetic methods used in preimplantation genetic diagnosis and non-invasive prenatal diagnosis, as well as new methods used in postnatal genetic diagnosis of thalassaemia and haemoglobinopathies.

CLINICAL INDICATIONS FOR USING NEW METHODS

Clinical indications for laboratory tests requested to investigate potential Hb variants are generally as follows **(7)**:

- Unexplained thalassaemia phenotype
- Family history compatible with the Hb variant
- Routine neonatal testing for common haemoglobinopathies (e.g., HbS, HbC, thalassaemias),
- Cyanosis due to inadequate oxygenation without cardiopulmonary disease
- Erythrocytosis with normal or elevated erythropoietin levels
- Unexplained haemolytic anaemia

NEXT GENERATION SEQEUNCING METHOD

NGS technologies have achieved the capacity to sequence the entire human genome at a level of ultra-high throughput, scalability and speed not possible using Sanger sequencing technology. Most NGS platforms have three general steps; first, library preparation using random fragmentation of DNA followed by ligation with specialised linkers. Second, library amplification using clonal amplification methods and Polymerase Chain Reaction (PCR). Third, sequencing using the incorporation of fluorescently labelled nucleotides by DNA polymerases or ligation processes.

NGS has provided researchers with the possibilities to identify complex diseases through WES, WGS or GPT. The targeted NGS approach is designed to cover the entire regions encoding globin genes, their essential regulatory regions and modifier genes such as KLF1, BCL11A, HBS1L and MYB **(8, 9)**. WGS and WES are important for the identification of non-coding causative mutations that may cause disruption of transcriptional factor occupancy sites and cis-regulatory elements **(10)**.

In order to address the question of whether it is necessary to validate NGS data with Sanger Sequence analysis, Sanger-based validation of NGS variants was studied using data obtained from the ClinSeq® project with a large-scale study and a systematic evaluation. In general, the validation rate of NGS variants using Sanger sequencing method was found to be 99.96%, thus it was emphasised that the validation of variants found in NGS using Sanger sequencing is very limited and leads to unnecessary time and economic loss **(11)**.

Various molecular techniques are used for point mutation detection in β-thalassaemia and large deletion detection in α-thalassaemia. All of these techniques have some advantages and disadvantages. Recently, screening of both α-thalassaemia and β-

thalassaemia genes with NGS has been introduced. With this technique, thalassaemias that cannot be diagnosed with other conventional techniques can be diagnosed. The biggest limitation of the use of NGS in thalassaemia screening is that its cost is still expensive **(12, 13)**.

THIRD GENERATION SEQUENCING METHOD IN ALPHA THALASSAEMIA

In a study demonstrating the importance of NGS and Third Generation Sequencing (THR) analysis in the diagnosis of alpha-thalassaemia, PCR-based reverse dot blotting (PCR-RDB) was used to identify known variants. Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) were used for unknown variants. As a confirmation test, NGS and UND were performed, and a new α-thalassemia deletion was identified in two families by UND and its importance in expanding the molecular spectrum of α-thalassemia was emphasised **(14)**.

SMRT METHOD IN ALPHA THALASSAEMIA

The α-globin fusion gene between the HBA2 and HBAP1 genes is of clinical importance in thalassaemia screening because this fusion gene can cause severe HbH when fused with α0-thalassaemia (α0-thal). Due to the rare rearrangement in the α gene cluster without dosage change, this fusion gene is difficult to detect by common molecular testing approaches used for the diagnosis of α-thal. One study used single molecule real-time (SMRT) sequencing to detect this fusion gene in 23 carriers identified by NGS among 16,504 screened individuals. According to YND results, 14 of 23 carriers were pure heterozygous, 8 were compound heterozygous and 1 was homozygous. Using SMRT, the fusion mutant was successfully detected in all 23 carriers, and SMRT identified one of the two "pure" heterozygotes as a compound heterozygote with anti-3.7 triplication and the other as a homozygote. The results emphasised the importance of SMRT in α fusion gene detection due to its efficient, accurate and one-step features **(15)**.

NGS IN DIAGNOSIS OF BETA THALASSAEMIA

The discovery and identification of new and rare pathogenic thalassaemia variants makes it possible to better prevent the disease, especially in areas of high prevalence.

In one study, in a Chinese thalassaemia family, blood samples were first screened by haematological analysis and clinical routine genetic screening. Then, HGP and Sanger sequencing used in NGS were performed to find and identify a novel deletion variant. The deletion identified by HGP was confirmed by real-time quantitative polymerase chain reaction (qPCR). It was emphasised that NGS analysis has the potential to find rare and new thalassemia mutants **(16)**.

NGS IN DIAGNOSIS OF FUSION (ΕΓΔΒ) THALASSEMIA

NGS used by genomics laboratories is well suited for the detection of small sequence changes but is more important for the detection of structural variants (SV), mainly due to its relatively short sequence reads. Because the SV inversion/deletion causing εγδβ-thalassaemia was identified using base pair resolution NGS and a powerful bioinformativ approach. The combined NGS and bioinformatics strategy proved to be highly successful and applicable to routine diagnoses **(17)**.

NGS IN DIAGNOSIS OF RARE HAEMOGLOBINOPATHIES

Unstable haemoglobinopathies (UHs) are rare anaemia disorders characterised by abnormal haemoglobin variants with reduced stability. UHs therefore precipitate easily, causing haemolysis and in some cases leading to dominant beta-thalassaemia (dBTHAL). The clinical picture of UHs is highly heterogeneous, with a predominant inheritance pattern rather than recessive as in the more common Hb syndromes.

Most cases of UH are not detected by conventional tests, so the diagnosis requires a high level of suspicion from the treating physician. In this situation, NGS is important for the diagnosis of patients with

dBTHAL and other less severe UH variants. Five clinical cases presenting with chronic haemolytic anaemia, three of which were severe blood transfusion-related anaemia, were studied. HGP was performed in three cases and TEA in two cases. Five different UH variants were identified in relation to the clinical symptoms of the patients. Four variants in beta-globin (Hb Bristol-Alesha, Hb Debrousse, Hb Zunyi and the new Hb Mokum) and one variant in the alpha-globin gene causing Hb Evans were identified. NGS has been proposed for the definitive diagnosis of severe forms leading to UH variants, especially dBTHAL **(18)**.

NGS IN PRE-MARITAL SCREENING

In a study to investigate the feasibility of combining Gap PCR with NGS for thalassaemia carrier screening in the Chinese population, blood samples were collected from 944 couples before pregnancy. Thalassaemia carrier screening was performed using a combination of routine haematological method, Gap PCR and NGS. It has been suggested that the combined Gap-PCR and NGS method is a cost-effective method especially in identifying α thalassaemia mutation carriers **(19)**.

NGS IN NEWBORN SCREENING

With the aim of screening thalassaemia in newborns, heel blood samples of 206 newborns were collected on dried papers and DNA was extracted from these samples and NGS analysis was performed. In a total of 206 newborns, mutations were detected in 22 cases including 11 α thalassaemia and 11 β thalassaemia. 7 of 11 α thalassaemia cases had 64% (7/11) deletions (4 αα/-α(3.7), 2 αα/-SEA, 1 αα/-α(4.2)) and the remaining 4 cases had point mutations (4/11, 36%): Hb Part-Dieu hybrid, Hb Quong Sze hybrid, Hb Westmead hybrid, HBA1: C. 95 + 9 c > T. In all 11 cases of β-thalassaemia, 7 different point mutations were detected. NGS technology has been published for use in newborn heel blood samples. It has been emphasised that the advantages of this method include easy sampling, obtaining definite results and its widespread use in clinical diagnosis, thus it is important for early diagnosis of thalassemia disease **(20)**.

ADDITIONAL METHODS IN NEXT GENERATION SEQUENCING

NGS4THAL

We developed NGS4THAL, a bioinformatics analysis pipeline that analyses NGS data to detect pathogenic variants for thalassemia and other haemoglobinopathies. NGS4THAL rearranged ambiguously mapped NGS reads in homologous Hb gene clusters for accurate detection of point mutations and small deletions and duplications. A combination of complementary SV and in-house control data database containing specific SVs was used to ensure accurate detection of complex SV types. Detected variants were matched against those in HbVar (Human Haemoglobin Variants and Thalassaemia Mutations Database), allowing recognition of known pathogenic variants, including disease modifiers. Tested on simulation data, NGS4THAL achieved high sensitivity and specificity.

Comparison with previously diagnosed pathogenic Hb variants in the laboratory showed that the targeted NGS sequencing data were 100% accurate. NGS4THAL has been published as a highly accurate and effective molecular diagnostic tool for thalassaemia and other haemoglobinopathies based on specific analysis of NGS data **(21)**.

NGS + Gap PCR

Thalassaemia syndromes result from complex mechanisms, including copy number variants (CNVs) and single nucleotide variants (SNVs), CNV types of thalassaemia are typically detected by Gap PCR. SNV types are detected by Sanger sequencing.

A new method that detects CNVs and SNVs simultaneously with Gap PCR and NGS has been developed **(22)**.

In a study conducted in China, blood samples from 15,807 individuals were screened by haematological tests and 3973 suspected thalassaemia carriers were found, and these samples were analysed by combined NGS and Gap-PCR. In the findings, 943 α-thalassaemia carriers, 708 β-thalassaemia carriers and 53 combined α and β-thalassaemia carriers were diagnosed as carriers (10.78%), and the rates

of α-thalassaemia, β-thalassaemia and combined α and β-thalassaemia were 5.97%, 4.48% and 0.34%, respectively. In addition, 19 α-thalassaemia variations and 21 β-thalassaemia variations were identified in thalassaemia carriers. It was emphasised that approximately 2.88% of thalassemia carriers could be missed by conventional genetic analysis, and therefore, the combination of NGS and Gap-PCR is an effective thalassemia screening method **(23)**.

In another study conducted in Vietnam, 5880 pregnant women were screened with a combination of NGS and Gap-PCR and the data showed that thalassaemia carriage was higher than previously reported. The combination of NGS and Gap-PCR was found to be an effective and large-scale thalassaemia screening method that can detect a wide range of mutations **(24)**.

COMPARISON OF NGS WITH TRADITIONAL DIAGNOSTIC METHODS

A comprehensive NGS test has greatly facilitated the screening and diagnosis of haemoglobin disorders. An NGS panel targeting the coding regions of haemoglobin genes and four modifier genes was designed.

In the carrier screening test consisting of a total of 20,222 people, haematological tests and NGS were performed at the same time, haematological tests showed that 3565 people were carriers, while NGS was positive in 4180 people. Cases who were positive in haematological tests were screened with conventional genetic tests and 2506 people were found positive and 1059 people were found negative. NGS test was performed in negative subjects and mutations were found in 186 subjects. 12.1% of pathogenic or probable pathogenic variants not identified by conventional methods were found. This study was published as the first large-scale population study systematically evaluating the application of NGS technique in molecular carrier screening and diagnosis of haemoglobinopathies **(25)**.

Another comparative study was conducted in pregnant women. Whole blood, haemoglobin electrophoresis, conventional thalassaemia gene screening and NGS were performed from the peripheral blood of 2858 pregnant women to evaluate the diagnostic value of NGS in screening for thalassaemia genes. The rate of missed diagnosis of α-thalassaemia and β-thalassaemia using NGS was 0.87% and 1.59%, respectively, while the rate of missed diagnosis of α-thalassaemia and β-thalassaemia using conventional screening models was 26.77% and 2.38%, respectively. The area under the ROC curve for α-thalassaemia and β-thalassaemia in the NCD was 0.994 and 0.991, respectively, while the area under the ROC curve for α-thalassaemia and β-thalassaemia screening with the conventional screening model was 0.866 and 0.988, respectively.

The sensitivity, missed diagnosis rate, Youden index and negative predictive value of α-thalassaemia and β-thalassaemia screening using NGS were found to be significant compared to conventional screening. NGS has shown that it has accurate and diagnostic value for thalassaemia screening and can be widely applied in the clinic **(26)**.

NEW METHODS IN PREIMPLANTATION GENETIC DIAGNOSIS

MDA and MALBAC method

A study aimed to evaluate the performance of two whole-genome amplification methods, multiple displacement amplification (MDA) and multiple annealing and loop-based amplification cycling (MALBAC), for β-thalassemia genotyping and single nucleotide polymorphism (SNP). As a method, MALBAC and MDA were performed on single-cell and five-cell samples from peripheral blood and discarded embryos. Differences in amplification efficiency, positive predictive value, sensitivity, allele dropout (ADO) rate, SNPs and CV values between the two methods were determined. Through Sanger sequencing at the single-cell and five-cell levels, it was shown that both the amplification speed and ADO ratio of MDA were better than those using MALBAC, and the sensitivity and positive predictive value obtained from MDA were higher than those obtained from MALBAC. In β-thalassaemia genotyping, using NGS at the single-cell level, MDA was confirmed to have better characteristics than MALBAC for SNP detection. How-

ever, MALBAC was found to be more stable and homogeneous than MDA using low-depth NGS at the single-cell level for CNV detection. It was concluded that MALBAC is a better option for CNV detection, while MDA is more suitable for SNV detection ranking **(27).**

Long Term Reading method

Another study aimed to evaluate long-term read sequencing for preimplantation haplotype linkage analysis. As a method, genetic material from three β-thalassemia mutation carrier pairs was sequenced using single-molecule real-time sequencing in the 7.7 kb region and partially overlapping 7.4 kb region of the HBB gene. Haplotypes consisting of 17 HBB gene mutations common in the Chinese population and a continuous sequence of single nucleotide polymorphisms associated with these mutations were analysed. Using the same method to analyse the multiple substitution amplification products of embryos from three families and comparing the results with those of the parents, it has been published that embryos can be found to carry disease-causing mutations without the need for a probe.

The HBB gene mutations of three pairs were accurately detected and the haplotype linked to the pathogenic site was successfully obtained without the need for a probe. A total of 68.75% (22/32) of embryos from the three families were successfully haplotype linkage analysed and were in agreement with the results of NGS-based mutation site detection.

This study supports long-read sequencing as a potential tool for pre-implantation haplotype linkage analysis **(28)**.

NGS METHOD IN NON INVASIVE PRENATAL DIAGNOSIS

With the discovery of extracellular free DNA (cf-DNA) circulating in maternal plasma, Non Invasive Prenatal Screening (NIPT) method can be used for the typing of all anoploidies, especially chromosome 21, 18, 13 and gonadal aneuploidies, as well as microdeletion, microduplication anomalies and RHD.

Studies are ongoing to test autosomal recessive disorders based on NGS analysis in NIPT. Diagnosis of haemoglobinopathies by NIPT is facilitated by methods such as targeted probe capture, haplotype analysis and development of bioinformative methods in order for the NGS method to provide healthier results **(29, 30, 31)**.

NEW AND INNOVATIVE METHODS IN NON INVASIVE PRENATAL TESTING

Haplotype-assisted analysis:

Invasive prenatal diagnosis (IPD) of thalassaemia syndromes is performed by sequence analysis MLPA, Gap PCR and reverse dot blot (RDB) analysis from fetal DNA obtained by invasive methods from chorionic villi, amnion or cord blood.

Successful results were obtained with the haplotype-assisted analysis method for the NIPT method in thalassaemia. A total of eight families with proband children with thalassaemia were included in the study during the next pregnancy. The sequence of thalassaemia genes of parents and probands was determined using NGS based on the thalassaemia AmpliSeq panel. The cfDNA from probands and pregnant women was analysed using the target gene platform (HGP).

Fetal haplotype has been confirmed using Gap PCR and RDB from parental haplotypes and invasively sampled amniotic fluid. A non-invasive prenatal diagnosis procedure from maternal plasma fetal DNA has been successfully developed based on haplotype analysis.

The haplotypes of eight foetuses were found to be identical to the IPD results with 100% accuracy. In this study, it was emphasised that NIPT is also important in the diagnosis of α and β thalassaemia by using haplotype-supported NGS analysis **(32)**.

Another study in Cyprus analysed ten maternal plasma samples from at-risk pregnancies, selecting four single nucleotide polymorphisms (SNPs) at the β-globin locus that show a high degree of heterozygosity in the Cypriot population. NIPT was performed in eight of the ten families by haplotype analysis. It was reported that NGS was effective in

detecting alleles transferred from the father to the foetus in maternal plasma **(33)**.

Father mutation analysis

A study in South East Asia aimed to detect paternal mutations using NGS in maternal plasma samples. The method used in this study extracted DNA from maternal plasma from 83 families in which both parents were carriers of the HbE mutation or one of the four common β-thalassaemia mutations. PCR amplicons covering each mutation were generated, pooled and sequenced using Illumina MiSeq, and Fastq files were analysed to detect inheritance of the paternal mutation.

In two cases where the fathers were compound heterozygous for HbE and -28A>G, the fetus was correctly diagnosed as inheriting one of the paternal mutations, with an overall sensitivity of 100% and specificity of 92.1% for the detection of paternal mutations. In conclusion, it has been shown that the detection of paternal mutations using NGS can be easily detected with high sensitivity and specificity and eliminates the need for invasive testing in 50% of pregnancies at risk of thalassaemia **(34)**.

Probe nested PCR approach analysis

The aim of this study was to develop and evaluate a non-invasive prenatal diagnosis approach for haemoglobinopathies using cfDNA in maternal plasma. Couples presenting for prenatal diagnosis of haemoglobinopathies with different maternal and paternal mutations were included in the study. As a method, peripheral blood was collected from the mother at different periods of pregnancy and fetal DNA was isolated before invasive fetal sampling. Nested polymerase chain reaction (PCR)-based protocols were developed to detect the presence or absence of the paternal mutation.

Overall, of the 30 pairs in which the parental mutations differed, no paternal mutation was found in 14 of the 30 pairs and a mutation was found in 16 cases. Using cfDNA from maternal plasma, the absence of a paternal mutation was correctly identified in 12 of 14 cases and the presence of a paternal mutation was correctly identified in 12 of 16 cases. Although the nested PCR approach makes it possible to amplify small amounts of cfDNA from maternal plasma at different gestational periods, it was possible to accurately diagnose the presence or absence of a paternal mutation in the foetus **(35)**.

Droplet Digital PCR analysis

In this study, we developed two NIPT assays based on an innovative and sensitive droplet digital PCR (ddPCR) technology to identify the two most common β-thalassaemia mutations in the Mediterranean region (β+IVSI-110 and β039), either maternal or paternal. As a result, a simple and sensitive diagnostic tool based on ddPCR was published for NIPT of β+IVSI-110 and β039 mutations, which can be applied to other single mutations inherited from the father and, for the first time, from the mother **(36)**.

Foetal NRBC analysis

The aim of this study was to evaluate and compare the diagnostic value of two noninvasive diagnostic methods for fetal thalassaemia using cf-DNA and nucleated RBCs (NRBCs) in a group of pregnant women. As a method, 10 ml of blood was collected in two k3EDTA tubes from 32 pregnant women whose partners and themselves were thalassaemia carriers. One tube was used for NRBC enrichment and the other for cf-DNA extraction.

NRBCs were isolated by MACS and immunohistochemistry, and the genome of stained cells was amplified by multiple displacement amplification (MDA). The beta-globin segments of these products were used as templates in PCR. cf-DNA was extracted by THP method and 300 bp areas were obtained from agarose gel as fetal DNA. This DNA was used as a template in touch down PCR to amplify the beta-globin gene.

Amplified beta-globin results were compared with CVS results. The data showed that the sensitivity and specificity of thalassaemia diagnosis with NRBC was 100% and 92%, respectively, while the sensitivity and specificity of thalassaemia diagnosis with cf-DNA was 100% and 84%, respectively. As a result, it was concluded that these methods with

high sensitivity could be used as screening tests but could not be used as diagnostic tests because their specificity was lower than CVS **(37)**.

Dense parallel alignment

The aim of this study was to identify fetal Sickle Cell Anaemia (SCA) for NIPT by a targeted massively parallel sequencing (MPS) method using cfDNA from maternal plasma, without the need for paternal or proband samples. A total of 64 women were included in the study, of whom 42 were OHA carriers, 15 were homozygous for OHA and seven were compound heterozygous (Hb SC). The clinical sensitivity and specificity of the NIPT results were 100% and 100%, respectively. This study demonstrated the suitability of NIPT for clinical use for OHA **(38)**.

Relative Mutation dosage analysis

A feasibility study was conducted in an antenatal clinic to assess whether HGP and relative mutation dosage (GMD) could be used to accurately identify β-thalassaemia mutations in cf-DNA. The study included couples in which both partners were carriers of β-thalassaemia or an HbE mutation and therefore at risk of carrying a β-thalassaemia-affected foetus.

As a method, 49 samples previously identified as inheriting a paternal β-thalassaemia mutation were amplified using nested polymerase chain reaction followed by sequencing. GMD was used to classify the new variant or mutant allele of the foetus from the mother.

As a result, the maternally inherited allele was detected by GMD in 48 out of 49 samples (98.0%) and fetal diagnosis was made in 44 out of 48 samples (91.7%). Therefore, the overall sensitivity and specificity for inheritance of the maternal genotype was 87.5% and 95.8%, respectively.

It has been emphasised that GMD analysis for the determination of the inheritance of maternal β-thalassemia mutations has an economic potential for clinical use, and it will be applicable to other single gene disorders **(39)**.

COMPARING NIPT AND IPD METHODS

One study aimed to use HPG analysis from cf-DNA to determine the fetal genotype in pregnant women at risk of Hb Bart hydrops fetalis. A total of 192,173 couples from 30 hospitals were included in the study, 878 couples (pregnant women and their partners) were found to be carriers of α-thalassaemia of the Southeast Asian type (-/ααα). All pregnant women underwent NIPT and IPD. In IPD, samples obtained by chorionic villus, amniocentesis or cordocentesis were evaluated by Gap PCR. HPG was used in NIPT. As a result, the sensitivity and specificity of the NIPT method were found to be 98.81% and 94.72%, respectively, and 100% and 99.31%, respectively, in the IPT set. In addition, a complete sample of mothers with Southeast Asian carriers was diagnosed with 100% sensitivity and 99.89% specificity at T13, T18 and T21. The importance of the method for both genotyping α-thalassaemia in a tube blood sample and early screening of foetal aneuploidies in the chromosome has been emphasised **(40)**.

Another study aimed to compare paternal beta-thalassaemia mutations in maternal cf-DNA with conventional IPT using ARMS and RT-PCR. A total of 26 couples with a history of children with beta-thalassaemia were selected and routine CVS and cf-DNA sample results were compared. When compared with conventional PCR, 11 cases (84.6%) were successfully matched, while two cases (15.4%) were not matched with conventional IPT. In conclusion, it was emphasised that the low amount of fetal DNA in maternal plasma is a limiting factor and further improvements are needed for enrichment of fetal cf-DNA to ensure full compatibility with conventional IPD **(41)**.

In another study, IPD and NIPT were compared in 102 pregnant women using re-sequencing technology (cSMRT). 29 homozygotes or compound heterozygotes, 54 heterozygotes and 19 normally homozygotes were identified by IPT. When NIPT was compared with IPT, 99 of 102 foetuses (97%) were identical on NIPT. Overall, the sensitivity and specificity of the NIPT test were 100% and 97.26%, respectively. This study demonstrated that the cSMRT-based NIPT test for β-thalassaemia is an

alternative to IPT for pregnant haemoglobinopathy carrier couples **(42)**.

In conclusion; new variants are added to the currently known 1866 variants of thalassaemia and abnormal haemoglobins every day. In order to identify such a wide range of molecular variations, besides conventional and molecular genetic diagnostic methods, NGS method has gained great importance in recent years.

With NGS, targeted panel tests, whole exome and whole genome analysis methods are used for rapid, high-throughput detection of genetic variants.

NGS methods have a very important place in the diagnosis and prevention of haemoglobinopathies in community screening, prenatal period, newborn screening, preimplantation genetic diagnosis and non-invasive prenatal screening with new techniques.

REFERENCES

1. Canatan D. α, β, δ and γ thalassaemias and related conditions . Diagnosis of Haemoglobinopathy (Translation Editor: Karakaş Z.) Barbara J. Bain: Diagnosis of Haemoglobinopahy Third Edition, 2020 John Wiley & Sons Ltd. pages 85-184
2. Farashi S, Harteveld CL. Molecular basis of α-thalassemia Blood Cells Mol Dis.2018;70:43-53.
3. Cao A and Galanello, R. Beta-thalassemia. Genet Med.2010:12(2):61-76.
4. Jaing TH, Chang TY, Chen SH, et.al. Molecular genetics of β -thalassemia: a narrative review. Medicine 2021; 100:45(e27522).
5. Clark BE, Shooter C, Smith F, Brawand D et. al. Next-generation sequencing as a tool for breakpoint analysis in rearrangements of the globin gene clusters. Review Int J Lab Haematol.2017 May;39 Suppl 1:111-120. doi: 10.1111/ijlh.12680.
6. Peng C, Zhang H, Ren J et.al. Analysis of rare thalassemia genetic variants based on third-generation sequencing. Sci Rep. 2022 Jun 14;12(1):9907. doi: 10.1038/s41598-022-14038-8.
7. Thom CS, Dickson CF, Gell DA, Weiss MJ. Haemoglobin variants: biochemical properties and clinical correlates. Cold Spring Harb Perspect Med. 2013 Mar 1;3(3):a011858. doi: 10.1101/cshperspect.a011858.
8. Munkongdee T, Chen P, Winichagoon P, Fucharoen F, Paiboonsukwong K. Update in Laboratory Diagnosis of Thalassemia Front Mol Biosci. 2020; 7:74. doi: 10.3389/fmolb.2020.00074. eCollection 2020.
9. Shang X, Peng Z, Ye Y, et. al. Rapid Targeted Next-Generation Sequencing Platform for Molecular Screening and Clinical Genotyping in Subjects with Haemoglobinopathies EBio Medicine. 2017; 23:150-159.
10. Russo R, Marra R, Rosato BE, Iolascon A, Andolfo I. Genetics and Genomics Approaches for Diagnosis and Research Into Hereditary Anaemias. Review Front Physiol. 2020; 11:613559.
11. Beck TF, James C. Mullikin JC and Biesecker LG. Systematic Evaluation of Sanger Validation of Next-Generation Sequencing Variants. Clinical Chemistry (2016) 62:(4)647-654.
12. Munkongdee T, Chen P, Winichagoon P. Update in Laboratory Diagnosis of Thalassemia Front Mol Biosci. 2020 May 27; 7:74. doi: 10.3389/fmolb.2020.00074. eCollection 2020.
13. Achour A, Koopmann TT, Baas F et.al.The Evolving Role of Next-Generation Sequencing in Screening and Diagnosis of Haemoglobinopathies. Front Physiol. 2021 Jul 27; 12:686689. doi: 10.3389/fphys.2021.686689. eCollection 2021.
14. Xu R, Li H, Yi S.et.al. Identification of a novel 10.3 kb deletion causing α0-thalassemia by third-generation sequencing: Pedigree analysis and genetic diagnosis. Clin Biochem. 2023 Mar; 113:64-69. doi: 10.1016/j.clinbiochem.2022.12.018. Epub 2023 Jan 4.
15. Zhou QM, Jiang F, Xu J. High accuracy of single-molecule real-time sequencing in detecting a rare α-globin fusion gene in carrier screening population. Ann Hum Genet. 2023 Mar;87(1-2):9-17. doi: 10.1111/ahg.12486. Epub 2022 Nov 1.
16. Yin ZZ, , Yao J Wei FX et al. Targeted Next-Generation Sequencing Reveals a Large Novel β-Thalassemia Deletion that Removes the Entire HBB Gene. Haemoglobin. 2022 Sep;46(5):290-295. doi: 10.1080/03630269.2022.2145964. Epub 2022 Nov 22.
17. Clark BE, Shooter C, Smith F et.al. Next-generation sequencing as a tool for breakpoint analysis in rearrangements of the globin gene clusters. Int J Lab Haematol. 2017 May;39 Suppl 1:111-120. doi: 10.1111/ijlh.12680.
18. Rizzuto V, Koopmann TT, Blanco-Álvarez A,. Usefulness of NGS for Diagnosis of Dominant Beta-Thalassemia and Unstable Haemoglobinopathies in Five Clinical Cases. Front Physiol. 2021 Feb 5; 12:628236. doi: 10.3389/fphys.2021.628236. eCollection 2021.
19. Zhao J, Li J, Qiaohong Lai Q et.al. Combined use of gap-PCR and next-generation sequencing improves thalassaemia carrier screening among premarital

adults in China. J Clin Pathol. 2020 Aug;73(8):488-492. doi: 10.1136/jclinpath-2019-206339. Epub 2020 Jan 24.

20. Tan M, Lu S, Wu LS et.al.Application of Next Generation Sequencing to Screen the Neonatal Thalassemia Genes. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2015 Oct;23(5):1404-9. doi: 10.7534/j.issn.1009-2137.2015.05.035.
21. Cao Y, Ha SY, So CC et.al. NGS4THAL, a One-Stop Molecular Diagnosis and Carrier Screening Tool for Thalassemia and Other Haemoglobinopathies by Next-Generation Sequencing. J Mol Diagn. 2022 Oct;24(10):1089-1099. doi: 10.1016/j.jmoldx.2022.06.006. Epub 2022 Jul 19.
22. Fan DM,Yang X, Huang LM et.al.Simultaneous detection of target CNVs and SNVs of thalassemia by multiplex PCR and next generation sequencing Mol Med Rep. 2019 Apr;19(4):2837-2848. doi: 10.3892/mmr.2019.9896. Epub 2019 Jan 24.
23. Zhang H, Li C, Li J et.al. Next-generation sequencing improves molecular epidemiological characterisation of thalassemia in Chenzhou Region, P.R. China J Clin Lab Anal.2019 May;33(4):e22845. doi: 10.1002/jcla.22845. Epub 2019 Feb 27.
24. 23.Lam TT, Nguyen DT, Le QT et.al Combined Gap-Polymerase Chain Reaction and Targeted Next-Generation Sequencing Improve α- and β-Thalassemia Carrier Screening in Pregnant Women in Vietnam. thal).Hemoglobin.2022 Jul;46(4):233-239. doi: 10.1080/03630269.2022.2096461. Epub 2022 Aug
25. Shang X, Peng Z, Ye Y et.al. Rapid Targeted Next-Generation Sequencing Platform for Molecular Screening and Clinical Genotyping in Subjects with Haemoglobinopathies EBioMedicine. 2017 Sep; 23:150-159. doi: 10.1016/j.ebiom.2017.08.015. Epub 2017 Aug 17.
26. Wang K, Dang YH, Li ZL et.al. Clinical Significance of Screening Thalassemia with High- Throughput Sequencing. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2019 Aug;27(4):1220-1226. doi: 10.19746/j. cnki.issn.1009-2137.2019.04.037.
27. He F, Zhou W, Cai R et.al. Systematic assessment of the performance of whole-genome amplification for SNP/CNV detection and β-thalassemia genotyping. J Hum Genet. 2018 Apr;63(4):407-416. doi: 10.1038/s10038-018-0411-5. Epub 2018 Feb 13.
28. Wu H, Chen D, Zhao Q et.al. Long-read sequencing on the SMRT platform enables efficient haplotype linkage analysis in preimplantation genetic testing for β-thalassemia. J Assist Reprod Genet. 2022 Mar;39(3):739-746. doi: 10.1007/s10815-022-02415-1. Epub 2022 Feb 9.
29. Erlich HA, López-Peña C, Carlberg KT et.al. Noninvasive Prenatal Testing for β-Thalassemia and Sickle Cell Disease Using Probe Capture Enrichment and Next-Generation Sequencing of DNA in Maternal Plasma J Appl Lab Med. 2022 Mar 2;7(2):515-531. doi: 10.1093/jalm/jfab118.
30. Wang W, Yuan Y, Zheng H et.al. A Pilot Study of Noninvasive Prenatal Diagnosis of Alpha- and Beta-Thalassemia with Target Capture Sequencing of Cell-Free Fetal DNA in Maternal Blood Genet Test Mol Biomarkers. 2017 Jul;21(7):433-439. doi: 10.1089/gtmb.2016.0411. Epub 2017 May 24.
31. Zhang J, Li J, Saucier JB, et.al. Non-invasive prenatal sequencing for multiple Mendelian monogenic disorders using circulating cell-free fetal DNA.Nat Med. 2019 Apr;25(4):701-702. doi: 10.1038/s41591-019-0391-9.
32. Yang X, Ye Y, Fan D et.al. Non invasive prenatal diagnosis of thalassemia through multiplex PCR, target capture and next generation sequencing. Mol Med Rep. 2020 Aug;22(2):1547-1557. doi: 10.3892/mmr.2020.11234. Epub 2020 Jun 15.
33. Papasavva T, J van Ijcken WF, Kockx CEM et.al. Next generation sequencing of SNPs for non-invasive prenatal diagnosis: challenges and feasibility as illustrated by an application to β-thalassaemia. Eur J Hum Genet. 2013 Dec;21(12):1403-10. doi: 10.1038/ejhg.2013.47. Epub 2013 Apr 10.
34. Xiong L, Barrett AN, Hua R. Non-invasive prenatal diagnostic testing for β-thalassaemia using cell-free fetal DNA and next generation sequencing. Prenat Diagn. 2015 Mar;35(3):258-65. doi: 10.1002/pd.4536. Epub 2014 Dec 29.
35. D'Souza E, Sawant PM,Nadkarni AH et.al. A Gorakshakar, K Ghosh, Detection of fetal mutations causing haemoglobinopathies by non-invasive prenatal diagnosis from maternal plasma.J Postgrad Med. 2013 Jan-Mar;59(1):15-20. doi: 10.4103/0022-3859.109483.
36. D'Aversa E, Breveglieri G, Boutou E et.al. Droplet Digital PCR for Non-Invasive Prenatal Detection of Fetal Single-Gene Point Mutations in Maternal PlasmaAffiliations expand. Int J Mol Sci. 2022 Mar 4;23(5):2819. doi: 10.3390/ijms23052819.
37. Shafei N, Hakhamaneshi MS, Houshmand M et.al. Diagnostic Value of Non-Invasive Prenatal Screening of β-thalassemia by Cell Free Fetal DNA and Fetal NRBC. Curr Mol Med. 2019;19(2):105-111. doi: 10.2174/1566524019666190226124135.
38. Campen JV,Silcock L,Yau S et.al. A novel non-invasive prenatal sickle cell disease test for all at-risk pregnancies Br J Haematol. 2020 Jul;190(1):119-124. doi: 10.1111/bjh.16529. Epub 2020 Feb 25.

39. Xiong L Barrett AN, R Hua R et.al. Non-invasive prenatal testing for fetal inheritance of maternal β-thalassaemia mutations using targeted sequencing and relative mutation dosage: a feasibility study BJOG. 2018 Mar;125(4):461-468. doi: 10.1111/1471-0528.15045. Epub 2018 Jan 9.
40. Yang J, Peng CF, Qi Y et.al.Noninvasive prenatal detection of haemoglobin Bart hydrops fetalis via maternal plasma dispensed with parental haplotyping using the semiconductor sequencing platform. Am J Obstet Gynecol. 2020 Feb;222(2):185.e1-185.e17. doi: 0.1016/j.ajog.2019.07.044. Epub 2019 Aug 5.
41. Afzal M, Naeem MA, Ahmed S et al. Noninvasive prenatal testing of beta-thalassemia for common Pakistani mutations: a comparative study using cell-free fetal DNA from maternal plasma and chorionic villus sampling Haematology.2022 Dec;27(1):353-359. doi: 10.1080/16078454.2022.2045052.
42. Lv W, Linpeng S, Liang D, et.al. Noninvasive prenatal diagnosis for pregnancies at risk for β-thalassaemia: a retrospective study. BJOG.2021 Jan;128(2):448-457. doi: 10.1111/1471-0528.16295. Epub 2020 May 28.

Correspondence Address:
Prof. Dr Duran Canatan
Paediatric Haematology and Genetics Specialist
Antalya Bilim University, Antalya, Türkiye
E-mail: duran.canatan@Antalya.edu.tr
durancanatan@gmail.com
Tel: +90.532.3641373

NEW TREATMENT METHODS FOR THALASSEMIA

Antonis Kattamis
Thalassemia Unit, First Department of Pediatrics, National and Kapodistrian University of Athens
The 'Aghia Sophia' Children's Hospital European Reference Network- EuroBloodNet Center,
Athens, Greece

ABSTRACT

Over the last years, different agents have been studied mainly focusing on the improvement of the hematological status of thalassemic patients. These agents are targeting different aspects of the pathogenetic mechanisms that are involved in thalassemia. Luspatercept was approved for marketing as the first in class pharmaceutical agent targeting ineffective erythropoiesis. It was shown that it can decrease transfusion burden in transfusion dependent thalassemia and improve the levels of hemoglobin in non-transfusion dependent patients. Other agents are in advanced stages of clinical development. The allosteric activators of pyruvate kinase have shown great promises in patients with hemoglobinopathies, including sickle cell anemia and thalassemia. Different agents targeting the disrupted iron metabolism have been studied, but the results, so far, have not verified the initial expectations, which were based in preclinical positive results.

Keywords: Novel agents, thalassemia, luspatercept, pyruvate kinase

INTRODUCTION

The pathogenesis of the thalassemia is complex and involves significant α/β- globin chain imbalance, extensive ineffective erythropoiesis, increase hemolysis, and deranged iron regulation.

Over the last years significant advances in the understanding of the pathogenesis of thalassemia have taken place. All this new knowledge has been transcribed to many attempts to develop new pharmaceutical agents. These agents are targeting different stages of the deranged erythropoietic development and the increased hemolytic susceptibility of the thalassemic red blood cells. In this chapter, we will review the new agents that have been developed, which more likely will be changing the way thalassemia is managed in the very near future **(1)**.

AGENTS TO IMPROVE INEFFECTIVE ERYTHROPOESIS

Studies have shown that the ligands of activin receptor IIB (ActRIIB), mainly the transforming growth factor-β (TGF-β) molecules, are involved in modulating the differentiation of late-stage erythrocyte precursors independently from erythropoietin. Blocking ActRIIB by using ligand traps led to improvement in hematological indexes, bone marrow density, and splenomegaly in β-thalassemic mice. The exact mechanisms by which these traps exert its full effects in β-thalassemia are yet to be fully understood. The first-in-class of these ligand traps is Luspatercept, a recombinant fusion protein consisting of a modified form of the extracellular domain of the human ActRIIB linked to the human immunoglobulin G1 fragment crystallizable domain. Luspatercept was evaluated in a Phase 3, randomized, double-blind, placebo-controlled, multicentre 'BELIEVE' study in transfusion-dependent β-thalassemic (TDT) patients.

The results of the BELIEVE (NCT02604433) study showed significant clinical benefit across multiple parameters, with the primary endpoint of a decrease ≥ 33% in transfusion burden from baseline during weeks 13-24 being reached by 21.4% of the luspatercept-treated compared to 4.5 % of placebo-treated patients **(2, 3)**. Overall, during the first 24 weeks of the trial, mean transfusion burden changes in the Luspatercept group was −2.20 RBC units compared with +0.72 units in the placebo group.

The change was heterogeneous and more pronounced in patients with less severe genotypes **(4, 5)**. Results from the extension phase trial have shown a sustained reduction in transfusion burden. Of note, similar changes in transfusion burden in β^0/β^0 patients compared with the full population for both endpoints of ≥ 33% and of ≥ 50% reductions during any 12-week interval were observed and with splenectomised patients showing a more robust response **(4, 6)**. The safety profile of Luspatercept was manageable, with the main adverse events being bone pain, arthralgia, dizziness, hypertension, and hyperuricemia. Of note, 3.6% of the patients compared to 0.9% in placebo-treated patients experienced a clinically confirmed thromboembolic event, while an increase in extramedullary hematopoietic sites was also rarely noted, emphasizing the need for close monitoring **(2)**.

Luspatercept has also been showed to be effective in non-transfusion-dependent β-thalassemia (NTDT) based on the results of BEYOND trial **(7)**. BEYOND (NCT03342404) was a phase 2, double-blind, randomized (2:1), placebo-controlled, multicentre trial evaluating the efficacy and safety of luspatercept in 145 adults (≥18 years) patients with NTDT. The trial met its primary endpoint with 74/96 (77.1%) of patients in the luspatercept arm vs 0/49 placebo patients achieving erythroid response, defined as an increase from baseline of ≥1.0 g/dL in mean hemoglobin level over a continuous 12-week interval during weeks 13-24, in the absence of transfusions. Fifty (52.1%) patients in the luspatercept group had a mean hemoglobin increase of at least ≥1.5 g/dL. It was notable that none of the patients developed thromboembolic event **(8, 9)**.

Luspatercept (Reblozyl®) is currently approved for the treatment of anemia in adult patients with β-thalassemia who require regular red blood cell transfusions (US Food and Drug Administration - FDA in 2019 and European Medicines Agency - EMA in 2020) and for the treatment for adult patients with anemia associated with NTDT (EMA, March 2023). It is administered subcutaneously every 3 weeks at a starting dose of 1mg/kg Body Weight (B.W.) which can be titrated up to 1.25 mg /kg B.W. Real-life data on the use of Luspatercept are slowly accumulating. These data are essential to establish its efficacy and toxicity profile in non-selected TDT patients outside the clinical setting of a study. Ongoing trials are evaluating its use in pediatric patients with thalassemia and in patients with α-thalassemia including transfusion and non-transfusion-dependent.

Increased erythropoietin levels in thalassemia drive erythroblast proliferation through JAK2-STAT5 pathway signalling. In preclinical studies, JAK2 inhibition resulted in improvement in splenomegaly in thalassemic patients **(10)**. Treatment with **Ruxolitinib**, a JAK1/2 inhibitor, in a small study of 30 TDT patients, resulted in sustained decrease in spleen size but in no clinically relevant changes in pre-transfusion hemoglobin levels or transfusion requirements. Thus, further development of this drug for thalassemia is not planned **(11)**.

AGENTS TO IMPROVE GLOBIN CHAIN IMBALANCE

Pharmacological reactivation of γ-globin expression can substitute for the reduced β-globin production and improve the globin chain imbalance in β-thalassemia. **Thalidomide** is an oral synthetic glutamic acid derivative with anti-inflammatory, anti-angiogenic, and immunomodulatory effects, which also increases fetal hemoglobin production through incompletely understood mechanisms. Several case series and small cohort studies have demonstrated some clinical benefits with a manageable toxicity profile, apart from the known teratogenicity. Randomized, controlled clinical trials are needed to better understand the safety and efficacy of the drug, but it may have a role in treatment, especially in resource-limited settings **(12, 13)**.

AGENTS TO DECREASE HEMOLYSIS

Thalassemic red blood cells (RBC) have substantially increased metabolic oxidative stress, leading to increased hemolysis and early cell death. Increasing the erythrocytic ATP levels will counterbalance the augmented intracellular energy demands and decrease the vulnerability of the thalassemic erythrocytes. The allosteric activator of pyruvate kinase, mitapivat, has been shown increases erythrocytic

ATP levels and improves both ineffective erythropoiesis and RBC survival in preclinical thalassemic models **(14)**. Data have recently become available from an open-label, multicentre, phase 2 study of mitapivat in 20 adults with NTDT and a hemoglobin level of ≤10 g/dL evaluating safety and efficacy in achieving a hemoglobin increase by ≥1.0 g/dL. Sixteen (80%) patients had an erythroid response (5/5 in α-thalassemia and 11/15 in β-thalassemia) **(15)**. Mitapivat is currently studied in phase 3 clinical trials for TDT (ENERGIZE-T, NCT04770779) or NTDT (ENERGIZE- NCT04770753). A phase 2 clinical trial with the use a second pyruvate kinase activator, Etavopivat, is also currently ongoing (NCT04987489) **(16)**.

AGENTS TO IMPROVE DERANGED IRON METABOLISM

Hepcidin which is produced mainly in the liver, serves as the master regulator of iron homeostasis. Hepcidin reduces iron absorption, circulation and availability. Iron homeostasis is deranged in thalassemia, with hepcidin levels being inappropriately low compared to the iron load. In thalassemic murine models, restoring iron homeostasis, either by increasing the hepcidin levels or enhancing its action mainly by blocking the cellular iron exporter ferroportin has been shown to restrict splenomegaly and improve erythropoiesis and anemia without interfering with iron chelation **(17-23)**. Different agents targeting iron dysregulation have been or are being evaluated. Studies with synthetic human hepcidin LJPC-401 failed to reach an optimistic endpoint of decreasing cardiac siderosis in TDT patients. The use of the hepcidin mimetic PTG-300 showed prolonged suppression of iron parameters in TDT, but studies were not advanced past phase 2 **(24)**. Because the subcutaneous injection of hepcidin caused a decrease in serum iron levels at 8 hours post-dose but returned to baseline levels within 48 hours and even rebounded after that. Clinical trials are currently evaluating two ligand-conjugated agents, an anti-sense oligonucleotide (IONIS-TMPRSS6-LRx) and a small interfering RNA (SLN124), which induce hepatic hepcidin production by targeting the metalloprotease, transmembrane serine protease 6 (TMPRSS6) **(17-23)**. Studies are also ongoing with the oral agent (VIT-2763) targeting ferroportin, the unique cellular iron exporter and receptor of hepcidin **(17-23, 25)**. Initial observations from this class of agents did not suggest a robust efficacy that can lead to a dramatic change in hematological status of the thalassemic patients.

CONCLUSIONS

The therapeutic options for thalassemic patients have evolved significantly over the last decades. The understanding of the pathogenesis of the disease is guiding the advent of new therapies. Luspartercept is the first novel agent to enter clinical practice and can lead to improvement in transfusion burden and anemia in thalassemic patients. Other agents are currently under different stages of development. Nevertheless, data on long-term efficacy and safety for these new agents and ways to reach universal accessibility for these novel therapies are of major significance. The future for the management of thalassemia looks brighter.

REFERENCES

1. Kattamis A, Kwiatkowski JL, Aydinok Y. Thalassaemia. Lancet 2022; 399: 2310-2324.
2. Cappellini MD, Viprakasit V, Taher AT et al. A Phase 3 Trial of Luspatercept in Patients with Transfusion-Dependent beta-Thalassemia. N Engl J Med 2020; 382: 1219-1231.
3. Cappellini MD, Taher AT, Piga A et al. Health-Related Quality of Life Outcomes for Patients with Transfusion-Dependent Beta-Thalassemia Treated with Luspatercept in the Believe Trial. Blood 2020; 136: 8-9.
4. Taher AT, Viprakasit V, Hermine O et al. Sustained Reductions in Red Blood Cell (RBC) Transfusion Burden and Events in β-Thalassemia with Luspatercept: Longitudinal Results of the Believe Trial. Blood 2020; 136: 45-46.
5. Cappellini MD, Hermine O, Piga A et al. Assessment of response to luspatercept by β-globin genotype in adult patients with β-thalassemia in the BELIEVE trial Hemasphere 2020; 4: 108
6. Hermine O, Cappellini MD, Taher AT et al. Longitudinal Effect of Luspatercept Treatment on Iron Overload and Iron Chelation Therapy (ICT) in Adult Patients (Pts) with β-Thalassemia in the Believe Trial. Blood 2020; 136: 47-48.
7. Piga A, Perrotta S, Gamberini MR et al. Luspatercept improves hemoglobin levels and blood transfusion

requirements in a study of patients with beta-thalassemia. Blood 2019; 133: 1279-1289.
8. Taher AT, Cappellini MD, Kattamis A et al. The BEYOND study: results of a phase 2, double-blind, randomized, placebo-controlled multicenter study of luspatercept in adult patients with non-transfusion dependent beta-thalassemia. Hemasphere 2021; 5: 1.
9. Musallam KM, Taher AT, Kattamis A et al. Profile of Luspatercept in the Treatment of Anemia in Adults with Non-Transfusion-Dependent beta-Thalassemia (NTDT): Design, Development and Potential Place in Therapy. Drug Des Devel Ther 2023; 17: 1583-1591.
10. Libani IV, Guy EC, Melchiori L et al. Decreased differentiation of erythroid cells exacerbates ineffective erythropoiesis in beta-thalassemia. Blood 2008; 112: 875-885.
11. Taher AT, Karakas Z, Cassinerio E et al. Efficacy and safety of ruxolitinib in regularly transfused patients with thalassemia: results from a phase 2a study. Blood 2018; 131: 263-265.
12. Chandra J, Parakh N, Sidharth et al. Efficacy and Safety of Thalidomide in Patients With Transfusion-Dependent Thalassemia. Indian Pediatr 2021; 58: 611-616.
13. Nag A, Radhakrishnan VS, Kumar J et al. Thalidomide in Patients with Transfusion-Dependent E-Beta Thalassemia Refractory to Hydroxyurea: A Single-Center Experience. Indian J Hematol Blood Transfus 2020; 36: 399-402.
14. Matte A, Federti E, Kung C et al. The pyruvate kinase activator mitapivat reduces hemolysis and improves anemia in a beta-thalassemia mouse model. J Clin Invest 2021; 131.
15. Kuo KHM, Layton DM, Lal A et al. Safety and efficacy of mitapivat, an oral pyruvate kinase activator, in adults with non-transfusion dependent alpha-thalassaemia or beta-thalassaemia: an open-label, multicentre, phase 2 study. Lancet 2022; 400: 493-501.
16. Kattamis A. An energy booster for thalassaemic red blood cells. Lancet 2022; 400: 470-471.
17. Nyffenegger N, Flace A, Doucerain C et al. The Oral Ferroportin Inhibitor VIT-2763 Improves Erythropoiesis without Interfering with Iron Chelation Therapy in a Mouse Model of beta-Thalassemia. Int J Mol Sci 2021; 22.
18. Manolova V, Nyffenegger N, Flace A et al. Oral ferroportin inhibitor ameliorates ineffective erythropoiesis in a model of beta-thalassemia. J Clin Invest 2019; 130: 491-506.
19. Casu C, Oikonomidou PR, Chen H et al. Minihepcidin peptides as disease modifiers in mice affected by beta-thalassemia and polycythemia vera. Blood 2016; 128: 265-276.
20. Gardenghi S, Ramos P, Marongiu MF et al. Hepcidin as a therapeutic tool to limit iron overload and improve anemia in beta-thalassemic mice. J Clin Invest 2010; 120: 4466-4477.
21. Schmidt PJ, Toudjarska I, Sendamarai AK et al. An RNAi therapeutic targeting Tmprss6 decreases iron overload in Hfe(-/-) mice and ameliorates anemia and iron overload in murine beta-thalassemia intermedia. Blood 2013; 121: 1200-1208.
22. Casu C, Aghajan M, Oikonomidou PR et al. Combination of Tmprss6- ASO and the iron chelator deferiprone improves erythropoiesis and reduces iron overload in a mouse model of beta-thalassemia intermedia. Haematologica 2016; 101: e8-e11.
23. Aleku M, Chivers S, Gille H et al. Non-clinical safety of sln124, a GalNAC conjugated 19-mer double-stranded sirna targeting TMPRSS6 facilitating evaluation in clinical studies. Hemasphere 2021; 5: 391.
24. Lai A, Voskaridou E, Flevari P et al. A hepcidin mimetic, PTG-300, demonstrates pharmacodynamic effects indicating reduced Iron availability in transfusion-dependent beta-thalassemia subjects. Hemasphere 2020; 4: 110.
25. Porter J, Taher A, Viprakasit V et al. Oral ferroportin inhibitor vamifeport for improving iron homeostasis and erythropoiesis in beta-thalassemia: current evidence and future clinical development. Expert Rev Hematol 2021; 1-12.

Correspondence Address:
Prof. Dr. Antonis Kattamis
Thalassemia Unit, First Department of Pediatrics, National and Kapodistrian University of Athens.
The 'Aghia Sophia' Children's Hospital European Reference Network- EuroBloodNet Center,
Athens, Greece
E-mail: ankatt@med.uoa.gr

CHAPTER 12
STEM CELL TRANSPLANTATION AND GENE THERAPY

HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THALASSEMIA

Prof. Dr. Osman Alphan Küpesiz, Assoc. Prof. Dr. Funda Tayfun Küpesiz
Akdeniz University School of Medicine
Dept. of Pediatrics, Pediatric Hematology Oncology Unit Antalya-Türkiye

ABSTRACT

Life expectancy of patients diagnosed with thalassemia major has increased with the developments in recent years. However, the increase in their quality of life was limited. Patients who may reach their fifties must still continue their lives dependent on hospitalization and transfusion. The only curative treatment method is hematopoietic stem cell transplantation. With the updating of supportive care and treatment protocols during the transplantation process, there have been significant decreases in morbidity and mortality rates. More than 1300 transplants are reported in our country. In this section, the section on stem cell transplantation in thalassemia patients is reported.

Keywords: Beta thalassemia major, hematopoietic stem cell transplantation

INTRODUCTION

Thalassemia, one of the most common hemoglobinopathies, is a serious public health problem for many countries. For these patients who survive with transfusion support and chelation treatments, the most common curative treatment option that is available to patients and supported by health authorities within the treatment spectrum extending to gene therapy is allogeneic hematopoietic stem cell transplantation (HSCT). It has been reported that more than 3000 allogeneic HSCTs have been performed for thalassemia worldwide **(2)**. The increase in transplantation experience and modern transplantation approaches have improved HSCT outcomes. As long-term life expectancy has been achieved for patients, improving the quality of life has become a focal point.

Pre-Transplantation Risk Factors

It has been shown that bone marrow transplantation outcomes in thalassemia patients are related to the patient's age and disease status at the time of HSCT. The Pesaro group developed a scoring system that predicts transplant outcomes with three independent prognostic factors identified for pediatric patients younger than 17 years of age (hepatomegaly, fibrosis status of the liver, and patients receiving effective chelation therapy) **(3, 4)**. According to these risk factors, patients are evaluated in three risk groups as shown in Table 1. Extensive Pesaro experience has shown that a thalassemia-free survival (TFS) is 85-90%, 80% and 65-70% for patients belonging to class 1, 2 and 3 groups, respectively, while the probability of transplant-related mortality (TRM) gradually increases from class 1 to class 3.

The Pesaro classification has been validated in children who received adequate chelation therapy before transplantation. Mathews et al. defined a high-risk subgroup in the Pesaro classification by identifying new prognostic factors (patient age and liver size) that affect transplant outcomes for children who cannot receive effective chelation therapy before transplantation **(1)**. European Society for Blood and Marrow Transplantation (EBMT) group analyzed 1493 transplanted thalassemia patients between 2000 and 2010, and they found the overall survival (OS) rate to be over 90% and the thalassemia-free survival (TFS) rate to be over 83% in patients transplanted before the age of 14, indicating that the ideal age limit for HSCT is 14 **(5)**. In a study evaluating 1469 patients who underwent transplantation in 25 pediatric centers in Türkiye, OS and TFS rates were found to be significantly higher in patients who underwent SCT before the age of seven **(6)**.

Table 1: *Pesaro Risk Classification [3, 4]*

Pesaro Risk factors

1. Hepatomegaly (more than 2 cm at the costal margin)
2. Hepatic fibrosis (in pre-transplant biopsies)
3. Iron chelation (those receiving regular iron chelation starting within 18 months after the first transfusion)

Pesaro Risk Groups

Low Risk Patients (Class I): No risk factors
Moderate Risk Patients (Class II): Those with risk factors 1 and 2
High Risk Patients (Class III): Those with all 3 risk factors.
(Class III)→ ***Very high risk group*** [1]
Typically ≥ 7 years old and Hepatomegaly ≥ 5 cm

Because patients in China do not accept liver biopsy, Li et al. defined the NF-08-TM classification, in which patient age, extent of hepatomegaly and serum ferritin level are considered prognostic markers, as an alternative to Pesaro scoring **(7)** **(Table 2)**.

Table 2: *NF- 08- TM classification [7]*

Group	Ferritin (mg/L)	Hepatomegaly (cm)	Age (years)
Low Risk	<3000	<2.5	<4
Moderate Risk	Those who are not in the low or high risk groups		
High Risk	> 5000	>4	>8

Donor selection

The first experiences with thalassemia patients undergoing allogeneic HSCT began with transplants using bone marrow-derived stem cells from human leukocyte antigen (HLA)-matched sibling donors (MSDs) and continued in the 1990s with the transplants performed by Lucarelli et al. with matched family donors (MFD) **(3)**. In these transplantations, where busulfan – cyclophosphamide (Bu-Cy) based conditioning regimens were used, OS and event-free survival (EFS) rates were above 90% and 80%, considering the Pesaro risk classification **(3, 8)**.

The percentage of patients who can find a matched family donor varies between 25% and 30% **(9)**. The first large series with matched unrelated donors (MUD) was by La Nasa et al. **(10)**. In their other series, the same group continued to improve the success of transplantation results by first adding thiotepa (TT) to the Bu-Cy regimens and then adding fludarabine (Flu) **(11, 12)**. The results obtained by Hongeng et al. in MFD and MUD transplantations were comparable **(13)**. Haploidentical transplantation is an option for patients who do not have a suitable donor, and haploidentical HSCT results are satisfactory in studies **(14-16)**.

Stem Cell Source

While bone marrow-derived stem cells were used in the first transplants performed in thalassemia patients, the use of peripheral blood-derived stem cells began with MUD transplantation **(9)**. Li et al., in their study presenting the results of transplantation using MUD and peripheral blood stem cells, stated that the frequency of acute graft versus host disease (GVHD) was comparable to the literature **(7)**. If an insufficient number of bone marrow-derived stem cells are collected due to the high weight difference between the recipient and donor, peripheral blood stem cells can be used as a stem cell source if volunteer donors prefer peripheral blood stem cell collection in MUD transplants. In β-TM, which is a benign disease, the preferred stem cell source is the bone marrow to avoid the risk of chronic GVHD. The use of peripheral blood stem cells also has advantages such as faster engraftment and lower risk of graft failure. Although the results are successful in studies using irrelevant cord blood as a stem cell source, the use of cord blood is not preferred as a stem cell source due to risks such as delayed engraftment and graft failure **(2, 17)**.

Conditioning Regimen

In TM, a benign hematological disease, the transplant conditioning regimen is structured to prevent graft failure. Due to the hyperplasia of the bone marrow and the presence of alloimmunization in these patients, a myeloablative conditioning regimen containing busulfan (Bu) and cyclophosphamide (Cy) has been accepted as the gold standard for years. Although good engraftment and graft function were achieved with this regimen, drug and dose changes were made in the search for a conditioning regimen with lower toxicity due to toxic adverse effects. Reducing the dose of cyclophosphamide from 200 mg/kg to 160-120 mg/kg reduced cardiotoxicity but increased the frequency of graft rejection. Therefore, fludarabine (Flu) and thiotepa (TT) were added to prevent graft rejection in patients whose Cy dose was reduced.

Busulfan has high hepatotoxicity and poses a particular risk for sinusoidal obstruction syndrome. Treosulphan is a structural analogue of Bu and is a drug with such potent myeloablative/immunosuppressive activity but lower extramedullary toxicity. The use of treosulphan in the conditioning regimen has been investigated as an alternative to Bu. However, it has been observed that the risk of mixed chimerism is higher in patients using treosulphan than in those using busulfan **(14, 18)**.

Treatment adjustments (dose reduction in Cy, use of treosulphan instead of Bu) have improved OS and thalassemia-free survival (TFT) by reducing transplant-related mortality (TRM) rates.

Graft versus Host Disease Prophylaxis

The most important factor determining the quality of life after transplantation in hemoglobinopathies is GVHD. Calcineurin inhibitors (CNIs) and short-term methotrexate (MTX) therapy (days +1, +3, +6, and +11) are usually used for GVHD prophylaxis. Cyclosporine A (CsA) is used primarily as a CNI. However, in some centers, tacrolimus is preferred because it may be stronger. The risk of graft rejection and the detrimental effect of graft-versus-host disease (GVHD) in patients with thalassemia cannot be ignored. The incidence of both graft failure and GVHD was reported to be lower with the addition of antithymocyte globulin (ATG) to the Bu/Cy conditioning regimen **(18, 19)**.

Table 3: *Historical data of HSCT characteristics in thalassemia major patients* [9]

Time	Transplantation feature	Conditioning regimen
1980s	BM MSD/ MFD	Bu, Cy
2004	BM MSD/ MFD	P 26
2005	BM MUD	Bu, Cy Bu, Cy,TT
2006	CB/ BM/ PSC MFD	Bu, Cy±ATG Bu, Flu, Cy, ATG, TLI
2008	BM MSD/ MFD	Treo, TT, Flu, ATG
2011	PSC (T cell depletion) Haploidentical	Hydroxyurea, Azat, Bu, Flu, Cy,TT ATG,
2012	PSC MUD	Hydroxyurea, Azat, Bu, Flu, Cy,TT ATG,
2013	BM RD	P 26.1
2021	PSC MUD	PTIS, HU Bu, Flu, Cy, TT, TBI, ATG, PTCy

ATG: *Anti-thymocyte globin,* ***Bu:*** *Busulfan,* ***Cy:*** *Cyclophosphamide,* ***Flu:*** *Fludarabine,* ***HU:*** *Hydroxyurea,* ***BM:*** *Bone marrow,* ***CB:*** *Cord blood,* ***MFD:*** *Matched family donor,* ***MSD:*** *Matched sibling donor,* ***MUD:*** *Matched unrelated donor,* ***P26:*** *Protocol 26,* ***PSC:*** *Peripheral stem cell,* ***PTCy:*** *Posttransplant cyclophosphamide,* ***PTIS:*** *Pretransplant immunosuppressive,* ***TBI:*** *Total body irradiation,* ***TLI:*** *Total lymphocyte irradiation*
Treo: *Treosulphan,* ***TT:*** *Thiotepa*

Post-transplant follow-up

Chimerism

Approximately 10% of transplants performed in patients with thalassemia result in stable mixed chimerism. In TM patients, even a small amount of donor hematopoietic cells can provide adequate erythropoiesis, saving the patient from transfusion dependence (2). There is no consensus yet on the factors that cause mixed chimerism in these patients. In their study where they analyzed transplantation data that resulted in mixed chimerism in 38 of 144 thalassemia pediatric patients transplanted between 2009 and 2012, Chen et al., like other previous studies, showed that MSD use was an independent risk factor for the development of stable mixed chimerism **(19, 20)**. The minor HLA compatibility between MSDs and recipient is higher than the compatibility between MUD donor and recipient. This causes immunotolerance, preventing immune-mediated destruction of recipient cells and may lead to mixed chimerism.

Another independent risk factor is the conditioning regimen used. Suppression of recipient T lymphocytes and recipient antigen-presenting cells by using immunosuppressive treatments before transplantation is important in achieving complete chimerism. Chen et al. found that, in addition to MSD and conditioning regimen, the rate of mixed chimerism was higher in transplants where ABO group compatible donors were used and the TNC count was below $10x10^8$/kg **(19)**.

It is known that donor lymphocyte infusion (DLI) is effective in increasing chimerism in patients who develop mixed chimerism. DLI seems to be more effective if it is performed before the patient's hemoglobin decreases, that is, before the donor cell rate decreases further **(19, 21)**. By activating T-reg, dendritic cells, and natural NK cells with cytokine therapy in patients who can not undergo DLI, the effectiveness of IL-2 and INF α treatments, which are based on creating immunotolerance between recipient and donor cells, did not reach the desired level **(19)**.

Survival

In the transplant experience forty years ago, busulfan and cyclophosphamide formed the basis of the conditioning regimen. The overall survival of transplants performed with this regimen was 81% and TS was 75%. Graft failure rates were high due to high alloimmunization brought about by transfusions. It was necessary to develop immunosuppression and myeloablative regimens in the pre-transplant period.

In the last 30 years, conditioning regimen changes have been made, taking into account the frequent transfusion in thalassemia and other additional diseases. Accordingly, the best results are provided by the use of bone marrow as stem cell source, selection of fully matched siblings, and the conditioning regimen applied according to the patient. In transplantations performed in this way, OS is achieved over 90% and TS is over 80%. The fact that treosulphan is less toxic and causes less veno-occlusive disease, the use of cyclophosphamide in lower doses, the addition of ATG/alemtuzumab to the regimen, and the inclusion of fludarabine and thiotepa in the protocol have a direct impact on the increase in survival.

Table 4: *Main studies describing conditioning regimens for HSCT in beta thalassemia*

Author	Year	Country	Number (N)	Median Age (years)	Risk Group	Conditioning Regimen	GVHD Prophylaxis	Stem cell	Donor Type	aGVHD I–II/ III–IV %	cGVHH %	TRM %	Graft failure	GS %	EFS %
Lucarelli [3]	1990	Italy	116	10.5	I-39 II-36 III-24	Bu 3,5 mg/kg/gx4, Cy 50 mg/kg/gx4	CSA, MTX, Cy	BM	MFD	4.3	0.8	5.1 19.4 37.5	0 8.3 16	94 80 61	94 77 53
Lucarelli [8]	1998	Italy	393	<17	I-121 II-272	Bu 3,5 mg/kg/gx4, Cy 50 mg/kg/gx4	CSA	BM	MFD	Ø	Ø	5 15	5 4	95 85	90 81
Lucarelli [8]	1998	Italy	125	<17	III	Bu 3,5 mg/kg/gx4, Cy 30-40 mg/kg/gx4	CSA, MTX	BM	MFD	Ø	Ø	19	33	78	54
La Nasa [10]	2002	Italy	32	14	I-4 II-11 III-17	Bu 4 mg/kg/gx4, Cy 30–50 mg/kg/gx4; Bu 4 mg/kg/gx4, TT 10 mg/kg, Cy 30-50 mg/kg/gx4	CSA, MTX	BM	MUD	41	25	19	12.5	79	66
Lawson [22]	2003	England	55	6.4	I-17 II-27 III-11	Bu 3,5–4 mg/kg/gx4, Cy 30–50 mg/kg/gx4, ± Flu ± ATG	CSA, MTX	Ø	MFD	84/ 15	16	5.4	13.2	94.5	81.8
La Nasa [11]	2005	Italy	68	15	I-14 II-16 III-38	Bu 4 mg/kg/gx4, Cy 50 mg/kg/gx4; Bu 4 mg/kg/gx4, TT 10 mg/kg, Cy 50 mg/kg/gx4; Bu 4 mg/kg/gx4, TT 10 mg/kg, Flu 40 mg/m^2/gx4	CSA, MTX	BM	MUD	40	18	20	13	79.3	65.8

Hongeng [13]	2006	Thailand	28	7.2	I-15 II-III13	Bu 4 mg/kg/gx4, Cy 50 mg/kg/gx4 ± ATG 40 mg/kg; Bu 8 mg/kg, Flu 175 mg/m^2, Cy 50 mg/kg/gx4, ATG 20 mg/kg, TLI 500 cGy	CSA, TCM, MTX, MTP	BM, PSC, CB	MFD	32/ 11	14	14.3	14	92	82
Hongeng [13]	2006	Thailand	21	4	I-13 II-III18	Bu 4 mg/kg/gx4, Cy 50 mg/kg/gx4 ± ATG 40 mg/kg	CSA, TCM MTX	BM	MUD	43/ 14	14	7.1	14	82	71
La Nasa [12]	2007	taly	45	33	I-14 II-18 III-13	Bu 3,5 mg/kg/gx4 TT 10 mg/kg Cy 50 mg/kg gx4 or Cy 60 mg/kg/gx2; Bu 3,5 mg/kg/gx4, TT 10 mg/kg Flu 40 mg/m^2/gx4	CSA-MTX	BM	MUD	44	Ø	13.3	15.6	86.7	71.4
La Nasa [12]	2007	Italy	53	12	I-18 II-21 III-14	Bu 3,5mg/kg/gx4, Cy 30-50mg/kg/gx4; Bu 3,5 mg/kg/gx4, TT 10 mg/kg, Flu 40 mg/m^2/gx4	CSA-MTX	BM	MUD	30	Ø	11.3	15.1	88.7	73.6
Bernardo [18]	2008	taly	20	13	I-7 II-4 III-9	Treo 14 g/m^2/ gx3, TT 8 mg/kg, Flu 40, mg/m^2/gx4, ATG 10 mg/kg/gx3	CSA, MTX	BM	MFD, MUD	10/ 5	5	5	10	95	85

Caocci [14]	2011	taly	28	10	II-4 III-24	Treo, TT, Flu; Bu, Cy Bu, TT, Cy	CSA-MTX	Ø	MFD, HAPLO MUD	21/ 14	18	10.9	14.3	89.3	78.6
Sodani [15]	2011	taly	31	Ø	Ø	HU 60 mg/kg/gx50; Azat 3 mg/kg/gx48, Flu 30 mg/m^2/gx5, Bu 3 mg/kg/gx4, Cy 50 mg/kg/gx4, TT 10 mg/kg, ATG 12,5 mg/kg/gx4	CSA	TCD PSC	HAPLO	0	0	6.5	22.5	93.5	71
Li [7]	2012	China	30	6	II	HU 30 mg/kg/g, Azat 3 mg/kg/g, Bu 2,8–4,4 mg/kg/gx 4, Cy 60 mg/kg/gx2, Flu 40 mg/m^2/ gx4, TT 10 mg/kg, ATG 15–30 mg/kg/gx4 or TG 2,5 mg/kg/gx4	CSA, MFM, MTX	BM, CB-BM	MFD	0/ 3.6	0	10	6.9	90	83.3
Li [7]	2012	China	52	6	II	HU 30 mg/kg/g, Azat 3 mg/kg/g, BU 2,8–4,4 mg/kg/gx 4, Cy 60 mg/kg/gx2, Flu 40 mg/m^2/ gx4, TT 10 mg/kg, ATG 15–30 mg/kg/gx4 or TG 2,5 mg/kg/gx4	CSA, MFM, MTX	PSC	MUD	0/ 9.6	0	7.7	1.9	92.3	90.4

Bernardo [23]	2012	taly	60	7	I-27 II-17 III-4	Treo 14 g/m^2/ gx3, TT 8 mg/kg, Flu 40mg/m^2/gx4, ATG 10 mg/kg/gx3	CSA, MTX	BM, PSC, CB	MFD, MUD	7/ 7	2	6.6	9	93	84
Mathews [1]	2013	ndia	193	>11	III-139 III c-54	Bu 4 mg/kg/gx4, Cy 50 mg/kg/gx4, ATG 30 mg/kg/gx3; Bu 150 mg/m^2/gx4, Cy 50 mg/kg/gx4	CSA, MTX	BM, PSC	MFD, MUD	44	18	28	12	53.6 39.4	57.3 32.4
Mathews [1]	2013	ndia	74	>11	III-50 III c-24	TT 8 mg/kg, Treo 14 g/m^2/gx3, Flu 30 mg/m^2/gx4	CSA, MTX	BM, PSC	MFD, MUD	35	11	12	8	37.4 36.6	78.8 77.8
Gaziev [24]	2013	taly	16	9.6	I-5 II-5 III-10	P26.1	CSA, MTX, PND, CY	BM	MRD	19/ 13	13	6	0	94	94
Gaziev [24]	2013	taly	66	10	II-31 III-35	Bu 3,5 mg/kg/gx4, Cy 50 mg/gx4± TT; Bu 3,5 mg/kg/gx4, Cy 50 mg/gx4; HU 30 mg/kg/g, Azat 3 mg/kg/g, Flu 20 mg/m ^{2}, Bu 3,5 mg/kg/gx4, Cy 22,5–40 mg/gx4 ± TT	CSA, MTX, PND, PTCy	BM	MSD	36/ 7	11	8	12	92	82

Anurathapan 25]	2014	Thailand USA	22	17	III [c]	PTIS [b] x2, Flu 35 mg/m^2/gx6, Bu 130 mg/m^2/gx4, ATG 1.5 mg/kg/gx3	CSA, TCM, BM, MFM PSC	MFD, MUD	9/ 14	18	9	0	90	93
Anurathapan 16]	2016	Thailand	31	10.1	I-7 II-9 IIIc-15	PTIS[b]x2, Flu 35 mg/m^2/gx6, Bu 130 mg/m^2/gx4, ATG 1.5 mg/kg/gx3	CY, TCM or TCD-SIL, MFM PSC	HAPLO	29/3.2	16.1	3.2	3.2	95	94
Caocci 26]	2017	taly	258	12	I-57 II-83 III-21	Bu- Cy 30–50 mg/kg/gx4; Bu –Cy 30–50 mg/kg/gx4, TT; Treo-TT-Flu, ATG	CSA, TCM, Ø MPD, MTX	MFD, MUD	23.6	12.9	13.8	6.9	82.6	77.8

ATG: Anti-thymocyte globin, Bu: Busulfan, Cy: Cyclophosphamide, Flu: Fludarabine, HU: Hydroxyurea, Treo: Treosulphan, Azat: Azothiopurine, BM: Bone marrow, CB: Cord blood, MFD: Matched family donor, MSD: Matched sibling donor, MUD: Matched unrelated donor, P26: Protocol 26, PSC: Peripheral stem cell, PTCy: Posttransplant cyclophosphamide, PTIS: Pretransplant immunosuppressive, TBI: Total body irradiation, TLI: Total lymphocyte irradiation, Ø: Not known, TCD: T cell depletion

Treo: Treosulphan, TT: Thiotepa

P26: HU 30 mg/kg/day, azathioprine 3 mg/kg/day, Flu 20 mg/m2/day x5, Bu 3.5 mg/kg/day x4, Cy 40 mg/kg/day x4, ATG;

P26.1 protocol 26.1: HU 30 mg/kg/day, azathioprine 3 mg/kg/day, Flu 30 mg/m2/day x4, Bu 3.5 mg/kg/day x4, TT 10 mg/kg, Cy 50 mg/kg/day x4 and ATG 10 mg/kg;

PTIS a: Flu 150 mg/m2/day x5, Cy 1 g/m2/day x1, dexamethasone 20 mg/m2/dayx5;

PTIS b: Flu 40 mg/m2/day x5, dexamethasone 25 mg/m2/day

Bu, busulfan; Cy, cyclophosphamide; TTP, thiothepa; Treo, treosulphan; PTCy, post-transplant Cy; Flu, fludarabine; Azat, azathioprine; ATG, anti-thymocyte globulin; TG, thymoglobulin; CSA, ciclosporin; TCM, tacrolimus; SIL, silorimus; ALE, alemtuzumab; DEX, dexamethasone; MTP, methylprednisolone; MTX, methotrexate; MEL, melphalan; MFM, mycophenolate mofetil; TBI, total body irradiation; TLI, total lymphoid irradiation; BM, bone marrow; PBSC, peripheral blood stem cells; CB, cord blood; TCD, T cell depletion; MFD, matched family donor; MUD, matched unrelated donor; MSD, matched sibling donor; NA, not applicable; IP, interstitial pneumonitis; IH, intracranial hemorrhage; S, septicemia; E, encephalitis; CLS, capillary leak syndrome; VOD, veno-occlusive disease; HF, hepatic failure; CHF, congestive heart failure; AHA, autoimmune hemolytic anemia; HC, hemorrhagic cystitis; Tx, transplant; IIIC, Class 3 very high risk, d, day.

HSCT and non-transplant medical treatment both have risks. Transplantation has a higher risk of transplantation-related morbidity and mortality. However, most surviving patients continue to receive treatment for thalassemia. Medical therapy must be continued throughout life and carries risks associated with chronic transfusions (alloimmunization, transfusion reactions) and chelation therapy (neurotoxicity, renal toxicity, and others). Each approach has different long-term consequences to consider.

Allogeneic hematopoietic stem cell transplantation is the only widely available curative treatment for thalassemia, although promising early clinical trials such as gene therapy and gene regulation are ongoing. Although transplantation is much cheaper than gene therapy, it carries many risks and costs. However, many individuals will not have a suitable donor, adequately resourced care facilities, and/or financial resources for transplantation. For this reason, in cases of appropriate age and meeting pre-transplant criteria, stem cell transplantation should be performed with a family or unrelated donor in a center experienced in thalassemia diagnosis, treatment, and HSCT.

REFERENCES

1. Mathews, V., et al., *A new stratification strategy that identifies a subset of class III patients with an adverse prognosis among children with beta thalassemia major undergoing a matched related allogeneic stem cell transplantation.* Biol Blood Marrow Transplant, 2007. **13**(8): p. 889-94.
2. Strocchio, L. and F. Locatelli, *Hematopoietic Stem Cell Transplantation in Thalassemia.* Hematol Oncol Clin North Am, 2018. **32**(2): p. 317-328.
3. Lucarelli, G., et al., *Bone marrow transplantation in patients with thalassemia.* N Engl J Med, 1990. **322**(7): p. 417-21.
4. Giardini, C. and G. Lucarelli, *Bone marrow transplantation for beta-thalassemia.* Hematol Oncol Clin North Am, 1999. **13**(5): p. 1059-64, viii.
5. Baronciani, D., et al., *Hemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000-2010.* Bone Marrow Transplant, 2016. **51**(4): p. 536-41.
6. Yesilipek, M.A., et al., *Thalassemia-free and graft-versus-host-free survival: outcomes of hematopoietic stem cell transplantation for thalassemia major, Tur-*

kish experience. Bone Marrow Transplant, 2022. **57**(5): p. 760-767.
7. Li, C., et al., *A novel conditioning regimen improves outcomes in beta-thalassemia major patients using unrelated donor peripheral blood stem cell transplantation.* Blood, 2012. **120**(19): p. 3875-81.
8. Lucarelli, G., et al., *Bone marrow transplantation in thalassemia. The experience of Pesaro.* Ann N Y Acad Sci, 1998. **850**: p. 270-5.
9. Mulas, O., et al., *Conditioning Regimens in Patients with beta-Thalassemia Who Underwent Hematopoietic Stem Cell Transplantation: A Scoping Review.* J Clin Med, 2022. **11**(4).
10. La Nasa, G., et al., *Unrelated donor bone marrow transplantation for thalassemia: the effect of extended haplotypes.* Blood, 2002. **99**(12): p. 4350-6.
11. La Nasa, G., et al., *Unrelated donor stem cell transplantation in adult patients with thalassemia.* Bone Marrow Transplant, 2005. **36**(11): p. 971-5.
12. La Nasa, G., et al., *Status of donor-recipient HLA class I ligands and not the KIR genotype is predictive for the outcome of unrelated hematopoietic stem cell transplantation in beta-thalassemia patients.* Biol Blood Marrow Transplant, 2007. **13**(11): p. 1358-68.
13. Hongeng, S., et al., *Outcomes of transplantation with related- and unrelated-donor stem cells in children with severe thalassemia.* Biol Blood Marrow Transplant, 2006. **12**(6): p. 683-7.
14. Caocci, G., et al., *Prospective assessment of health-related quality of life in pediatric patients with beta-thalassemia following hematopoietic stem cell transplantation.* Biol Blood Marrow Transplant, 2011. **17**(6): p. 861-6.
15. Sodani, P., et al., *T cell-depleted hla-haploidentical stem cell transplantation in thalassemia young patients.* Pediatr Rep, 2011. **3 Suppl 2**: p. e13.
16. Anurathapan, U., et al., *Hematopoietic stem cell transplantation for homozygous beta-thalassemia and beta-thalassemia/hemoglobin E patients from haploidentical donors.* Bone Marrow Transplant, 2016. **51**(6): p. 813-8.
17. Yesilipek, M.A., *Hematopoietic Stem Cell Transplantation in Patients with Hemoglobinopathies.* Hemoglobin, 2020. **44**(6): p. 377-384.
18. Bernardo, M.E., et al., *Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in patients with thalassaemia major.* Br J Haematol, 2008. **143**(4): p. 548-51.
19. Chen, H., et al., *Prediction, management, and prognosis of mixed chimerism after hematopoietic stem cell transplantation in transfusion-dependent pediatric thalassemia patients.* Pediatr Transplant, 2020. **24**(8): p. e13876.
20. Fouzia, N.A., et al., *Long-term outcome of mixed chimerism after stem cell transplantation for thalassemia major conditioned with busulfan and cyclophosphamide.* Bone Marrow Transplant, 2018. **53**(2): p. 169-174.
21. Karasu, G.T., et al., *The value of donor lymphocyte infusions in thalassemia patients at imminent risk of graft rejection following stem cell transplantation.* Pediatr Blood Cancer, 2012. **58**(3): p. 453-8.
22. Lawson, S.E., et al., *Bone marrow transplantation for beta-thalassaemia major: the UK experience in two paediatric centres.* Br J Haematol, 2003. **120**(2): p. 289-95.
23. Bernardo, M.E., et al., *Allogeneic hematopoietic stem cell transplantation in thalassemia major: results of a reduced-toxicity conditioning regimen based on the use of treosulfan.* Blood, 2012. **120**(2): p. 473-6.
24. Gaziev, J., et al., *Bone marrow transplantation for thalassemia from alternative related donors: improved outcomes with a new approach.* Blood, 2013. **122**(15): p. 2751-6.
25. Anurathapan, U., et al., *Outcomes of thalassemia patients undergoing hematopoietic stem cell transplantation by using a standard myeloablative versus a novel reduced-toxicity conditioning regimen according to a new risk stratification.* Biol Blood Marrow Transplant, 2014. **20**(12): p. 2066-71.
26. Caocci, G., et al., *Long-term survival of beta thalassemia major patients treated with hematopoietic stem cell transplantation compared with survival with conventional treatment.* Am J Hematol, 2017. **92**(12): p. 1303-1310.

Correspondence Address:
Prof. Dr. Alphan Küpesiz
Akdeniz University School of Medicine
Dept. of Pediatrics, Pediatric Hematology Oncology Unit
Antalya- Türkiye
E-mail: akupesiz@akdeniz.edu.tr

HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN WITH THALASSEMIA MAJOR IN TÜRKİYE

M. Akif Yesilipek
Professor of Pediatrics
Medicalpark Antalya Hospital
Head of Pediatric Hematology & Oncology and Pediatric Stem Cell Transplantation Unit
Antalya, Türkiye

ABSTRACT

It is estimated that millions of people worldwide are affected by hemoglobinopathies, the most prevalent single-gene disorders. The most common disease in this group is thalassemia. Despite the fact that fewer newborns are being diagnosed with hemoglobinopathies nowadays, it is still a significant health issue for many nations. Allogeneic haematopoietic stem cell transplantation is the sole curative option for patients with hemoglobinopathies to stop irreversible organ damage, despite the fact that frequent red cell transfusions, enhanced iron chelation, and supportive therapy alternatives have increased life expectancy. Hematopoietic stem cell transplantation is currently carried out in a significant number of patients with hemoglobinopathies around the world. Modern transplantation techniques and diligent post-transplant patient follow-up have improved survival outcomes. Nowadays, quality of life has gained importance, as has thalassemia-free survival after HSCT. Haematopoietic stem cell transplantation is advised in instances of thalassemia in light of recent findings.

In this article, the experience gained from the clinical studies conducted by our team is interpreted and presented together with the published study results.

Keywords: Thalassemia, children, stem cell transplantation

REVIEW

The most prevalent illnesses caused by a single gene are hemoglobinopathies. They are thought to affect millions of individuals worldwide and pose a serious health issue for numerous nations. In Türkiye, the prevalence of b-thal carriers is 2.3% overall; however, there are geographical variations, with rises up to 10.0–13.0% in the southern regions. Türkiye is thought to have more than 5,000 thalassemia patients. Along with routine transfusions, advancements in oral chelation and supportive therapies have increased patients' life expectancy, quality of life, and ability to transition from a fatal childhood illness to a chronic condition that lasts into adulthood. However, the only curative treatment is hematopoietic stem cell transplantation (HSCT). Excessive iron overload as a result of regular blood transfusions in transfusion-dependent thalassemia patients can lead to various morbidities and mortality. The goal of allogenic HSCT in thalassemia major (TM) patients is to stop irreversible organ damage brought on by iron overload that might arise during the course of the illness naturally.

Since the first HSCT for a patient with TM was successfully completed forty years ago, allogeneic HSCT from a matched donor is considered standard clinical practice in thalassemia patients and is being used more often all over the world. Baronciani and colleagues **(1)** retrospectively examined the outcomes of 1493 consecutive patients with thalassemia major who underwent HSCT between 2000 and 2010 at 127 centers worldwide (14% of them from Türkiye), using data from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry. After a median observation period of 2 years, they reported 2-year OS and TFS of 88± 1% and 81±1%, respectively. The HLA

MSD donors had the best results in that study, and they also noted that the threshold age for the best transplant outcomes was roughly 14 years old, with an OS of 90–96% and an EFS of 83–93% when transplants were carried out before the patient was 14 years old. Only 133 of the 18-year-old patients in this analysis had transplants after 2000. It demonstrates that the majority of EBMT centers have agreed with the Pesaro group's advice that thalassemia patients undergo transplantation as soon as possible.

There have been reports of the good survival of patients who have undergone HSCT in several publications globally **(1, 2)**. Today, HSCT is performed on approximately 900 pediatric patients annually in more than 30 pediatric bone marrow transplant centers in Türkiye. A significant portion of these transplants are performed on patients with thalassemia major. In Türkiye, HSCT was started for the first time in patients with thalassemia towards the end of the nineties, and with the increase in the number of transplant centers in the following years, the number of transplanted patients today has reached approximately 2000. Firstly, in 2012, we had reported the outcomes of HLA-matched family hematopoietic stem cell transplantation in Turkish children with beta-TM **(3)**. In this study, 245 beta-TM kids who underwent their first allogeneic HSCT in Türkiye and were monitored for at least a year after the transplant were included. The patients ranged in age from 1 to 22 years, with a median age of 6.6. Twenty cord blood (CB) stem cells, 137 peripheral blood (PB) stem cells, and 88 bone marrow (BM) stem cells were given to patients. The donors were parents or siblings with matching HLA. Thirty-three children (13.5%) were found to have grade II-IV acute GvHD, whereas 28 kids (12.5%) were found to have cGvHD, eight of whom had the extensive variety. In 43 (17%) of the transplant patients, thalassemic reconstitution was seen. After 100 days, 17 patients passed away, the main causes of death being infections, GvHD, and hemorrhage. OS rates were 85.0 percent (95% CI, 80.2-89.8), and thalassemia-free survival rates were 68% (95% CI, 61.8-74.2). The findings of this study support the notion that HSCT can provide thalassemic patients with a cure, and it was determined that HSCT should be carried out in infancy, before iron overload and disease-related problems have developed.

GVHD-free survival has become important recently because thalassemia major is a non-malignant condition **(4)**. Long-term follow-up is crucial to track transplant problems and quality of life after the success of HSCT for the treatment of thalassemia in many nations **(5-7)**. One of the most crucial elements affecting quality of life following HSCT is chronic GVHD. So, in our opinion, TGFS should be specified alongside OS and TFS. In 2021, Turkish national data on HSCT results in thalassemia patients were published in the journal "Bone Marrow Transplantation" on behalf of the Turkish Pediatric Stem Cell Transplantation Group **(8)**. This study included 1469 TM patients who underwent their first HSCT between January 1988 and August 2020 in 25 pediatric centers in Türkiye. All of the patients needed blood transfusions. This study's objective was to compare GVHD-free survival to other survival metrics. By low- or high-resolution typing, family donors were perfectly HLA-matched (6/6 or 10/10) in the trial. High-resolution HLA typing was used for unrelated donors to assess potential donors, and those who had 9/10 and 10/10 matches were approved. A regimen of myeloablative conditioning based on BU/Treo was given to all patients. Patients receiving HSCT used bone marrow, peripheral blood stem cells, and umbilical cord blood as stem cell sources. The use of bone marrow was favored in cord blood transplants. In none of the transplantations, a haploidentical donor was employed. The primary outcomes were overall survival (OS), thalassemia-free survival (TFS), and thalassemia-GVHD-free survival (TGFS). Being alive without thalassemia, or GVHD, is referred to as TGFS. The longest follow-up length was 402 months, with a 62-month average for the patients. The age range of the patients was 1–29 years, with a median age of 7 years at the time of HSCT. In 2.7% of the patients, the primary graft failed. Additionally, 7.3% of the patients suffered secondary graft rejection, and 1.3% had a poor graft. 1249 patients had received their final chimerism findings at the time of this study, and 84.4% of them were being monitored for full donor chimerism. In 15.5% of the patients who were still alive without a blood transfusion, stable mixed chimerism was discovered.

The percentage of patients who were still alive in our study group who had cGVHD was 8.3%. In our study, mild or limited chronic GvHD was present in 2.2% of patients at their most recent visit. The TGFS rate of 80.8% was determined to be a marker for being alive and free of GVHD and thalassemia. Because donor/recipient immunological tolerance is induced, it is anticipated that patients with stable MC will experience a lower risk of cGVHD. In our investigation, there was a statistically significant difference between the rates of cGVHD in the MC and the group with complete donor chimerism ($p< 0.001$). This finding suggests that MC may be cGVHD-protective. The TGFS was somewhat greater in the MC group; however, the difference was not statistically significant **(8)**.

The success of HSCT is believed to be directly influenced by recent advancements in HSCT applications and the transplant center's expertise. In a hospital with experience doing more than 100 thalassemia transplants, univariate analysis revealed considerably improved TF and TGFS for patients who received their transplant. The relationships between the survival rates of TF and TGF and the transplant center's experience appear plausible. The results of the HSCT greatly improved over time, as stated by various authors **(1, 2, 9)**. For patients who received their transplant after 2010 in this study, the OS, TFS, and TGFS were much superior; they improved significantly over time. According to our research, both characteristics are improved by cautious patient selection, complete donor matching with high-resolution HLA typing, the preparation of an ideal conditioning regimen, and efficient GVHD prophylactic measures.

The results of the MUD and MSD transplants in our study were similar, with the MUD having slightly better results. In addition to the fact that all unrelated transplants were completed after 2010, we think that rigorous patient and donor selection also contributed to these results. Another multicenter study's authors recently observed comparable event-free and overall survival rates for patients with TM following transplants from related and unrelated donors who were HLA-matched **(10)**. Additionally, they advised beginning a concurrent search for HLA-matched related and unrelated donors early in the course of the illness and carrying out the transplantation using an HLA-matched unrelated donor if an HLA-matched sibling is not available. A 20% to 25% absolute reduction in event-free and overall survival was observed in that study when transplantation was postponed for longer than 15 years **(10)**. An international panel also stated that allogeneic HSCT is a suitable option for a child with lifelong control of iron overload and the absence of iron-related tissue complications prior to the development of iron overload if a well-matched donor (related or unrelated) is available as soon as possible **(2)**.

It is widely known that when the mismatch in the HLA loci rises, the success of transplantation declines and complications rise in HSCT. According to what is known, TFS and TGFS were considerably lower for transplants from 9/10 compatible donors than those from 10/10 donors among unrelated donors in this study. In patients with thalassemia major, haploidentical donor transplants have shown promising results in recent years **(11, 12)**. We do not think that enough experience has been gained, nevertheless, and that it is still too early for routine applications. In our registry, no haploidentical transplantation was seen.

The bone marrow is the preferred source of stem cells for thalassemia major patients. However, there are numerous examples of favorable outcomes using umbilical cord blood and peripheral blood stem cells **(13–15)**. In our previous publication, we used PBSCs as the graft source in 55.9% of thalassemia patients, and we found no statistically significant differences between PBSCs and BMSCs in terms of mortality rate or prevalence of acute GVHD **(3)**. Despite the positive engraftment outcomes, using PBSCs from an MSD or MFD is not recommended for patients with TM due to the potential increase in hazards of both acute and chronic GvHD **(16)**. Although it is commonly recognized that the risk of GVHD has decreased, there have been reports that UCB in thalassemia patients is linked to a higher risk of graft failure **(17–19)**. In our study, peripheral blood was used in 26% of cases, bone marrow in 65%, and cord blood from the same sibling who also had bone marrow in 8% of cases. We observed that the use of bone marrow from sibling donors

improved the course of life. Co-transplantation of a UCB unit and BM cells obtained from the same sibling donor has had positive results, according to reports **(20)**. The goal of a combined infusion is to retain the cord blood's protective impact against the development of GVHD while increasing the number of transplanted cells and enhancing hematopoietic recovery. The combination of UCB and BM improved all three of the survival measures in our study. We found that using peripheral blood as a source of stem cells for transplants from unrelated donors dramatically increased TFS. When all transplants were examined for outcome characteristics, the stem cell source had an impact on OS and TGFS but not TFS. This result demonstrates how complication-free survival is influenced by stem cell source selection. All survival parameters in this study were considerably better for transplants performed after 2010 and for patients under the age of seven, according to the multivariate analysis. At facilities with more than 100 TM patient transplants under their belts, the TFS and TGFS rates improved. The use of peripheral blood as the stem cell source had no effect on any of the three-survival metrics. The univariate analysis revealed a detrimental effect on OS; however, the multivariate analysis revealed no effects of cGVHD on OS and TFS **(8)**.

Different thalassemia patient outcomes may be caused by regional, ethnic, and genotypic characteristics. The results of HSCT for patients with thalassemia are reported in some recent papers from various parts of the world in Table 1. With a larger patient population and a longer follow-up period compared to past studies, we think our work significantly adds to the body of literature. In conclusion, our study established that allo-HSCT is a successful treatment for people with TM and that its results have been dramatically improving over time. If a patient with thalassemia major has an HLA-matched donor, we can advise that HSCT be performed before the patient turns 7 years old to prevent organ damage from iron overload and improve the patient's quality of life after the transplant.

Our analysis also revealed that institutions with experience and a history of performing more than 100 transplants for TM patients had higher TFS and TGFS rates. While TFS was noticeably greater for transplants from MUD donors, there was no difference in OS or TGFS rates between MSD and MUD. Transplants from 9/10 matched donors had much lower TFS and TGFS rates than transplants from 10/10 unrelated donors. In comparison to the complete donor chimerism group, the cGVHD rate was considerably lower in the MC group. This study is significant since it presents transplant outcomes for individuals with thalassemia for a whole nation with high case involvement. However, it is crucial to take into account survival without cGVHD, which affects the quality of life and the factors impacting it. Currently, the criteria of thalassemia-free survival employed for the evaluation of HSCT results are insufficient. Our study's findings, we are confident, will help clarify this matter and make a big difference in the thalassemia major patients' post-HSCT quality of life.

The monitoring of iron overload is one of the issues that should be taken into account in the follow-up of patients with thalassemia following HSCT. Although patients with b-TM no longer require transfusions following HSCT, the pre-transplantation burden raises the risk of cirrhosis, cardiomyopathy, and hepatic fibrosis. According to a study by the Pesaro group, cirrhosis manifested in a sizable part of these patients over the post-transplant period, while 22.0% of patients with b-thal advanced with symptoms of hepatic fibrosis **(27)**. The Pesaro group also claimed that by using phlebotomy to draw 6 mL/kg of blood at 2-week intervals, iron load may be decreased **(28)**. In a different study conducted by this team, liver cirrhosis was discovered through serial pathological biopsy assessments, and liver cirrhosis was found to reduce following the reduction of the iron burden with phlebotomy and chelation **(29)**. When phlebotomy is not an option, oral chelators have emerged as a helpful substitute for lowering the iron burden. Deferasirox (DFX) was demonstrated in one study to effectively lower the blood ferritin level **(30)**. The use of DFX following HSCT was demonstrated to be safe and effective in our multicenter prospective trial **(31)**. The use of 20 mg/kg of DFX in patients with high iron burdens may be suggested in light of the findings of this investigation, at least six months fol-

lowing HSCT and after the cessation of immunosuppressive medication.

The significance of QoL after transplantation is demonstrated by the recent TFS rate after HSCT of 90.0%. The primary element influencing QoL in the post-transplant era is chronic graft vs. host illness. Patients with severe cGVHD, such as those who have bronchiolitis obliterans, are significantly restricted in their physical activity, despite the fact that this complication is challenging to cure. It may not be reasonable to draw a firm conclusion about whether late-stage endocrine issues following transplantation are due to HSCT because iron overload in the pre-transplant phase may produce endocrine disorders in patients with b-thal. Weight, height standard deviation score values, femur-vertebra Z scores, insulin resistance, and the frequency of gonadal insufficiency were all shown to be lower in patients who got HCST before the age of seven in a study we previously conducted to examine late endocrine side effects **(32)**. Regarding hypothyroidism, hypoparathyroidism, and adrenal insufficiency, no differences were discovered in the same study. We also assessed a total of 328 b-TM patients, 169 of whom underwent HSCT before the age of 7, and 159 of whom were older than the age of 7, in order to comprehend the effect of age on transplantation success **(33)**. According to the study's findings, patients who underwent HSCT after turning 7 had greater rejection rates and worse rates of thalassemia-free and thalassemia/GVHD-free survival. In another study, we compared the quality of life (QoL) of HSCT patients to those who received supportive care as follow-up. We discovered that patients who underwent HSCT had a higher QoL. This study stressed that cGVHD is the most significant consequence to affect quality of life, making GVHD prevention the primary goal. According to La Nasa et al. **(35)**, the general population's health-related quality of life (QoL) was similar to that of 109 patients who had received HSCT 20 years earlier in terms of their mental health, level of education, employment position, living arrangements, and birth rates. The age- and sex-matched group getting conventional supportive care in the same trial showed inferior results for these criteria. In this study, the presence of GVHD and older age (age of transplant >15 years) were identified as two characteristics that negatively impacted QoL. The use of ATG was suggested as a solution to lessen the complications of GVHD. Growth following HSCT was normal, according to Li et al. **(36)**, although girls required hormonal assistance since gonadal functions affected them more than boys did. Numerous studies in the field of health economics have demonstrated that the cost of care for transplant recipients is lower than for b-TM patients receiving conventional transfusion-chelation therapy **(37–39)**. When considering the difficulties that could arise as a result of iron overload, hematopoietic stem cell transplant applications are significantly more cost-effective. In light of the available research, we advise applying HSCT early on, before organ damage develops, if a patient with b-TM has a fully matched sibling, family member, or unrelated donor.

Families lacking a fully compatible related donor may be given the option of a fresh pregnancy using the pre-implantation genetic diagnostic (PGD) technique to develop a healthy donor. Many patients with b-TM have been given the option to undergo HSCT thanks to this approach, which has been successfully used in Türkiye **(40–42)**. However, the actual number of cells in the cord blood obtained and kept after the birth of a sibling with a perfect match is typically lower. Patients who have b-TM may experience graft failure due to a low cell count. It is advised to wait until the infant is old enough to harvest enough stem cells from the bone marrow in addition to those in the cord blood because of this danger **(20)**. PGD is debatable in numerous nations due to ethical and religious concerns. As a result, it is not utilized globally. When no eligible family donor is available and PGD is not an option, a compatible, unrelated donor may be employed. Recent developments in high-resolution human leukocyte antigen (HLA)-typing technologies and the inclusion of more than 45 million donors in the voluntary donor registration system of Bone Marrow Donors Worldwide (BMDW) have led to the publication of successful findings from unrelated donors **(1, 8, 43)**. Our possibilities have also grown as a result of Türkiye's substantial increase in records of Turkish volunteer donors (Türkök).

REFERENCES

1. Baronciani D, Angelucci E, Potschger U, Gaziev J, Yesilipek A, Zecca M, et al. Hemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000-2010. Bone Marrow Transplant. 2016 Apr;51(4):536-41.
2. Angelucci E, Matthes-Martin S, Baronciani D, Bernaudin F, Bonanomi S, Cappellini MD, Dalle JH, Di Bartolomeo P, de Heredia CD, Dickerhoff R, Giardini C, Gluckman E, Hussein AA, Kamani N, Minkov M, Locatelli F, Rocha V, Sedlacek P, Smiers F, Thuret I, Yaniv I, Cavazzana M, Peters C; EBMT Inborn Error and EBMT Paediatric Working Parties. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. Haematologica. 2014 May;99(5):811-20.
3. Yesilipek MA, Ertem M, Cetin M, Öniz H, Kansoy S, Tanyeli A, et al. HLA-matched family hematopoetic stem cell transplantation in children with beta thalassemia major: the experience of the Turkish Pediatric Bone Marrow Transplantation Group. Pediatr Transplant. 2012 Dec;16(8):846-51.
4. Angelucci E. Complication free survival long-term after hemopoietic cell transplantation in thalassemia. Haematologica. 2018 Jul;103(7):1094-1096.
5. La Nasa G, Caocci G, Efficace F, Dessì C, Vacca A, Piras E, et al. Long-term healthrelated quality of life evaluated more than 20 years after hematopoietic stem cell transplantation for thalassemia. Blood 2013;122:2262–70.
6. Rahal I, Galambrun C, Bertrand Y, et al. Late effects after hematopoietic stem cell transplantation for beta-thalassemia major: the French national experience. Haematologica. 2018;103(7):1143-1149.
7. Uygun V, Tayfun F, Akcan M, Karasu GT, Kupesiz A, Hazar V, Yeşilipek A. Quality of life assessment in hematopoietic stem cell transplantation performed on thalassemia major patients. Pediatr Hematol Oncol. 2012 Aug;29(5):461-71.
8. Yesilipek A, Uygun V, Kupesiz A, Karasu G, Ozturk G, Ertem M, et al. Thalassemia-free and graft-versus-host-free survival: outcomes of hematopoietic stem cell transplantation for thalassemia major, Turkish experience. Bone Marrow Transplant. 2022 May;57(5):760-767.
9. Galambrun C, Pondarre C, Bertrand Y,Loundou A, Bordigoni P, Frange P, et al. French multicenter 22-year experience in stem cell transplantation for beta-thalassemia major: lessons and future directions. Biol Blood Marrow Transplant. 2013;19(1):62-8.
10. Li C, Mathews V, Kim S, et al. Related and unrelated donor transplantation for b-thalassemia major: results of an international survey. Blood Adv. 2019;3(17):2562–2570.
11. Anurathapan U., Hongeng S, Pakakasama S, Songdej D., Sirachainan N., Pongphitcha P., et al. Hematopoietic Stem Cell Transplantation for Severe Thalassemia Patients from Haploidentical Donors Using a Novel Conditioning Regimen. Biol Blood Marrow Transplant 26 (2020) 1106-1112.
12. Oevermann L, Schulte JH, Hundsdörfer P, Hakimeh D, Kogel F, Lang P, Corbacioglu S, et al. HLA-haploidentical hematopoietic stem cell transplantation in pediatric patients with hemoglobinopathies: current practice and new approaches. Bone Marrow Transplant. 2019 Aug;54(Suppl 2):743-748.
13. Yesilipek MA, Hazar V, Küpesiz A, Kizilörs A, Uguz A, Yegin O. Peripheral blood stem cell transplantation in children with beta-thalassemia. Bone Marrow Transplant. 2001 Dec;28(11):1037-40.
14. Ghavamzadeh A, Kasaeian A, Rostami T, Kiumarsi A. Comparable Outcomes of Allogeneic Peripheral Blood versus Bone Marrow Hematopoietic Stem Cell Transplantation in Major Thalassemia: A Multivariate Long-Term Cohort Analysis. Biol Blood Marrow Transplant. 2019 Feb;25(2):307-312.
15. Sun L, Wang N, Chen Y, Tang L, Xing C, Lu N, Shi Y, Ma Y, Lin F, Yu K, Feng J. Unrelated Donor Peripheral Blood Stem Cell Transplantation for Patients with beta-Thalassemia Major Based on a Novel Conditioning Regimen. Biol Blood Marrow Transplant. 2019 Aug;25(8):1592-1596.
16. Strocchio L, Locatelli F. Hematopoietic Stem Cell Transplantation in Thalassemia. Hematol Oncol Clin North Am. 2018 Apr;32(2):317-328.
17. Locatelli F, Rocha V, Reed W, Bernaudin F, Ertem M, Grafakos S,et al. Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. Blood. 2003 Mar 15;101(6):2137-43.
18. Kanathezhath B, Walters MC. Umbilical cord blood transplantation for thalassemia major. Hematol Oncol Clin North Am. 2010 Dec;24(6):1165-77.
19. Ruggeri A, Eapen M, Scaravadou A, Cairo MS, Bhatia M, Kurtzberg J, Wingard JR, Fasth A, Lo Nigro L, Ayas M, Purtill D, Boudjedir K, Chaves W, Walters MC, Wagner J, Gluckman E, Rocha V; Eurocord Registry; Center for International Blood and Marrow Transplant Research; New York Blood Center. Umbilical cord blood transplantation for children with thalassemia and sickle cell disease.

Biol Blood Marrow Transplant. 2011 Sep;17(9):1375-82. doi: 10.1016/j.bbmt.2011.01.012. Epub 2011 Jan 28.

20. Tucunduva L, Volt F, Cunha R, Locatelli F, Zecca M, Yesilipek A, Caniglia M, Güngör T, Aksoylar S, Fagioli F, Bertrand Y, Addari MC, de la Fuente J, Winiarski J, Biondi A, Sengeloev H, Badell I, Mellgren K, de Heredia CD, Sedlacek P, Vora A, Rocha V, Ruggeri A, Gluckman E; Eurocord and the EBMT Paediatric working party. Combined cord blood and bone marrow transplantation from the same human leucocyte antigen-identical sibling donor for children with malignant and non-malignant diseases. Br J Haematol. 2015 Apr;169(1):103-10.
21. Alonso L, González-Vicent M, Belendez C, Badell I, Sastre A, Rodríguez-Villa A, et al. Hematopoietic stem cell transplantation in pediatric patients with thalassemia and sickle cell disease: An experience of the Spanish Working Group for Bone Marrow Transplantation in Children (GETMON). Med Clin (Barc) 2019 Feb 15;152(4):135-140.
22. Choudhary D, Doval D, Sharma SK, Khandelwal V, Setia R, Handoo A. Allogenic Hematopoietic Cell Transplantation in Thalassemia Major: A Single-center Retrospective Analysis From India. J Pediatr Hematol Oncol. 2019 Jul;41(5):e296-e301.
23. Caocci G, Orofino MG, Vacca A, Piroddi A, Piras E, Addari MC,et al. Long-term survival of beta thalassemia major patients treated with hematopoietic stem cell transplantation compared with survival with conventional treatment. Am J Hematol. 2017 Dec;92(12):1303-1310.
24. Ramprakash S, Agarwal R, Dhanya R, Marwah P, Soni R, Yaqub N, et al. Low-cost matched sibling bone marrow transplant for standard-risk thalassemia in a limited-resource setting. Pediatric Hematology Oncology Journal 2 (2017) 107-113.
25. Li Q, Luo J, Zhang Z, Liu L, Luo L, Yang G, et al. G-CSF-Mobilized Blood and Bone Marrow Grafts as the Source of Stem Cells for HLA-Identical Sibling Transplantation in Patients with Thalassemia Major. Biol Blood Marrow Transplant. 2019 Oct;25 (10):2040-2044.
26. Lai X, Liu L, Zhang Z, Shi L, Yang G, Wu M,et al. Hepatic veno-occlusive disease/sinusoidal obstruction syndrome after hematopoietic stem cell transplantation for thalassemia major: incidence, management, and outcome. Bone Marrow Transplant. 2021 Feb 19. doi: 10.1038/s41409-021-01233-w. Online ahead of print. PMID: 33608657.
27. Angelucci E, Muretto P, Nicolucci A, et al. . Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. Blood. 2002; 100:17-21.
28. Angelucci E, Muretto P, Lucarelli G, et al. Treatment of iron overload in the "ex-thalassemic". Report from the phlebotomy program. Ann N Y Acad Sci. 1998 Jun 30; 850:288-93.
29. Muretto P, Angelucci E, Lucarelli G. Reversibility of cirrhosis in patients cured of thalassemia by bone marrow transplantation. Ann Intern Med. 2002 May 7;136 (9):667-72.
30. Yesilipek MA, Karasu G, Kazik M, et al. Posttransplant oral iron-chelating therapy in patients with beta-thalassemia major. Pediatr Hematol Oncol. 2010 Aug;27(5):374-9.
31. Yesilipek MA, Karasu G, Kaya Z, et al. A Phase II, Multicenter, Single-Arm Study to Evaluate the Safety and Efficacy of Deferasirox after Hematopoietic Stem Cell Transplantation in Children with beta-Thalassemia Major. Biol Blood Marrow Transplant. 2018 Mar;24 (3):613-618.
32. Aldemir-Kocabas B, Tezcan-Karasu G, Bircan I, et al. Evaluating the patients with thalassemia major for long-term endocrinological complications after bone marrow transplantation. Pediatr Hematol Oncol. 2014 Oct;31(7):616-23.
33. Karasu G, Uygun V, Hazar V, et al. Hematopoetic stem cell transplantation in patients with thalassemia major: Better in younger [Meeting Abstract]. Bone Marrow Transplant. 2019 Jul; 54:128-129.
34. Uygun V, Tayfun F, Akcan M, et al. Quality of life assessment in hematopoietic stem cell transplantation performed on thalassemia major patients. Pediatr Hematol Oncol. 2012 Aug;29 (5):461-71.
35. La Nasa G, Caocci G, Efficace F, et al. Long-term health-related quality of life evaluated more than 20 years after hematopoietic stem cell transplantation for thalassemia. Blood. 2013 Sep 26;122 (13):2262-70.
36. Li CK, Chik KW, Wong GW, et al. Growth and endocrine function following bone marrow transplantation for thalassemia major. Pediatr Hematol Oncol. 2004 Jul-Aug; 21 (5):411-9.
37. Mohamed SY. Thalassemia Major: Transplantation or Transfusion and Chelation. Hematol Oncol Stem Cell Ther. 2017 Dec; 10 (4):290-298.
38. John MJ, Jyani G, Jindal A, et al. Cost Effectiveness of Hematopoietic Stem Cell Transplantation Compared with Transfusion Chelation for Treatment of Thalassemia Major. Biol Blood Marrow Transplant. 2018 Oct;24(10):2119-2126.
39. Sruamsiri R, Chaiyakunapruk N, Pakakasama S, et al. Cost utility analysis of reduced intensity

hematopoietic stem cell transplantation in adolescence and young adult with severe thalassemia compared to hypertransfusion and iron chelation program. BMC Health Serv Res. 2013 Feb 5;13:45.
40. Kahraman S, Beyazyurek C, Yesilipek MA, et al. Successful haematopoietic stem cell transplantation in 44 children from healthy siblings conceived after preimplantation HLA matching. Reprod Biomed Online. 2014 Sep; 29 (3):340-51.
41. Kurekci E, Kupesiz A, Anak S, et al. Hematopoietic Stem Cell Transplantation Using Preimplantation Genetic Diagnosis and Human Leukocyte Antigen Typing for Human Leukocyte Antigen-Matched Sibling Donor: A Turkish Multicenter Study. Biol Blood Marrow Transplant. 2017 May; 23 (5):790-794.
42. Yesilipek MA, Karasu G, Ercelen N, et al. Successful hematopoietic SCT from non-identical twins to two sisters with beta-thalassemia major by using pre-implantation genetic diagnosis and HLA typing. Bone Marrow Transplant. 2011 Dec; 46 (12):1581-2.
43. La Nasa G, Argiolu F, Giardini C, et al. Unrelated bone marrow transplantation for beta-thalassemia patients: The experience of the Italian Bone Marrow Transplant Group. Ann N Y Acad Sci. 2005; 1054: 186-95.

Correspondence Address:
M.Akif Yesilipek
Professor of Pediatrics
Medicalpark Antalya Hospital
Head of Pediatric Hematology & Oncology and Pediatric Stem Cell Transplantation Unit
Antalya, Türkiye
ORCID 0000-0002-4514-8637
E-mail: yesilipek@gmail.com
Tel: +90 5322836237

Table 1: *Some recent publications which report the outcomes after HSCT in patients with thalassemia*

Author	Country	Publishing Year	Transplant interval	n	Donor type	Follow-up Median	OS %	TFS %	TGFS %	Comment
Alonso[21]	Spain	2019	1989-2014	43	MSD,MRD,MUD	3 years	92	81	NA	In 6 centers in Spain
Choudhary[22]	India	2019	2008-2017	203	MSD,MRD,MUD, Haplo	29 months	88.5	82	NA	12.9% cGVHD
Li[10]	China,India,USA	2019	2000-2016	1110	MSD,MRD,MUD, Haplo	5 years	≤6 years old 90	86		An International study, 90% of patients in the last decade
							7-15 years old 84	80		
							16-25 years old 63	63	NA	
Galambrun[9]	France	2013	1985-2007	108	MSD,MRD,MUD	12 years	86.8	69.4	NA	96 siblings, 12 cGVHD
Caocci[23]	Italy	2017	1987-2016	258	MSD, MUD	11 years	82.6	77.8	NA	Adult OS 70,TFS 67.3 , cGVHD 12.9%
Ramprakash[24]	India, Pakistan, Sri Lanka	2017	2013-2016	71	MSD	17.5 months	93	83	74.6	BU oral,CY, ATG, cGVHD 4%
Li[25]	China	2019	2007-2018	184	MSD	3 years	97.8	97.3	89.5	G-CSF-Mobilized Blood and BM Grafts
Lai[26]	China	2021	2007-2019	521	MSD, MUD, Haplo	3 years	94.3	92.5	86.9	VOD 10.4%
Yesilipek[8]	Türkiye	2021	1988-2020	1469	MSD,MRD,MUD	62 months	92.3	82.1	80.8	Only first transplantation results, whole country results from 25 pediatric centers

GENE THERAPY METHODS IN THALASSEMIA AND HEMOGLOBINOPATHIES

Devrim Demir-Dora[1,2,3]
Akdeniz University Faculty of Medicine
Department of Medical Pharmacology
2 Department of Gene and Cell Therapy
3Department of Medical Biotechnology
Antalya-Türkiye

ABSTRACT

Hemoglobinopathies are autosomal recessive disorders that are caused by quantitative and qualitative mutations of hemoglobin (Hb). Quantitative mutations cause defects in the synthesis of the α- and β-globin chains in adult Hb. The two most common monogenic diseases, sickle cell disease and β-thalassemia, are both β-globin gene disorders that are potentially curable after allogeneic hematopoietic stem cell transplantation (HSCT) or autologous HSCT after genetic modification. The aim of genetically corrected autologous HSCT is to provide a one-time, universal treatment that overcomes the main drawbacks of allogeneic HSC transplantation. Gene therapy is a promising treatment option for these diseases. Gene addition, gene editing, and gene silencing can be done by ex vivo or in vivo methods. Many gene therapy medicinal products (GTMPs) are being investigated in clinical trials for the treatment of β-thalassemia and sickle cell disease. Generally, CRISPR-Cas9 was used for gene editing, and lentiviral vectors were used for transducing autologous CD34+ cells. Zynteglo is the first and only EMA- and FDA-approved product for the treatment of pediatric and adult patients with β-thalassemia. Zynteglo (betibeglogene autotemcel), an autologous CD34+ cell encoding the βA-T87Q-globin gene, is a one-time ex vivo gene therapy medicinal product, transduced by a lentiviral vector. Even though gene therapy has advanced significantly, there are still many problems to be solved for evaluating the efficacy and safety profile of GTMPs. Gene therapy for thalassemia and hemoglobinopathies is still in its early stages.

Keywords: Gene therapy, β-thalassemia, sickle cell disease, hemoglobinopathy, hematopoietic stem cells, gene addition, gene editing, viral vector, clinical trials

INTRODUCTION

Gene therapy is a fast developing area of pharmaceutical industry with a lot of potential for treating a variety of inherited and acquired illnesses. It is a therapeutic method in which functional genes are transplanted into a human cell or the faulty gene is removed or silenced in order to correct a genetic defect or to give the cell a new function. Delivering genes such as 'DNA, RNA, mRNA, siRNA, antisense oligonucleotides, the CRISPR/Cas9 system, ZFNs, and TALENs to patients in order to repair disease-causing damaged genes' is referred to as gene therapy by European Medicines Agency **(1, 2)**.

The European Medicines Agency (EMA) defines gene therapy medicines as 'those that contain genes that have therapeutic, prophylactic, or diagnostic effects and are used to repair tissue damage, replenish nutritional deficiencies to maintain bodily functionality, and prevent the expression of undesirable genes' **(1)**.

Hemoglobinopathies including sickle cell disease and thalassemia show promise as possible targets for gene therapy. These diseases are brought on by genetic abnormalities that interfere with the produc-

tion of hemoglobin, the protein in red blood cells that carries oxygen. To fix these genetic mutations and return hemoglobin production to normal, gene therapy is used as a new treatment. The aim of genetically corrected autologous hematopoietic stem cell (HSC) transplantation is to provide a one-time, universal treatment that overcomes the main drawbacks of allogeneic HSC transplantation **(3, 4)**.

GENE THERAPY METHODS

Gene therapy can be classified as, ex vivo (outside the body) or in vivo (inside the body), according to their administration method **(5)**. Ex vivo gene therapy involves removing cells from the patient, modifying them in the laboratory, and then reintroducing them into the patient by transplantation or transfusion **(6)**. On the other hand, in vivo gene therapy is the replacement of missing gene or the administration of therapeutic genes to the patient in a drug delivery system, thus providing treatment without removing the patient gene from the cell **(Figure 1)**.

Therapeutic genes are delivered to the target cells or tissues via a variety of delivery methods **(Table 1) (7, 8)**. Due of their effectiveness in transferring genes into cells, viral vectors like adenoviruses and lentiviruses are frequently used **(9, 10)**.

Principles of Gene Therapy

The following are the main principles of gene therapy:

a. **Gene Correction:** Gene therapy occasionally aims to correct genetic changes that cause diseases. This might require techniques like gene editing, in which the DNA is precisely modified to correct or replace the faulty gene with a functional one. CRISPR-Cas9 is one of the most widely used gene-editing tools at the moment **(11, 12)**.
b. **Gene Addition:** It is another method of gene therapy that involves adding a functional copy of a gene to the patient's cells. The therapeutic gene is transferred into the patient's cells either by viral vectors or non-viral techniques **(8)**.
c. **Gene Silencing:** When a gene's excessive activity causes a disease, gene therapy may also involve blocking the expression of a specific gene. RNA interference (RNAi) is a method for silencing specific genes **(13, 14)**.

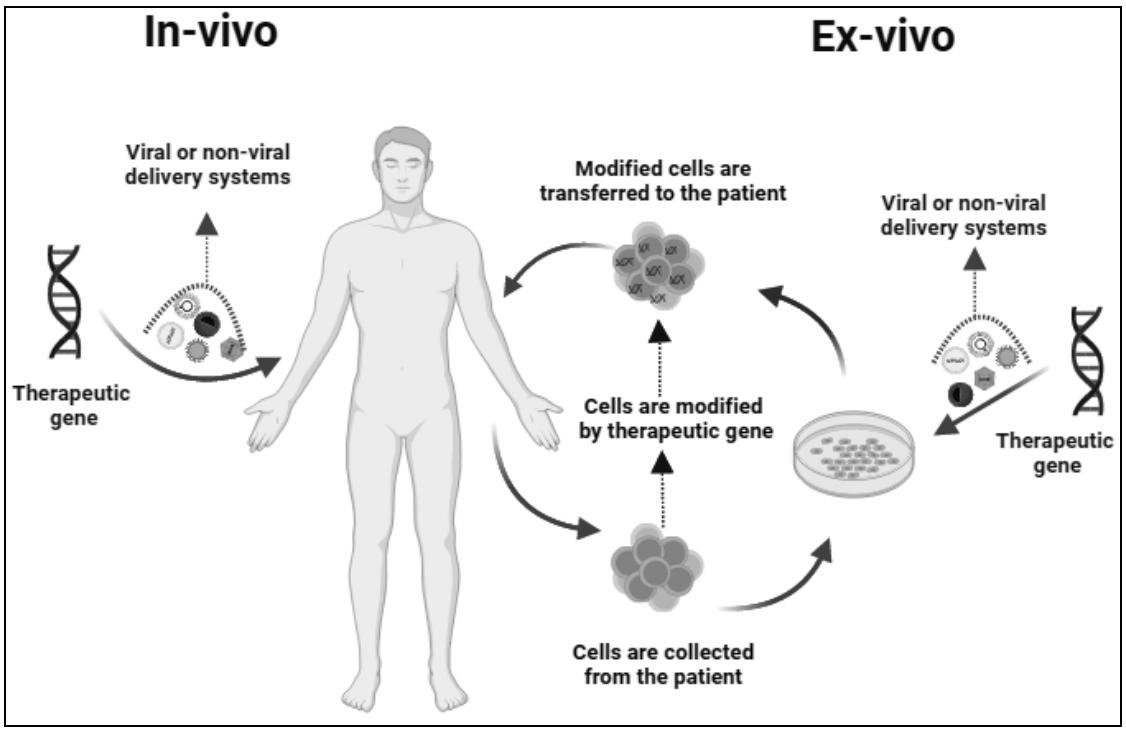


Figure 1: *Ex-vivo and in-vivo gene therapy*

Table 1: *Viral and non-viral gene delivery systems* [7]

VİRAL DELIVERY SYSTEMS	
• Retrovirus • Adenovirus • Adeno-associated Virus (AAV) • Herpes Simplex Virus (HSV)	
NON-VIRAL DELIVERY SYSTEMS	
Physical Methods	
• Naked DNA Injection • Electroporation • Microinjection • Particle bombardment	
Chemical Methods	
• Calcium phosphate and DEAE-Dextran	
• Inorganic Particles	Silica, gold, carbon nanotubes, quantum dots
• Drug Delivery Systems	- Lipid-based: cationic lipid complexes, liposomes, lipid nanoparticles, niosomes, emulsions - Polymer-based: cationic polymeric complexes, dendrimers, micels, polymeric nanoparticles

By transfer of nucleic acids such as DNA, RNA, mRNA, siRNA, antisense oligonucleotide, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR Associated (Cas9) system, zinc finger nucleases (ZFN), Transcription Activator Like Effector Nuclease (TALEN); gene silencing, exon skipping, gene editing, gene knockout and nucleotide modifications may ocur; making repair, insertion or deletion of a genetic sequence possible **(1, 7, 15)**.

Gene therapy medicinal products (GTMPs), consist of a vector or delivery formulation/system. Gene delivery vector or formulation of the product contains a genetic construct designed to express a particular transgene (therapeutic sequence) for the regulation, repair, replacement, addition or deletion of a genetic sequence. The active substance is the nucleic acid sequence(s), or genetically modified microorganism(s), virus(es) or cells **(1, 5)**.

Gene Therapy Methods in Thalassemia and Hemoglobinopathies

Hemoglobinopathies are autosomal recessive disorders which are caused by quantitative and qualitative mutations of hemoglobin (Hb). Quantitative mutations cause defects in the synthesis of the α- and β-globin chains in adult Hb. The HBB gene, which encodes β-globin, or its regulatory elements can have deletions or point mutations that lead to a reduction in the formation of β-globin chains, causing quantitative deficiencies known as β-thalassemia **(16)**. Qualitative mutations cause changes in the amino acid structure of the globin chain, such as those found in Hb S (HBB: c.20A>T), Hb C (HBB: c.19G>A), and Hb E (HBB: c.79G>A) **(16, 17)**.

Thalassemia

Thalassemia is an inherited blood illness brought on by abnormalities in the genes essential for producing hemoglobin. Thalassemias are caused by a large number of heterogeneous mutations that result in abnormal globin gene expression, which completely eliminates or considerably lowers globin chain synthesis. Mutations in the α- or β-globin gene lead to α- and β- thalassemia, respectively. α-Thalassemia is typically caused by deletions within the α-globin gene cluster, resulting in the loss of function of one or both α-globin genes in each locus. Along with anemia, life-threatening complications such as bone

deformities, fatigue, jaundice and, in severe cases, organ damage may ocur **(17, 18)**.

- **Beta-thalassemia**

At least 250 mutations in the beta-globin gene cause reduction (beta+ genotype) or absence (beta0 genotype) of β-globin chain expression, leading in β-thalassemia, an inherited hemoglobin (Hb) condition **(16-18)**. The two most common monogenic diseases, sickle cell disease and β-thalassemia, are both β-globin gene disorders that are potentially curable after allogeneic hematopoietic stem cell transplantation (HSCT) or autologous HSCT after genetic modification **(3, 4, 19-21)**. Anemia is caused by the ensuing alpha/beta chain imbalance, primarily due to inefficient erythropoiesis **(19)**. Due to anemia that has existed since infancy, patients with transfusion-dependent thalassemia (TDT), the most severe form of beta-thalassemia, need lifelong transfusions and iron chelation therapy. β-thalassemia is also known as beta-thalassemia major or Cooley's Anemia.

TDT has been treated using allogeneic hematopoietic stem cell transplantation (allo-HSCT) for over 40 years, however, due to a lack of related or unrelated donors, it is limited for a large number of patient **(19)**. Recently, gene therapy has become a promising therapeutic option for young people with thalassemia who lack HLA-identical donors **(17-19)**. It offers the potential for phenotypic correction either through improved production of gamma chains that join with alpha chains to form fetal hemoglobin or through expression of a functioning copy of the beta-globin gene in hematopoietic stem cells **(4)**.

The following gene therapy approaches are being investigated in clinical trials for the treatment of β-thalassemia **(3, 22-24)**.

a. **Gene Addition**: In order to restore regular hemoglobin synthesis, this method entails inserting a functioning copy of the defective gene (often the therapeutic or wild-type β-globin gene) into the patient's cells. Gene addition therapy is frequently applied for treatment of thalassemia by using viral or non-viral vectors **(Table 1)**.
b. **Gene Editing**: Thalassemia-causing genetic defects can be directly corrected using gene editing techniques like CRISPR-Cas9. This strategy focuses on and fixes the patient's particular DNA mutation, enabling the generation of normal hemoglobin. To achieve this, CRISPR-Cas9 can be administered directly to HSCs or bone marrow cells **(16, 25-27)**.
c. **Induction of Fetal Hemoglobin (HbF):** Inducing the formation of fetal hemoglobin (HbF) in thalassemia patients is another gene therapy strategy. HbF can carry more oxygen than adult hemoglobin with a mutation. Thalassemia symptoms can be lessened in adult patients by reactivating the generation of HbF utilizing drugs or gene therapy methods **(28)**.
d. **Ex Vivo Gene Therapy**: This process involves the removal of the patient's HSCs or bone marrow cells. These cells are subsequently genetically modified outside the body using gene addition or editing techniques to correct the mutation or enhance the synthesis of HbF. The altered cells will be reintroduced into the patient's body and will be able to produce healthy red blood cells. HSC modification is based on gene addition (ß-globin wildtype, therapeutic ß-globin, ɣ-globin or shRNA) or DNA editing (BCL11A enhancer disruption, generation of hereditary persistence of fetal hemoglobin mutations and ß-globin gene correction) **(29)**.

Ongoing clinical trials for treatment of β-Thalassemia by gene-addition or gene-editing techniques are given in Table 2 and Table 3 respectively.

Approved Gene Therapy Medicinal Product for β-Thalassemia

Zynteglo (betibeglogene autotemcel) is a one-time ex vivo gene therapy, uses a lentiviral vector and made specifically for each patient, using the patient's own haematopoietic stem cells. It is autologous CD34+ cells encoding βA-T87Q-globin gene for treatment of adult and pediatric patients with ß-thalassemia who require regular red blood cell (RBC) transfusions.

It is manufactured by Bluebird Bio and approved by EMA in May 2019, but was withdrawn by its manufacturer, in March 2022 due to its pricing policy. It

is approved by FDA on August 17, 2022 and it is on the market in infusion bags containing 2.0 - 20 × 106 cells/mL (1.7 to 20 × 106 CD34+ cells/mL), frozen in approximately 20 mL of solution. The minimum dose is 5.0 × 106 CD34+ cells/kg patient weight **(30-32)**.

National Clinical Trial (NCT) numbers of the trials related with Zynteglo are NCT 01745120; 02140554; 02151526; 02633943; 02906202; 03207009; 04293185; and 04628585 **(32, 33)**.

The choice of thalassemia gene therapy depends on a number of variables, including the particular genetic mutation, the patient's age, and the severity of the illness. Advances in the realm of gene therapy for thalassemia may provide new hope for those who are stricken by the disorder.

Sickle Cell Disease

Sickle Cell Disease (SCD) is a congenital blood condition, that is characterized by hemoglobin S, an abnormal form of the protein. It is caused by a single missense mutation that causes valine to replace glutamic acid in the sixth position of the hemoglobin β-globin chain; which encodes for the beta-globin subunit of hemoglobin This change in protein level provides HbA with sickle hemoglobin (HbS). Sickle cell disease (SCD) is a collective term referring to any β-hemoglobinopathy containing the HbS allele. Homozygosity for this mutation results in sickle cell anemia (SCA) which is the most severe type of SCD **(16)**. This abnormal HbS can result in stiff, sticky, and sickle-shaped red blood cells, which can cause a various complications like acute vaso-occlusive crises (VOC), pain crises, hemolysis, anemia, organ damage, acute renal failure, acute chest syndrome, stroke, rethinopathy and heightened susceptibility to infections. The severity of sickle cell disease might vary, with some people having milder symptoms and others possibly dealing with more serious problems **(3, 16, 34)**.

Gene therapy, which combines autologous hematopoietic stem cell transplant (HSCT) with genetically altered cells, has emerged as a viable treatment option for sickle cell disease. The following gene therapy approaches are being investigated in clinical trials for the treatment of SCD **(24, 35)**.

a. **Gene Addition:** In this method, a functioning β-globin gene is transferred into the patient's hematopoietic stem cells (HSCs), to make normal hemoglobin and stop forming of sickle-shaped red blood cells. Hematopoietic stem cells (HSCs) can be genetically modified using viral vectors to introduce globin genes, such as γ-globin, γ/β-globin hybrids, and anti-sickling β-globin. γ-retroviruses are frequently used due to their advantages such as easy manipulation to create replication-incompetent vectors and stable packaging cell lines, flexible target cell types that can be transduced, and stable integration. Lentiviral vectors (LV), derived from HIV-1, have shown great potential for clinical applications due to their ability to deliver more complex DNA cassettes. They are more safe than retroviruses and they can transduce non-dividing HSCs **(9, 36)**.
b. **Gene Editing**: Site-specific nucleases (SSNs) that are genetically created have the ability to direct editing, ideally to a single base pair throughout the whole genome. These SSNs include CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats–CRISPR-associated nuclease 9) and ZFNs (zinc finger nucleases) and TALENs (transcription activator-like effector nucleases) **(20, 26, 27, 37)**. The β-globin gene mutation can be corrected by gene editing technologies such as CRISPR-Cas9 and Cas12 (NCT 04853576 and NCT 05444894). It completely replaces the patient's DNA and restores healthy hemoglobin synthesis. The BCL11A gene is a well-researched candidate for gene editing in SCD **(11, 20)**.
c. **Induction of Fetal Hemoglobin (HbF):** An alternative strategy is reactivating the production of fetal hemoglobin (HbF), for preventing red blood cells from deforming, by increased HbF levels. It is the most frequent strategy in clinical trials.

The tech,niques used to increase ɣ-globin expression are:

- downregulation of BCL11A via disruption of enhancer elements
- post-transcriptional regulation of BCL11A by short-hairpin RNA
- Y-globin promoters using base-editing

- ɣ-globin gene addition
- ɣ-globin gene addition with post-transcriptional regulation of BCL11A and
- recreating mutations in the β-globin associated with hereditary persistence of fetal hemoglobin.

Lentiviral vectors encoding the βA-T87Q-Globin and βAS3-Globin genes are used in clinical trials for induction of fetal hemoglobin **(38)**.

d. **Ex Vivo Gene Therapy**: HSCs are genetically modified ex vivo, by removing them from the patient. Cells are modified by using gene addition or editing techniques, and transferred into the patient's body to produce healthy red blood cells **(39)**.

Ongoing clinical trials for treatment of sickle cell disease by gene-addition or gene-editing techniques are given in Table 4 and Table 5 respectively.

FUTURE PERSPECTIVES

Even though gene therapy has advanced significantly, there are still many problems to be solved for evaluating the efficacy and safety profile of the GTMPs. Immunogenicity is the main problem for gene therapy medicinal products and long-term safety studies should be done. Development of gene delivery systems is very important for efficient gene therapy and patient safety. Gene therapy for thalassemia and hemoglobinopathies is still in its early stages, and while clinical trials have yielded promising results, it may be some time before these treatments become widely available. The exact mutation and the patient's condition determine the best strategy, and each has its own set of difficulties and considerations. Clinical trials and ongoing research in the field give hope for improved treatments and may be cures for a number of diseases.

Acknowledgement

I wish to thank Prof. Dr. Salih Şanlıoğlu for his valuable suggestions.

REFERENCES

1. European Medicines Agency, Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products, EMA/cAT/80183/2014, committee for Advanced Therapies (cAT), 22 March 2018.
2. Cesur-Ergün, B, & Demir-Dora, D. Gene therapy in cancer. *The Journal of Gene Medicine*. 2023; e3550. Advance online publication. https://doi.org/10.1002/jgm.3550
3. Lidonnici, MR, Scaramuzza, S, & Ferrari, G. Gene Therapy for Hemoglobinopathies. *Human gene therapy* 2023;34(17-18): 793–807. https://doi.org/10.1089/hum.2023.138
4. Ikawa Y, Miccio A, Magrin E, et al. Gene therapy of hemoglobinopathies: progress and future challenges. *Hum Mol Genet*. 2019; 28(R1): R24-R30.
5. Demir-Dora D. ALS Hastalığında İleri Tedaviler: Gen ve Hücre Tedavileri, In: Uysal H, Ed. Nadir Hastalık Olarak ALS, 1. Basım ed. Koza Matbaacılık, Palme Yayınevi; 2020: 273-290, ISBN: 978-605-282-639-3, Ankara
6. Naldini l. Ex vivo gene transfer and correction for cell-based therapies. *Nature Reviews Genetics* 2011;12:301-315.
7. Demir-Dora D. Gen tedavisinde kullanılan viral olmayan taşıyıcı sistemler, In: Bağcı G, Ed. Gen ve Hücre Tedavisinde Güncel Gelişmeler. 1. Baskı. Ankara: Türkiye Klinikleri; 2021. p.20-6.
8. Leask F. Technology digest: non-viral vectors. *Regenerative medicine* 2022; 17(11): 789–791. https://doi.org/10.2217/rme-2022-0115
9. Maetzig T, Galla M, Baum C, et al. Gammaretroviral vectors: biology, technology and application. *Viruses.* 2011;3:677–713.
10. Lundstrom K. Viral Vectors in Gene Therapy: Where Do We Stand in 2023?. *Viruses* 2023;15(3):698. https://doi.org/10.3390/v15030698
11. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science.* 2013;339:819–823.
12. Li T, Yang Y, Qi H, et al. CRISPR/Cas9 therapeutics: progress and prospects. *Signal transduction and targeted therapy*. 2023; 8(1): 36. https://doi.org/10.1038/s41392-023-01309-7
13. Patterson J. Small interfering RNA (siRNA)-based therapeutics. *Drug and therapeutics bulletin.* 2023; 61(5):72–76. https://doi.org/10.1136/dtb.2023.000004
14. Traber GM, & Yu AM. RNAi-Based Therapeutics and Novel RNA Bioengineering Technologies. *The Journal of pharmacology and experimental therapeutics*. 2023;*384*(1):133–154. https://doi.org/10.1124/jpet.122.001234
15. Gaj T, Gersbach CA, Barbas CF. 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol*. 2013;31:397–405.

16. Papizan JB, Porter SN, Sharma A, et al. Therapeutic gene editing strategies using CRISPR-Cas9 for the β-hemoglobinopathies. *The Journal of Biomedical Research*. 2021; 35(2): 115–134.
17. Saeidnia M, Fazeli P, Farzi A, et al. An Expert Overview on Therapies in Non-Transfusion-Dependent Thalassemia: Classical to Cutting Edge in Treatment. *Hemoglobin*. 2023;47:2:56 –70. DOI: 10.1080/03630269.2022.2158099
18. Finotti A, Breda L, Lederer CW, et al. Recent trends in the gene therapy of β-thalassemia. *Journal of Blood Medicine*. 2015; 69-85. DOI: 10.2147/JBM.S46256
19. Thuret I, Ruggeri A, Angelucci E, et al. Hurdles to the Adoption of Gene Therapy as a Curative Option for Transfusion-Dependent Thalassemia. *Stem Cells Translational Medicine*, 2022, 11, 407–414, https://doi.org/10.1093/stcltm/szac007
20. Germino-Watnick P, Hinds M, Le A, Chu R, Liu X and Uchida N. Hematopoietic stem cell gene-addition/editing therapy in sickle cell disease. Cells 2022; 11(11): 1843. doi: 10.3390/cells11111843. PMID: 35681538; PMCID: PMC9180595.
21. Lattanzi A, Camarena J, Lahiri P, et al. Development of β-globin gene correction in human hematopoietic stem cells as a potential durable treatment for sickle cell disease. *Science Translational Medicine.* 2021; *3*(598):eabf2444. https://doi.org/10.1126/scitranslmed.abf2444.
22. Segura EER, Ayoub PG, Hart KL, et al. Gene Therapy for β Hemoglobinopathies: From Discovery to Clinical Trials. *Viruses.* 2023; 15:713. https://doi.org/10.3390/v15030713
23. Karponi G & Zogas N. Gene Therapy For Beta-Thalassemia: Updated Perspectives. *The Application of Clinical Genetics*. 2019;167-180, DOI:10.2147/TACG.S178546
24. Ros FA, Couto SCF, Milhomens J, et al. A systematic review of clinical trials for gene therapies for beta-hemoglobinopathy around the world. *Cytotherapy*. 2023;00 1-7.
25. Gamage U, Warnakulasuriya K, Hansika S, et al. CRISPR Gene Therapy: A Promising One-Time Therapeutic Approach for Transfusion-Dependent β-Thalassemia-CRISPR-Cas9 Gene Editing for β-Thalassemia. *Thalass.Rep.* 2023; 13, 51–69. https://doi.org/10.3390/thalassrep13010006
26. Hoban MD, Bauer DE. A genome editing primer for the hematologist. *Blood*. 2016; 127(21):2525-2535.
27. Rahimmanesh I, Boshtam M, Kouhpayeh S, et al. Gene Editing-Based Technologies for Beta-hemoglobinopathies Treatment. *Biology*. 2022;11:862. https://doi.org/10.3390/biology11060862
28. Breda L, Rivella S, Zuccato C, et al. Combining gene therapy and fetal hemoglobin induction for treatment of beta-thalassemia. *Expert Rev Hematol*. 2013;6:255–264.
29. Cavazzana M, Antoniani C, Miccio A. Gene therapy for b-hemoglobinopathies. *Mol Ther* 2017; 25(5):1142–54.
30. https://www.fda.gov/vaccines-blood-biologics/zynteglo (accessed on 15.10.2023)
31. https://www.ema.europa.eu/en/medicines/human/orphan-designations/eu-3-12-1091 (accessed on 15.10.2023)
32. Asghar AA, Khabir Y, Hashmi MR. Zynteglo: Betibeglogene autotemcel – An innovative therapy for β-thalassemia patients. *Annals of Medicine and Surgery*. 2022; Volume 82, 104624, ISSN 2049-0801, https://doi.org/10.1016/j.amsu.2022.104624
33. https://www.clinicaltrials.gov/ (accessed on 15.10.2023)
34. Abdel-Hadi L, Carmenate YV, Castillo-Aleman YM, et al. Treatment of sickle cell disease - options and perspective. *Am J Blood Res* 2023;13(2):61-70.
35. Abraham AA and Tisdale JF. Gene therapy for sickle cell disease: moving from the bench to the bedside. *Blood*. 2021, Vol 138, Number 11, 932-941.
36. White SL, Hart K, and Kohn DB, Diverse Approaches to Gene Therapy of Sickle Cell Disease. *Annu. Rev. Med*. 2023;74:473–87.
37. Ikwuka AO, Musa S, Udeh FC, et al. CRISPR-Cas9 Genomic Editing as an Innovation in the Management of Sickle Cell Disease: A Systematic Review. *American Journal of Medical Science and Innovation*, 2023;*2*(2):44–56. https://doi.org/10.54536/ajmsi.v2i2.1760
38. Kanter J, Walters MC, Krishnamurti L, et al. Biologic and clinical efficacy of LentiGlobin for sickle cell disease. *N. Engl. J.Med.* 2022;386:617–28.
39. Rosanwo TO and Bauer DE. Editing outside the body: Ex vivo gene-modification for β-hemoglobinopathy cellular therapy. *Molecular Therapy*. 2021; 29:11). https://doi.org/10.1016/j.ymthe.2021.10.002.

Correspondence Address:
Devrim Demir-Dora 1, 2, 3
Akdeniz Üniversitesi Tıp Fakültesi
1Tıbbi Farmakoloji Anabilim Dalı
2Gen ve Hücre Tedavisi Anabilim Dalı
3Tıbbi Biyoteknoloji Anabilim Dalı
Antalya
Tel: 0-532-4133477
E-mail: dedemir@yahoo.com
devrimdemirdora@akdeniz.edu.tr
ORCID: orcid.org/0000-0002-6610-2507

Table 2: *Ongoing gene-addition clinical trials for β-Thalassemia (from ClinicalTrials.gov, assessed: 15.10.2023)*

Clinical Trial ID	Study Start	GTMP	Strategy	Vector	Phase	Status	Sponsor
NCT01639690	2012-07	Autologous CD34+ cells transduced with TNS9.3.55	β-Globin Addition	Lentiviral	1	Active, not recruiting	Memorial Sloan Kettering Cancer Center
NCT02633943	2014-01	Autologous CD34+ cells transduced with βA-T87Q-globin	β-Globin Addition	Lentiviral	Observational	Active, not recruiting	Bluebird Bio
NCT03275051	2017-10-04	Autologous CD34+ cells transduced with lentiviral vector encoding the human beta globin gene OTL-300 (Formerly GSK2696277)	β-Globin Addition	Lentiviral	Interventional	Active, not recruiting	IRCCS San Raffaele
NCT04592458	2020-12-01	β-globin restored autologous hematopoietic stem cells with LentiHBBT87Q.	β-Globin Addition	Lentiviral	Early Phase 1	Recruiting	Shenzhen Hemogen
NCT05015920	2021-04-01	BD211 Drug Product: CD34+ HSCs, undergo ex vivo transduction with lentiviral vector encoding βA-T87Q-globin	β-Globin Addition	Lentiviral	1	Recruiting	Shanghai BDgene Co., Ltd.
NCT05762510	2023-02-22	GMCN-508B (LentiRed): Autologous CD34+ Stem Cells Transduced Ex Vivo With a LentiRed Lentiviral Vector	β-Globin Addition	Lentiviral	Early Phase 1	Recruiting	First Affiliated Hospital of Guangxi Medical University

***GTMP:** Gene Therapy Medicinal Product

Table 3: *Ongoing gene-editing clinical trials for β-Thalassemia (from ClinicalTrials.gov, assessed: 15.10.2023)*

Clinical Trial ID	Study Start	GTMP	Strategy	Gene editor	Phase	Status	Sponsor
NCT04208529	2021-01-20	CTX001	BCL11A enhancer disruption	CRISPR-Cas9	Observational	Enrolling by invitation	Vertex Pharmaceuticals
NCT04211480	2020-04-01	γ-globin reactivated autologous hematopoietic stem cells	γ-globin expression	CRISPR-Cas9	Interventional	Active, not recruiting	Bioray Laboratories
NCT03655678	2018-09-14	CTX001	BCL11A enhancer disruption	CRISPR-Cas9	2/3	Active, not recruiting	Vertex Pharmaceuticals Incorporated

***GTMP:** Gene Therapy Medicinal Product

Table 4: *Ongoing gene-addition clinical trials for sicle cell disease (from ClinicalTrials.gov, assessed: 15.10.2023)*

Clinical Trial ID	Study Start	GTMP	Strategy	Vector	Phase	Status	Sponsor
NCT02140554	2014-08	bb1111	βA-T87Q addition	Lentiviral	1/2	Active, not recruiting	Bluebird Bio
NCT04293185	2020-02-14	bb1111	βA-T87Q addition	Lentiviral	3	Recruiting	Bluebird Bio
NCT02186418	2014-07	ARU-1801	γ Addition	Lentiviral	1/2	Active, not recruiting	Children's Hospital Medical Center, Cincinnati
NCT03282656	2018-02-13	autologous CD34+ HSC cells transduced with the lentiviral vector containing a short-hairpin RNA targeting BCL11a	γ Induction via addition of shRNA silencing BCL11A	Lentiviral	1	Active, not recruiting	DavidWilliams (BCH)
NCT03964792	2019-11-12	DREPAGLOBE drug product	βAS3 Addition	Lentiviral	1/2	Active, not recruiting	Assistance Publique Hôpitaux de Paris
NCT02247843	2014-12	βAS3-FB vector transduced peripheral blood CD34+ cells	βAS3 Addition	Lentiviral	1/2	Recruiting	Donald B. Kohn (UCLA)
NCT05353647	2022-07-12	Autologous CD34+ HSC cells transduced with the lentiviral vector containing a shRNA targeting BCL11a	γ Induction via addition of shRNA silencing BCL11A	Lentiviral	2	Recruiting	David Williams

GTMP: Gene Therapy Medicinal Product

Table 5: *Ongoing gene-editing clinical trials for sicle cell disease (from ClinicalTrials.gov, assessed: 15.10. 2023)*

Clinical Trial ID	Study Start	GTMP	Strategy	Gene editor	Phase	Status	Sponsor
NCT03745287	2018-11-27	CTX001	Erythroid lineage-specific enhancer of the BCL11A disruption	CRISPR-Cas9	2/3	Active, not recruiting	Vertex Pharmaceuticals
NCT03653247	2019-03-06	BIVV003	BCL11A locus targeting CD34+HSPC transfected ex vivo with ZFN mRNAs targeting the B-cell lymphoma/leukemia 11A (BCL11A) locus.	Zinc Finger Nuclease	1/2	Recruiting	Sangamo Therapeutics
NCT04443907	2020-08-26	OTQ923	Two genome-edited HSPC products to reduce the biologic activity of BCL11A and increase HbF	CRISPR-Cas9	1/2	Recruiting	Novartis Pharmaceuticals
NCT04774536	2024-06-01	CRISPR_SCD001	Autologous HSPCs with sickle allele modified by the CRISPR-Cas9 ribonucleoprotein	CRISPR-Cas9	1/2	Not recruiting	MarkWalters
NCT04819841	2021-11-15	GPH101 Drug Product	CRISPR-Cas9 edited and sickle mutation-corrected HSPCs to convert HbS to HbA	CRISPR-Cas9	1/2	Terminated	Graphite Bio
NCT04853576	2021-05-04	EDIT-301	Autologous CRISPR gene-edited CD34+ HSPCs and Progenitor Cells	CRISPR-Cas9	1/2	Recruiting	Editas Medicine
NCT05456880	2022-08-30	BEAM-101 Autologous CD34+ HSPCs edited ex-vivo.	BEAM-101 cells are engineered *ex vivo* with an ABE that incorporates A → G base edits in the *HBG1* and *HBG2* gene promoters, which regulate the expression of HbF (1st base-edited)	CRISPR-Cas9	1/2	Recruiting	Beam Therapeutics Inc.
NCT05951205	2024-01	Exa-cel	Autologous CD34+ hHSPCs modified with CRISPR-Cas9 at the erythroid lineage-specific enhancer of the BCL11A gene	CRISPR-Cas9	3	Recruiting	Vertex Pharmaceuticals

***GTMP:** Gene Therapy Medicinal Product

CHAPTER 13
THALASSEMIA AND HEMOGLOBINOPATHIES PREVENTION PROGRAMS

PUBLIC EDUCATION FOR THE PREVENTION OF THALASSEMIA AND HEMOGLOBINOPATHIES

A.Sabri Kemahlı,M.D.
Professor of Pediatrics and Ped. Hematology
College of Medicine, Alfaisal University, Riyadh, S. Arabia

ABSTRACT

Hemoglobinopathies are considered as a public health problem in Türkiye and The National Hemoglobinopathy Control program has been implemented since 2003. Providing community education about thalassemia and hemoglobinopathies, especially in regions where the prevalence is high, is mandatory for the success of the control program. Studies conducted in Türkiye and around the world have shown that the level of knowledge about thalassemia and hemoglobinopathies in the societies with high prevalence of these disorders is not at the desired level. On the other hand, it has been observed that effective education increases knowledge in societies and leads to behavioral changes such as interest and participation in screening and prenatal diagnosis activities. Education should be provided to target societies and groups, under the leadership of specialist physicians and with the participation of allied health personnel. The clinical features, hereditary aspects of hemoglobinopathies, how screening is performed, and the importance of premarital screening should be emphasized in the training program. Although face-to-face methods are the most effective, the addition of promotional tools such as books, brochures, and methods such as videos, slides and posters increases the effectiveness of training.

Keywords: Thalassemia, hemoglobinopathies, prevention public education

INTRODUCTION

Thalassemia, or as more broadly defined, hemoglobinopathy, is a common public health problem in the region that includes Türkiye. While it is mostly seen in the Mediterranean and Aegean regions, patients and carriers are found in every part of the country. In addition to being an important public health issue, the ease and widespread applicability of screening tests makes it possible to conduct community screening. In this way, carriers in the community can be identified and counseling can be provided to couples.

As a result of thalassemia screening studies starting from the 1970s, thalassemia was accepted as a public health problem in 1993 and the hemoglobinopathy control program was implemented in 33 provinces in 2003 (Çavdar & Arcasoy, 1971;Aydinok et al., 2018; Canatan, 2014) . The birth of sick individuals is prevented in regions with high frequency of carriers, by providing genetic counseling and necessary medical assistance to couples who are found to be carriers during screening.

In addition to academic studies, voluntary organizations have also contributed to reach this stage. Thalassemia societies communicating with patients and their families, became the driving force for community screening and education, and often initiated the first studies in their regions. Non-governmental organizations such as societies, associations and clubs have contributed to academic studies and the activities of thalassemia associations (Arcasoy A, Turan F, Yeşil N, Kemahlı S, Uysal Z, Canatan D, 1994).

STUDIES ON NEEDS ASSESSMENT FOR EDUCATION

Even if hemoglobinopathies are common in a society, it is important to know the level of knowledge about thalassemia and hemoglobinopathies in the

general population to determine the target groups to be reached as well as the content and level of training programme.

Several studies have been published evaluating level of knowledge in various societies about thalassemia and hemoglobinopathies and showing how it can be modified with education.

In a survey conducted in Denizli, Türkiye, on couples applying for premarital hemoglobinopathy screening, 91% of the participants appreciated the importance of premarital screening, but only 57.7% reported that they had previously heard of this program. It was determined that 53.2% of the participants thought thalassemia trait to be a disease, and 82.5% had no idea whether thalassemia was contagious or not. It has been observed that the correct answers to various questions about the hereditary transmission and treatment of thalassemia are between 41.6-50% (Balci et al., 2014).

The frequency of thalassemia carriers in Bangladesh is between 6-12%. A study was conducted among 1578 university students from different colleges, and it was observed that 67% of the students had never heard of thalassemia. There were however differences between students in different colleges: while 82% of science students had heard of thalassemia, this rate was found to be 21.9% for humanities students and 16.2% for business students. The level of knowledge about thalassemia was found to be parallel to these rates: the knowledge level was found to be 4.72 out of a total of 12 points for all students, 5.03 for science students, 3.66 for humanities students, and 4.05 for business students (Hossain et al., 2020).

Similarly, in a study conducted on 920 university students in Saudi Arabia, it was observed that only 48% of the students had heard of thalassemia, and even though premarital screening was mandatory, only 50% of the married students had heard of thalassemia. When knowledge levels were questioned, it was observed that the general average was 4.4 out of a total score of 12 points, 5.2 for science students, 4.7 for art students, and 3.9 for dentistry students (Olwi et al., 2018).

In an interesting study showing the effect of public education and screening, the knowledge levels of Italians living in Sicily were compared with those individuals of Italian descent in USA (Italian-Americans) and non-Italian Americans (other Americans) living in the USA. While 85% of Italians in Sicily had previously heard of thalassemia, this rate was found to be 19% in Italian-Americans and 21% in other Americans. It was observed that the knowledge level of Italians living in Sicily was significantly higher than the other two groups. Correct response rates were found to be 54.9% in Italians, 16.5% in Italian-Americans, and 23.6% in other Americans. An important point is that while 4% of the Italians participating in the study had higher education, these rates were 44% and 48% in the other two groups (Armeli et al., 2005). On the other hand, it was observed that all three groups had heard of Down syndrome at a rate of 96-97%, and their correct answers to the questions about Down syndrome were close to each other (59.3%, 58.4% and 60.2%). These results show that community screening and education increases awareness in the society, independent of the general education level.

When examples of education programs on thalassemia and hemoglobinopathies are examined, the positive effects of the training can be seen.

When a video education given to high school students in Indonesia, their previously insufficient knowledge about thalassemia increased and the knowledge was retained as shown in the tests 2 weeks after the education (Rakhmilla et al., 2017).

In a study conducted with focus groups in Malaysia, it was observed that 68.6% of the participants had previously heard of thalassemia and knew that it was hereditary. However, it was observed that their level of information on thalassemia was parallel with their level of education. Almost all groups perceived a lack of information to be the main cause of unawareness about thalassemia in the public. Some groups had the opinion that religious permission or fatwa is required regarding prenatal diagnosis and termination of pregnancies. Although groups generally believe that terminating pregnancy is unethical, a change in attitude was observed in some, when the effects of the disease on patients and families were discussed (Wong et al., 2011).

A comparison of the pre-test and post-test in an education program given to 8th grade students in Adana, Türkiye showed that the information was well received (Arpaci et al., 2003).

In a study conducted in rural areas of Cambodia, it was found that the knowledge and attitudes of those who received education regarding the prevention and control of thalassemia increased significantly (Cheng et al., 2018).

Families of patients with thalassemia were also included in the knowledge level assessments in some studies.

A study in Pakistan addressed parents of thalassemic children; only 44.6% of parents knew that thalassemia is a hereditary disease, and 33% knew that the disease could occur if both parents were carriers. The correct answers to questions about prenatal diagnosis varied between 8.7-86.1% (Ishaq et al., 2012).

In a study in Indonesia, it was reported that parents' have a good knowledge of thalassemia and increased even more with online education (Asa et al., 2021).

An important point is the necessity of monitoring and follow-up after educating the target audience. It has been determined that the proportion of knowledgeable pregnant women who received training for sickle cell anemia in Tanzania has increased. from 12.4% to 85.9% after education. They were screened and informed about the importance of neonatal screening. Pregnant women who were determined to be carriers of sickle cell anemia took their babies for screening, while the majority of those who were determined to be Hb AA usually did not. (Tutuba et al., 2023).

All these studies show that thalassemia education is necessary for societies, and that education can increase the level of knowledge and result in behaviour and attitude changes.

TO WHOM AND WHERE SHOULD TRAINING BE PROVIDED?

It is a necessity to provide public education, with priority in regions with high rate of carriers.

In some cases initial education started with couples with sick children, however the effectiveness of this strategy for education and screening was limited. Nowadays the first targets for screening and education are couples who are about to get married and newlyweds (Cao & Kan, 2013).

Community education is an important part of thalassemia control programmes and, unlike education given to susceptible families for genetic counseling and prenatal diagnosis, it should cover the entire target population.

To reach as wide part of a society as possible, different segments of society should be informed through education in designated groups. Groups such as students, teachers, community leaders, soldiers, non-governmental organizations at all levels are among the target audiences.

Priority should be given to providing training in places where the target audiences are located. So, schools, military units, workplaces, villages, events led by non-governmental organizations, etc. can be counted among these.

Larger events that will include wider segments of society can also be organized.

The primary institution responsible for health education at the national level is the Ministry of Health, and their support must be received for public education programmes. Additionally, it is important to receive support and contributions from the Ministries of National Education, National Defense and Internal Affairs as well as universities, Red Crescent, and non-governmental organizations.

Since education will be mainly provided to adult populations, it should be planned and carried out by taking into account adult education principles, which can be listed as follows (Norman, 1999):

Adults want to learn what they need.

1. Their requirements determine the training content.
2. Adults' individual characteristics and circumstances must be taken into account.
3. Training should be carried out within a plan with determined goals and objectives.
4. They want to participate actively in education.
5. They require positive feedback.

Face-to-face education is a priority in community education.

The advantages of face-to-face education are instant communication with the audience and the opportunity to answer questions instantly and after the training.

In addition, changes can be made in the content and depth of the education according to the characteristics of the target audience and their level of prior knowledge.

The presence of individuals or families with hemoglobinopathy/thalassemia in the target society with their problems seen or heard by the society, creates a need and motivation for education. Thus, what the society wants to learn about thalassemia can be determined. In relatively small communities (such as villages, towns), obtaining information about the needs of the society in advance makes education more effective. It would be appropriate to make an announcement before the education sessions, announcing content, lecturers and duration of the activity.

It may be appropriate to start the education by emphasizing that there are individuals with hemoglobinopathy in the society and that their problems are known by the society.

Being open to questions and contributions at every stage of the educational activity is an important point that will ensure the active participation of individuals in the meeting.

In events held in schools, military units and workplaces, the training content can be determined according to the level of knowledge of the participants, if possible, in advance, or by asking questions at the beginning of the event.

WHO SHOULD DELIVER THE EDUCATION?

Community education should be provided by healthcare personnel, especially by knowledgeable and experienced physicians. Primarily specialists in hematology, pediatrics and genetics should deliver the education. Contribution of family physicians and allied health personnel, who work with this group of patients and their families, should also be sought. Announcing the competency of educators in advance will increase participation and interest in the event. In Sardinia, 70 % of the target population was informed by physicians (family physicians, obstetricians and genetic counselors) (Cao & Kan, 2013).

CONTENT OF THE EDUCATION PROGRAM

The education should cover the following topics:

- Frequency of thalassemia and hemoglobinopathies in the population
- Clinical features and findings
- Characteristics of hereditary transmission-differences between carriers and patients
- Treatment and challenges
- Who should be screened?
- The necessity of premarital screening and the way to follow for carriers
- Screening methods
- Suggestions for married and soon-to-be-married couples
- Recommendations for patient families (as a separate session)

Face-to-face conference training with visual materials such as posters, pictures, slides and videos increases effectiveness. Distributing educational materials such as brochures and booklets during these events is very effective in ensuring the retainment of information. Although some studies have shown that both lecturing and booklets increase knowledge levels, it is known that using different methods together is more effective (Dehkordi & Heydarnejad, 2008).

Innovative methods can be used to reach wider audiences. An example of this is the “Talotır” project implemented within the scope of the national

hemoglobinopathy control program in Türkiye. With this project, 62682 people received education in 23 provinces in 7 months by the use of a mobile truck (TIR) unit (Canatan et al., 2013).

Mass media such as radio, TV, newspapers and social media can also be used effectively for large scale public information.

CONCLUSION

Based on the results of screening for thalassemia and hemoglobinopathies, information and education should be provided to the public, with priority given to target regions and groups. Although face-to-face methods are most effective, the additional use of promotional tools such as books, brochures and audio-visual methods increases the effectiveness.

REFERENCES

1. Arcasoy A, Turan F, Yeşil N, Kemahlı S, Uysal Z, Canatan D, A. N. (1994). Muğla ili ve çevresinde thalassemia ve anormal hemoglobin sıklığının taranması. *Pediatride Yönelişler*, *1*, 78–80.
2. Armeli, C., Robbins, S. J., & Eunpu, D. (2005). Comparing knowledge of β-thalassemia in samples of Italians, Italian-Americans, and non-Italian-Americans. *Journal of Genetic Counseling*, *14*(5), 365–376. https://doi.org/10.1007/s10897-005-1123-5
3. Arpaci, A., Aytaç, N., Yüregir, G. T., Tuli, A., & Aksoy, K. (2003). An education programme on sickle cell anemia and β-thalassemia for the 8th grade students. *Turkish Journal of Haematology*, *20*(1), 19–24.
4. Asa, P., Indiastuti, D. N., Andarsini, M. R., Fauziah, J. N., & D'Arqom, A. (2021). Empowering Thalassemia Patients and Family to Increase Public Knowledge on Thalassemia. *Jurnal Pengabdian Kepada Masyarakat (Indonesian Journal of Community Engagement)*, *7*(4), 228. https://doi.org/10.22146/jpkm.69349
5. Aydınok, Y., Oymak, Y., Atabay, B., Aydoğan, G., Yeşilipek, A., Ünal, S., Kılınç, Y., Oflaz, B., Akın, M., Vergin, C., Evim, M. S., Çalışkan, Ü., Ünal, Ş., Bay, A., Kazancı, E., İleri, T., Atay, D., Patıroğlu, T., Kahraman, S., … Karakaş, Z. (2018). A national registry of thalassemia in Türkiye: Demographic and disease characteristics of patients, achievements, and challenges in prevention. *Turkish Journal of Hematology*, *35*(1), 12–18. https://doi.org/10.4274/tjh.2017.0039
6. Balci, Y. I., Ergin, A., Polat, A., Atilgan, T., Uzun, U., & Koyuncu, H. (2014). Thalassemia premarital screening program: Public view, what has been done, what needs to be done? *UHOD - Uluslararasi Hematoloji-Onkoloji Dergisi*, *24*(4), 247–252. https://doi.org/10.4999/uhod.14569
7. Canatan, D. (2014). Hemoglobin international journal for hemoglobin research Thalassemias and Hemoglobinopathies in Türkiye MINI REVIEW Thalassemias and Hemoglobinopathies in Türkiye. *Hemoglobin*, *38*(5), 305–307. https://doi.org/10.3109/03630269.2014.938163
8. Canatan, D., Aydinok, Y., Kilinç, Y., Karakaş, Z., Şaşmaz, I., Apak, H., & Sarper, N. (2013). Ulusal Talasemi önleme Kampanyasi: Talotir Projesi. *Turkish Journal of Hematology*, *30*(1), 91–92. https://doi.org/10.4274/tjh.2012.0121
9. Cao, A., & Kan, Y. W. (2013). The Prevention of Thalassemia. *Cold Spring Harb Perspect Med*, *3*(a011775), 1–15. https://doi.org/10.1101/cshperspect.a011775
10. Çavdar, A. O., & Arcasoy, A. (1971). The Incidence of Thalassemia and Abnormal Hemoglobins in Türkiye. *Acta Haematologica*, *45*(5), 312–318. https://doi.org/10.1159/000208641
11. Cheng, K., Fucharoen, S., Sanchaisuriya, K., Fucharoen, G., Sanchaisuriya, P., & Jetsrisuparb, A. (2018). Effect of health education on severe thalassemia prevention and control in communities in Cambodia. *Archives of Public Health*, *76*(1), 1–8. https://doi.org/10.1186/s13690-018-0259-3
12. Dehkordi, A. H., & Heydarnejad, M. S. (2008). Effect of booklet and combined method on parents' awareness of children with β-thalassemia major disorder. *Journal of the Pakistan Medical Association*, *58*(9), 485–487.
13. Hossain, M. S., Hasan, M. M., Raheem, E., Islam, M. S., Al Mosabbir, A., Petrou, M., Telfer, P., & Siddiqee, M. H. (2020). Lack of knowledge and misperceptions about thalassaemia among college students in Bangladesh: A cross-sectional baseline study. *Orphanet Journal of Rare Diseases*, *15*(1), 1–10. https://doi.org/10.1186/s13023-020-1323-y
14. Ishaq, F., Abid, H., Kokab, F., Akhtar, A., & Mahmood, S. (2012). Awareness among parents of β-Thalassemia major patients, regarding prenatal diagnosis and premarital screening. *Journal of the College of Physicians and Surgeons Pakistan*, *22*(4), 218–221. https://doi.org/04.2012/JCPSP.218221
15. Norman, G. R. (1999). The adult learner: A mythical species. *Academic Medicine*, *74*(8), 886–889. https://doi.org/10.1097/00001888-199908000-00011
16. Olwi, D. I., Merdad, L. A., & Ramadan, E. K. (2018). Thalassemia: a prevalent disease yet unknown term among college students in Saudi

Arabia. *Journal of Community Genetics*, *9*(3), 277–282. https://doi.org/10.1007/s12687-017-0351-3

17. Rakhmilla, L. E., Larasati, R., & Sahiratmadja, E. K. (2017). Assessing Knowledge About Thalassemia Among Reproductive Age Population After Video Media Education. *Journal of Biomedical and Clinical Science*, *2*(2), 30–32.
18. Tutuba, H. J., Jonathan, A., Lloyd, W., Masamu, U., Marco, E., Makani, J., Ruggajo, P., Kidenya, B. R., Minja, I. K., & Balandya, E. (2023). The efficacy of maternal health education and maternal screening on knowledge and the uptake of infant screening for sickle cell disease in Dar-Es-Salaam, Tanzania; a quasi experimental study. *BMC Public Health*, *23*(1). https://doi.org/10.1186/s12889-022-14859-2
19. Wong, L. P., George, E., & Tan, J. A. M. A. (2011). A holistic approach to education programs in thalassemia for a multi-ethnic population: Consideration of perspectives, attitudes, and perceived needs. *Journal of Community Genetics*, *2*(2), 71–79. https://doi.org/10.1007/s12687-011-0039-z

Correspondence Address:
A.Sabri KEMAHLI,M.D.
Professor of Pediatrics and Ped. Hematology
College of Medicine, Alfaisal University, Riyadh, S. Arabia
E-mail: kemahlisabri@gmail.com

GENETIC COUNSELING IN THALASSEMIA AND HEMOGLOBINOPATHIES

Öğr. Gör. Elif Gözde Gökkaya
Antalya Bilim University, Vocational School of Health Services, Department of Medical Laboratory Techniques, Antalya, Türkiye

ABSTRACT

Hemoglobin, vital for oxygen transport, comprises two α-like and two β-like globin chains. Over 1700 mutations affect globin synthesis (thalassemia) or hemoglobin's structure, causing autosomal recessive monogenic disorders. Hemoglobinopathies denote changes in the structure of globin genes. Mutations in α and β globin chain genes result in hemoglobinopathies characterized by abnormal globin synthesis or hemoglobin properties. These mutations are genetically inherited, and the presence of these mutations can be detected in people with some tests. Genetic counseling can be especially important for individuals with a family history of genetic disorders, couples considering having children, or anyone who wants to gain insight into their genetic health. These professionals play a crucial role in helping individuals and families make informed choices about their healthcare. Genetic counseling can start even before a pregnancy occurs. Couples or individuals may seek genetic counseling before conceiving to understand their risk factors for passing on genetic conditions to their future children. This allows for informed family planning decisions. Success in genetic counseling and screening programs is multifaceted and requires a holistic approach. It hinges on the competence, compassion, and dedication of genetic counselors, as well as effective collaboration with healthcare teams and the active participation of the individuals being screened. Ultimately, the goal is to identify and manage genetic risks to promote healthier lives and prevent the onset of genetic conditions.

Keywords: Genetic counseling, prenatal screening, premarital screening

BACKGROUND

Hemoglobinopathies and Thalassemias

Hemoglobin (Hb), the protein that transports oxygen from the lungs to tissues, is a tetramer composed of two α-like globin chains and two β-like globin chains. Throughout development in humans, β-like genes are expressed sequentially. The fetal Aγ- and Gγ-globin genes are silenced around the time of birth, and adult β-globin gene expression predominates; this process is known as γ-to-β Hb switching. Strong chromatin opening and DNA enhancer elements (DNase hypersensitive sites [HSs]) are found in a 16-kb-long locus control region (LCR), which is situated 40–60 kb upstream of the β-globin genes and is required for high-level globin gene expression **(1)**.

Hemoglobinopathies refer to structural alterations in globin genes. Some of these variations are linked to clinical conditions such as sickle cell anemia and related disorders, hemolysis due to unstable hemoglobins, changes in hemoglobin oxygen affinity, and hemoglobins that cannot maintain iron in the ferrous (Fe^{2+}) state. However, the majority of structural variants don't exhibit clinical symptoms and are often discovered incidentally, typically during the measurement of hemoglobin A_{1c} in diabetic patients through methods like high-performance liquid chromatography (HPLC) or capillary zone electrophoresis. Furthermore, if a variant's amino acid change doesn't alter its charge, it might go undetected by chromatographic techniques **(2)**.

Hemoglobinopathy is the most common group of autosomal recessive monogenic disorders. It is characterized by mutations or deletions in the genes

encoding the alpha (α) and beta (β) globin chains of the hemoglobin molecule. This condition is caused by more than 1700 different mutations that either affect the synthesis of globin chains (thalassemia) or change the structure and properties of hemoglobin (hemoglobin variants or abnormal hemoglobins). When they contain homozygous and compound heterozygous mutations, they result in 4 main clinical conditions, each exhibiting varying phenotypic features and disease severity. These are:

- α-thalassemia syndrome,
- β-thalassemia syndrome; including δβ-thalassemia and Hb E/β thalassemia
- Sickle cell syndromes (major genotypes Hb S/S, Hb S / C and Hb S/β-thal and less common genotypes Hb S/ D Punjab, Hb S O Arab and Hb S/Lepore)
- Such as hemolytic anemia, polycythemia and, less commonly, Hb variants that result in cyanosis **(3).**

In each human diploid cell, you can find four copies of the α -globin gene situated on chromosome 16. While α-thalassemia is typically a result of one or more deletions in the α-globin chain locations, it's important to note that not all cases of α-thalassemia stem from gene deletions. Clinically, there are four distinct α-thalassemia syndromes: silent carrier, α-thalassemia trait, HbH disease, and hydrops fetalis syndrome. These conditions arise due to the inheritance of molecular mutations that impact the activity of one, two, three, or all four of the alpha-globin genes **(4)**.

Thalassemia mutations either completely eliminate (β^0) or greatly decrease (β^+) the production of hemoglobin ß-chains, leading to an imbalance between hemoglobin a- and ß-chains. This imbalance causes ineffective red blood cell production, potentially leading to extramedullary hematopoiesis. Most patients with two inactivated ß-globin genes have thalassemia major, requiring lifelong red blood cell transfusions. Roughly 10% to 15% of individuals possessing mutations that permit some ongoing ß-globin expression, genetic enhancers increasing γ-globin and fetal hemoglobin (HbF) levels beyond infancy, or co-inherited α-thalassemia mutations exhibit the thalassemia intermedia condition. These patients may occasionally need red blood cell transfusions but typically experience skeletal alterations and iron accumulation later in life due to significant erythroid hyperplasia **(5)**.

The beta-gene cluster is located on chromosome 11, and beta-thalassemia encompasses various forms. β^0 thalassemia involves a complete absence of β-chain production, while β^+ thalassemia results in partial deficiency. Both forms exhibit hypochromia and microcytosis. Thalassemia major, where beta chain synthesis is severely inhibited, leads to significant anemia around 3 to 6 months of age. This causes stress in the bone marrow, continuing HbF synthesis but insufficiently compensating for anemia. Irregularly distributed HbF results in anisochromasia. Ineffective erythropoiesis, primarily due to accelerated apoptosis, is driven by excess alpha chains in erythroid precursors. Hemoglobin patterns vary, with HbF making up 8% to 90% of total hemoglobin. Severity ranges from β-thalassemia major to minor or carrier **(4)**.

In δβ+ thalassemia, an abnormal hemoglobin called Hb Lepore is produced. Hb Lepore consists of a normal alpha chain paired with a nonalpha chain, formed by the fusion of the N-terminal residue of the delta chain with the C-terminal residue of the beta chain. There are various forms of Hb Lepore with different fusion points in the amino acid sequences. While Hb Lepore has limited clinical significance, it's of significant genetic interest. Its presence highlights the proximity of delta- and beta-chain loci on the same gene, with the delta locus preceding the beta locus. Individuals with such genes may have up to 25% Hb Lepore in their blood and increased HbF levels (5%–70%). Hb Lepore occurs sporadically in most racial groups and rarely leads to significant anemia **(4)**.

Sickle hemoglobin (HbS) results from a genetic change in the β-globin subunit, specifically the substitution of glutamic acid to valine. Roughly 8% of African Americans carry this variant, known as sickle cell trait (HbAS). In equatorial Africa, where malaria is common, HbAS prevalence can exceed 30% due to its protective effect against severe malaria complications. HbAS individuals typically have 40% HbS and 56%–58% HbA and are usually

asymptomatic unless exposed to severe hypoxia, leading to sickle cell disease symptoms (sickling).

In individuals with sickle cell anemia (HbSS), a condition where both genes are affected, deoxy-HbS polymerization leads to the formation of multistranded fibers, altering red blood cell (RBC) shape from biconcave discs to elongated crescents. This process is reversible upon hemoglobin reoxygenation, allowing for repeated cycles of sickling and unsickling. The main consequences of sickling are RBC membrane damage, causing abnormal permeability and dehydration, leading to premature RBC destruction and chronic hemolytic anemia. Additionally, sickled RBCs are rigid, increasing blood viscosity, obstructing capillary flow, and causing tissue hypoxia. Prolonged tissue hypoxia can result in cell death, tissue necrosis/infarction, and organ damage, often referred to as vaso-occlusive pain crises during acute episodes **(6)**.

Genetic Counseling

Genetic counseling is a process in which medical conditions such as having a genetic disease, being at risk for the disease or determining the risk in the family are evaluated, information is provided to the client, the status of the genetic disease is evaluated. If necessary, the tests and treatment methods can be shaped **(7)**.

In 1975, the American Society of Human Genetics explained the concept of genetic counseling as follows. This definition is one of the most comprehensive definitions made. According to this, genetic counseling is a communication process which deals with the human problems associated with the occurrence, or the risk of occurrence, of a genetic disorder in a family. This process involves one or more individuals who have received adequate training attempting to assist the individual or family, which:

(1) comprehend medical facts including diagnosis, likely course of the disorder, and appropriate management,

(2) evaluate the way heredity contributes to the disease and the risk of recurrence in specific relatives,

(3) understanding alternative ways to cope with the risk of recurrence,

(4) choose appropriate behavior and act accordingly, taking into account risky situations, family goals, ethical and religious standards, and

(5) to make the most appropriate arrangement for the family member affected by the disease and/or at risk of recurrence of the disease.

A genetic specialist can help in several ways, including official or informal consultations, genetic counseling sessions, and genetic evaluations. A genetic specialist can confirm the diagnosis of a hereditary condition or offer a more precise risk assessment. Genetic testing alone, or in conjunction with testing, a clinical examination, and family history, may be used to make a diagnosis. When necessary, genetic specialists can offer management choices or specialized referrals. They can also advise general practitioners regarding a genetic condition's prognosis, course of therapy, and long-term results as well as suggest educational materials for patients and their families. A genetic referral is made by looking at the patient information, the name of the referrer, the reason for the referral, information about the suspected diagnosis if known, and family history **(8)**.

The depression levels of family members who received genetic counseling were investigated. The majority of patients reported moderate to severe levels of depression prior to getting genetic counseling. After obtaining genetic counseling, most participants reported modest levels of depression, and five persons even reported no depression. Most participants overall reported favorable changes that suggested lower depression levels. Findings show how genetic counseling sessions might help parents of children with thalassemia major feel less depressed by providing emotional support and illness education **(9)**.

Situations That Require Genetic Counseling

According to genetic counselors, we can divide situations requiring genetic counseling into three headings. Patients who meet one or more of the

following conditions should be considered for referral to genetic specialists.

- **Family History**
  * A member or members who have a birth defect, an inherited disorder, or mental retardation.
  * Early deaths of one or more members from known or unknown illness circumstances
  * A member or members who have adult-onset diseases including cancer, dementia, or cardiovascular disease, especially if they developed when they were young
  * Couples interested in genetic testing or in learning more about illnesses that are more common in their ethnic group

  Three generations should be included in a basic family history. Ask the patient about their medical history before moving on to inquiries about their siblings and parents to start gathering information for the family history. Questions should include general information such as names and dates of birth, family origin or racial/ethnic background, health status, age at death and cause of death for each family member, pregnancy outcomes of the patient and genetically related relatives.
- **Developmental Delay/Growth**
  * Those who have developmental delays in their child or are worried that their child may have them as a result of an inherited illness or birth defect
  * Parents whose newborn was identified by regular newborn screening as having a genetic illness
- **Reproductive Issues**
  * Women above the age of 35 who are pregnant or intend to become pregnant
  * Women who have had several miscarriages, including those resulting in infant deaths
  * People who are worried that the result of their pregnancy may be at risk due to their occupations, habits, or medical histories. Common reasons for worry include radiation exposure, pharmaceuticals, illicit drugs, chemicals, or diseases.
  * First cousins or other near blood relatives
  * Pregnant women whose ultrasound results or blood tests show that they could be more likely to experience certain difficulties or give birth to birth defects **(8)**.

Prenatal Diagnosis

The major goal of screening is to enhance patient outcomes through early disease detection and care, as well as the opportunity to provide genetic counseling to parents of affected children **(10)**. Pregnancy is the best moment to determine a person's carrier status, estimate their genetic risk, and receive genetic counseling. Young individuals who are carriers should receive genetic counseling, which should cover topics such whether prenatal diagnoses are available, potential dangers to offspring, and reproductive alternatives. Population screening in conjunction with genetic counseling is very beneficial since it enables at-risk couples to make educated decisions about their reproductive options. Such a couple has the choice to choose to carry to term only those pregnancies in which the fetus is unaffected through genetic counseling and prenatal testing **(11)**. Prenatal screening is now accessible in many low-resource nations, some of which have also developed carrier-screening programs. Prenatal screening is rapidly gaining popularity worldwide **(12)**.

All at-risk couples should be given the opportunity to meet with experts in the area for a consultation to go over the various reproductive options and the likelihood of getting a prenatal diagnosis. Genetic counseling conversations should be open-ended, free of jargon, and if feasible, take the couple's cultural views into consideration. The couple must make the final choice independently of anyone else. To provide the couple enough time to make an informed decision and to not rule out the possibility of access to some prenatal diagnosis procedures, counseling should be provided before conception and/or during the first few weeks of pregnancy. If the couple hasn't had any prior testing, the request for screening tests should be made at the first obstetric appointment **(13)**.

Prenatal diagnosis methods were also used to struggle thalassemia disease. The finding that β-globin

chain synthesis in the cord blood of β-thalassemia heterozygotes was much lower than normal and that adult hemoglobin synthesis could be observed in fetuses at midtrimester initially raised the prospect of prenatal diagnosis of hereditary hemoglobinopathies. The β^S-chain was later detected in the blood of the fetuses. Fetal blood sampling by fetoscopy or placentocentesis was established. Then, prenatal diagnosis using globin chain synthesis on fetal blood was introduced. By using the molecular hybridization approach, the first molecular diagnosis was made for the diagnosis of α-thalassemia. For a brief time, the diagnosis of β-thalassemia by DNA analysis was accomplished either directly by mutation-specific oligonucleotide hybridization on electrophoretically separated DNA fragments or indirectly by study of DNA polymorphisms connected to the β-globin gene **(14)**.

In Taiwan, a prenatal thalassemia screening program resulted in a significant reduction in the incidence of β-thalassemia. While before the program approximately 20 children with thalassemia were born each year, after seven years of the screening programme, the number of cases had fallen to only three to six affected children each year. Another prenatal screening program has been running in Guangdong, China, for 11 years. More than 95% of detected carrier couples were diagnosed prenatally, and only one β-thalassemia patient was born due to misdiagnosis during prenatal diagnosis. All affected fetuses identified in the screening program were terminated. In the UK, most potential parents choose to terminate their pregnancies if the fetus is affected by β-thalassemia. Some countries do not provide prenatal diagnostic services to couples because termination of pregnancy is prohibited on religious grounds. Before Iran approved prenatal diagnosis and pregnancy termination for high-risk couples, almost half of the carrier couples discovered by the premarital screening program started dating and the other half separated **(15)**.

Premarital Screening

Early in the 20th century, the idea of a premarital health examination was developed. It was initially implemented as a health measure in the USA and Scandinavian nations **(16)**. A important preventive measure to lower the prevalence of several genetic disorders and sexually transmitted diseases is premarital screening. Premarital screening is a screening program made available to engaged couples in order to detect those who may be inherited diseases or carriers. Although these carriers are typically asymptomatic, if both couples are carriers, their future children could contract these disorders **(17)**.

A premarital screening program was launched in Türkiye in 1995. There was a decrease in the number of babies diagnosed with thalassemia within a 5-year period. According to a study conducted in Denizli province, two out of 15 couples diagnosed were consanguineous. 6 couples who had children applied to the centers for prenatal diagnosis. Prenatal screening revealed 1 fetus to be normal, 4 to have thalassemia minor, and 1 to have thalassemia major; this pregnancy was terminated by selective abortion **(18)**.

Premarital genetic screening became mandatory in Saudi Arabia in 2004. It was decided that couples should receive genetic counseling before marriage, if necessary. Following counseling advice was left optional. The consequences of this decision taken in 2009 were investigated. At the end of 6 years, it was revealed that 26.5% of at-risk couples cancelled the marriage. In just 2009, 51.9% of at-risk couples cancelled their marriage **(19)**.

Older studies conducted in Saudi Arabia were also examined. In a study conducted in 2007, couples who were considering getting married between 2005 and 2006 were investigated. While 90% of high-risk couples got married despite being aware of the risk, the rest gave up on getting married. The reason why high-risk couples insist on not getting married has been interpreted as religious and cultural **(20)**. Compared to other sources, it can be said that trust in premarital screening programs has increased over time in Saudi Arabia and the number of sick child births has decreased.

Premarital screening became mandatory in Kuwait in September 2009. A study conducted in Kuwait investigated the preventive and deterrent effects of premarital screening programs on the prevalence of hemoglobinopathies. Data obtained in the last 11 years were used in the research. At the end of the

study, it was found that 3.8% of high-risk couples ended their marriage, and 46.5% of couples in a different group ended their marriage due to disagreements due to hemoglobinopathy. Thus, it can be said that the premarital screening program managed to end the decision to getting married and continue marriage in 50.4% of high-risk couples in 11 years **(21)**.

Achieving Success of Genetic Counseling in Screening Programs

Genetic counseling is not only conveying some information to people, but also educating them. For this reason, the subject must be explained within the education system and presented as a usable service. Considering that those who have previously received some basic information about genetic diseases will understand genetic counseling more easily, educating the public in primary and secondary schools and through various media should be adopted as a basic principle. Preventing genetic diseases such as thalassemia and hemoglobinopathy requires an organized effort. Detection of carriers is possible if all levels of the public health system know and care about the issue. Since genetic counseling requires one-to-one contact, accurate information transfer, time and communication skills, healthcare professionals should be trained in counseling through professionally prepared training programs. Since medical knowledge and diagnostic possibilities increase over time, training should be repeated at periodic intervals. Improving the knowledge of healthcare personnel about genetic problems will intensify the need to be aware of the problem **(12, 22)**.

Knowledge and understanding of thalassemia carrier screening can be increased if public education is provided. Some thalassemia carrier screening programs educate participants before the screening process; however, different countries may provide training to participants in different ways. Successful society-wide training programs are run in countries with high carrier rates, such as Greece, Italy and Cyprus. One study stated that 85% of study participants in Italy had heard of thalassemia, but only 19% of Italian-Americans living in the United States had heard of thalassemia. Native Italians showed significantly higher levels of thalassemia knowledge compared to Italian Americans. Training has been reported to be provided through mass media, public lectures, training of health professionals, as well as posters and brochures in marriage registry offices and medical clinics **(15, 26)**.

In Thailand, 12 hospitals providing thalassemia services from different regions were selected for the prevention and control of thalassemia. National plans have been developed to provide information about thalassemia to hospital administrators in order to identify the problem, thereby informing the public about thalassemia and genetic counseling. These plans include the use of easily understandable media and language for communication in high-risk populations, financial support from the government, and the addition of thalassemia education to high school and university curricula **(23)**.

Despite all the controls, it was thought that education, counseling and screening for risk groups alone would not be sufficient to prevent the birth of sick babies due to the high incidence of thalassemia in the Turkish Republic of Northern Cyprus (TRNC). For this purpose, premarital screening became legally mandatory in 1980 and it was decided that all couples who were going to get married would be screened free of charge under state control. These practices have been widely accepted by the public and supported by the state. After 2001, no babies with thalassemia were born in TRNC. In this context, education in preventive health services, prevention of a disease, follow-up and rehabilitation of patients are considered to be very important. TRNC has successfully brought an important problem under control **(24)**.

Genetic counseling can be provided to patients using today's latest technologies. In this context, an application called Cyber Gen was developed by Setiawan and his colleagues for thalassemia patients. Patients registered in the hospital can log in to the application and interact with nurses, patients and caregivers. This makes it easier to track patients' tests and exchange information. Additionally, a large disease-specific database can be obtained **(25)**. This large database will shed light on new research. With such applications like Cyber Gen,

access to genetic counseling services can be facilitated.

Considering all this information, we can say that through science, we can control hereditary diseases like thalassemia and hemoglobinopathy. At this stage, it is necessary for scientists and the public to come together in awareness and to organize. When we add today's technologies to the equation, we can further facilitate public education and access to healthcare services. We must keep our minds open and be open to new developments in the light of science.

REFERENCES

1. Cavazzana M, Antoniani C, Miccio A. Gene Therapy for β-Hemoglobinopathies. Vol. 25, Molecular Therapy. American Society of Gene and Cell Therapy; 2017. p. 1142–54.
2. Sabath DE. Molecular diagnosis of thalassemias and hemoglobinopathies: An ACLPS critical review. Vol. 148, *Am J Clin Pathol*. Oxford University Press; 2017. p. 6–15.
3. Ozbolat G, Tuli A. Talasemi ve ilgili hemoglobinopatilerin Moleküler Tanı Yöntemleri: Günümüz ve Gelecek. *Adıyaman Üni Sağlık Bil Derg*. 2017;3(3):599–616.
4. Marengo-Rowe AJ. The thalassemias and related disorders. *Proc (Bayl Univ Med Cent),* 2007;20. P. 27-31.
5. Kunz JB, Kulozik AE. Gene Therapy of the Hemoglobinopathies. Vol. 4, *HemaSphere. Wolters Kluwer Health*; 2020. p. E479.
6. Forget BG, Franklin Bunn H. Classification of the disorders of hemoglobin. Cold Spring Harb Perspect Med. 2013 Feb;3(2).
7. Kaplan Ü. Türkiye'de Genetik Danışmanlık ve Biyoetik. Doctoral thesis Ankara: *Ankara Uni, The Institute of Biotech*. 2018.
8. Terry SF. (Editor). Understanding Genetics: A New England Guide for Patients and Health Professionals. Washington (DC): Genetic Alliance. 2010
9. Setiawan H, Ediati A, Indah Winarni T. Genetic Counseling to Reduce the Level of Depression in Parents of Children with Thalassemia Major. 2017. *2nd International Conference on Sports Science, Health and Physical Education (ICSSHPE).* p. 102-106.
10. Canatan D, Corrons JLV, Piacentini G, et al. Immigration and screening programs for hemoglobinopathies in Italy, Spain and Türkiye. *Acta Biomedica*. 2021 Sep 2;92(4).
11. Cao A, Galanello R. Beta-thalassemia. Vol. 12, *Genetics in Medicine*. 2010. p. 61–76.
12. Petrou M. Preveniton of Thalassemias and Other Hemoglobin Disorders: Vol. 1 Principles. Thalassemia International Federation. Chapter 5. 2013.
13. Mandrile G, Barella S, Giambona A, et al. First and Second Level Haemoglobinopathies Diagnosis: Best Practices of the Italian Society of Thalassemia and Haemoglobinopathies (SITE). *J Clin Med*. 2022 Sep 1;11(18).
14. Cao A, Kan YW. The prevention of thalassemia. *Cold Spring Harb Perspect Med*. 2013 Feb;3(2).
15. Cousens NE, Gaff CL, Metcalfe SA, et al. Carrier screening for Beta-thalassaemia: A review of international practice. Vol. 18. *Eur J Hum Genet*. 2010. p. 1077–83.
16. Lanczová I. History Of Mandatory Premarital Health Examinations. *The Lawyer Quarterly.* 2021. 11(4). p. 682-690.
17. Al-Shafai M, Al-Romaihi A, Al-Hajri N, Islam N, Adawi K. Knowledge and Perception of and Attitude toward a Premarital Screening Program in Qatar: A Cross-Sectional Study. *Int J Environ Res Public Health*. 2022;19(7).
18. Keskin A, Türk T, Polat A, et al. Premarital Screening of Beta-Thalassemia Trait in the Province of Denizli,Türkiye. *Acta Haematol*. 2000. p. 31-33.
19. Memish ZA, Saeedi MY. Six-Year Outcome Of The National Premarital Screening And Genetic Counseling Program For Sickle Cell Disease And Beta-Thalassemia İn Saudi Arabia. *Ann Saudi Med*. 2011. 31(3). p. 229-235.
20. AlHamdan NAR, AlMazrou YY, AlSwaidi FM, et al. Premarital screening for thalassemia and sickle cell disease in Saudi Arabia. *Genetics in Medicine*. 2007. 9(6). p. 372–3777.
21. Aldeen NR, Osman AA, Alhabashi MJ et al. The Prevalence Of B-Thalassemia And Other Hemoglobinopathies In Kuwaiti Premarital Screening Program: An 11-Year Experience. *J Pers Med*. 2021;11(10).
22. Gülleroğlu KS, Sarper N, Gökalp AS. Public Education For The Prevention Of Hemoglobinopathies: A Study Targeting Kocaeli University Students. *Turk J Hematol*. 2007.24. p. 164-170.
23. Dhamcheree V, Romyanan O, Ninlagarn T. Genetic Counseling for Thalassemia in Thailand: Problems and Solutions. *Southeast Asıan J Trop Med Publıc Health.* 2001. 32(2). p. 413-418
24. Şahin-Tarım H, Öz F. A Hematologic Disease in the Turkish Republic of Northern Cyprus: Thalassemia Major. *Mediterranean Nursing and Midwifery*. 2023 Apr 1;3(1):43–7.

25. Setiawan H, Setiawan D, Suhanda, Mustopa AH. Development of Android-based Mobile Application "Cyber Gen" for Genetic Counselling Implementation among Thalassemia Patients. Vol.2111 *Journal of Physics: Conference Series*. 2021.
26. Armeli C, Robbins SJ. Eunpu D. Comparing Knowledge of β-Thalassemia in Samples of Italians, Italian-Americans, and Non-Italian-. *J Genet Counsel*, 2005.14. p.365-376.*-0

Corresponadence Address:
Elif Gözde GÖKKAYA
Antalya Bilim University, Vocational School of Health Services, Department of Medical Laboratory Techniques, Antalya, Türkiye.
E-mail: gozde.gokkaya@antalya.edu.tr

PRENATAL DIAGNOSIS METHOD APPLICATIONS

Emine Kırtıs[1], Gul Alkan Bülbül[1], İnanç Mendilcioğlu[2]
[1] Fellow in Perinatology, Department of Obstetrics and Gynecology, Akdeniz University, Antalya, Türkiye
[2] Professor and Chair, Department of Obstetrics and Gynecology, Akdeniz University, Antalya, Türkiye

ABSTRACT

Hemoglobinopathies is one of the most common monogenic diseases in the world. Pregnant individuals who are at risk of having a fetus with hemoglobinopathy during pregnancy should be offered a fetal diagnostic test after a detailed examination. Fetal DNA can be obtained through prenatal diagnostic methods such as chorionic villus sampling, amniocentesis, or cordocentesis. Chorionic villus sampling is typically performed between the 10th and 13th weeks of pregnancy, offering early diagnosis opportunities. Amniocentesis can be conducted starting from the 15th week of pregnancy. Cordocentesis is commonly used for fetal blood sampling after the 18th week of pregnancy. Due to the availability of early screening methods and more sensitive and cost-effective polymerase chain reaction techniques in the first half of pregnancy, there is rarely a need for fetal blood analysis for thalassemias. Celocentesis is a technique used for very early prenatal diagnosis of hemoglobinopathies, but given the potential adverse fetal effects, extensive randomized studies are required before its routine clinical use. The use of noninvasive prenatal testing for monogenic autosomal recessive diseases such as sickle cell disease and thalassemia presents several challenges. In autosomal recessive cases, because 50% of the fetal genome is inherited from the mother, distinguishing whether the fetus is affected by hemoglobinopathy requires the identification of the pathogenic allele from the father through noninvasive prenatal testing and distinguishing it from the maternally inherited pathogenic allele. Noninvasive prenatal testing remains a screening test, and results should be confirmed through an invasive procedure before any intervention. When prenatal diagnosis confirms the presence of fetal hemoglobinopathy, parents should be provided with detailed information about the natural course of the disease, potential effects on the child's health, available treatment options, and reproductive alternatives.

Keywords: Hemoblobinopathy, prenatal diagnosis, chorionic villus sampling, amniocentesis, fetal blood sampling

BACKGROUND

Hemoglobinopathies, the prevailing cluster of severe autosomal recessive monogenic conditions in humans, arise from mutations occurring within or near the globin genes. More than 1500 mutations in globin genes have been described, leading either to reduced synthesis of the globin chains of hemoglobin (thalassemia syndromes) or altered structure (hemoglobin variants) **(1, 2)**.

EPIDEMIOLOGY

Hemoglobinopathies are estimated that 7% of the population worldwide with an annual birth rate of approximately 300,000 to 400,000 individuals affected by these conditions **(3)**. Hemoglobinopathies are prevalent in malaria-endemic regions (the Mediterranean, Asia, and sub-Saharan Africa) owing to natural selection **(4)**. Moreover, the prevalence of hemoglobinopathies has increased in non-endemic regions such as Europe, North America and Australia due to population migrations. Consequently, these conditions have evolved into a significant global health concern **(5)**.

SCREENING GOALS, POTENTIAL BENEFITS, AND HARMS

Prenatal screening and genetic counseling play a significant role, especially in regions with a high prevalence of thalassemia. These measures are cru-

cial for the early detection and informed decision-making of couples at risk of passing on thalassemia to their children. However, it is important to emphasize the growing global concern, exacerbated by the patterns of global migration that bring together diverse populations, highlighting the need for accessible and widespread genetic counseling and screening programs worldwide **(5, 6)**.

The primary objectives of preconception hemoglobinopathy screening are to identify couples whose offspring are at risk for having an inherited hemoglobinopathy, provide them with information about the disorder, and discuss their reproductive alternatives. Couples may choose to proceed with pregnancy with or without a prenatal diagnosis for fetal hemoglobinopathy. Alternatively, they might opt for in vitro fertilization combined with preimplantation genetic testing for monogenic disorders with implantation of likely unaffected embryos **(7)**. Providing early diagnosis and information about the disease for couples who are currently pregnant and have a risk of inheriting a genetic hemoglobin disorder is crucial. This information can be obtained through prenatal diagnostic methods such as chorionic villus sampling (CVS), amniocentesis, or cordocentesis. The possibility of having an affected child allows couples to prepare for postnatal care or consider pregnancy termination **(7)**. However, in cases where the results are uncertain and predicting the clinical condition of the affected child is challenging, screening and diagnosis may increase parental anxiety.

IDENTIFYING TRAITS AT RISK

Universal (non-selective) hemoglobinopathy screening is considered the best approach whenever possible, especially given the increasing ethnic and geographic diversity of hemoglobinopathy genotypes. Universal screening allows for the identification of a larger number of hemoglobinopathy carriers compared to selective screening based solely on race and ethnicity **(8)**. Recognizing that individuals may have limited awareness of their own racial/ethnic heritage and family history of hemoglobinopathies, the American College of Obstetricians and Gynecologists recommends universal hemoglobinopathy testing for individuals planning to have children or during their initial prenatal consultation if prior test results are unavailable **(9)**.

As a fundamental protocol, all pregnant individuals should undergo a complete blood count, which is readily available even in resource-limited countries and provides baseline hemoglobin/hematocrit values **(10)**. In cases where there is no iron deficiency but the mean corpuscular volume (MCV) and/or mean corpuscular hemoglobin (MCH) are abnormally low (e.g., MCV <80 femtoliters [fL], MCH <27 picograms [pg]), maternal hemoglobin analysis can be conducted using protein chemistry methods. These methods typically include high-performance liquid chromatography (HPLC) and, less commonly, capillary electrophoresis, isoelectric focusing, or cellulose acetate electrophoresis **(11)**.

In situations where obtaining test results promptly is crucial for maximizing reproductive options, it is recommended to screen both partners simultaneously. This may involve the use of DNA-based tests for a fast and effective definitive diagnosis, particularly if the male partner is unavailable or declines screening, especially in ethnic groups at high risk of hemoglobinopathies. Discussing and offering fetal diagnostic tests is particularly important in such cases **(12)**.

PRE-PROCEDURAL COUNSELING

Pregnant individuals at risk of having a fetus with hemoglobinopathy should be offered comprehensive counseling followed by fetal diagnostic testing. The counseling process involves collecting the patient's familial background, ethnic heritage, and previous genetic, obstetric, medical, and surgical history, furthermore understanding the underlying for the decision to pursue diagnostic fetal testing **(13)**. When the prerequisites for proposing invasive prenatal testing for a pregnancy at risk have been satisfied, the counseling process should include a description of the most suitable procedure for the prenatal genetic diagnosis, taking into account the stage of gestation and the associated procedural risks. Blood samples from both parents should be obtained to confirm carrier status of the thalassemia mutation before invasive procedure and to serve as a reference DNA source for use in subsequent prenatal molecular analysis **(14)**. While the primary indication for invasive testing is thalassemia, it is prudent to advise

karyotype analysis, ensuring a comprehensive evaluation of genetic information **(15)**.

FETAL DNA OBTAINING BY INVASIVE TEST IN PRENATAL DIAGNOSIS

1- Chorionic Villus Sampling

CVS is a medical technique involving the collection of small samples of the placenta for prenatal genetic diagnosis, typically conducted within 10 and 13 weeks of gestation **(16)**. Ultrasound-guided CVS and a schematic representation of the image are shown in figure 1. Chorionic tissue retrieval can be achieved either through a transabdominal or transcervical approach. Although operator preference plays a role in this determination, technical considerations, especially regarding placental positioning, may influence the choice between the two methods **(17)**.

Approximately 2 mm samples of chorionic villus tissue are sufficient for polymerase chain reaction (PCR)-based analysis and backup culture. However, when chorionic villi remain closely associated with maternal decidua, fetal and maternal cell types are invariably separated within the CVS tissue specimen. Consequently, CVS cultures manifest the highest potential for maternal cell contamination **(18)**. Therefore, culturing of the sample should be avoided unless a sufficient quantity of the sample has been obtained.

The most serious complications associated with CVS are fetal loss or injury. Additionally, other concerns relate to bleeding, infection, and uncertain results. A comprehensive analysis, involving a systematic review of 16 cohort studies focusing on CVS complications, revealed a cumulative fetal loss rate of 0.7 percent occurring within a 14-day timeframe after transabdominal CVS (TA-CVS) procedures **(19)**.

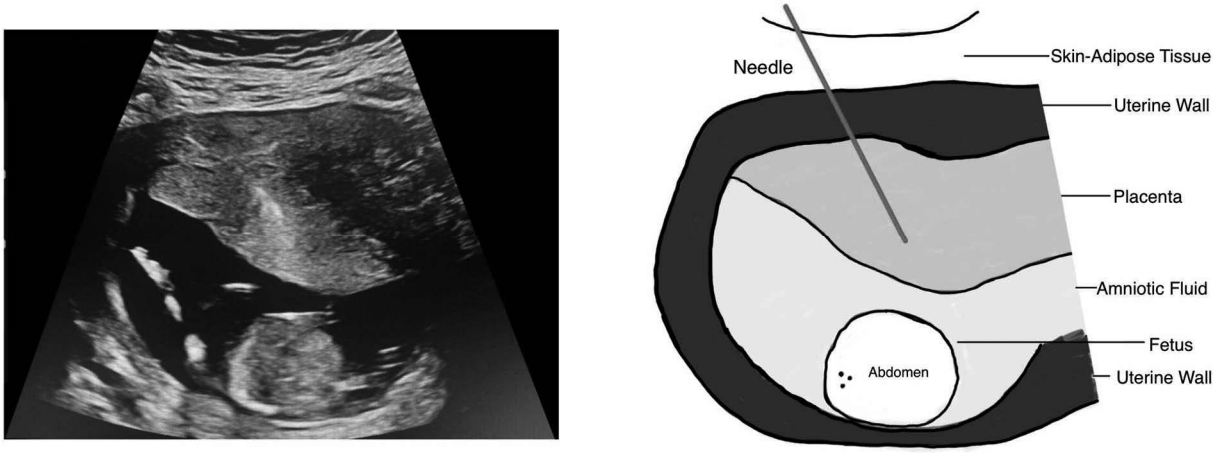


Figure 1: *Ultrasound image showing the needle entry point during chorionic villus sampling and the schematic representation of the image. Under real-time sonographic guidance, the needle is directed through the abdominal wall to the uterus and placenta, collecting a small cell sample from the chorionic villus.*

2. Amniocentesis

Amniocentesis entails a diagnostic procedure involving the extraction of amniotic fluid from the uterine cavity using a transabdominal approach with a needle **(20)**. Ultrasound-guided amniocentesis and a schematic representation of the image are shown in figure 2. Amniocentesis can be performed from 15 weeks of pregnancy onwards, with various chromosomal, biochemical, molecular and microbial studies being performed on the amniotic fluid sample **(21)**. A significant drawback associated with this method is the delayed availability of prenatal diagnostic results, typically occurring between the 17th and 20th weeks of gestational age. In cases where a genetic anomaly is detected in the fetus and the patient opts for termination of pregnancy, a late abortion carries more emotional stress and physical risks for the woman compared to abortion performed in the first trimester **(22, 23)**.

Amniotic cells, upon centrifugation of the amniotic fluid, can be directly utilized for molecular analysis.

Although the DNA yield from amniotic cells is generally lower than that obtained from CVS samples, it remains sufficient for PCR-based analysis as long as 10 ml of fluid is procured. Direct analysis of uncultured amniotic fluid requires careful evaluation as fetal cells may be contaminated with maternal cells. These are best avoided by careful inspection for the presence of blood in the aspirated amniotic fluid, along with discarding the first 1-2 ml sample containing maternal skin fibroblasts **(20)**. Amniotic cell culture significantly increases the amounts of fetal DNA, with the advantage of minimizing maternal contamination risk due to the suboptimal growth of maternal lymphocytes in this culture. However, the disadvantage of amniotic cell culture is the delay in reporting time **(24)**. The primary complications of amniocentesis are rupture of membranes, direct fetal injury, indirect fetal injury, and infection. The procedure has a risk of fetal loss of approximately 0.5% **(21)**.

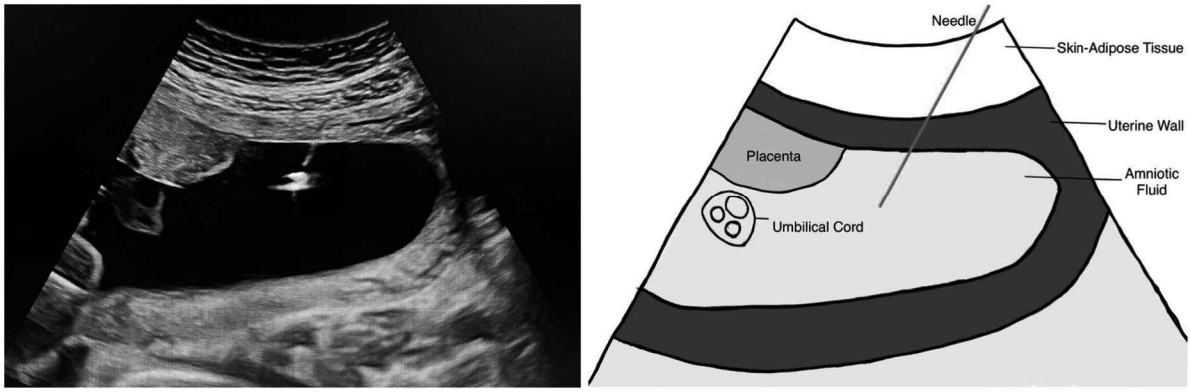


Figure 2: *Ultrasound image showing the needle entry point away from the fetus during amniocentesis and the schematic representation of the image. Under real-time sonographic guidance, the needle is directed through the abdominal wall to the uterus, and 10-20 cc of amniotic fluid sample is taken for analysis.*

3- Fetal Blood Sampling

Fetal blood sampling includes three techniques: cordocentesis, alternatively termed percutaneous umbilical blood sampling, along with intrahepatic blood sampling and cardiocentesis **(25)**. Ultrasound-guided cordocentesis and a schematic representation of the image are shown in figure 3. In contemporary practice, fetal blood analysis is rarely used for prenatal testing for thalassemia, due to the availability of early screening and more accurate, cost-effective PCR methods and more reliable prenatal diagnosis in the first half of pregnancy. In certain settings where PCR-based techniques are unavailable, the assessment of cord hemoglobin A (Hb A) levels remains a strategy for prenatal diagnosis of severe β-thalassemia **(26)**. Cordocentesis can be used to obtain fetal blood, usually after 18 weeks. In the second-trimester cord blood of fetuses affected by β-thalassemia major, the levels of Hb A typically exhibit a range spanning from 0 to 0.2 percent. These levels are discernible from heterozygous fetuses, where Hb A levels were found to be>1.3 percent in various studies conducted via HPLC or hemoglobin (Hb) electrophoresis **(27, 28)**. The utilization of fetal blood for β-thalassemia globin chain synthesis is no longer used by most centers as it is technically more demanding than current DNA diagnosis. Fetal blood analysis is now primarily considered in relatively late stages of gestation, especially when α-thalassemia hydrops fetalis has already been identified through ultrasound **(29)**. In these cases, a rapid diagnosis can be achieved within a matter of minutes through the use of HPLC to analyze fetal blood, as Hb F will be absent if the fetus is affected. Maternal contamination is a serious issue in the application of fetal Hb analysis in prenatal diagnosis **(30)**. To ascertain potential contamination with maternal blood, fetal blood should undergo separation, utilizing techniques such as the Kleihauer Betke staining method or electronic blood tests **(31)**. Cordocentesis carries the risk of major fetal complications, including bleeding, bradycardia, and infection, all of which may pose life-threatening risks. Maternal complications unrelated to the pregnancy are rare. The risk of postprocedure pregnancy loss appears to be 1.4 to 1.9 percent **(25, 32, 33)**.

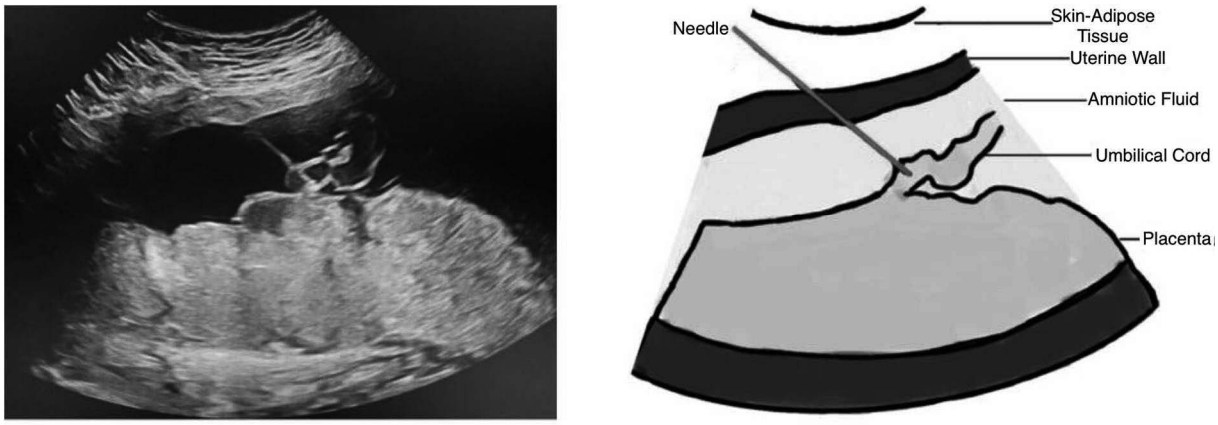


Figure 3: *Ultrasound image showing the needle entry point during cordocentesis and the schematic representation of the image. Under real-time sonographic guidance, the needle is directed through the abdominal wall to the uterus and the umbilical vein, collecting a fetal blood sample for analysis from the umbilical vein.*

4- Celocentesis

During the first 12 weeks of pregnancy the amniotic sac is surrounded by celomic fluid in the extraembryonic celomic cavity. Celocentesis involves the ultrasound-guided aspiration of celomic fluid during the gestational period of 6 to 10 weeks, presenting the opportunity for exceedingly early prenatal diagnosis **(34)**. The celomic fluid, originating from the extraembryonic mesoderm, contains cells of embryo-fetal origin. While the cultivation of celomic cells for fetal karyotyping remains unfeasible, molecular biology methodologies can be employed to analyze DNA extracted from these cells, enabling the diagnosis of hemoglobinopathies and other genetic disorders **(35)**. Maternal contamination is overcome by employing targeted antibodies to selectively isolate fetal cells and, in cases of very high maternal contamination, using a micromanipulator to pick up individual cells **(36)**. However, potential risks to the developing fetus, including procedural miscarriage and limb abnormalities, underscore the need for comprehensive and randomized trials before integrating this experimental methodology into standard clinical protocols **(37)**.

NONINVASIVE SCREENING

Non-invasive prenatal testing (NIPT) using cell-free DNA from maternal plasma, has reshaped the current system of prenatal care used to screen for common chromosomal aneuploidies **(38)**. The fragmented DNA is obtained from apoptotic cells of fetal origin, mainly trophoblastic placental cells **(39)**. This collection of freely circulating fetal DNA must constitute a minimum of 2% to 4% of the overall pool of cell-free DNA in maternal plasma to provide a NIPT result. A simple blood sample collection is conducted during pregnancy, as early as 9 weeks gestation **(40)**.

NIPT protocols to detect paternally derived genetic loci in cell-free DNA are relatively uncomplicated. Presently, there are multiple techniques to detect inherited paternal β-thalassemia mutations (PIB). The absence of PIB implies that the fetus inherited the paternal normal β-globin allele, and, thereby remains unaffected by β-thalassemia disease. On the other hand, a positive PIB may indicate that the at-risk fetus either carries the PIB trait or is affected by β-thalassemia **(41, 42)**. For autosomal recessive disorders, the detection of maternally inherited alleles by NIPT is much more challenging due to the fact that 50% of the fetal genome shares an identical composition with the mother's genome. While progress has been made in this field, there are only limited reports detailing methodologies for the detection of maternally inherited alleles, such as the utilization of relative mutation dosage or relative haplotype dosage via next-generation sequencing and droplet digital PCR, for instance **(43, 44)**. This breakthrough in NIPT has the substantial advantage of reducing the necessity for invasive procedures by half. However, despite these advances, the incorporation of NIPT for hemoglobinopathies into the clinical diagnostic setting has not yet been realized, except for a few instances where it is available sole-

ly for screening purposes. In such cases, invasive follow-up procedures remain a requirement **(45)**.

ULTRASOUND MARKERS

Ultrasound markers can also be used to screen fetuses of couples at risk, particularly in cases of alpha thalassemia major **(46)**. The cardiothoracic ratio, middle cerebral artery peak systolic velocity, and placental thickness are most commonly employed. The cardiothoracic ratio, in particular, appears to be most effective for detecting fetal alpha thalassemia major during early gestational weeks. These ultrasound markers offer a valuable non-invasive approach for identifying cases of fetal alpha thalassemia major, providing critical information for timely intervention and management **(47)**.

POSTDIAGNOSTIC MANAGEMENT

When prenatal diagnosis reveals the presence of fetal hemoglobinopathy, parents should be thoroughly counseled about detailed information about the specific disorder's natural progression, potential impacts on the child's health, available treatment options, and reproductive alternatives **(48)**. Fetal hemoglobinopathy generally does not exert adverse effects on either the fetus, the mother, or the course of the pregnancy. However, in the case of alpha thalassemia major, often leads to hydrops fetalis, and without intervention, it can lead to fetal demise during the later stages of the second trimester through the mid-third trimester. An intervention option for fetuses affected by alpha thalassemia major is serial intrauterine transfusions. This approach has demonstrated success in achieving live births, occasionally even at full term. However, it entails the infant's dependence on transfusions and the associated consequences, unless postnatal allogeneic hematopoietic cell transplantation is performed **(49)**.

In utero hematopoietic cell transplantation is a promising investigational strategy offered at selected fetal therapy centers. This method involves introducing donor cells into a developing host before immune maturation occurs. This process aims to establish donor-specific tolerance and avoid potential adverse effects commonly linked to postnatal hematopoietic stem cell transplantation **(50)**.

ACKNOWLEDGEMENT

We thank Dr. Mustafa Özer for his substantive contributions in preparing illustrations.

REFERENCES

1. Williams TN, Weatherall DJ. World distribution, population genetics, and health burden of the hemoglobinopathies. Cold Spring Harb Perspect Med. 2012;2(9):a011692.
2. Traeger-Synodinos J. Preimplantation genetic diagnosis, an alternative to conventional prenatal diagnosis of the hemoglobinopathies. Int J Lab Hematol. 2013;35(6):571-9.
3. Traeger-Synodinos J, Harteveld CL. Preconception carrier screening and prenatal diagnosis in thalassemia and hemoglobinopathies: challenges and future perspectives. Expert Rev Mol Diagn. 2017;17(3):281-91.
4. Taylor SM, Parobek CM, Fairhurst RM. Haemoglobinopathies and the clinical epidemiology of malaria: a systematic review and meta-analysis. Lancet Infect Dis. 2012;12(6):457-68.
5. Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood. 2010;115(22):4331-6.
6. Cao A, Rosatelli MC, Monni G, Galanello R. Screening for thalassemia: a model of success. Obstet Gynecol Clin North Am. 2002;29(2):305-28, vi-vii.
7. De Wert GM, Dondorp WJ, Knoppers BM. Preconception care and genetic risk: ethical issues. J Community Genet. 2012;3(3):221-8.
8. Kaufmann JO, Demirel-Güngör G, Selles A, Hudig C, Steen G, Ponjee G, et al. Feasibility of nonselective testing for hemoglobinopathies in early pregnancy in The Netherlands. Prenat Diagn. 2011;31(13):1259-63.
9. ACOG Practice Bulletin No. 78: hemoglobinopathies in pregnancy. Obstet Gynecol. 2007;109(1):229-37.
10. Sirichotiyakul S, Maneerat J, Sa-nguansermsri T, Dhananjayanonda P, Tongsong T. Sensitivity and specificity of mean corpuscular volume testing for screening for alpha-thalassemia-1 and beta-thalassemia traits. J Obstet Gynaecol Res. 2005;31(3):198-201.
11. Hartwell SK, Srisawang B, Kongtawelert P, Christian GD, Grudpan K. Review on screening and analysis techniques for hemoglobin variants and thalassemia. Talanta. 2005;65(5):1149-61.
12. Phadke SR, Puri RD, Ranganath P. Prenatal screening for genetic disorders: Suggested guidelines for the Indian Scenario. Indian J Med Res. 2017;146(6):689-99.

13. Douglas Wilson R. Genetic considerations for a woman's pre-conception evaluation. J Obstet Gynaecol Can. 2011;33(1):57-64.
14. Traeger-Synodinos J, Harteveld CL, Old JM, Petrou M, Galanello R, Giordano P, et al. EMQN Best Practice Guidelines for molecular and haematology methods for carrier identification and prenatal diagnosis of the haemoglobinopathies. Eur J Hum Genet. 2015;23(4):426-37.
15. Mendilcioglu I, Yakut S, Keser I, Simsek M, Yesilipek A, Bagci G, et al. Prenatal diagnosis of β-thalassemia and other hemoglobinopathies in southwestern Türkiye. Hemoglobin. 2011;35(1):47-55.
16. Ledbetter DH, Martin AO, Verlinsky Y, Pergament E, Jackson L, Yang-Feng T, et al. Cytogenetic results of chorionic villus sampling: high success rate and diagnostic accuracy in the United States collaborative study. Am J Obstet Gynecol. 1990;162(2):495-501.
17. Jackson LG, Zachary JM, Fowler SE, Desnick RJ, Golbus MS, Ledbetter DH, et al. A randomized comparison of transcervical and transabdominal chorionic-villus sampling. The U.S. National Institute of Child Health and Human Development Chorionic-Villus Sampling and Amniocentesis Study Group. N Engl J Med. 1992;327(9):594-8.
18. Saura R, Roux D, Taine L, Maugey B, Laulon D, Laplace JP, et al. Early amniocentesis versus chorionic villus sampling for fetal karyotyping. Lancet. 1994;344(8925):825-6.
19. Mujezinovic F, Alfirevic Z. Procedure-related complications of amniocentesis and chorionic villous sampling: a systematic review. Obstet Gynecol. 2007;110(3):687-94.
20. Cruz-Lemini M, Parra-Saavedra M, Borobio V, Bennasar M, Goncé A, Martínez JM, et al. How to perform an amniocentesis. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2014;44(6):727-31.
21. ACOG Practice Bulletin No. 88, December 2007. Invasive prenatal testing for aneuploidy. Obstet Gynecol. 2007;110(6):1459-67.
22. Hull D, Davies G, Armour CM. Survey of the Definition of Fetal Viability and the Availability, Indications, and Decision Making Processes for Post-Viability Termination of Pregnancy for Fetal Abnormalities and Health Conditions in Canada. J Genet Couns. 2016;25(3):543-51.
23. DiMiceli-Zsigmond M, Williams AK, Richardson MG. Expecting the Unexpected: Perspectives on Stillbirth and Late Termination of Pregnancy for Fetal Anomalies. Anesth Analg. 2015;121(2):457-64.
24. Schrijver I, Cherny SC, Zehnder JL. Testing for maternal cell contamination in prenatal samples: a comprehensive survey of current diagnostic practices in 35 molecular diagnostic laboratories. J Mol Diagn. 2007;9(3):394-400.
25. Ghidini A, Sepulveda W, Lockwood CJ, Romero R. Complications of fetal blood sampling. Am J Obstet Gynecol. 1993;168(5):1339-44.
26. Sanguansermsri T, Thanarattanakorn P, Steger HF, Tongsong T, Chanprapaph P, Wanpirak C, et al. Prenatal diagnosis of beta-thalassemia major by high-performance liquid chromatography analysis of hemoglobins in fetal blood samples. Hemoglobin. 2001;25(1):19-27.
27. Rao S, Saxena R, Deka D, Kabra M. Use of HbA estimation by CE-HPLC for prenatal diagnosis of beta-thalassemia; experience from a tertiary care centre in north India: a brief report. Hematology. 2009;14(2):122-4.
28. Srivorakun H, Fucharoen G, Sae-Ung N, Sanchaisuriya K, Ratanasiri T, Fucharoen S. Analysis of fetal blood using capillary electrophoresis system: a simple method for prenatal diagnosis of severe thalassemia diseases. Eur J Haematol. 2009;83(1):57-65.
29. Liao C, Zhou JY, Xie XM, Li R, Li DZ. Cord blood analysis for rapid prenatal confirmation of Hb Bart's disease using the Sebia Capillary electrophoresis system. Hemoglobin. 2012;36(2):186-91.
30. Sanguansermsri T, Thanaratanakorn P, Steger HF, Tongsong T, Sirivatanapa P, Wanapirak C, et al. Prenatal diagnosis of hemoglobin Bart's hydrops fetalis by HPLC analysis of hemoglobin in fetal blood samples. Southeast Asian J Trop Med Public Health. 2001;32(1):180-5.
31. Forestier F, Cox WL, Daffos F, Rainaut M. The assessment of fetal blood samples. Am J Obstet Gynecol. 1988;158(5):1184-8.
32. Tongsong T, Wanapirak C, Kunavikatikul C, Sirirchotiyakul S, Piyamongkol W, Chanprapaph P. Fetal loss rate associated with cordocentesis at midgestation. Am J Obstet Gynecol. 2001;184(4):719-23.
33. Tongsong T, Wanapirak C, Piyamongkol W, Sirirchotiyakul S, Tongprasert F, Srisupundit K, et al. Second-trimester cordocentesis and the risk of small for gestational age and preterm birth. Obstet Gynecol. 2014;124(5):919-25.
34. Jurkovic D, Jauniaux E, Campbell S, Pandya P, Cardy DL, Nicolaides KH. Coelocentesis: a new technique for early prenatal diagnosis. Lancet. 1993;341(8861):1623-4.
35. Jauniaux E, Cirigliano V, Adinolfi M. Very early prenatal diagnosis on coelomic cells using quantita-

tive fluorescent polymerase chain reaction. Reprod Biomed Online. 2003;6(4):494-8.
36. Giambona A, Damiani G, Leto F, Jakil C, Renda D, Cigna V, et al. Embryo-fetal erythroid cell selection from celomic fluid allows earlier prenatal diagnosis of hemoglobinopathies. Prenat Diagn. 2016;36(4):375-81.
37. Makrydimas G, Damiani G, Jakil C, Cigna V, Orlandi M, Picciotto F, et al. Celocentesis for early prenatal diagnosis of hemoglobinopathy. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2020;56(5):672-7.
38. Allyse M, Minear MA, Berson E, Sridhar S, Rote M, Hung A, et al. Non-invasive prenatal testing: a review of international implementation and challenges. Int J Womens Health. 2015;7:113-26.
39. Zafari M, Kosaryan M, Gill P, Alipour A, Shiran M, Jalalli H, et al. Non-invasive prenatal diagnosis of β-thalassemia by detection of the cell-free fetal DNA in maternal circulation: a systematic review and meta-analysis. Ann Hematol. 2016;95(8):1341-50.
40. Hui L, Bianchi DW. Fetal fraction and noninvasive prenatal testing: What clinicians need to know. Prenat Diagn. 2020;40(2):155-63.
41. Ramezanzadeh M, Salehi M, Farajzadegan Z, Kamali S, Salehi R. Detection of paternally inherited fetal point mutations for β-thalassemia in maternal plasma using simple fetal DNA enrichment protocol with or without whole genome amplification: an accuracy assessment. J Matern Fetal Neonatal Med. 2016;29(16):2645-9.
42. Galbiati S, Monguzzi A, Damin F, Soriani N, Passiu M, Castellani C, et al. COLD-PCR and microarray: two independent highly sensitive approaches allowing the identification of fetal paternally inherited mutations in maternal plasma. J Med Genet. 2016;53(7):481-7.
43. Jiang F, Liu W, Zhang L, Guo Y, Chen M, Zeng X, et al. Noninvasive prenatal testing for β-thalassemia by targeted nanopore sequencing combined with relative haplotype dosage (RHDO): a feasibility study. Sci Rep. 2021;11(1):5714.
44. Debrand E, Lykoudi A, Bradshaw E, Allen SK. A Non-Invasive Droplet Digital PCR (ddPCR) Assay to Detect Paternal CFTR Mutations in the Cell-Free Fetal DNA (cffDNA) of Three Pregnancies at Risk of Cystic Fibrosis via Compound Heterozygosity. PLoS One. 2015;10(11):e0142729.
45. Scotchman E, Chandler NJ, Mellis R, Chitty LS. Noninvasive Prenatal Diagnosis of Single-Gene Diseases: The Next Frontier. Clin Chem. 2020;66(1):53-60.
46. Thammavong K, Luewan S, Wanapirak C, Tongsong T. Ultrasound Features of Fetal Anemia Lessons From Hemoglobin Bart Disease. J Ultrasound Med. 2021;40(4):659-74.
47. Li X, Zhou Q, Zhang M, Tian X, Zhao Y. Sonographic markers of fetal α-thalassemia major. J Ultrasound Med. 2015;34(2):197-206.
48. Deans Z, Hill M, Chitty LS, Lewis C. Non-invasive prenatal testing for single gene disorders: exploring the ethics. Eur J Hum Genet. 2013;21(7):713-8.
49. Muncie HL, Jr., Campbell J. Alpha and beta thalassemia. Am Fam Physician. 2009;80(4):339-44.
50. Derderian SC, Jeanty C, Walters MC, Vichinsky E, MacKenzie TC. In utero hematopoietic cell transplantation for hemoglobinopathies. Front Pharmacol. 2014;5:278.

Correspondence Address:
Prof. İnanç Mendilcioğlu
Department of Obstetrics and Gynecology,
School of Medicine,
Akdeniz University,
Dumlupinar Boulevard 07070
Kampus, Antalya, Türkiye
E-mail: imendilcioglu@gmail.com

PREIMPLANTATION GENETIC TESTING FOR HEMOGLOBIN DISORDERS

Anver Kuliev and S Rechitsky
Reproductive Genetic Innovations, Chicago, Illinois-USA

ABSTRACT

Haemoglobin disorders, including thalassemia and sickle cell disease, are among the most frequent in the indication profile for preimplantation genetic testing of monogenic disorders (PGT-M), performed already for thirty-three years, and represents an important alternative to prenatal diagnosis, which involves difficult decision of pregnancy termination of an affected foetuses, expected in 25% of cases. We present here our over 30 years' experience of PGT-M for these conditions, resulting in birth of hundreds of unaffected children, which is a part of our overall PGT-M series of up to ten thousand cases resulting in births of thousands of children free of genetic disorders, which represents the world largest experience of PGT-M. The accumulated experience demonstrates considerable progress in using PGT-M for prevention of thalassemia and sickle cell disease, as the basis for primary prevention of the conditions of public health importance at the community level.

Keywords: Preimplantation Genetic Testing for monogenic disorders (PGT-M)/ thalassemia/ sickle cell disease/ Preimplantation HLA typing (PGT-HLA)/ assisted reproduction/ blastocyst biopsy/ polar body sampling.

INTRODUCTION

Despite the progress in treatment of thalassemia and sickle cell disease, allowing a normal lifespan, good quality of life, and possibility to reproduce, it involves the long-term social, familial, and financial consequences. So, it also became a routine practice to prevent the new affected births, with also an option of preimplantation genetic testing (PGT) to avoid the previously required need for termination of pregnancy, which is the major limitation of the control measures, restricting the choices of the at risk couples **(1-3)**. In those population and ethnic groups, or families that cannot accept the preventative approaches, reflecting their social and religious differences, it has also become realistic to provide radical treatment through stem cell transplantation, using the exactly matched sibling donors obtained by preimplantation HLA typing (PGT-HLA) **(4-9**, currently performed for hundreds of patients (10-**15)**. The available framework of avoiding the birth of affected children with haemoglobin disorders involves prospective identification of couples at risk and providing option for them to avoid the birth of an affected child, and also to have an opportunity of having an unaffected child serving a tissue compatible donor for a radical treatment of the affected child in the family by bone marrow transplantation.

Ideally, in the population with high frequency of hemoglobinopathy genes, the interventions are taken before pregnancy, so the at-risk couples could not only avoid the birth of an affected child, but most importantly, have an unaffected offspring. Accordingly, it is reasonable that this information is available to the couples at risk detected through an expanded carrier screening **(16, 17)**, so they could make their choices from all the available options, such as prenatal diagnosis, still the most widespread choice, or PGT, if couples cannot accept prenatal diagnosis due to a potential pregnancy termination they have to face when an affected pregnancy is detected. As mentioned, in addition, they have also an option to use PGT to produce an unaffected HLA matched donor for their older affected sibling, to perform a radical treatment by HLA matched stem cell transplantation, as an attractive approach in avoiding a life-long blood transfusion which itself

presents a risk for life threatening complications. So, this paper will describe the present status of PGT for hemoglobin disorders, as a primary prevention tool, as well as an alternative approach for improving access to HLA-compatible stem cell transplantation treatment, based on our personal experience for more than twenty-five years, since we performed the first PGT for thalassemia in Cyprus (1).

PRESENT EXPERINCE

As presented in **Table 1**, hemoglobin disorders are one of the components of PGT for hematology conditions, including coagulopathies, hemolytic and aplastic anemia. Thalassemia is one of the largest group of monogenic disorders for which PGT-M was performed in our experience **(Table 2)**. In some communities, such as in Cyprus, Greece, and Türkiye, PGT is becoming a routine procedure for couples carrying thalassemia mutations who cannot accept prenatal diagnosis and termination of pregnancy**.** As mentioned, introduced first in Cyprus (1), thousands of cycles have now been performed worldwide. At present, the proportion of PGT for hemoglobin disorders in our overall PGT-M experience is close to 10%. A total of 474 PGT-M cycles were performed for 264 patients, resulting in transfer of 679 thalassemia free embryos in 373 cycles (1.82 average embryos per transfer), resulting in birth of 160 unaffected children **(Table 2)**. Of more than 60 thalassemia mutations tested, IVS I-110 were most prevalent, common in populations of Eastern Mediterranean origin. Among other prevalent mutations tested were Gln39Stop codon, IVS1-5 and IVS1-6 **(Table 3)**.

While PGT-M cycles were mainly performed for heterozygous carriers, 15 cycles were done for couples with homozygous or compound heterozygous male or female patients at 50% risk of bearing an affected offspring. In these couples, PGT involved testing for either three different mutations or, in the majority of cases, for two different mutations or the same maternal and paternal mutation. Analysis of these mutations was done either simultaneously or in sequence by testing the maternal mutations in PB1 and PB2, and the paternal ones by embryo biopsy.

With progress in the treatment of thalassemia, PGT-M is requested by affected and well-treated patients who wish to reproduce. Life expectancy has been significantly improved for individuals with thalassemia, who may be treated radically by stem cell transplantation. Our strategy in such cases depends on whether the affected partner is male or female, because testing may be entirely based on oocytes if the affected partner is male, or in contrast, embryo testing when the female partner is affected. However, if a male partner is affected, male factor infertility is usually involved; thus, male partners often need a special treatment prior to PGT-M, and undergo testicular biopsy. In affected females a limited number of oocytes may be available after stimulation regiments, making PGT in some cases quite challenging **(15)**.

A total of 332 PGT cycles were done for 219 couples at risk for producing offspring with for sickle cell disease. This resulted in transfer of 465 sickle cell disease free embryos in 266 cycles (1.74 average embryos per transfer), resulting in birth of 166 unaffected children **(Table 2)**.

The smaller PGT series was for alpha thalassemia, of which 40 cycles were performed for 27 patients, resulting in transfer of 52 alpha thalassemia free embryos in 31 cycles (1.67 average embryos per transfer), resulting in birth of 19 unaffected children **(Table 2)**.

PGT cycles were performed using a standard IVF protocol coupled with micromanipulation procedures of polar body (PB) or embryo biopsy, described in detail elsewhere **(18)**. Details of PGT guidelines were reported previously **(19, 20)**. The present standards of the procedure involve whole genome amplification (WGA) of biopsied PB or blastocyst samples, followed the multiplex nested PCR analysis of the mutations in question and linked markers in a multiplex heminested system **(18, 21)**. The majority of cases are currently performed by blastocyst biopsy followed by WGA **(18, 21)**.

However, not all couples can accept traditional PGT because of their objection to micromanipulation and potential discard of the tested embryos. To avoid this all the tested oocytes can be frozen at the pronuclear stage immediately after fertilization and extrusion of PB2, although freezing of the mutation free oocytes may be avoided, if they are detected well before the

pronuclear fusion and prior to when a decision to discard could not be avoided. This has become realistic because of DNA analysis being completed within less than 9 hrs., prior to pronuclei fusion, providing a possibility for application of PGT for couples who are unable to accept any intervention and discard of the human embryos. The first such couple whom we applied preconception testing was one at risk for producing offspring with sickle cell disease, resulting in the transfer of two unaffected embryos yielding a singleton pregnancy and birth of unaffected child, following confirmation of PB diagnosis by chorionic villus sampling (CVS) **(15)**. This shows that preconception testing is a realistic option for couples who cannot accept traditional PGT because of their objection to micromanipulation and potential discard of the tested embryos.

To perform PGT-M together with HLA typing, HLA genes were tested using the short tandem repeats in the HLA region. In a multiplex heminested PCR system we used closely linked polymorphic short tandem repeat (STR) markers located throughout the HLA region: D6S426, D6S291, Ring 3 CA, TAP1, G51152, D6S2447, LH1,DN, D6S273, 9N-2, TNF a,b,c,d; 62, MIC A, MIB, D6S276, D6S439, D6S1624, D6S265, D6S510; D6S248, RF, MOG a,b,c,d, D6S 258, D6S306, D6S464, D6S299, D6S461 **(18)**. The choice of alleles and markers was based on information provided concerning the presence of maternal and paternal matching or non-matching chromosome 6 alleles. For each family, heterozygous alleles and markers (haplotypes) were selected that were not to be shared by parents. That is, haplotype analysis for the father, mother, and affected child was performed for each family prior to PGT-HLA. This allowed a strategy for detecting and avoiding misdiagnosis due to preferential amplification and allele dropout (ADO), potential recombination within the HLA region, and a possible aneuploidy or uniparental disomy of chromosome 6. All of these pitfalls could adversely affect diagnostic accuracy of HLA typing of the embryo.

Among conditions requiring HLA-compatible stem cell transplantation, thalassemia is one of the most prevalent. Approximately, 40% of 485 PGT-HLA cycles in our experience were performed for thalassemia, because bone marrow transplantation is the only option for their radical treatment. Although considerable progress was achieved in the treatment of the disease by bone marrow transplantation, its application is still limited to the availability of HLA-matched stem cells. Thus, PGT-HLA is clearly an attractive option applied already for over two decades **(15)**. In 54% of these cycles unaffected HLA-matched embryos were detected for transfer, of which 1.54 embryos on the average predicted to be either unaffected carriers or normal and HLA-identical to the affected siblings, were transferred, which is not significantly different from the expectation. As a result, a total of 32 unaffected HLA-identical children were born, serving the HLA compatible donors for the affected siblings. Umbilical cord blood or bone marrow from the children obtained from PGT-HLA were transplanted, resulting in a successful hematopoietic reconstitution of the affected siblings. The other large and extremely successful experience was reported from Istanbul Center, where 626 PGT-HLA cycles were performed, with the majority of them done for thalassemia, resulting in birth of 128 thalassemia-free HLA matched children **(10-14)**. Stem cells of 66 of these children have already been used for stem cell transplantation treatment, with successful bone marrow reconstitution in almost all of them. ESHRE Consortium collected series of 127 babies produced by PGT-HLA from Europe, reporting the 76.2% success rate of stem cell transplantation treatment in this series **(12)**. A chance to identify unaffected embryos fully matched to siblings with hemoglobinopathies is 18.75%. As there is also, approximately, 50% chance of chromosome aneuploidy in patients of advanced reproductive age, the chance of identification of hemoglobinopathy free, HLA matched and euploid embryos is only under 10% **(15)**.

Despite some remaining ethical issues, there has been an increase in the attractiveness of PGT- HLA for couples with affected thalassemia children requiring HLA-compatible stem cell transplantation, providing a practical option, as these couples wish to have another unaffected child anyway. This makes PGT a realistic alternative to conventional prenatal diagnosis, as couples are provided the important prospect not only to avoid an inherited risk without facing termination of pregnancy, but also to

establish that pregnancy with genetic parameters, that may benefit the affected member of the family. Thus, couples at risk of having children with thalassemia will benefit from information provided them about presently available options not only of avoiding the birth of an affected child but also for selecting a suitable stem cell donor for their affected siblings.

REFERENCES

1. Kuliev A, Rechitsky S, Verlinsky O, et al. Preimplantation diagnosis of thalassemia. J Assist Reprod Genet. 1998; 15:219–225.
2. Kuliev A, Rechitsky S, Verlinsky O, et al. Birth of healthy children following preimplantation diagnosis for thalassemias. J Assist Reprod Genet. 1999; 16:219–225.
3. Kuliev A, Packalchuk T, Verlinsky O, Rechitsky S. Preimplantation diagnosis for hemoglobinopathies. Hemoglobin. 2011; 35 5(6:5) 47–55. doi:10.3109/03630269.2011.608457. Epub 2011
4.. Verlinsky Y, Rechitsky S, Schoolcraft W, Strom C, Kuliev A. Preimplantation diagnosis for Fanconi anemia combined with HLA matching. JAMA, 2001; 285:3130-3133.
5. Kahraman S, Karlilaya G, Sertyel S, Karadayi H, Findicli N, Oncu N. Clinical aspects of preimplantation genetic diagnosis of single gene disorders combined with HLA typing. Reprod Biomed Online. 2004; 9:529-532.
6. Van de Velde H, Georgiou I, De Rycke M, et al. Novel universal approach for preimplantation genetic diagnosis of β-thalassemia in combination with HLA matching of embryos. Hum Reprod 2004; 19:700–708.
7. Kuliev A, Rechitsky S, Verlinsky O, Tur-Kaspa I, Kalakoutis G, Angastiniotis M, Verlinsky Y. Preimplantation diagnosis and HLA typing for hemoglobin disorders. Reprod Biomed Online, 2005; 11:362-370.
8. Kuliev A, Verlinsky O, Rechitsky S. Preimplantation HLA typing for stem cell transplantation treatment of hemoglobinopathies. Thalassemia Reports, 2014. DOI: http://dx.doi.org/10.4081/thal.2014.1853
9. Rechitsky S, Kuliev A, Leigh D et al. Single molecule sequencing: a new approach for preimplantation testing and noninvasive prenatal diagnosis confirmation of fetal genotype. The Journal of Molecular Diagnostics, 2020; 22:220-227
10. Kahraman S. PGD for HLA: Clinical Outcomes of HLA compatible transplantation following PGD. Reprod Biomed Online, 2013; 26 (Suppl 1):S9-10.
11. Kahraman S, Ogur C, Turkiye Klinikleri T. Prenatal, Non-invazive Prenatal and Preimplantation Genetic Diagnosis for Hemoglobinopathies. J Med Genet. Special Topics. 2017; 2(1):31-9
12. Kakourou G, Kahraman S, G. C. Ekmekci GC et al, The clinical utility of PGD with HLA matching- a collaborative multicentre ESHRE study. Human Reproduction, 2018; 33(3):520-530. doi: 10.1093/humrep/dex384pp.
13. Umay KB, Gavaz M, Kumtepe ÇY, Yelke H, Pirkevi Çetinkaya C, Çetinkaya, M, Kahraman S. Successful hemapoietic stem cell transplantation in 62 children from healthy siblings conceived from preimplantation HLA Matching: a clinical experience of 327 cycles. Reprod Biomed Online, 2019; 39(Suppl. 1):e13-e14
14. Kahraman S, Cetinkaya M, Kara B. Prenatal Diagnosis and Preimplantation Genetic Approach in Fanconi Anemia. Fanconi Anemisi. Türkiye Klinikleri; 2021; p.139-44.
15. Kuliev A**,** Rechitsky S, Simpson JL. Practical Preimplantation Genetic Testing. Third Edition. Springer Nature Switzerland, 2020; doi.org/10.1007/978-3-030-43157-0
16. Simpson JL, Rechitsky S, Kuliev A. Before the beginning: the genetic risk of a couple aiming to conceive. Fertil Steril 2019; 112:622-630
17. Chen SC, Zhou XY, Li SY *et al.* Carrier burden of over 300 diseases in Han Chinese identified by expanded carrier testing of 300 couples using assisted reproductive technology. *J Assist Reprod Genet* 2023; 40, 2157–2173
18. Kuliev A, Rechitsky S, Verlinsky O. Atlas of Preimplantation Genetic Diagnosis. An Illustrated Textbook. Third Edition. Taylor & Francis. London, 2014
19. Preimplantation Genetic Diagnosis International Society (PGDIS). Guidelines for good practice in PGD: program requirements and laboratory quality assurance. Reprod BioMed Online 2008; 16:134-147
20. ESHRE PGT Consortium good practice recommendations for the organization of PGT Human Reproduction Open, Volume 2020, Issue 3, 2020
21. Rechitsky S, Pakhalchuk T, Goodman A, San-Ramos J, Zlatopolsky Z, Kuliev A. First systematic experience of combined PGD for single gene disorders and/or Preimplantation HLA typing with 24-chromosome aneuploidy testing, Fertility & Sterility, 2015; 103(2): 503-512

Correspondence Address:
Prof. Dr. Anver Kuliev
2910 MacArthur Blvd, Chicago, IL 60062
E-mail: anverkuliev@hotmail.com

Table 1: *List of Hematologic Disorders and Coagulopathies for which PGT was performed*

Disease	Gene	Omim
Hemoglobinopathies		
HEMOGLOBIN--BETA LOCUS; HBB	*HBB*	141900
HEMOGLOBIN--ALPHA LOCUS 1; HBA1	*HBA1, HBA2*	604131
Anemias (Hemolytic anemias)		
ANEMIA, NONSPHEROCYTIC HEMOLYTIC, DUE TO G6PD DEFICIENCY	*G6PD*	300908
PYRUVATE KINASE DEFICIENCY OF RED CELLS + HLA	*PKLR*	266200
RHESUS BLOOD GROUP	*RHD*	111680
BLOOD GROUP--KELL-CELLANO SYSTEM	*KEL*	110900
PORPHYRIA, CONGENITAL ERYTHROPOIETIC	*UROS*	263700
Aplastic anemia		
SHWACHMAN-DIAMOND SYNDROME; SDS	*SBDS*	
DIAMOND-BLACKFAN ANEMIA 1; DBA1 DIAMOND-BLACKFAN ANEMIA 2; DBA2 DIAMOND-BLACKFAN ANEMIA 3; DBA3 DIAMOND-BLACKFAN ANEMIA 5; DBA5 DIAMOND-BLACKFAN ANEMIA 9; DBA9	*RPS19,* *RPS20,* *RPS24,* *RPL35A,* *RPS10*	105650 606129 610629 612528 613308
FANCONI ANEMIA, COMPLEMENTATION GROUP A; FANCA FANCONI ANEMIA, COMPLEMENTATION GROUP C; FANCC FANCONI ANEMIA, COMPLEMENTATION GROUP D2; FANCD FANCONI ANEMIA, COMPLEMENTATION GROUP F; FANCF FANCONI ANEMIA, COMPLEMENTATION GROUP I; FANCI FANCONI ANEMIA, COMPLEMENTATION GROUP J; FANCJ	*FANCA* *FANCC* *FANCD2* *FANCF* *FANCI* *BRIP1*	 227650 227645 227646 603467 609053 609054
Coagulopathies (disorders of bleeding and coagulation)		
BLEEDING DISORDER, PLATELET-TYPE, 16; BDPLT16	*ITGB3*	187800
AMEGAKARYOCYTIC THROMBOCYTOPENIA, CONGENITAL; CAMT	*MPL*	604498
PROTHROMBIN DEFICIENCY, CONGENITAL	*F2*	613679
FACTOR V DEFICIENCY	*F5*	227400

FACTOR VII DEFICIENCY	*F7*	227500
HEMOPHILIA A	*F8*	306700
HEMOPHILIA B	*F9*	306900
THROMBASTHENIA OF GLANZMANN	*ITGA2B*	273800
THROMBOTIC THROMBOCYTOPENIC PURPURA, CONGENITAL; TTP	*ADAMTS13*	274150
WISKOTT-ALDRICH SYNDROME; WAS	*WAS*	301000
THROMBOCYTHEMIA 1; THCYT1	*SH2B3*	187950
THROMBOCYTOPENIA-ABSENT RADIUS SYNDROME; TAR	*RBM8A*	274000
THROMBOPHILIA DUE TO PROTEIN S DEFICIENCY, AUTOSOMAL DOMINANT; THPH5	*PROS1*	612336

Table 2: *PGT for hemoglobinopathies in our overall PGT-M experience*

HEMAGLOBINOPATHIES	**PATIENTS**	**Cycles**	**Transfers**	**Embryos transferred**	**Pregnancy**	**SAB**	**Delivery**	**Babies born**
Thalassemia (HBB locus)	***264***	***474***	***373 (78.7%)***	***679 (1.82)***	***161 (43%)***	***26 (16%)***	***135 (84%)***	***160***
Sickle cell anemia (HBB locus)	***219***	***332***	***266 (80%)***	***465 (1.74)***	***180 (67.7%)***	***24 (13%)***	***156 (87%)***	***166***
subtotal	***483***	***806***	***639***	***1144***	***341***	***50***	***291***	***326***
Hemoglobin-alpha locus (HBA1/HBA2)	***27***	***40***	***31 (77,5%)***	***52 (1.67)***	***19 (61%)***	***2 (11%)***	***17 (89%)***	***19***
TOTAL	***510***	***846***	***670 (79%)***	***1196 (1.78)***	***360 (53.7%)***	***52 (14%)***	***308 (86%)***	***345***

Table 3: *List of thalassemia mutations for which PGT was performed in our experience*

1. Beta-Thalassemia
1.1 Transcriptional mutations
nt -90 C>T
nt -88 C>T
nt -87 C>G
nt -79 A>G
nt -42 C>G
nt -32 C>T
nt -31 A>C
nt -30 T>A
nt -29 A>G

nt -28 A>G
*32 A>C
c.*113 A>G
CAP +1 (A->C) silent nt 1 A>C
Pro5Ala
Pro5Ser
Glu6Lys
Glu6Val
Leu11Pro
Arg12Ser
Trp15Stop
Lys18Stop
Glu26Lys
Ala27Ser
Arg31Thr
Gln39Stop
Gly70Ser
Glu121Ala
Glu121Val
Poly A (A->G)
1.2. Splicing Mutations
IVS-I-1 (G->T)
IVS-I-5 (G->C)
IVS-I-6 (T->C)
IVS-I-110 (G->A)
IVS-I-116 (T->G)
IVS-II-1 (G->A)
IVS-II-2 (A>C)
IVSII-654 (C->T)
IVS-II-745 (C->G)
IVS-II-848 (C->A)
c.316-130 (T>C)
1.3. Insertions/Deletions
Codons 7/8 (+G)
Codons 27/28 (+C)
45 kb deletion; the Filipino deletion beta0
Codon 5 (-CT)
Codon 6 (-A);
Codon 8 (-AA)

Codons 36/37 (-T)
Codons 41/42 (-TTCT)
Codon 44 (-C)
Codon 76 (-C)
619 bp deletion beta0
Hb Lepore-Hollandia
Sicilian (deltabeta)0-Thal
Chinese ^{G}gamma(^{A}gammadeltabeta)0-Thal
c.93-22_95 (24 bp deletion)
c.112 DELETION "T"
2. Alpha Thalassemia
- -(MC); a deletion of at least 46 kb involving both alpha genes and zeta gene alpha-Thal-1
- -(THAI); a deletion of 34-38 kb involving the alpha1, alpha2, and zeta genes alpha-Thal-1
- -(FIL); a deletion of 30-34 kb involving the alpha1, alpha2, and zeta genes alpha-Thal-1
- -(MED-I); deletion of ~17.5 kb including both alpha-globin genes alpha-Thal-1
- -(SEA); deletion of ~20 kb including both alpha-globin genes alpha-Thal-1
Codon 30(31) deletion - GAG
c.95+2_+6 DELETION, IVS1+2_+6 DELETION
c.187 DELETION "G"
Codon 30(31) deletion - GAG

PREMARITAL HEMOGLOBINOPATHY SCREENING PROGRAM IN TURKIYE

Specialist Dr. Yüksel Hakan Aydoğmuş, Specialist Odyolog Rahşan Çınar,
Specialist Nurse Eda Çağrı Olcar, Prof. Dr. Sedat Kaygusuz
Ministry of Health, General Directorate of Public Health – Ankara-Türkiye

ABSTRACT

Hereditary blood diseases, especially thalassemia and sickle cell anemia, are an important public health problem in our country and in the world. The majority of thalassemia carriers do not know that they have this disease, but they only learn when they have a child with thalassemia or have a special blood test. In our country, the frequency of beta-thalassemia carriers is 2.1%, and it is known that there are approximately 1,300,000 carriers and around 4513 patients. In order to combat hereditary blood diseases, the "Law on Combating Hereditary Blood Diseases" numbered 3960 was enacted in our country on 30.12.1993. In this context, thalassemia centers were established in Antalya, Antakya, Mersin and Muğla under our Ministry in 1994. Hemoglobinopathy Control Program was launched in 33 high risk provinces where is high risk in 2003, and 8 more provinces were added to the program in 2013.

As of November 1, 2018, the Hemoglobinopathy Control Program has been implemented in 81 provinces under the name "Premarital Hemoglobinopathy Screening Program". The purpose of the screening program is; The aim of the program is to identify carrier couples for hemoglobinopathies in the premarital period and to carry out the necessary studies to help them have healthy babies, and to prevent morbidity and mortality due to hemoglobinopathies. Within the scope of the program; counseling service is provided to prospective spouses who apply to family physicians to obtain a pre-marriage report, and then a blood sample is taken from the prospective male spouse for screening, blood samples are sent to the public health laboratory in the province or with which the province has an agreement, and screening tests are performed. If the male partner is found to be a carrier or suspect for hemoglobinopathy, a screening test is also performed on the female partner. Couples who are both carriers are directed to centers to receive genetic counseling, and when they think about having children, they are given the necessary guidance to have a healthy baby. In the screenning program; Complete Blood Count (CBC), HPLC (High Performance Liquid Chromatography), Capillary Electrophoresis methods are used. Screening data is processed by family physicians into the system they use and is followed up. In the program, which started to be implemented in 81 provinces in November 2018, 779,091 people (female + male) were screened in 2022.

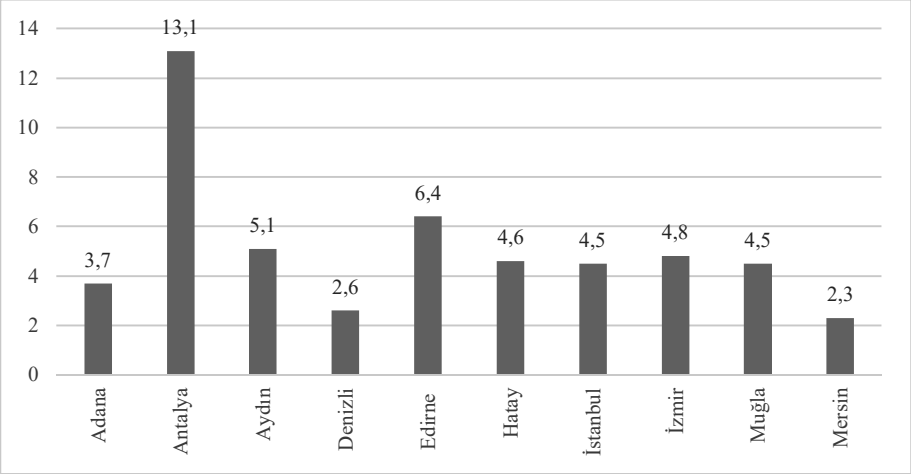
Keywords: Hemoglobinopathy, thalassemia, sickle cell anemia, screening, screening program

Hereditary blood diseases, especially thalassemia and sickle cell anemia, are an important public health problem in our country and in the world. The majority of thalassemia carriers do not know that they have this disease, but they only learn when they have a child with thalassemia or have a special blood test. In our country, the frequency of beta-thalassemia carriers is 2.1%, and it is known that there are approximately 1,300,000 carriers and around 4513 patients. Due to the high number of consanguineous marriages and 70% of these consanguineous marriages being made between first degree relatives, the incidence of hemoglobinopathies, which is a genetically transmitted disease, increases in the society, hundreds of sick children are born every year, and the sick child suffers from this con-

dition, causing financial losses to the family, society and the state. morale is negatively affected.

In order to combat hereditary blood diseases, the "Law on Combating Hereditary Blood Diseases" numbered 3960 was published in our country on 30.12.1993. In this context, thalassemia centers were established in Antalya, Antakya, Mersin and Muğla under our Ministry in 1994. Under the coordination of our Ministry's General Directorate of Maternal and Child Health-Family Planning and Treatment Services, all centers, foundations and associations working on thalassemia and hemoglobinopathy established the National Hemoglobinopathy Council (NHC) in our country on 23.06.2001

Screening studies carried out by the Ministry of Health and UHK in 16 centers in the Marmara, Aegean and Mediterranean regions in the 5 years before 2000 were collected and evaluated. According to the results of this study, a total of 377,399 healthy people were screened, and the distribution of the number of people screened and the frequency of thalassemia and abnormal hemoglobin by province was revealed **(Figure 1)**. In this study, the average thalassemia carrier frequency was found to be 4.3%.



Figurel 1: *Hemoglobinopathy Screening Results of 2000 year*

In 2002, a form prepared by our General Directorate was sent to the Health Directorates of 81 provinces and they were asked to report hemoglobinopathy cases in their provinces. Provincial Health Directorates sent the standard form to all health institutions in their provinces and forwarded the results they obtained to our General Directorate.

A system has been created to collect clinical information about hemoglobinopathies and to monitor patient individuals in a healthy manner.

As a result of transferring the forms from all provinces to the database, how many cases are in which province, diagnosis of the cases, age group, gender, social security status, etc. Their distribution could be determined accordingly. The number of cases detected in this study covering 81 provinces is 4513 and these cases are; It is distributed as 57% thalassemia major, 23% sickle cell anemia, 11% thalassemia intermedia, 5% thalassemia trait, 2% sickle cell anemia + thalassemia major and 2% other abnormal hemoglobins.

As a result of this study, Türkiye's hemoglobinopathy map was created. The purpose of creating this map was to identify risky provinces and direct studies accordingly **(Figure 2)**.

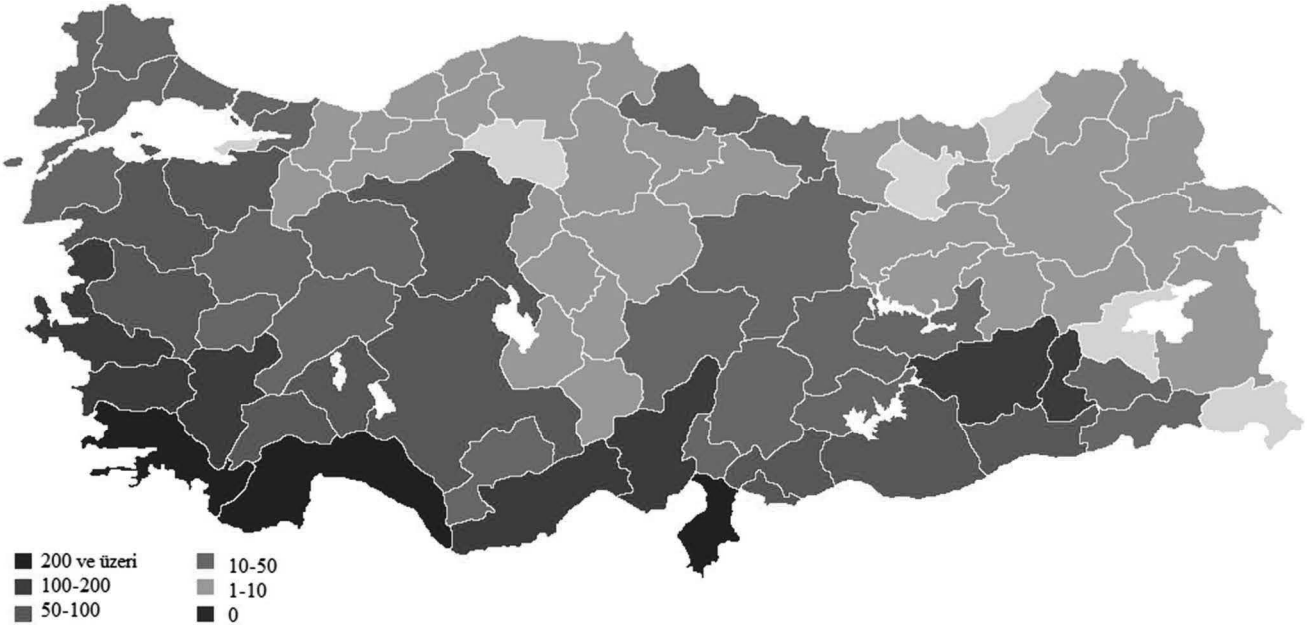


Figure 2: *Türkiye hemoglobinopathy map of 2002*

The Law on Combating Hereditary Blood Diseases aims to ensure that measures to prevent and combat abnormal hemoglobins, especially thalassemia and sickle cell anemia, which are common hereditary blood diseases in our country, within the scope of preventive health services, and to ensure that activities for the diagnosis and treatment of these diseases are carried out at specified standards and to regulate the procedures and principles. Based on the Official Gazette dated 24 October 2002 and numbered 24916, "Regulation on Hemoglobinopathy Control Program and Diagnosis and Treatment Centers from Hereditary Blood Diseases" was published.

This regulation; For hemoglobinopathies, one of the hereditary blood diseases of public institutions and organizations, natural and private legal entities; It covers education, screening, genetic counseling, pre- and postnatal diagnosis, all kinds of activities related to the treatment of patients, diagnosis and treatment centers, registration, notification, referral and permission procedures.

In addition, a Hemoglobinopathy Scientific Board was established based on this regulation.

Then, "Hemoglobinopathy Control Program (HCP)" has been started in 33 cities such as Aydın, Batman, Bilecik, Burdur, Bursa, Çanakkale, Denizli, Diyarbakır, Düzce, Edirne, Erzurum, Eskişehir, Gaziantep, Hatay, Mersin, Isparta, İstanbul, İzmir, Kahramanmaraş, Karaman, Kayseri, Kırklareli, Kocaeli, Konya, Kütahya , Manisa, Muğla, Sakarya, Şanlıurfa, Tekirdağ in order to organize and disseminate the services provided in this field, in 2003 by the General Directorate of Maternal Child Health and Family Planning and the General Directorate of Treatment Services of the Ministry of Health, taking into account the frequency of the disease in Türkiye.

Figure 3: *33 Provinces Where Hemoglobinopathy Control Program Is Implemented in 2003*

With the launch of the program, 3-level Hemoglobinopathy Diagnosis and Treatment Centers started to be opened in 33 provinces. In these centers, all couples were screened for hemoglobinopathies before marriage by taking the decision of the Provincial Hygiene Board. The aim of the screening program is to conduct carrier screening for couples before marriage and to prevent the birth of new patients by identifying couples who are both carriers and benefiting from genetic counseling before having children.

All newly diagnosed cases and screening numbers in the 33 provinces where the program was carried out were collected every 3 months with the "Hereditary Blood Diseases Notification Form". With the increase in the number of Primary Hemoglobinopathy Diagnostic Centers, the number of people screened for hemoglobinopathies before marriage has also increased.

Within the framework of the program, social activities such as "National Thalassemia Youth Camp", "International Thalassemia Summer School" and "International Painting Competition for Children with Thalassemia" have been carried out with many organizations to solve the problems of people with hemoglobinopathies.

May 8 is celebrated as "World Thalassemia Day" in the world, and within the scope of awareness activities, various trainings, meetings, seminars, panels, etc. are held in our country for healthcare personnel and the public. events, educational speeches and programs were held in mass media.

With the Decree Law No. 663 on March 19, 2012, the General Directorate of Maternal and Child Health and Family Planning (MCHAP) was closed and these services were transferred to the Public Health Institution of Türkiye (THSK). In this context, screening studies previously carried out in MCHFP centers have started to be carried out by the Public Health Laboratory. In 2016, the Hemoglobinopathy Diagnostic Guide was published to guide our biochemistry experts within the framework of algorithms in the evaluation and interpretation of screening test results.

In line with the guide; Biochemistry specialists serving in primary care provide data obtained by HPLC and hemoglobin electrophoresis, complete blood count, iron-ferritin, etc. Training was given to evaluate it together with tests.

THSK was restructured as the General Directorate of Public Health with the restructuring of the Ministry of Health in 2017 and is still run by the same general directorate.

Considering the prevalence of the disease in Türkiye, HCP was started in the first 33 provinces, and in 2013, 8 more provinces (Afyonkarahisar, Kilis, Mardin, Osmaniye, Siirt, Şırnak, Uşak, Yalova) were included and started to be implemented in 41 provinces. It has been carried out as the "Premarital Hemoglobinopathy Screening Program", which has become widespread in 81 provinces as of November 1, 2018.

With the spread of the program in 81 provinces, the Premarital Hemoglobinopathy Screening Program Field Guide was published. The guide includes the implementation steps of the program and the responsibilities of provincial health directorates and health institutions.

In line with the flow in Scheme 1 of the national program, counseling services are provided to prospective spouses who apply to family physicians to obtain a pre-marriage health report, and then a blood sample is taken from the prospective male spouse for screening and screening tests are performed by sending blood samples to the laboratory in the province or to the province affiliated to it.

If the male partner is found to be a carrier or suspect for hemoglobinopathy, a screening test is also performed on the female partner. Couples who are both carriers are directed to centers to receive genetic counseling, and when they think about having children, they are given the necessary guidance to have a healthy baby.

The program aims to prevent morbidity and mortality caused by hemoglobinopathies. In the screening program; Complete Blood Count (CBC), HPLC (High Performance Liquid Chromatography), Capillary Electrophoresis methods are used. Data regarding the screening are processed by family physicians into the system they use.

With the spread of the program over the years, screening rates increased from 32% to 83% of marriages in the program provinces, the number of detected carrier couples increased from 472 in 2003 to 1419 in 2018, and the number of sick babies born has fallen below from 300 to 100.

Since 2003, more than 500 thousand carriers have been detected in the program provinces, and the number of babies born with hemoglobinopathy has decreased by 2/3 over the years. While thalassemia carrier status is the most common carrier (61%), thalassemia major is in the first place in the distribution of patients, sickle cell anemia is in the second place, and other hemoglobinopathies are in the third place.

The distribution of patients and carriers in 2018 is given in Figure 4.

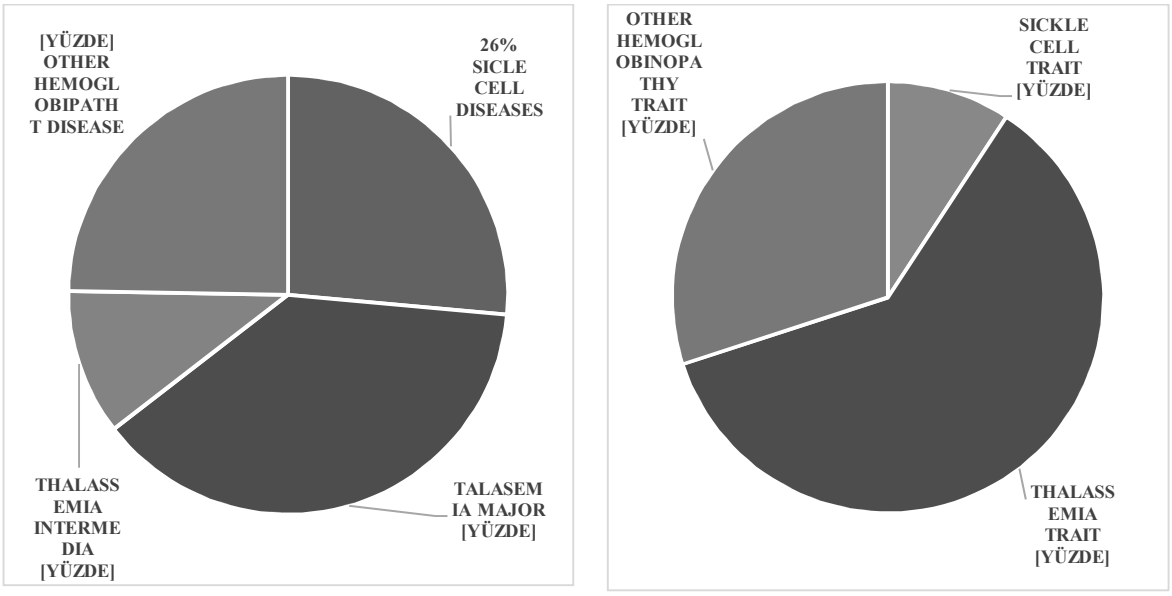


Figure 4: *Distribution of carriers and patients according to HCP data*

It started to be implemented in 81 provinces in November 2018, and 779,091 people (women + men) were screened in 2022. The screening rate is 79.9.

Correspondence Address
Specialist Dr. Yüksel Hakan Aydoğmuş,
Ministry of Health, General Directorate of Public Health, Ankara-Türkiye
E-mail: yukselhakan.aydogmus@saglik.gov.tr

MEDITERRANEAN BLOOD DISEASES FOUNDATION (AKHAV) SERVING THALASSAEMIA AND HEMOGLOBINOPATHIES SINCE 1996

The Mediterranean Blood Diseases Foundation (AKHAV) was established in 1996 with the participation of patients' relatives, businessmen and physicians in order to carry out implementation, education and scientific research at world standards, especially in the fields of Thalassemia and Haemoglobinopathies.

A summary of the scientific studies and services carried out by our Foundation in this field since its establishment is as follows;

In 1996, with the cooperation of Antalya Governorship, Antalya Municipality and Directorate of Health, the premarital screening programme launched with the slogan "**First Thalassemia Test then Marriage**" resulted in a 95% decrease in the birth rate of patients with thalassemia in Antalya city centre. This campaign was started to be implemented in 33 provinces with the Thalassemia Prevention Project in 2003, in 41 provinces in 2013 and became a routine service in 81 provinces in 2018.

In 1998, with the cooperation of Antalya Governorship, Health Directorate and Press/Media, "**Don't fade thalasemia flowers**" campaign was initiated. With this campaign, the "Blood Mother and Blood Father" programme was implemented in order to raise public awareness and provide continuous blood donation to every Thalassemia patient. The campaign was handed over to the Turkish Red Crescent in 2006 and was implemented throughout the country by the Turkish Red Crescent.

In 1998, Antalya Talasemia Prevention Project was initiated in the centre and districts of Antalya in cooperation with Akdeniz University Faculty of Medicine, Department of Public Health and Antalya Health Directorate. The project, which aims to increase public awareness and consciousness through education and information, continues today with the cooperation of all public and non-governmental organisations. As a result of these studies, the number of newborn patients in Antalya has been prevented by ninety-five per cent.

In 2000, under the auspices of our 9th President Süleyman Demirel, the 1st International Thalassemia Summer School was organised in Antalya.

In 2002, under the auspices of President Rauf Denktaş, the 2nd International Thalassemia Summer School was organised in TRNC.

In 2004, **AKHAV Hemoglobinopathy Diagnosis Centre** was established with the licence of the Ministry of Health. Thalassemia tests are still being performed in this centre for married couples. Abnormal haemoglobins detected in the tests performed in this centre for twenty years are published in scientific journals.

In 2004, the 3rd International Thalassemia Summer School was organised in Antalya in cooperation with the Ministry of Health, Turkish Blood Centres, Transfusion Association and European Transfusion Committee.

In 2006 and 2008, the 4th and 5th International Thalassemia Summer School was organised in Antalya in cooperation with the Ministry of Health and the Turkish Thalassemia Federation, of which our foundation president is the president.

In 2008, Türkiye's first Thalassemia and Blood Centre was established in Antalya Training and Research Hospital under the leadership of our Foundation, together with the philanthropist businessman Adem Tolunay and the Ministry of Health. Antalya Training and Research Hospital Adem Tolunay Thalassemia and Blood Centre has been serving not only Antalya but also patients from 81 provinces since its establishment.

In 2010, with the support of the Governorship of Antalya, a training programme was organised for muhtars. A total of 540 headmans and municipal marriage officers were trained in 18 districts.

In 2011, the 12th World Thalassemia Congress was organised in Antalya in cooperation with the Ministry of Health, the Thalassemia International Federation and the Turkish Thalassemia Federation, of which our foundation president is the president.

In 2013, with the permission of the Ministry of Health, in cooperation with Antalya Provincial Public Health Directorate, Antalya Metropolitan Municipality and Zeytinköy Development Association, the project "TOUCHING THE HUMAN" was carried out in Zeytinköy for three months, aiming free public examination and screening.

In 2013, the 6th International Thalassemia Summer School and Congress was organised in cooperation with the Ministry of Health and the Turkish Thalassemia Federation, of which our foundation president is the president.

In 2014, "Pre-Marital Examination and Counselling Training" was provided to 30 midwives and nurses from Antalya, Isparta and Burdur provinces at Muratpaşa Reproductive Health Training Centre in cooperation with Antalya Governorship and Antalya Provincial Directorate of Public Health.

In 2015, "Thalassemia Awareness Project" was carried out with the students of the Department of Medical Biology and Genetics of Akdeniz University Medical Faculty on 14 March Medical Day.

In 2016, with the Mediterranean Blood Diseases Association, within the scope of PRODES supported by the Department of Associations of the Ministry of Internal Affairs, within the scope of the "Talas Emmi Hand in Hand with Local Administrators" project, Local Administrators and Mukhtars in 19 districts were trained on Thalassemia

In 2016, within the scope of the EU Erasmus+ KA1- Individuals' Learning Mobility Youth Mobility project, a thalassemia awareness programme was carried out in Antalya with 50 young people from seven countries in the project named "THALAS UNCLE-TALAS EMMI" with the number 2016-2 TR01-KA105-035664.

In 2017-2018, the EU Erasmus+ Vocational Education Programme 2016 Main Action-2 Strategic Partnerships Vocational Education innovation development project "Diagnosis and Treatment of Endocrine Complications to Provide a Better Quality of Life for Thalassemic Young People", short name "EQUALITY", was carried out under the coordination of our foundation, in cooperation with Antalya Governorship, Antalya Training and Research Hospital, Antalya Thalassemia Association, Ferrara Quisiana Hospital in Italy and Joseph Carrera Institute of Barcelona University in Spain.

In 2019-2022, under the coordination of our Foundation, in cooperation with the General Directorate of Public Health of the Ministry of Health, Ferrara Quisiana Hospital in Italy and Joseph Carrera Institute of the University of Barcelona in Spain, "Thalassaemia Problem and Precautions in Migrants".

EQUALITY PLUS project was realised. In this project, 411 Syrian doctors were trained together with the team of the General Directorate of Public Health and provincial health managers of Adana, Mersin, Hatay, Gaziantep, Kilis and Şanlıurfa.

Since its establishment, our foundation has been regularly carrying out training and screening activities on hereditary blood diseases, especially thalassemia, in radio and television stations,

schools, municipalities, non-governmental organisations and villages in and around Antalya.

We continue our work as an example of how a non-governmental organisation in our country should carry out its mission in cooperation with the public sector.

Mediterranean Blood Diseases Foundation
Board of Directors

Chairman: Prof. Dr Duran Canatan
Deputy Chairman: Av. Ali Rıza Tıraş
Secretary General Mustafa Ülgüt
Treasurer: Hayri Oruç
Project Responsible Member: Dr Özlem Zümrüt
Member of Institutions Communication: Dr Zekiye Özdemir
Press and Public Relations Member: Gürsel Kaya

29 October 2023