Pediatric Hematology and Oncology Chapter

Indian Academy of Padiatrics

Evidence-based IAP-PHO Guidelines for the Diagnosis and Management of Thalassemia - 2023

1st Edition

Dr Mamta Manglani
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Dr Nita Radhakrishnan
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Dr Jagdish Chandra
Chair, InPHOG Subcommittee on Hemoglobinopathies

Published by: Pediatric Hematology Oncology Chapter of the Indian Academy of Pediatrics

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Foreword

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15 March 2023

Dear Friends,

It is my privilege to write the foreword to the IAP-PHO Guidelines on Diagnosis and Management of Thalassemia. These guidelines will offer beneficial information to all healthcare professionals involved in the treatment of thalassemia. It includes updated data on new approaches and an overview of progress achieved to date, towards a complete cure using methods such as stem cell transplantation and gene therapy.

The inherited hemoglobin disorders pose a paramount health problem placing an indeterminable emotional, psychological and economic burden on millions of people around the world. Although there has been tremendous progress towards the management of thalassemia, the truth remains that the therapeutics of thalassemia are costly and treatment is an excruciating process.

Over the last 50 years, we have witnessed that inherited hemoglobin disorders like Thalassemia and Sickle cell disease from being disorders with poor outcomes and early fatality are now considered chronic ailments with improving survival and quality of life.

There is enough published data now to prove that early diagnosis and comprehensive care can improve the quality of life of those living with Thalassemia. Prevention strategies in the form of mass screening, high-risk population screening and prenatal testing have all proved to be beneficial tools to decrease new births with hemoglobinopathy in an attempt to reduce the disease burden. Breakthrough in genetic research has made prevention a reasonable option everywhere.

The availability of hematopoietic stem cell transplant (HSCT) and advances in this field in the last two decades has immensely changed the outlook of families with children afflicted with Thalassemia and given them hope regarding a permanent cure for the disease. With the help of the public health system, private organizations, and NGOs, the scenario has changed from despair to a structured management plan and support system for those living with Thalassemia. However, this type of change is visible only in urban areas of the country causing a discrepancy in the health of these children based on their residential location and socio-economic status.

Initiatives towards the availability of more daycare transfusion centres, comprehensive clinics, safe blood products, chelating agents, centres offering prenatal diagnosis and advanced care centres offering HSCT as well as novel therapies wherever indicated, are the need of the hour.

These guidelines have been aptly and precisely framed after scientific deliberations amongst the experts in the field from our country over a period of nearly six months. I am sure the wide circulation of these guidelines will ensure that every child and adult living with thalassemia receives appropriate care and benefits from the advances in the field, which will translate into a better quality of life.

Guidelines would also work as an educational tool in training medical students and health professionals catering to children living with Thalassemia, updating their knowledge and strengthening their skills to improve the quality of care being offered to these individuals. With advances in safe blood transfusion practices and the availability of chelating drugs, the goal of achieving an average life span as close to the general population for those living with thalassemia would be possible if these patients are managed comprehensively by a multidisciplinary team including experts from various subspecialties such as cardiology, endocrinology, hepatology, psychiatry etc. and allied health professionals like physiotherapists, dieticians, counsellors and medical social workers.

IAP-PHO Chapter has always actively participated in and supported all initiatives towards treatment and prevention of thalassemia through collaboration and dialogues with government authorities, NGOs, patient support groups, and welfare organizations at the regional and national levels. Through this foreword, I would appeal to everyone to take an effort to ensure these evidence-based guidelines formulated under the aegis of the Pediatric Hematology-Oncology (PHO) Chapter of the Indian Academy of Pediatrics (IAP) reach every care provider in the country and benefit all individuals living with thalassemia.

I take this opportunity to thank all the authors, reviewers and editors who took the effort to put the guidelines together and I am truly honoured to write the foreword to such an excellent scientific compilation on a topic so close to my heart. I wish the readers a great reading and learning experience and I am truly happy for the patients who will surely witness the positive change in their health with the advances in the field.

Madhukar Lokeshwar

Preface

02 May 2023

This book is a labour of love, dedication and passion of a lot of individuals. We are fortunate to have conceptualized the writing of this guideline and to have brought together the collective experience of many experts in the country.

We, as a country, are proud of the diversity we offer; diversity of landscape, diversity of culture, cuisine and language. However, the diversity in healthcare services is distressing, to say the least. We are witness to the fact that the standard of treatment differs widely in the healthcare pyramid of our country. Despite being a rapidly developing economy with booming technological expertise, transforming the healthcare referral pathway is a distant dream. Hence, unfortunately, even today, the outcome of each child depends on where he/she was born and how efficient the family was in getting to the top of this pyramid. This is equally true for thalassemia.

The need for uniformity in care for these children prompted us to initiate this guideline as a collaborative project of Pediatric Hematology-Oncology (PHO) Chapter of Indian Academy of Pediatrics (IAP) and the Indian Pediatric Hematology Oncology Group (InPHOG) Subcommittee on Hemoglobinopathies. For decades, care providers have relied on guidelines published by other countries to manage their patients. The gaps in knowledge, care and logistics are many. However, we are fortunate that there is a revolutionary change in government support for children with thalassemia, sickle cell anaemia, hemophilia, cancers and many other diseases needing chronic care. The gaps between administrative intent and grass-root level functioning have to be bridged with appropriate manpower training and implementation of various programs rolled out by the government. This guideline brings together the senior-most teachers with vast clinical and administrative experience and enthusiastic middle-order faculty with expertise in technology and advances in care. We bring together doctors from all specialities needed for comprehensive care of these children, administrators, civil societies and allied health professionals like counselors and dieticians to ensure completeness in what we discuss. And finally, we discuss not only published evidence but also what is the standard practice which incorporates both logistics and science.

We are glad that what was just a thought in our minds 2 years ago, has now translated into a well-written guideline book, ready for rollout. We are grateful to all experts, who have spent time contributing to various chapters as well as editing this guideline. This herculean task would not have been possible without their support and expertise.

We thank Dr Amita Trehan and the executive board of the PHO Chapter of IAP, for being supportive of this initiative and the team from Central IAP led by Dr Upendra Kinjawadekar and Dr Vineet Saxena for their constant encouragement. We are also indebted to InPHOG Subcommittee on Hemoglobinopathies, for their guidance.

We hope this book reaches where it is needed.

We hope it reaches every pediatrician, every postgraduate in pediatrics, transfusion medicine and general medicine, every health professional involved in the care of a thalassemic child and every administrator who organizes support for their treatment.

We hope it helps bring in what we aimed for – "Uniformity in Care".

Mamta Manglani **Nita Radhakrishnan**

Pooja Dewan **Jagdish Chandra**

सत्यमेव जयते

डॉ. राजीव बहल, एमडी, पीएचडी
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Secretary, Government of India
Department of Health Research
Ministry of Health & Family Welfare &
Director-General
Indian Council of Medical Research

MESSAGE

The thalassemias and structural haemoglobin variants are the commonest monogenic disorders globally. India has the largest number of children with Thalassemia major in the world, about 1 to 1.5 lakhs, and about 10,000-15,000 children having Thalassemia major are born every year. Current treatment given is repeated blood transfusions, followed by regular iron chelation therapy to remove the excessive iron overload, consequent to the multiple blood transfuions. The only cure available for such children is bone marrow transplantation (BMT). Preventive measures include carrier screening, genetic counselling and prenatal diagnosis.

I am pleased to see a PHO Chapter's Thalassemia Guideline Book for the delivery of care to patients with thalassaemia developed by the Indian Academy of Paediatrics. This document should be an invaluable source of information for medical professionals with a clear, comprehensive guide to the optimal treatment of thalassaemia, based on scientific evidence, clinical studies and observations.

Rajiv Bahl

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Dear All,

With a birth cohort of about ten thousand children of Thalassemia Major getting added to our population every year, the responsibility of we pediatricians increases manyfold in providing standardized comprehensive care to such children keeping our unique socioeconomic and geographic conditions in mind.

Working for an effective, nationally coordinated PREVENTIVE program, sharing essential information and knowledge to support best possible growth and development of these children and providing equitable access to excellent medical treatment under one roof are some of the areas that we can definitely address while working towards ZERO thalassemia.

I'm sure these guidelines prepared by the Pediatric Hematology Oncology Chapter of the Indian Academy of Pediatrics under the leadership of Dr Mamta Manglani and Dr Nita Radhakrishnan will go a long way in providing uniformity in care of children born with Thalassemia.

I heartily congratulate all the team members for bringing out these country specific guidelines.

Best Regards,

Dr Upendra Kinjawadekar
National President 2023
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INDIAN ACADEMY OF PEDIATRICS
PEDIATRIC HEMATOLOGY-ONCOLOGY CHAPTER

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Dear friends,

India has a huge burden of hemoglobin disorders, thalassemia and sickle cell disease being dominant. At present a stem cell transplant is the only cure available in India. Patients with thalassemia can have a reasonable quality of life with good transfusion practice and chelation, transplant for all being a distant dream in our country.

An ounce of prevention is better than a pound of cure. The need of the hour is to disseminate awareness and promote screening programs. In addition, the number of government centers where antenatal testing is available needs to expand. We need to work together to achieve this goal. We are a large populous country and to achieve a 'zero thalassemia' status requires inputs from the Government, NGOs, thalassemia associations and CSR support.

It is concerning that the management of patients with thalassemia is inadequate. Over the years transfusion services are available in most civil hospitals across states and a large number of state governments provide chelating agents. However, medical management is often wanting owing to lack of trained personnel.

These guidelines are intended to act as a ready reference for providing standard care management across all thalassemia centers in the country.

I complement the editors for undertaking this task and am sure the good work will be a large step in enhancing the care of our patients.

Amita Trehan
Chairperson, PHO chapter of IAP
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16 March 2023

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1 IAP PHO Guidelines on Diagnosis and Management of Thalassemia Syndromes

The why and the how

Mamta Manglani, Jagdish Chandra, Nita Radhakrishnan

Thalassemia syndromes are a group of preventable genetic disorders affecting hemoglobin due to reduced or absent production of globin chains. The severe forms of these syndromes that result from homozygous or compound heterozygous states, require regular blood transfusions to sustain life and are associated with chronic morbidity and sequelae that can lead to disability or death.

There are two main types of thalassemias: α and β thalassemia based on the type of globin chain affected. Approximately 5% and 1.5% of the world population are α and β thalassemia carriers, respectively. Although the prevalence of α thalassemia carriers is higher, since α chains are represented by 2 genes from each parent, it takes 3 or 4 defective genes to have a symptomatic child with α thalassemia, making it less common than β-thalassemia major (one gene from each parent) [1].

The overall prevalence of β-thalassemia carrier state in India is about 3-4%, with an estimated 40 million carriers and over 100,000 patients living with Thalassemia major [1]. Some ethnic groups like Sindhis, Kutchis, Lohanas, Punjabis, Bhanushalis, Agris, Mahars, Saraswats, Lingayats, Gowdas, Reddys, Neobuddhists, as well as a few tribal populations, have a higher (5-17%) prevalence of β-thalassemia carrier status [2,3].

The main components of the management of thalassemia include

i. Care

Conventional management includes appropriate diagnosis with investigations, safe packed red cell transfusions, chelation therapy, regular monitoring and follow-up. The management of thalassemia needs a multidisciplinary team consisting of pediatricians, hematologists (pediatric and adult), transfusion medicine specialists, endocrinologists, cardiologists, gastroenterologists, nutritionists etc for comprehensive care of these patients. There is a need to develop new thalassemia treatment centres and equip the existing ones with the appropriate manpower, and facilities for diagnosis and treatment of thalassemia.

ii. Cure

The current curative treatment option for thalassemia major viz. stem cell transplantation, although, being offered at many centres in India, is still

inaccessible for the majority of patients due to the high cost and apprehensions surrounding it. We need to work towards providing these facilities at the lowest cost possible by engaging all stakeholders such as central and state governments and non-governmental organisations. Gene therapy, which has recently received US FDA approval for adults and children with Transfusion Dependent Thalassemia (TDT), is beyond the reach of Indian patients at present as it is not indigenously available and prohibitively expensive if imported.

iii. Control

It is important to prevent the birth of a thalassemia homozygous or compound heterozygous child through public awareness programmes, population and cascade screening, premarital and antenatal screening followed by genetic counseling of at-risk couples and prenatal diagnosis where necessary.

Unfortunately, a large number of children continue to be born with thalassemia every year in our country due to a lack of awareness and lack of consistent screening programs in addition to deficient systematic strategies to prevent them. There is a dire need to strengthen these efforts at the national level with the involvement of government and professional bodies such as the Indian Academy of Pediatrics and Federation of Obstetricians and Gynecologists of India, as most preventive efforts are presently done by NGOs or individuals (medical personnel, patients, parents etc) who are passionate about the cause. Together with all these concerted efforts, the aim should be to achieve a "zero-thalassemia birth rate".

The Need

Even though large strides have been achieved in the care of patients with thalassemia in our country, a national policy for thalassemia is still under deliberation. At present, we are dependent on the following three documents for the management of these patients:

1 Indian Academy of Pediatrics National Guidelines, 2006 [4]

2. National Health Mission Guidelines for Prevention and Control of Hemoglobinopathies in India, 2016 [5]

3. Thalassemia International Federation (TIF) Guidelines, 4th edition, 2021 [6]

The present guidelines have been conceptualized by the Pediatric Hematology Oncology (PHO) Chapter of the Indian Academy of Pediatrics (IAP), to frame the standards of care for Indian patients with thalassemia, while incorporating the recent advances in thalassemia care. It will also help centres treating thalassemia in various health sectors of the country to follow uniform management and prevention strategies. We believe that the PHO Chapter of the IAP with more than 1000 pediatric hematologist members and the IAP with more than 40,000

pediatricians in its fold can together improve the standard of care for these children immensely. We hope this document remains a reference for generations of pediatricians, physicians and policymakers involved in thalassemia care and will help us establish the best standards of care in our country.

The Process

It was proposed to develop these guidelines under the aegis of the PHO Chapter of the IAP. On receiving approval from the Chapter, a consensus meeting of senior thalassemia specialists was organized on an online platform, where the concept note and timelines were discussed. The guidelines were conceptualized as two documents. The main document was envisaged in the form of a book, to be circulated to all major thalassemia treatment centers and to be available at all training workshops and conferences organized by the Chapter. The abridged version with all crucial recommendations, to be published in a widely circulated Indian journal after peer review.

After the initial meeting, a potential list of experts, who have been involved in the care of thalassemia and who have contributed to it significantly emerged. This required inviting experts from various specialities, in order to ensure a comprehensive document which dealt with all aspects, from diagnosis to management to prevention. Throughout the process, the focus was to provide evidence-based management guidelines, with an emphasis on literature that emerged from low and middle-income economies (LMIC) such as India, so that it is easily adaptable as well as sustainable. The group also discussed common issues such as difficulties in diagnosis and common queries in management and monitoring. There were limitations in developing evidence-based guidelines in certain aspects due to gaps in knowledge and poor quality of evidence i.e. lack of randomized controlled trials (RCT). Also, globally and even within India, there existed a wide range of treatment standards, which we had to be cognizant of. While providing T-cell-depleted mismatched stem cell transplantation was possible in select centres, a vast majority of patients received poor-quality blood for transfusion, less than adequate pre-transfusion hemoglobin and minimal or no chelation.

The collective effort began in August 2021, upon receiving approval from both Pediatric Hematology-Oncology (PHO) Chapter of Indian Academy of Pediatrics (IAP) and Central IAP. After finalizing the 23 topics in this subject, 2-3 experts were assigned for each topic. Three rounds of discussion with each of these experts were done initially over email and later, between March to October 2022 on an online platform, weekly. Each round aimed at refining the topic, addressing common questions, simplifying concepts and searching for contemporary literature. All discussions were recorded and links to the discussion were provided to the experts while finalizing the respective chapters. For difficult questions, for which literature evidence was poor, consensus was sought from senior experts.

The literature search by experts, who were assigned the topic, was restricted to English language and human-only subjects. No geographic exclusion was made. A search for recent articles, especially RCTs and experience from India and LMICs was looked into separately [6]. No expert/author has received any external funding for these guidelines and all have contributed voluntarily.

The version submitted after these discussions was edited by a team consisting of 10 writing group members or the editorial board, who prepared the final document. The level of evidence (LOE) of each recommendation was graded as per Oxford Centre for Evidence-Based Medicine (OCEBM) 2011 guidelines [7]. The final draft guidelines were circulated to all the editorial board members for comments, modifications and final approval. The statements in this document are the consensus recommendations of the expert group of 62 authors. We acknowledge that since the existing literature was limited, the recommendations were not strong in many instances. Also, in view of recent drugs and therapeutics entering this field, sufficient information is not yet available and this may change in the coming years with additional research. As knowledge grows, the information available will become more evaluable, permitting more stringent methodology to be applied for future editions.

We are happy to note a few major changes from the existing standard of care in recommendations on the chelator of first choice, folic acid supplementation and age at first T2*MRI among many others in this document. Also, this document serves as a practice manual with clear guidelines on how to set up a day-care centre, when to transition to the care of an adult physician, on aids for thalassemia patients including disability certification in India and so on. We also provide a bird's eye view of the main centres involved in thalassemia care in India, with the services they provide, basic minimum data sheets for transfusion, chelation, monitoring, how to give chelation etc to make this document wholesome and ready-to-use. We aim to develop self-paced video modules with certification for each of these topics to make them more accessible to doctors in this digital era soon.

As a group, we encourage centres to use this document in routine clinical practice. We urge young Indian doctors and researchers to conduct randomized studies on topics that have poor LOE currently and contribute to improving the quality of evidence. This document is primarily intended for healthcare professionals. No aspect of this should be used by patients with thalassemia or patient organisations directly without medical expertise.

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2 Epidemiology and Burden of Thalassemia

Amit Jain, Nitin Shah, Vineeta Gupta

Thalassemia is widely spread over the globe, stretching from the Mediterranean basin and parts of Africa, throughout the Middle East, Trans-Caucasus, the Indian subcontinent, Southeast Asia, Melanesia into the Pacific Islands and the Far East [1-3]. Migration and intermixing of the population have also spread it into non-endemic areas such as North America as well as Northern and Western Europe [2].

Out of the 270 million carriers of abnormal hemoglobinopathies, 80 million are β -thalassemia carriers. 300,000 to 400,000 babies are born with critical hemoglobin disorders every year, of which 23,000 constitute β-thalassemia homozygous [3]. Low to middle-income countries contribute to 90% of these total births of hemoglobinopathies [4]. It has been estimated that around 7% of pregnant women carry beta or alpha thalassemia, or hemoglobin S, C, D Punjab or E gene across the globe, and over 1.1% of couples are at-risk; amongst them [5], approximately 1.5% are β-thalassemia carriers [3,6].

Thalassemia is prevalent across India, with an average frequency of carriers being 3-4% (varies from 3 to 17%), which amounts to 35 to 45 million carriers in our diverse population [7-9]. One, out of every 2700 births, is a child with thalassemia homozygous. About 10,000 - 15,000 babies are born every year with severe hemoglobinopathy in India [10]. These numbers are based on data collected by individuals and groups maintaining databases from literature reports.

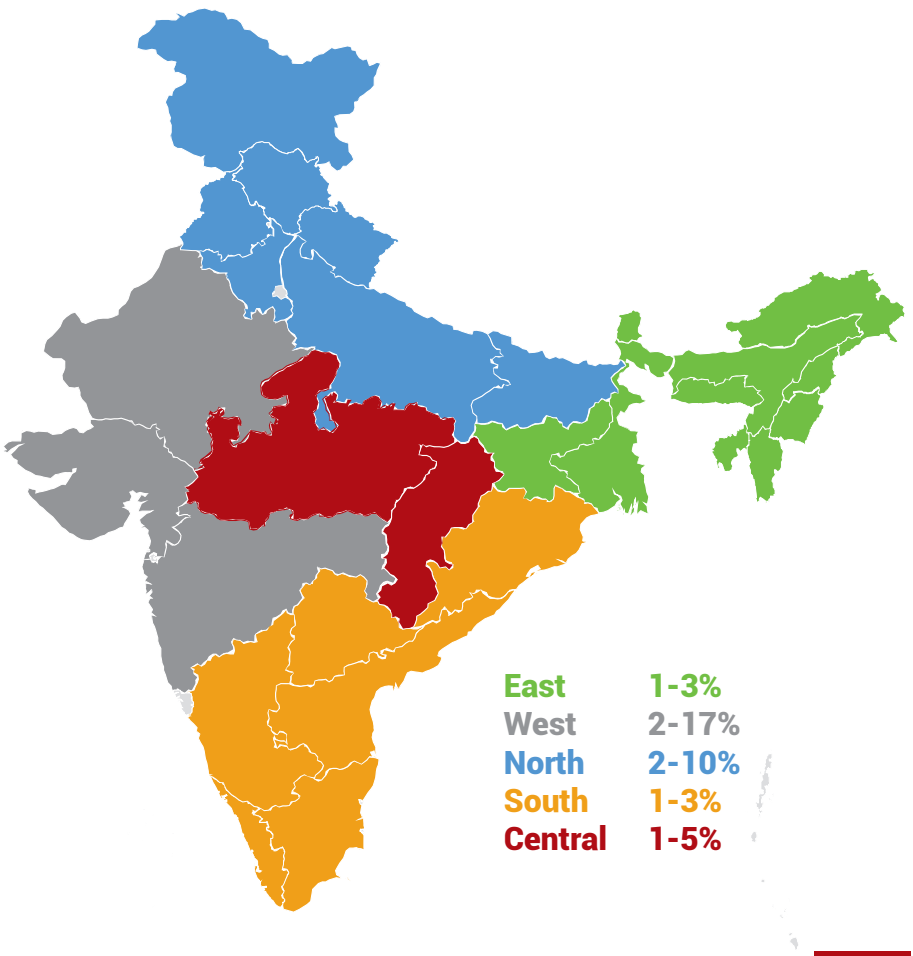
Thalassemia is more prevalent in certain Indian communities as shown in Table 1. HbD is present in about 2% of the population in Punjab [9]. HbE is common in the North Eastern states, and has a carrier frequency as high as 50%, in some areas; it is found in lower frequencies in the Eastern states of West Bengal, Bihar and Uttar Pradesh [11]. Figure 1 depicts the regional distribution of thalassemia across states in India.

Table 1. Prevalence of β thalassemia trait in high-risk communities [9]

Sindhis, Lohanas, Bhanushalis, Vellalas	8-17%
Vasava, Chaudhry, Gamit, Kokana tribes from south Gujarat	12-15%
Aroras, Khatris, Jaths,	6-10%
Prajapatis, Banias, Jains, Patels, Mayavanshis	6-8%
Bhuyan, Pik, Dudh Kharia tribes from Sundargarh, Odisha and Rohit tribe from Surat, South Gujarat	6-8%
Kayastha, Mandols	5-9%
Menons, Shiyas, Fakirs	5-7%
Neobuddhists, Mahars	4-6%

Adapted from: Colah et al. Burden of thalassemia in India: The road map for control. PHOJ; 2017: 79 – 84.

Figure 1. Region-wise burden of Thalassemia in India [9-14]



Financial, Psychological, and Emotional Burden

The average annual treatment expense of managing a person living with thalassemia in India is INR 74,948 (ranging from INR 41,514 to INR 151,800) [15]. The realistic cost of treatment incurred per year at current rates without subsidies is estimated to be INR 167,750 per patient as per analysis done by Kantharaj, et al. [16].

Beta-thalassemia is a chronic illness that causes enormous psychological issues to children and their families which include daily fears of getting pricks, spending time in hospital, taking medications, fear of future complications and overall compromized quality of life. Various strategies like cognitive behavioral therapy and counseling have shown to be effective in children with thalassemia helping them to adhere to chelation and transfusion protocols and improve their quality of life [17]. Refer to Chapter 17 on "Psychosocial Counseling in Thalassemia" for detailed discussion regarding the psychological impact in thalassemia.

Shortage of blood and need for transfusion centres

A lack of voluntary non-remunerated blood donor pool, coupled with a lack of awareness about thalassemia with the non-uniform implementation of national blood policies, and fragmented blood services contribute to a significant gap between the demand and timely supply of safe blood.

The approximate annual requirement of packed red cells for patients with thalassemia in our country is 3 million units annually and the collection is estimated at about one million units annually. Thus, demand constantly exceeds supply (considering 30 units/year/patient for a total of 100,000 patients). The demand and supply disparity reiterates the need for raising awareness about repeat voluntary unremunerated blood donation, promoting modern principles of patient blood management and strengthening capacities of human resources in the blood transfusion system, to ensure universal access to blood and components in India.

Approximately 1% of India's population presently donates blood annually. Of this, 25-30% is being utilized for patients with thalassemia, considering that currently, there are 100,000 to 150,000 transfusion-dependent thalassemia patients in the country. With the failure to implement the thalassemia prevention program effectively, these numbers would rise to 2,75,000 by 2026. This would impose a huge load on providing packed red cells for these patients and approximately 66% of total blood donation would be then utilized for thalassemia patients alone, posing a relative and significant shortage of blood products [18]. Many measures are being taken to plug this gap. In 2014, the National Blood Cell under the National Health Mission (NHM), launched by the government of India, set up a mission to ensure accessibility, adequacy, safety and quality of blood across the country with planning, networking and development of new policies [10].

E-raktkosh is an integrated electronic information and management website for implementing Drug & Cosmetic Act, National blood policy standards and guidelines ensuring proper collection & donation, effective management and monitoring the quality and quantity of the donated blood [19,20]. This web-based mechanism interconnects all the state's blood banks into a single network. However, it is yet to be fully functional and updated, with limited utility so far. Once operational, it would reduce the logistic burden on the mobilization of blood products. On similar grounds, there are state-wise portals for the same [21].

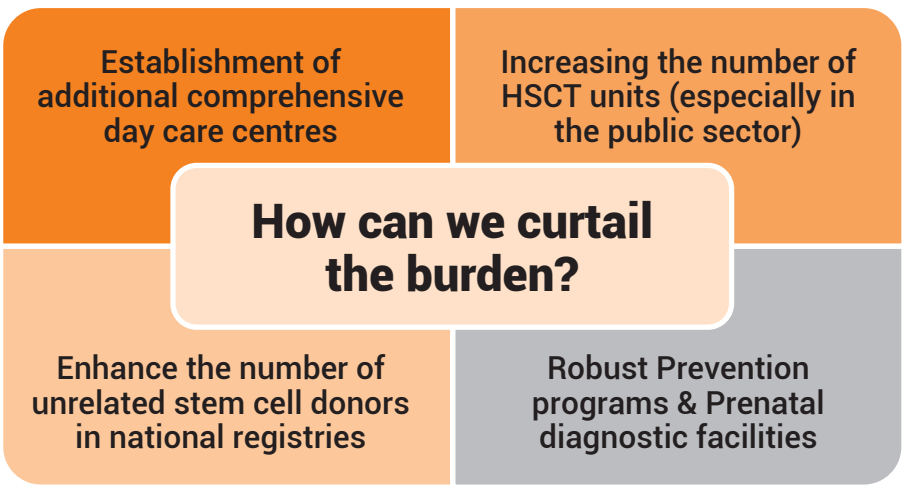
Need for Thalassemia Prevention Programs in India

The prevention programs in Cyprus, Sardinia, Latium and Montreal, demonstrated that an effectively implemented prevention and control program can successfully bring down the births of children affected with homozygous thalassemia to almost zero over time [22-24]. This would reduce the disease burden and enable better lifelong care for those affected and surviving with the disease.

As per the cost analysis done by Kantharaj, et al. [16], the cost of preventing new births of children with thalassemia by mass screening of pregnant women is 6,175 million, which is significantly lower than the cost of treating these patients over an estimated life span of 40 years which amounts to 66,800 million.

Thus, management of patients with thalassemia poses a great financial burden on families as well as nation. Hence, it is crucial for governments to devise policies for providing financial support and subsidizing the expenses of treatment including comprehensive care and hematopoietic stem cell transplants, along with a robust program for prevention. Figure 2 highlights the steps which if implemented can curtail the burden of thalassemia.

Figure 2. Suggested steps to curtail the burden of thalassemia



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3 Definitions, Terminologies and Genotype-phenotype Correlations in Thalassemia

Roshan Colah, Prateek Bhatia, Prashant Sharma

Several terms, entities and concepts related to thalassemia syndromes are defined in Table 1.

Table 1. Definitions and terminologies in thalassemia [1–8]

Terminology	Standard Definition
α-thalassemia silent carrier state	An asymptomatic form of thalassemia due to a single α globin gene deletion (α+/α^0). The patients have normal RBC indices and are non-anemic.
α-thalassemia trait	A clinically milder form of thalassemia primarily due to deletions and rarely due to point mutations of two α-globin genes in cis (α0; – –/αα) or trans (α+; –α/–α) manner.
β-thalassemia intermedia	Defined clinically as an intermediate severity β-thalassemia not requiring or requiring only occasional transfusional support due to reduced β-globin chain production. Genotypically, most cases are homozygotes or compound heterozygotes for a β^+ or β^0 and β^+ alleles, though rarely dominant forms due to β^+ allele and α triplication or exon-3 mutations can also be encountered. They are included under the larger rubric of non-transfusion dependent thalassemia (NTDT).
β-thalassemia major	Conventionally known as Cooley's anemia or Mediterranean anemia, it is defined clinically as the most severe form of β-thalassemia requiring regular transfusions to maintain normal hemoglobin levels due to complete or near complete absence of β-globin chain production. Genotypically, cases are homozygotes or compound heterozygotes for β^0 or β^+ alleles. They are included under the larger rubric of transfusion dependent thalassemia (TDT).

Terminology	Standard Definition
β-thalassemia minor	Also known as β-thalassemia carrier or β-thalassemia trait, it is defined clinically as the least severe form of thalassemia with mild anemia and usually an asymptomatic course due to mild reduction in β-globin chain output. Genotypically, cases are heterozygous with β^{+} or β^{++} mutations involving only a single allele.
Delta thalassemia	A rare form of thalassemia characterized by reduced output (δ^{+}) or complete absence of (δ^{0}) delta globin chains resulting in very low or undetectable HbA2 (normal range is less than 3.0%).
Delta-beta thalassemia	A rare form of hemoglobinopathy characterised primarily by large deletions involving β-globin and δ-globin genes resulting in markedly reduced or total absence of these globin chains and a compensatory increase in HbF or γ-globin chain output.
Ex--transfusion dependent thalassemia*	Refers to formerly transfusion-dependent patients whose disease has been "permanently and fundamentally modified" by curative/novel therapies, resulting in "sustained transfusion independence".
Hb Bart's hydrops fetalis	Defined clinically as the most severe form of α-thalassemia resulting in severe fetal anemia and usually death ***in-utero*** due to complete absence of all four α-globin chains. The condition is characterized by formation of Hb Bart's, an abnormal tetramer of γ-globin (γ4) chains.
Hb Lepore	A structural hemoglobin variant resulting from fusion/rearrangement of δ-globin and β-globin genes that can result in moderate to severe transfusion dependent anemia in homozygous or co-inheritance state with β-thalassemia.
HbA	Adult hemoglobin that normally replaces fetal hemoglobin after birth and reaches normal adult concentration of 97-98% in RBCs by 6 months of age. Structurally, it comprises of two α-globin and two β-globin chains and is denoted as HbA α2β2.

Terminology	Standard Definition
HbA2	A minor component (2-3%) of adult hemoglobin composed structurally of two α-globin and two δ-globin chains and denoted as HbA2 α2δ2.
HbE	A structural hemoglobin variant with thalassemic properties of reduced β-globin chain output (25-30% in carriers) resulting from a single amino acid substitution (lysine for glutamic acid) at position 26 of the β-globin chain. It is the most common abnormal hemoglobin variant in Southeast Asia.
HbF	Fetal hemoglobin which constitutes predominant form of hemoglobin for oxygen carriage during fetal life but reduces after birth to normal adult levels of around 1-1.5%. Structurally it comprises of two α-globin and two γ-globin chains and is denoted as HbF α2γ2.
HbH disease	Defined clinically as intermediate form of α-thalassemia resulting in moderate degree of hemolytic anemia and splenomegaly with a high risk for acute hemolytic crisis following exposure to infections and oxidant stress/drugs. The condition is characterised by deletional or non-deletional mutations involving three of the four α-globin genes.
HbS	A structural hemoglobin variant due to single amino acid substitution (glutamic acid to valine) at position 6 of the β-globin chain. This results in reduced solubility and precipitation of the abnormal β-globin chains during periods of hypoxia/low oxygen tension resulting in characteristic sickle-shaped RBCs. It is the commonest hemoglobin variant in the world.
Hereditary persistence of fetal hemoglobin (HPFH)	A relatively benign/asymptomatic hemoglobinopathy arising due to either large deletions in β-globin gene (deletional HPFH) or point mutations in promoters of γ^A or γ^G globin genes (non-deletional HPFH) with resultant increase in HbF levels.

Terminology	Standard Definition
it-TDT*	Standing for "intermittently-transfused TDT", these are formerly transfusion-dependent patients whose disease has been "permanently and fundamentally modified" resulting in reduction in, but not in obliteration of transfusion requirement.
Neo-transfusion dependent thalassemia*	Formerly non-transfusion-dependent patients who have subsequently become definitively dependent on transfusions to sustain life for the remainder of their disease. The designation should be employed judiciously keeping in view the long-term (and not a short-term) period of observation of the clinical course of the patient.
Non-transfusion dependent thalassemia (NTDT)	This terminology encompasses phenotypically less severe thalassemia cases either not requiring transfusions, or only occasionally requiring them. It primarily includes patients with genotypes of β-thalassemia intermedia, HbE/β-thalassemia and HbH disease (or α-thalassemia intermedia).
Transfusion-dependent thalassemia	This terminology encompasses phenotypically severe thalassemia cases requiring regular blood transfusion support and includes patients with genotype of β-thalassemia major, severe HbE/β-thalassemia, transfusion dependent HbH disease or HbH and surviving Hb Bart's hydrops.

*These recently introduced terms described in a correspondence by Musallam et al (2021) constitute expert opinion and may currently be considered non-standard terminology [8].

Genotype-Phenotype Correlation in β-thalassemia

β-thalassemias result from impaired synthesis of β-globin chains. The clinical severity depends on the degree of imbalance between α- and β-globin chain synthesis. Any factor that reduces this imbalance leads to a lesser extent of α-globin chain precipitation in erythroid precursors. This reduces ineffective erythropoiesis and hence ameliorates the severity of the disease [9,10].

Although, β-thalassemias are monogenic disorders, they behave clinically as polygenic traits with several factors affecting their wide clinical spectrum [4,11–14]. The phenotypes range from the non-transfusion-dependent

β-thalassemia intermedia (β-TI), a relatively milder disorder to the severe transfusion-dependent β-thalassemia major (β-TM) [15]. A commonly used classification of disease modifiers is given in Table 2.

The clinical classification of thalassemias is relatively fluid, as severe cases improve with definitive treatment and those with intermediate disease may worsen during periods of increased demand like pregnancy or growth spurts [8].

Table 2. Genetic modifiers of phenotypic variability in β-thalassemias [15–20]

Primary modifiers	Nature of mutations in the β-globin (HBB) gene (β^0, β^+, β^{++})
Secondary modifiers	Co-inherited defects in the α-globin genes (HBA1 and HBA2) (α-thalassemia, α-globin gene triplications or quadruplications) Enhancement of fetal hemoglobin (HbF) production Mutations / polymorphisms in the β-globin gene cluster SNPs in QTLs in other non-globin genes
Tertiary modifiers	Genes unrelated to hemoglobin production. These influence the onset and severity of other complications like jaundice, gallstones, iron overload, bone disease and thrombosis.

SNP: Single nucleotide polymorphisms; QTL: Quantitative trait loci

Primary Modifiers

Majority of the β-thalassemias are caused by point mutations in the β-globin gene or its flanking regions and a few occur due to deletion or insertion of a few nucleotides. Large deletions are rare except for the 619 bp deletion. The primary determinant of severity is the nature of the β-thalassemia mutations inherited from the parents. There are 5 to 6 common mutations causing β-thalassemia in every population and a larger number of rarer ones. Among Indians, most of these are severe mutations with absence of synthesis of β-globin chains (β^0) or significant impairment in β-globin production (severe β^+) while few mutations are milder or silent with some reduction in β chain synthesis (mild β^+ or β^{++}) [9,10,15]. The salient Indian mutations are listed in Table 3.

Table 3. Common mutations accounting for severe and mild phenotype in β-thalassemia in Indians [9,10,15,19,21,22]

	HGVS nomenclature	Severity
Severe mutations		
IVS 1-5 (G>C)	HBB:c.92+5G>C	Severe β^{+}
IVS 1-1 (G>T)	HBB:c.92+1G>T	β^{0}
Codons 8/9 (+G)	HBB:c.27_28insG	β^{0}
Codons 41/42 (-TCTT)	HBB:c.126_129delCTTT	β^{0}
Codon 15 (G>A)	HBB:c.48G>A	β^{0}
619 bp deletion	NG_000007.3:g.71609_72227del619	β^{0}
Mild / Silent mutations		
Capsite+1 (A>C)	HBB:c.-50A>C	β^{++}
-88 (C>T)	HBB:c.-138C>T	β^{+}
Poly A (T>C)	HBB:c.*110T>C	β^{++}

IVS1-5 (G>C) is the most common severe mutation accounting for 38% to over 80% of mutant alleles, frequencies being the highest in southern and eastern India [23,24]. The 6 severe mutations make up around 90% of all β-thalassemia alleles [23,24]. The Capsite+1 (A>C) and the -88 (C>T) are more frequently seen in north India and are often associated with near-normal MCH, MCV and HbA2 levels in heterozygotes [24–26]. Homozygosity or compound heterozygosity for two silent or mild β-thalassemia mutations (β^{++}/β^{++}) or co-inheritance of a severe β-thalassemia allele with a mild allele (β^{0}/β^{++}, β^{+}/β^{++}) often leads to a non-transfusion-dependent β-TI phenotype [17,18].

Overall, the prevalence of milder mutations is higher in β-TI than β-TM (8.0% versus 1.0%) [27]. Sometimes β-thalassemia heterozygotes also have a β-TI phenotype due to inheritance of a very rare mutation in exon 3 leading to highly unstable β-globin chains which precipitate in erythroid precursors causing ineffective erythropoiesis (dominant β-thalassemia) [5]. HbE-β-thalassemia and HbS-β-thalassemia also have variable phenotypes, partly depending on the type of the β-thalassemia mutation inherited [28,29]. However, β-thalassemia mutations alone cannot always explain the clinical phenotype and other genes are also involved [15].

Secondary Modifiers

1. Fetal hemoglobin (HbF) production

There are several genetic determinants that increase γ-globin chain production and fetal hemoglobin (HbF) levels that can ameliorate the severity of β-thalassemia (Table 4). The γ-globin chains combine with the excess α-globin to form HbF with selective survival of cells with a higher percentage of HbF.

Table 4. Genetic modifiers of HbF production [5,10,30]

Within the β-globin gene cluster	Unlinked to the β-globin gene cluster
Co-inherited HPFH	HBS1L-MYB intergenic region on chromosome 6q23
Co-inherited δβ-thalassemia	BCL11A gene polymorphisms on chromosome 2p16
^{G}γ -158 (C>T) - Xmn1 polymorphism	KLF1 gene variations on chromosome 19p13
β-thalassemia mutations in the promoter region	

Deletional and non-deletional hereditary persistence of fetal hemoglobin (HPFH) and δβ-thalassemia mutations, associated with a high HbF output in carriers, when co-inherited with severe β-thalassemia alleles, can result in a milder TI phenotype [31]. Some thalassemic deletions in the promoter of the HBB gene are associated with increased HbF levels which can adequately compensate for the absence of HbA. Heterozygotes with these mutations have unusually high HbA2 levels [13].

Under conditions of stress erythropoiesis as in homozygous β-thalassemia, presence of the T allele in the Xmn1 polymorphism leads to a high HbF response, delaying transfusion requirements in these patients. The T allele was more common in β-TI patients than in β-TM patients (68% vs 29%) [32,33]. The presence of the -158(C>T) mutation in the ^{G}γ promoter also increases the HbF levels in HbE-β-thalassemia and HbS-β-thalassemia [19]. HbF is a complex genetic trait where, apart from the Xmn1 polymorphism, there are 2 other major quantitative trait loci (QTLs) mapping outside the β-globin cluster where single nucleotide polymorphisms (SNPs) are strongly associated with HbF production (HBS1L-MYB, BCL11A) leading to a mild TI phenotype and delayed transfusion requirements in patients with homozygous β^0 thalassemia. BCL11A gene, when

downregulated, could lead to increased γ-globin gene expression. Common variants at these 3 loci could explain around 50% of variation in HbF levels [34]. The γ-globin gene promoter polymorphisms [−158 (C>T), + 25 (G>A)] together with BCL11A [rs1427407(G>T)] and HBS1L-MYB [rs66650371 (-3bp)] and [rs9399137(T>C)] polymorphisms were associated with higher HbF in a group of Indian patients with a lower disease severity score, milder clinical presentation and delayed need for transfusions [30,34,35].

Krüppel-like factor-1 (KLF1) has emerged a major erythroid specific transcription factor. Many KLF1 variations are associated with increased HbF and were more common in β-TI patients (14%) than in β-TM patients in a recent report. Further, 12% of β-thalassemia heterozygotes with HbF levels ranging from 1.3 to 15.9 % showed KLF1 gene variations [35,36]. These primary and secondary modifiers when taken together would be useful to develop a predictive score of severity to support the clinical decision to grade the patients as β-TI or β-TM.

2. Co-inherited defects in the α-globin (HBA) genes

A relative excess of unpaired α-globin chains that precipitate in the developing erythroblast causing oxidative membrane damage and ultimately apoptosis, is a major cause of ineffective erythropoiesis and anemia in β-TM and β-TI. Coinheritance of α-thalassemia, by reducing this imbalance, ameliorates the disease [5,10,13].

Deletional α-thalassemia (depicted as a hyphen, for e.g., --/αα) is commoner than point mutations or small indels (depicted as α^{ND} or α^{T}, or non-deletional α-thalassemia). The latter is typically more severe [37].

The commonest molecular genetic cause of α-thalassemia in India is a 3700 base-pair deletion that produces chromosomes with a single hybrid α2-α1 gene. The second commonest cause is a 4200 base-pair deletion resulting in loss of the HBA2 gene [38].

The α^{+} thalassemia genotype (-α/αα, -α/-α or $-\alpha^{3.7}$/αα) with deletion (or mutation) of one of the α-globin genes (HBA1 or HBA2) on a single chromosome 16 leads to reduced but not absent production of α-globin chains. This genotype is commoner in India [37,38].

The less frequent occurrence of α^{0} thalassemia (--/αα) with deletion of both the α-globin genes (HBA1 and HBA2) explains why HbH disease and Hb Bart's hydrops fetalis are encountered less frequently among Indians as compared to many south Asian countries [37–39].

Clinical severity in β-TM or β-TI is inversely proportional to the number of α-genes deleted. Deletion of a single α-gene only mildly reduces the severity of β-TM, but individuals with two α-gene deletions and homozygous β^{0}/β^{+} thalassemia display a milder form of β-TI [4,10,27].

Rare patients who co-inherit HbH (the equivalent of only one functioning α-gene) and homozygous β^0-thalassemia (expected to behave as β-TM), behave clinically as β-TI.

Inheritance of an increased number of α-globin chains in β-thalassemia trait exacerbates the globin chain imbalance, worsening the clinically asymptomatic trait state to β-TI [40–42].

Excess α-globin gene dosage may result in unusually early presentations of homozygous β-thalassemia, or in conversion of non-transfusion-dependent thalassemia to a transfusion-dependent state [41].

Tertiary modifiers of phenotype and their influence on disease complications in β-thalassemia

Tertiary modifiers play a pivotal role in disease progression, phenotype modification and the development of complications. They include mutations involved in the loci other than those responsible for globin chain synthesis and globin chain imbalance [12,14]. Various metabolic derangements resulting from the coinheritance of these tertiary modifiers have a significant impact on the life of thalassemic patients [10,16,27]. The salient such modifiers are listed in Table 5.

Table 5. Tertiary Modifiers of Phenotype in β-thalassemia

Organ system or metabolic pathway affected (reference)	Tertiary genetic modifiers of phenotype including commonly studied variants	Observed phenotypic variation(s) or complication(s)
Bilirubin metabolism [43,44]	Polymorphism of the promoter region of bilirubin UDP-glucuronosyl transferase (UGT1A1) [TA7/TA7 genotype]; rs3064744	Jaundice Hyperbilirubinemia (increased indirect bilirubin) Cholelithiasis
Iron metabolism [45,46]	Homozygous mutation of the HFE gene (C282Y, H63D); rs1800562 and rs1799945 HAMP and HFE2 genes; rs10421768 and rs74315323	Iron overload Hypogonadism Cardiac failure

Organ system or metabolic pathway affected (reference)	Tertiary genetic modifiers of phenotype including commonly studied variants	Observed phenotypic variation(s) or complication(s)
Bone metabolism [47–49]	Polymorphisms of the genetic loci of transforming growth factor β1 (TGFβ1), vitamin D receptor (VDR), and COL1A1 gene; rs2228570, rs1800012	Diffuse bone pain, low backache Vertebral fractures with pressure symptoms due to cord compression, spontaneous fractures, femoral head necrosis
Myocardial dysfunction [50]	Apolipoprotein E, ε4 allele Genes modulating free radical formation (i.e., cytochrome C oxidase), scavenger enzymes (superoxide dismutase, catalase), genes controlling replication of mitochondrial DNA, structural genes involved in the synthesis of membrane lipoproteins and genes involving DNA repair.	Left ventricular cardiac failure
Cardiac iron overload [51]	Glutathione S-transferase M1 (GSTM1) null (deleted) genotype [tagged by the pseudoSNP rs366631]	Myocardial iron overload.
Response to viral infections [52]	Interleukin-28B (IFNL3) gene polymorphisms in relation to HCV infection; rs12979860, rs8099917, rs12979860 HLA haplotype polymorphisms (rs7453920)	Spontaneous clearance, treatment response and stage of fibrosis in HCV positive patients

Organ system or metabolic pathway affected (reference)	Tertiary genetic modifiers of phenotype including commonly studied variants	Observed phenotypic variation(s) or complication(s)
Coagulation pathways [53]	Factor V Leiden (rs6025), MTHFR C677T, (rs1801133) and prothrombin G20210A (rs1799963)	Hemostatic changes including those in platelets, endothelium, leukocytes, the clotting cascade, and, the natural anticoagulant systems Increased frequency of thromboembolic events (cerebrovascular, DVT, PE etc.) especially in β-TI.

Rare syndromic associations of thalassemia with other manifestations

β-thalassemia: X-linked thrombocytopenia with thalassemia due to mutations in GATA1; Xp11.23, and β-thalassemia-trichothiodystrophy due to mutations in genes encoding TFIIH (transcription factor II human) subunits [54,55].

α-thalassemia: α-thalassemia / intellectual disability syndrome, chromosome 16-related (ATR-16 syndrome) as well as the acquired α-thalassemia (α-thalassemia-myelodysplastic syndrome; ATMDS) [56,57].

Genotype-Phenotype Correlation in α-thalassemia

HbH disease comprises the only symptomatic form of α-thalassemia that is compatible with extra-uterine life. It presents clinically as mild to moderate thalassemia intermedia [37,38].

Non-deletional α-thalassemia is typically more severe than deletional variants. Deletions of the HBA2 gene are more severe than those of HBA1 [37].

Hb Bart's hydrops fetalis most often results from large deletions on both chromosomes (--/--), and only rarely from a non-deletion variant (--/α^{ND}-).

HbH disease usually occurs due to a large α^0 deletion of one allele in trans with either a single α-globin-gene deletion (--/-α) or a non-deletion null variant (--/$\alpha^{ND}\alpha$ or --/$\alpha\alpha^{ND}$) [58,59].

Rarely, HbH disease occurs due to homozygosity for non-deletional HBA2 mutations ($\alpha^{ND}\alpha/\alpha^{ND}\alpha$), or due to homozygosity or compound heterozygosity for highly unstable α-globin gene variants. A commonly encountered non-deletional α-thalassemic mutation in India is HBA2:c.314G>A, p.Cys>Tyr (Hb Sallanches) [60–62].

The tertiary modifiers are largely common across β- and α-thalassemias.

Genotypic testing in thalassemic patients

In clinically typical cases of transfusion-dependent thalassemia, genetic testing to determine the precise HBB mutations (primary modifiers) is not essential to plan and guide treatment, as long as the diagnosis has been confirmed by the index case's HPLC/CE showing predominantly HbF, markedly reduced adult Hb, and HPLC/CE studies confirm heterozygous carrier status of both parents [21,63]. However, genetic testing is essential if prenatal diagnostic testing is envisaged in the future [3,64].

Determination of the primary modifiers i.e., the HBB mutations is desirable, if practically feasible, for a more detailed understanding of the likely clinical course of the patient [4,14].

Persons with β-TI typically benefit from confirmatory molecular testing, with the DNA analysis clarifying the clinical picture, and guiding genetic counseling. Such testing should begin with determination of the HBB mutation(s), followed by PCRs or MLPA for α-globin gene deletions and/or triplications [15,17–19,33].

Symptomatic individuals in whom only a single β-globin gene mutation is detected may have inherited supernumerary α-globin genes, mutations in HBB exon 3 (dominant β-thalassemia), unstable hemoglobins etc. Alternatively, they may have another contributing cause for anemia (for e.g., G6PD deficiency, hereditary spherocytosis) or an entirely different cause for their symptoms (for e.g., congenital dyserythropoietic anemia) with the β-thalassemia trait being an incidental finding [10,40–42,65].

In all of the above genetic testing scenarios, availability of parental screening results, as well as availability of parental DNA specimens, if possible, is valuable in elucidating the cause of symptoms in the proband [3,64].

Persons with a $\beta^0\beta^0$ genotype with unexpectedly milder symptoms should undergo testing for concomitant α-thalassemia and the $Xmn1^G\gamma$ -158(C>T) polymorphism status. The latter often shows linkage of the T genotype to the common IVS 1-1 (G>T) mutation [17,18].

While genetic testing is not essential to diagnose HbH disease, it can help guide management decisions by distinguishing deletional (mild) forms from non-deletional (typically moderate to severe) forms [37,66].

Initial testing in HbH disease should include targeted deletion analysis for common HBA1 and HBA2 deletions (done by GAP-PCR or MLPA), followed by sequence analysis of the α-globin genes. If the above are normal, the regulatory region multispecies conserved sequence 2 (MCS-R2; previously called HS-40) should be screened for uncommon deletions [37,66].

Key messages

1. Genetic modifiers of phenotypic variability in β-thalassemias include primary modifiers (beta globin gene mutations), secondary modifiers in the form of co-inherited alpha globin gene defects like α-thalassemia and supernumerary α-globin genes as well as regulators of fetal hemoglobin levels, and tertiary modifiers that affect the onset and severity of other systemic complications.
2. IVS1-5(G>C) is the commonest β-thalassemia mutation in India, accounting for 38% to over 80% of mutant alleles in India. This, and five other severe mutations, together constitute over 90% of all β-thalassemic alleles
3. The Capsite+1 (A>C) and -88 (C>T) mutations are commoner in north India and are associated with near-normal MCH, MCV and HbA2 levels in heterozygotes. They can hence be missed by hemogram and/or HPLC/CE-based screening.
4. Homozygosity or compound heterozygosity for two silent or mild β-thalassemia mutations (β^{++}/β^{++}) or co-inheritance of a severe β-thalassemia allele with a mild allele (β^{0}/β^{++}, β^{+}/β^{++}) leads to a non-transfusion-dependent β-thalassemia intermedia phenotype. Milder mutations are more prevalent in thalassemia intermedia than in thalassemia major.
5. β-thalassemia traits (heterozygotes) may rarely have more severe symptoms due to the presence of supernumerary α-globin, inheritance of an HBB exon 3 mutation leading to highly unstable β-globin chains or the presence of other co-inherited common hemolytic anemias like G6PD deficiency.
6. In clinically typical cases of transfusion-dependent thalassemia, genetic testing to determine the precise HBB mutations (primary modifiers) is desirable, but not essential, provided that the diagnosis has been confirmed by HPLC/CE of the index case as well as her/his parents.
7. DNA analysis in persons with non-transfusion-dependent β-thalassemia clarifies the clinical picture and guides genetic counseling.
8. Genetic testing is essential in all cases where prenatal diagnostic testing is envisaged in the future.

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4 Diagnosis of Beta Thalassemia Syndromes

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Thalassemia is a heterogeneous group of disorders. It was classified as thalassemia minor, thalassemia intermedia, and thalassemia major depending on the number of absent β chains and the severity of the clinical implications of the mutation. However, the current classification is based on transfusion requirement, which implies the clinical severity of the various known mutations along with other genetic modifiers if any. Diagnosis of thalassemia can be established with the help of a detailed history, thorough physical examination, complete hemogram with red cell indices, peripheral smear examination and analysis of hemoglobin (Hb) variants of the child and parents by HPLC. Confirmatory genetic tests can be done to find out the mutation responsible for the phenotypic manifestations by DNA analysis [1-6].

The clinical phenotype can vary from β thalassemia trait (heterozygous b thalassemia/ thalassemia minor/ carrier state) to transfusion-dependent thalassemia (TDT/ homozygous b thalassemia/ thalassemia major) [1-4]. The various phenotypes are depicted in Figure 1.

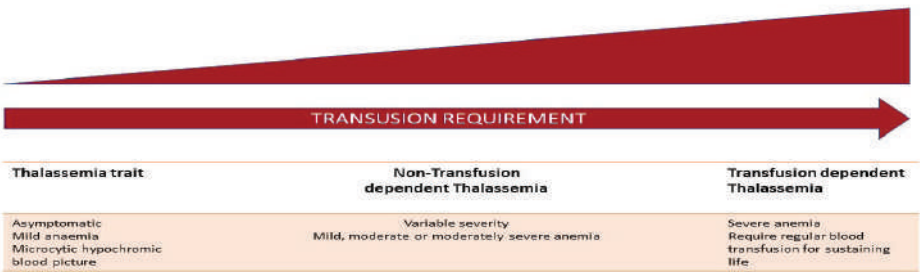

Figure 1. Spectrum of thalassemia syndromes

Individuals with b-thalassemia trait (BTT) are asymptomatic, have mild anemia and do not require transfusions. The hallmark finding in individuals with BTT is microcytic hypochromic anemia not responsive to iron supplementation. Patients with Transfusion Dependent Thalassemia (TDT) usually present in the first 2 years of life with progressive pallor, failure to thrive, and abdominal distension. They require transfusions to sustain normal growth and development. In between these two phenotypes are some individuals with thalassemia who do not require regular blood transfusion and are termed Non-transfusion Dependent Thalassemia (NTDT), previously called thalassemia intermedia.

These children, are not diagnosed early, present at an older age with anemia requiring none or occasional transfusions, growth retardation, splenomegaly, and bony deformities. Some patients with the homozygous defect may be diagnosed in the first two years of life when investigated for pallor but may have a phenotype of NTDT.

With the advent of newer therapies, the clinical classification between TDT and NTDT appears to be dynamic. Patients with NTDT may require regular transfusions as they grow older and those on regular transfusions may experience a reduction in transfusion requirement due to newer therapies [1,2,4].

Transfusion-dependent thalassemia

Individuals with transfusion-dependent thalassemia usually present between 6 months to 2 years of age as g globin chain production declines and a concomitant increase in b globin does not occur due to reduced/ absent production. Children present with progressive pallor, irritability, failure to thrive, abdominal distension, jaundice and feeding difficulties. There may be a history of recurrent fever due to repeated infections. Examination reveals a lethargic, irritable child with poor growth and development, anemia, mild jaundice, and hepatosplenomegaly due to extramedullary hematopoiesis. Untreated or poorly transfused patients may present with growth retardation, poor musculature, non-healing leg ulcers, and extramedullary masses. Bony deformities of long bones and thalassemic facies (prominent forehead, depressed nasal bridge, maxillary prominence, irregular dentition) are detected in an inadequately transfused child. If a regular transfusion regimen is initiated (maintaining pretransfusion Hb between 9 to 10.5 g/dL) and iron chelation is given optimally, patients grow well. Inadequate iron chelation leads to features of iron overload, the most common being delayed puberty and growth failure. The other manifestations may include cardiomyopathy, pericarditis, and arrhythmias due to myocardial hemosiderosis. Endocrine disorders like hypothyroidism, hypoparathyroidism and insulin-dependent diabetes may present in the second decade in poorly chelated individuals [1-5].

Non-transfusion-dependent thalassemia

NTDT involves a varied group of individuals who may have mild to moderately severe anemia. Symptomatic anemia during intercurrent illness is usually the first manifestation in patients with NTDT. The clinical severity of NTDT varies widely. On one end of the spectrum, there are children between 2-6 years of age who can maintain Hb > 7 g/dL without any transfusions but are growth retarded and have features of extramedullary hematopoiesis and at the other end, there are asymptomatic adults who are diagnosed incidentally when they seek medical help for other disorders or are planning their families. The transfusion

requirement also varies from no transfusion, infrequent transfusions needed during periods of stress like infection, and pregnancy to frequent transfusions.

Patients with NTDT may present with complications such as growth failure, delayed puberty, gall stones and leg ulcers. Cardiac manifestation in NTDT differs from TDT with high cardiac output state and pulmonary hypertension being the prominent manifestations. Splenomegaly is more prominent in patients with NTDT, which led to splenectomy being done in most of these patients in the past. Hypercoagulable states in NTDT are associated with a higher risk of deep venous thrombosis, portal vein thrombosis, stroke, and pulmonary embolism, which is seen even more in splenectomised NTDT patients. Patients with NTDT may present with paraparesis due to spinal cord compression secondary to extramedullary hematopoiesis. Pseudoxanthoma elasticum, a connective tissue disorder characterized by degeneration of elastic lamina in the arterial walls and calcium deposition has been documented in NTDT [7]. Individuals with NTDT may need regular transfusions later in life due to complications like pulmonary hypertension, stroke, and leg ulcers. NTDT is a dynamic condition and its severity may vary with time. Individuals with NTDT are at a higher risk of developing alloantibodies to transfused red cells and hence, the decision to start regular transfusions must be taken with careful deliberation [4,8,9].

Laboratory investigations

Complete blood counts, reticulocyte count and peripheral smear

A complete blood count (CBC) done by calibrated automatic electronic cell counters and peripheral smear (PS) examination is the preliminary investigation in a suspected case of thalassemia. It also helps differentiate beta thalassemia trait (BTT) and iron deficiency anemia (IDA), the two most common causes of microcytic hypochromic anemia in children. CBC in BTT shows low hemoglobin, erythrocytosis, microcytosis (low MCV = hematocrit/RBC count), low mean corpuscular hemoglobin (MCH=hemoglobin /RBC number), and a red cell distribution width (RDW) within or very close to the reference interval, reflecting uniformly small red cell size (microcytes). Carriers of β+ mutations (milder) have usually higher values of MCV and MCH than β° carriers, although lower than normal. The most widely used cut-off values of MCV and MCH for indicating thalassemia are 79 fL and 27 pg, respectively [5]. Reticulocytes are normal or slightly increased. Carriers of very mild or silent β-mutations may not show any consistent hematologic change [10].

The iron status should always be considered when evaluating MCH and MCV values since iron deficiency is the most common condition responsible for microcytosis. Iron deficiency anemia results in low hemoglobin, low RBC count with microcytosis and wide RDW, which helps differentiate it from BTT. It is important to know that patients with BTT could have concomitant iron deficiency

anemia resulting in ambiguous results, hence hematologic parameters should be repeated after iron supplementation before making a definitive diagnosis.

Peripheral smear in BTT without concomitant iron deficiency shows uniform microcytosis and hypochromic red cells, basophilic stippling, and the presence of some target cells and therefore RDW is in the normal range. In individuals with concomitant iron deficiency anemia, there is anisopoikilocytosis (variation in the size and shape of red cells), with increased RDW in addition to the above findings. Low serum iron, ferritin, and transferrin saturation with increased total iron-binding capacity (TIBC) are characteristic of iron deficiency anemia whereas serum iron/ferritin may be normal or increased in a patient with TDT.

CBC in homozygous beta-thalassemia shows significantly low hemoglobin (<7 g/dL), microcytosis, very low MCH (<20 pg), and low reticulocyte count (often <1%) suggestive of ineffective erythropoiesis. Peripheral smear shows an increased number of nucleated RBCs in addition to marked poikilocytosis (speculated tear-drop cells), schistocytes, micro spherocytes, polychromasia, and target cells.

High Performance Liquid Chromatography (HPLC) for Hb variants

HPLC is a powerful diagnostic tool for the direct identification of hemoglobin variants and has a high degree of precision and reproducibility. The most important parameters are the accurate quantitation of fetal Hb (HbF) and HbA2. In normal adults, the levels of HbF are <1% and HbA2 is 2.5 to 3.5%. The pathognomonic finding of BTT is the HbA2 >4% and typically does not exceed 8%.

Values of HbA2 between 3.5 and 3.9% are equivocal and require detailed evaluation for underlying concomitant iron deficiency or silent carrier state. Iron deficiency may lead to a lowering of HbA2 and hence in borderline values, the HPLC should be repeated after correcting concomitant iron deficiency [11]. Silent BTT cases also may have HbA2 values in the normal or borderline range between 3.6 and 3.9% [11].

Considerations for interpretation of Hb variants by HPLC include:

1. Correlate the HPLC results with the clinical picture (age, clinical presentation and community of origin).
2. Ensure that the blood sample of the index case is a pre-transfusion sample.
3. Family screening is extremely important to interpret the index case findings.
4. Silent BTT cases will be extremely difficult to pick up during screening programs.
5. Complex situations and prenatal diagnosis require molecular confirmation of the mutations.

6. HPLC cannot differentiate HbE from HbA2, and HbE is suspected when the HbA2% is reported to be ≥ 20%. Separation of peaks between HbA2 and HbE is possible with capillary electrophoresis.

Mutation analysis

More than 300 point mutations and few deletions are known for the β globin gene. Mutation analysis using an Amplification Refractory Mutation System (ARMS-PCR) for beta point mutations, a Gap-PCR technology for alpha thalassemia or hereditary persistence of fetal hemoglobin and, direct DNA sequencing can confirm the diagnosis of thalassemia. It gives clarity where a definitive diagnosis cannot be established by HPLC. It is also essential to have mutation analysis for prenatal testing during the next pregnancy and for preimplantation genetic testing [12].

Differences between TDT/NTDT

Differentiation between TDT and NTDT requires careful clinical examination along with several haematological parameters. The baseline hemoglobin levels in the steady state may help differentiate between TDT and NTDT. Patients should be followed up for 3-6 months after initial presentation to observe clinical severity before diagnosing TDT or NTDT.

Recommendations

1. Hypochromic and microcytic red cell indices and, quantification of hemoglobin variants by using automated HPLC are recommended for diagnosis of β-thalassemia heterozygotes or other Hb variants (Level of Evidence: 1).
2. Screening for identification of carriers of hemoglobinopathies should be done before marriage or before conception (Level of Evidence: 1).
3. In the suspected case of α thalassemia, reverse dot blot or GAP-PCR methods should be used as first-level screening investigation (Level of Evidence: 1).
4. DNA analysis should always be performed in couples at risk where the screening investigations are not decisive (Level of Evidence: 1).

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5a Transfusion Therapy in Thalassemia – Clinical Considerations

Deepak Bansal, Nita Radhakrishnan

Blood and blood products are categorized as drugs as per the Drugs and Cosmetics Act, Government of India, and hence the processing, issuing, and transfusion of blood and blood products should comply with the rules therein. Informed consent for each transfusion, taken in a language the patient/ legal guardian understands, is recommended by the National Blood Transfusion Council of the Ministry of Health and Family Welfare [1]. During informed consent, the benefits and risks of transfusion should be explained to the patient and/or the parent (Appendix A3). A good hemovigilance program is the key to providing a safe and effective transfusion to those needing it regularly.

Transfusion therapy is one of the pillars of the management of transfusion-dependent thalassemia (TDT). In severe homozygous and compound heterozygous states with transfusion dependency, 85% of children will die by the age of 5 years, if not initiated on adequate transfusions. Regular transfusion therapy compensates for chronic anemia, allows normal growth and activity, prevents bony deformities and allows patients to have a good quality of life. It has however been estimated that more than 22,500 deaths occur globally in thalassemia patients because of inadequate transfusions every year [2]. The aim of transfusion therapy is to provide safe blood to the patient at intervals that replicates the normal physiology and causes the least burden to family.

Initiation of transfusion therapy

It is not uncommon to face a clinical dilemma in deciding if a patient should be treated with regular transfusions as a case of TDT or managed with an occasional transfusion as in non-transfusion dependent thalassemia (NTDT). Most children with TDT manifest with hemoglobin (Hb) levels much lower than 7 g/dL before the age of 2 years. The laboratory criteria to define TDT is a Hb <7 g/dL on 2 occasions, > 2 weeks apart, provided contributory causes such as infections are excluded [3]. There is no single investigation that can distinguish TDT from NTDT. On the first visit, there is no urgency to label a child as TDT or NTDT. The second transfusion is given only after hemoglobin falls again to <7 g/dL and regular transfusion therapy with target pre-transfusion Hb is recommended here after.

Also, children considered initially as NTDT can be reclassified as TDT (neoTDT) on follow-up if there is growth failure, significant bone changes, or symptomatic

anemia [3,4]. The decision to transfuse in NTDT is not based on hemoglobin alone. NTDT patients may be started on a regular transfusion regimen in these circumstances and can be shifted back to NTDT once the desired clinical benefit is achieved [3].

A. Pre-transfusion hemoglobin

Blood transfusions in TDT are typically administered every 2-5 weeks to maintain the pre-transfusion Hb level of 9.5-10.5 g/dL [2]. This goal of maintaining a good pre-transfusion Hb is to allow normal growth, good energy levels, suppression of bone marrow erythroid hyperplasia, and suppression of extramedullary erythropoiesis. The pre-transfusion Hb is targeted higher at 11 to 12 g/dL in patients with heart disease and in those with clinically significant extramedullary hematopoiesis leading to symptomatic pseudotumors or massive splenomegaly with hypersplenism.

It is not uncommon to encounter patients with TDT who are unable to maintain a pre-transfusion Hb goal of >9.5 g/dL in India. Often the Hb is inappropriately allowed to drop to 7-9 g/dL or lower before a blood transfusion. The reasons for maintaining a lower pre-transfusion Hb would include a) perception among the treating physician/pediatrician and nursing staff that blood is to be transfused for anemia and not to maintain a 'normal' Hb, b) lack of availability of adequate blood, c) desire to postpone the transfusion visits for ease and convenience, d) erroneous concept that less blood transfused would avoid iron overload. This is an erroneous practice and persistently low pre-transfusion Hb results in chronic anemia that contributes to short stature, extramedullary erythropoiesis, skeletal changes, hemolytic facies, and increased iron absorption from the gut thus worsening the health and outcomes in thalassemia. The median survival of children who are maintained on low pre-transfusion Hb of 7-8 g/dL is just around 17 years [2]. Hence, all efforts should be made to ensure adequate pre-transfusion hemoglobin in the care of thalassemia patients.

B. Volume of Packed Red Cells

There is no role of whole blood transfusion in thalassemia care. The volume of packed cell transfusion is decided based on the weight of the child, pre-transfusion Hb and time since the last transfusion. In routine practice, 15-20 mL/kg of packed RBCs transfused would result in a rise of 2-3 g/dL, assuming the hematocrit of the bag to be near 60%. This is rounded off to the nearest bag size, in order to avoid wastage. In infants and young children, pedi-bags that provide 100 mL or customised volumes may be used if possible. We recommend the use of PRBC units that are stored for less than 14 days in order to circumvent the deleterious effects of prolonged storage [3].

C. The transition from 1 to 2 units of PRBCs

A unit of PRBC-SAGM (Saline, Adenine, Glucose and Mannitol) measures approximately 250 to 350 mL, and PRBC-CPDA-1 (citrate phosphate dextrose adenine solution) measures 200 to 300 mL. The blood volume to be transfused at each visit is 15-20 mL/kg. Therefore, for patients weighing more than 20 kg, one would need to transfuse >1 PRBC unit. However, to avoid discarding blood from a unit, it is common to start transfusing 2 units at each visit when the patient attains a weight of around 30 kg.

D. Post-transfusion Hb

A pre-transfusion Hb of 9.5-10.5 g/dL is usually targeted to increase the post-transfusion Hb to 13-15 g/dL. However, it is not necessary to document a post-transfusion Hb at each transfusion. If the fall in Hb is faster than expected, one may document it to look for delayed hemolysis, alloimmunization or hypersplenism.

E. Frequency of transfusion of blood

PRBCs are transfused at a variable frequency of 2-5 weeks, the interval decreasing with increasing age. Most patients with TDT will need to be transfused at approximately 3 weekly intervals to maintain the desired pre-transfusion Hb. This time to next transfusion can be calculated based on the time from the prior transfusion, pre-transfusion hemoglobin and the volume of PRBC given. The frequency of transfusion is often predictable, in the absence of infections, alloimmunization or splenomegaly.

G. Role of whole blood in thalassemia care

PRBC is recommended for transfusion. However, PRBCs may not be available in several health facilities. Whole blood (WB) may be transfused as the next best alternative. The approximate volume of a whole blood unit is 399 ml (350 mL blood + 49 mL CPDA-1) or 513 mL (450 mL blood + 63 mL CPDA-1). A whole blood bag is typically allowed to hang to let the red cells settle by gravity to the bottom of the bag. The supernatant plasma is not transfused. The volume of blood transfused should not exceed 20 mL/kg. The transfusion should be completed within 4 hours of release from the blood centre. However, it is important to emphasize that transfusing WB in place of PRBC is not ideal and should not be practiced as far as possible. Access to a standard blood bank that provides PRBCs is vital in the care of thalassemic patients.

F. Rate of transfusion

The rate of infusion of PRBCs should be 5 mL/kg/hour i.e. 15 to 20 mL/kg should be transfused over 3 to 4 hours. In patients with cardiac failure, it should be reduced to 1 to 3 mL/kg/hour. PRBCs should be transfused in adults at 1 to 2 mL/min for the first 15 minutes and then increased to 3-5 mL/min.

G. Myths and Misconceptions

1. Warming of blood before transfusion

There is no need to bring the red cell unit to room temperature before a transfusion [3]. The reasons are a) the initial rate of transfusion is maintained slowly, and the bag is expected to reach room temperature over the time it is transfused; b) to prevent the risk of bacterial overgrowth; and c) the risk of hypothermia exists primarily in neonates receiving an exchange transfusion and with older children receiving a massive transfusion, e.g., in trauma with blood loss. Warming using blood warmers is recommended for infusion rates greater than 50 mL/minute in adults or greater than 15 mL/kg/hour in children which is usually not followed in patients with thalassemia and in children with cold-antibody-mediated autoimmune hemolytic anemia.

2. The use of frusemide before/during a transfusion

There is no need to administer frusemide in most patients with TDT before or during a transfusion. Frusemide is indicated only when the pre-transfusion Hb is low (e.g., <7g/dL) in which case, PRBC is transfused over 4-6 hours in small aliquots of 5-10 mL/kg to prevent volume overload. Unnecessary administration of frusemide will result in diuresis and avoidable inconvenience to the patient.

3. Premedication for each transfusion

The use of routine premedication is not necessary as most units are leukodepleted and carries a very low risk of febrile non-hemolytic transfusion reaction. It may however be necessary in those who develop allergic transfusion reactions (mentioned in next section).

4. Antimalarials

It is not recommended to give antimalarials after each transfusion.

H. Leukodepletion of PRBCs

It is recommended to transfuse leukodepleted blood to all patients with thalassemia [3]. This is to a) reduce the risk of non-hemolytic febrile transfusion reactions and b) reduce the risk of human leukocyte antigen (HLA) alloimmunization, thereby reducing the risk of graft rejection following hematopoietic stem cell transplantation (HSCT) that may be performed in the future. Details on leukodepletion are provided in the next section.

I. Use of routine blood filters

Blood is always administered through a filter to remove small aggregates and blood clots that may have formed during storage. The usual filter is 170 to 260 microns. A routine blood filter does not provide leukoreduction and we recommend the use of blood filters only in those PRBC units that have been leukodepleted at source (blood bank).

J. Irradiated blood

Irradiation of blood is indicated in the peri-transplant period as patients as it reduces the risk of transfusion-associated graft versus host disease in immunocompromised recipients, besides reducing the chances of HLA alloimmunization and CMV infections in the patient.

K. Monitoring while on transfusions

Patients receiving transfusion should be monitored for symptoms and signs of potential adverse events/complications of transfusion. The vital signs [temperature, pulse rate, respiratory rate, blood pressure, and oxygen saturation (if available)] should be measured and recorded before the start of each transfusion, 15 minutes after the commencement, hourly after that, and at the conclusion.

M. Adverse effects of transfusion therapy

Transfusion reactions are adverse events associated with blood transfusion or its components. Patients who receive chronic transfusions are prone to a range of minor to potentially life-threatening adverse reactions, which, if not prevented, diagnosed early, and managed appropriately, can hamper their outcome. WHO recommends the development of national-level regulatory agencies and hospital transfusion committees to avoid unnecessary and unsafe transfusion practices and improve the safety of the transfusion process [1]. There should be a robust mechanism to report adverse events (hemovigilance).

Classification of adverse reactions to transfusions

Transfusion reactions range from relatively common reactions such as mild allergy, febrile non-hemolytic transfusion reactions (especially in non-leukodepleted products) to rare such as anaphylaxis, acute hemolysis, or sepsis to fatal ones such as Transfusion Related Acute Lung Injury (TRALI) and Transfusion Associated Circulatory Overload (TACO) [2].

Based on the time of onset of adverse events, they can be classified as (a) immediate or (b) delayed, as shown in Table 1.

Table 1. Classification of adverse reactions to transfusions based on time of onset

Immediate	Delayed
1. Allergic reactions 2. Anaphylaxis 3. Febrile non-hemolytic transfusion reactions 4. Sepsis 5. Acute hemolytic transfusion reactions 6. TACO 7. TRALI	1. Delayed hemolysis 2. Transfusion-associated graft versus host disease (TA-GvHD) 3. Transfusion transmitted infections (TTIs) 4. Red cell alloimmunization

A.1. Allergic reactions [3]

Clinical presentation: Usually mild (itching, hives, flushing)

Etiology: Allergic reactions are due to plasma proteins in blood product

Treatment: Symptomatic – Pheniramine maleate, other antihistamines

Prevention: Described under A.2

A.2. Anaphylaxis

Clinical presentation: Similar to a mild allergic reaction at the start. But this can progress to a severe and potentially life-threatening reaction. Fever, skin rash, coughing, difficulty breathing, stridor, wheezing, hypotension, etc., can be manifestations of anaphylaxis.

Etiology: Usually associated with plasma proteins in the blood product. Patients with IgA deficiency have alloantibodies against IgA and can develop anaphylaxis when they receive blood products containing IgA [5].

Treatment: Emergency care and adrenaline, steroids, antihistaminics, as per need.

Prevention: Recurrent allergic reactions may be prevented by washing red blood cells. However, this is required very rarely, for patients with IgA deficiency and severe allergic reactions. These patients may also be given blood from IgA-deficient donors, although this is often not practically feasible.

A.3. Febrile Non-Hemolytic Transfusion Reaction

Clinical presentation: Fever, rash, chills, rigors, body aches, nausea, vomiting

Etiology: It is caused by the release of cytokines from the breakdown of blood donor leukocytes. These inflammatory mediators accumulate during blood storage; hence, it is more with products that are stored for longer duration and are not leukoreduced [6].

Treatment: Stop transfusion. Rule out a hemolytic reaction and bacterial contamination. Management is symptomatic with antipyretics and antiallergic medications. Restart the blood at a slower rate, if hemolysis has been ruled out.

Prevention: The incidence of FNHTR has reduced in recent times following leukoreduction. FNHTR are best prevented with pre-storage leukoreduction than bedside leukoreduction.

A.4. Sepsis

Clinical presentation: Fever, chills, rigors, hypotension, and DIC within a few hours of having received a transfusion.

Etiology: Caused by bacterial or bacterial endotoxins in the blood product [4]

Treatment

1. Stop transfusion immediately.
2. Two wide-bore cannulas are to be inserted. Manage as per sepsis protocol.
3. Blood culture from patient
4. Obtain a culture of blood from the blood bag.
5. Send the blood bag back to the blood centre as described in A.5.
6. Broad spectrum antibiotics with gram-negative cover.

Prevention: Inspect the blood bag for any visible color change/ bubbling/ distention of bag. If these signs are present, do not transfuse the bag and report to the blood centre. Transfuse PRBCs within the prescribed time, do not leave PRBC units in ward refrigerators, do not use units after 4 hours of issue from the blood centre.

A.5. Acute Hemolytic Transfusion Reactions

Clinical presentation: Fever, chills, backache, dark/black-colored urine, difficulty in breathing, and hemodynamic compromise. Usually, symptoms start within a few minutes of the transfusion [3].

Etiology: Clerical errors, mismatched transfusions, antibodies to donor red cell antigens, non-immune due to heat/ poor product preparation

Treatment

1. Stop transfusion immediately. 2 wide-bore cannulae are to be secured.
2. Start IV Fluids – Normal saline (bolus may be required if hemodynamic compromise)
3. Symptomatic care- Paracetamol
4. Forced diuresis with frusemide may be needed, if the patient has reduced urine output.

5. Send the remaining bag, with 2 ml of patient's blood each in EDTA, and plain vacutainers may be sent to the blood centre with an adverse reaction reporting form immediately.
6. The blood centre will check for clerical errors, perform cross-match again and look for alloantibodies that may have been missed earlier.

Prevention

1. Documentation should be done appropriately at the time of sampling, cross-match and issue of blood from the blood centre, as well as prior to starting transfusion at the transfusion centre.
2. The same nurse/ phlebotomist who samples the patient should label the vials and fill out the request form used for cross-match. Double-check with the name, age, and registration number of the patient. Since there may be two or more patients with the same first name, including the surname in all documents is a good practice. Radio Frequency Identification (RFID) tags on blood bags may help reduce clerical errors.
3. Two staff members (one doctor and one nurse) should always check the blood unit before starting a transfusion.
4. Standard complete crossmatch techniques should be done in the blood centre.

A.6. Transfusion Associated Circulatory Overload

Clinical presentation: Fast breathing, difficulty in breathing with intercostal and subcostal retractions, tachycardia, hypoxia. This is commonly seen in those with severe anemia and pre-existing cardiac compromise.

Etiology: Volume overload due to more volume or faster than recommended transfusion. At times, underlying cardiac dysfunction, which may have been missed earlier, can precipitate TACO. As per National Health Care Safety Network, TACO is defined as pulmonary edema primarily related to circulatory overload which results in 3 or more of the following findings within 6 hours of transfusion: acute respiratory distress, radiographic pulmonary oedema, elevated central venous pressure, evidence of left heart failure, elevated B-type natriuretic peptide (BNP), and a positive fluid balance [7].

Treatment

1. Stop transfusion promptly.
2. Management of cardiac failure - Propped up position, Oxygen, Diuretics if there is no hypotension, Inotropes if hypotensive, Chest X-ray/ECHO for features of pulmonary oedema and/or ICU Care with cardiology consultation may be required.

Prevention: Adherence to transfusion volume and duration as prescribed based on baseline Hb and cardiac function.

A.7. Transfusion-Related Acute Lung Injury

Clinical presentation: Fast breathing, difficulty in breathing with intercostal and subcostal retractions, tachycardia, hypoxia, hypotension, fever, within 6 hours of having received a transfusion.

Etiology: Acute lung injury due to anti-HLA or anti-neutrophil antibodies in the donor product (human leukocyte antigen or human neutrophil antigen) that activates the patient's neutrophils resulting in the release of cytokines that cause non-cardiogenic pulmonary oedema. TRALI is defined as pulmonary edema after transfusion in the absence of circulatory overload or alternate risk factors for ARDS as per Canadian Consensus Criteria [8]. The presence of systemic inflammation also contributes to the pathogenesis.

Treatment

1. Stop transfusion promptly.
2. Initiate basic resuscitation measures – Oxygen therapy, IV Fluids, and assisted ventilation.
3. Chest X-ray/ ECHO for features of pulmonary oedema to rule out TACO.
4. ICU Care is usually required.

Prevention: Mitigation strategies suggested include reducing plasma in the product and/or using additive solutions, which may not be practical in thalassemic patients [7].

B.1. Delayed hemolysis

Clinical presentation: Unexplained anemia, low-grade fever, malaise, jaundice, and dark-colored urine that usually starts around 4-5 days after a transfusion [3].

Etiology: Delayed hemolysis occurs due to an alloantibody or autoantibody that was not detectable at the time of transfusion or the development of a new antibody. It is suspected when there is an increase in transfusion requirement in patients who were maintaining the desired pre-transfusion Hb earlier on a fixed schedule.

Treatment

1. Investigate the cause
2. Manage allo/autoantibody as per protocol (described later) – steroids, IVIG, immunosuppressants
3. Use only compatible units based on alloantibody (negative for the corresponding antigen)

Prevention: Regular alloantibody & autoantibody screen, especially in NTDT patients who have started transfusions at an older age, and extended matching of blood

units, if an antibody is detected [3]. There is no data on the cause of autoantibody formation; hence, no measures can be suggested to prevent the same.

B.2. Transfusion-associated Graft versus Host Disease (GVHD)

Clinical presentation: Fever, skin rash, jaundice, and diarrhea, as is seen in GVHD following HSCT. In addition, transfusion-associated GVHD may also be associated with pancytopenia and bone marrow failure, which is often fatal.

Etiology: GVHD is usually caused by the presence of viable donor lymphocytes that react with the immune-compromised hosts. In patients with thalassemia, it may occur in the post-transplant period or the in the case of secondary immunosuppression. It may occasionally be seen in immunocompetent recipients in case of family donations.

Treatment: Management is similar to GVHD post HSCT. It requires referral to a tertiary care center with hematology services.

Prevention: No directed donations from family members and transfusing irradiated blood components in the peri-transplant period.

B.3. Transfusion Transmitted Infections

Clinical presentation: These are usually detected on routine screening, and is recommended at the first presentation and annually thereafter. Development of jaundice or sudden increase in liver enzymes or ferritin warrants evaluation for Hepatitis B and C infection.

Etiology: Details of Hepatitis B and C are discussed in Chapter 14. HIV once detected is managed as per regular treatment guidelines issued by the government of India.

Prevention: HIV, Hepatitis B, Hepatitis C, Syphilis, and Malaria testing as per current government norms. NAAT testing of donor blood units for HIV, Hepatitis B and C are preferable [8].

B.4. Red cell alloimmunization

Alloimmunization is a complication encountered in 10-20% of thalassemic patients on chronic transfusion therapy. It is more common in NTDT patients, and the risk of alloimmunization is high if the first transfusion is received after the age of 1 year. Higher rates of up to 70% have been reported in sickle cell disease and NTDT, probably due to infrequent transfusions. Anti-E, anti-C, and anti-Kell alloantibodies account for 80% of the alloantibodies observed. 5 to 10% of patients may present with alloantibodies against rare red cell antigens or unidentified antigens.

Clinical presentation: It can manifest as acute or delayed haemolytic transfusion reactions.

Prevention: Extended red cell phenotyping has been reported to reduce the incidence of red cell alloimmunization to 0 to 7%. Therefore, it is recommended

to perform an extended phenotype of patient's RBC antigens at the first presentation and provide phenotype-matched red cells for ABO, D, Cc, Ee, and Kell, wherever feasible, to reduce the risk of allo-sensitization [9,10]. Leukoreduction to prevent alloimmunization has discordant results. However, universal leukoreduction is still being advised for all patients with thalassemia on regular transfusion therapy.

Treatment: It is recommended to provide corresponding antigen-negative red cell units in case an alloantibody is identified. Steroids, immunosuppressive drugs, and intravenous immunoglobulin have been tried, although with little benefit. Rituximab has shown limited effectiveness too [11,12].

Prevention: Extended phenotyping of ABO, D, Cc, Ee, and K antigens for all thalassemia patients at first presentation followed by phenotype-matched red cell transfusions are ideal.

Recent increase in transfusion frequency

Any sudden/ insidious increase in PRBC requirement should warrant investigation to ascertain the aetiology. See Figure 1.

Recommendations

1. It is recommended to initiate regular transfusion therapy in children who present with a diagnosis of TDT and 2 records of hemoglobin less than 7 g/dL, at 2 occasions that are more than 2 weeks apart (Level of evidence: 2).
2. We recommend the use of leukodepleted packed red blood cells at a volume of 15-20 ml/kg body weight once in 2-5 weeks in order to maintain pre-transfusion hemoglobin of 9.5 to 10.5 g/dL (Level of evidence: 1).

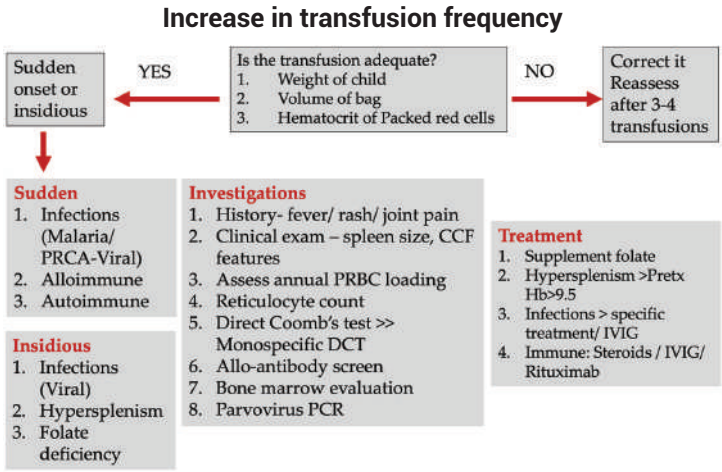

Figure 1. Evaluation of a patient with a recent increase in PRBC requirement

3. Patients with β-thalassemia should preferably receive leukodepleted packed red blood cell (PRBCs) units with a leukocyte count of less than 5 x 106 per unit with appropriate quality control measures (Level of evidence:1).
4. Packed red blood cells should be used within 14 days of collection. Voluntary donors are to be encouraged. Units donated by immediate family members are not to be transfused (Level of evidence 2).
5. We recommend that the safety of blood transfusions for thalassemia patients should be regulated by the hospital transfusion committee or licensed blood centres where the former does not exist (Level of Evidence: 1). All standard precautions to prevent clerical errors should be taken while collection, labelling of samples and transfusion forms, while checking of blood and before transfusion.
6. A record of transfusion reactions, antibody screening and annual red cell requirement is to be maintained for all patients (Level of evidence: 1).

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5b Transfusion Therapy in Thalassemia – Technical Considerations

Ashish Jain, Satyam Arora, Vinita Srivastava

Blood component preparation for thalassemia

Thalassemia patients receive regular transfusions at an interval of 15-20 days. As recipients of a huge number of transfusions, these patients are also prone to numerous types of transfusion reactions. Transfusion of blood components is preferred (Packed Red Blood Cells, PRBCs) over whole blood (WB) transfusions. There are many blood component modifications done to provide a safe blood component for transfusion as well as to prevent transfusion reactions.

1. Anticoagulant and additive solutions used for storage

Anticoagulants are used to store the red cells without significant loss of metabolic integrity and function. Citrate is a very important ingredient of these solutions since it inhibits the coagulation process by chelating available calcium from the whole blood. The maximum duration of storage of blood components varies depending on which type of anticoagulant solutions are used. Table 1 depicts the shelf-life of red blood cells based on the type of anticoagulant used [1]. All of these storage solutions should be able to achieve a mean 24-hour post-transfusion survival of at least 75% of the transfused red cells at the end of their shelf life [1]. The storage temperature for WB and PRBCs is 2 to 6°C as per the regulatory requirements in India [2].

Table 1. Shelf life of the red cells based on the type of anticoagulant used

Anticoagulation solution	Shelf-Life (days)
CPD (Citrate, Phosphate, Dextrose)	21
CP2D (Citrate, Phosphate, Dextrose)	21
CPDA-1 (Citrate, Phosphate, Dextrose, Adenine)	35

The oxygen release function of hemoglobin (Hb) in transfused blood is extremely important as it gets impaired during normal storage due to progressive loss of 2,3-biphosphoglycerate (2,3-BPG), previously known as 2,3-diphosphoglycerate, DPG. However, the rapid repletion of 2,3-BPG after transfusion generally compensates for the loss of function during storage [1]. The introduction of additives solutions such as AS-1 (Adsol), AS-3 (Nutricell) and AS-5 permits the storage of PRBCs for up to 42 days [1].

In patients with transfusion-dependent thalassemia (TDT), pretransfusion Hb levels should be 9.5 to 10.5 g/dL, and post-transfusion Hb levels should not exceed 14–15 g/dL.

2. Leukodepletion

It has been estimated that a freshly collected WB unit contains roughly 109 leukocytes, and their concentration continues to decrease with subsequent component processing [3,4]. Leukodepletion is the process of reducing the number of leukocytes to less than 1 x 106 as per the European standards or less than 5 x 106 as per the US FDA standards. Leukodepletion can be achieved by passing the blood through a leukocyte reduction filter, which can achieve a three to four-log (99.9 to 99.99%) reduction in the leukocyte count of the unit. This level of leukodepletion is considered the critical threshold for eliminating adverse reactions attributed to contaminating white cells like HLA-alloimmunization of recipients and cell-associated infectious agents such as cytomegalovirus, in addition to avoiding febrile non-hemolytic transfusion reactions (FNHTRs) [3].

Leukodepletion can be performed at the time of blood collection/ processing known as pre-storage leukodepletion or leukofiltration) or at the time of transfusion (bedside leukodepletion). Pre-storage leukocyte reduction or filtration is more effective than bedside leukocyte reduction, results in lower levels of inflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor) during storage, and hence, is quite efficient in the prevention of FNHTRs. It can also promote ready access to an adequate inventory of leukodepletion components.

A total Hb content of at least 40 g per unit of PRBC is required. The US-FDA requires that leukodepleted RBC units must not lose more than 15% of their starting Hb content [3]. In contrast, the use of bedside leukocyte reduction filters may not yield an appropriate reduction of leukocytes as well as the techniques may not be standardized. This bedside filtration has also been associated with dramatic hypotension in some individuals, often in the absence of other symptoms [1,3].

3. Washed PRBCs

It is indicated for patients having repeated severe allergic transfusion reactions or for patients with immunoglobulin A (IgA) deficiency, in which the recipient's pre-formed antibody to IgA may result in an anaphylactic reaction. Washing of the donor product removes plasma proteins that constitute the target of antibodies in the recipient. Washing may be accomplished using manual or automated techniques. Washed red cells that are not suspended in storage solution hence must be transfused within 24 hours, The use of a closed system, semiautomated cell processors and modern additive solutions (SAGM) can

extend the post-wash storage for shelf life as long as 14 days. Washing alone usually does not result in adequate leucocyte reduction and should not be used as a substitute for leukoreduction. In addition, washing of red cell units removes some RBCs from the transfusion product, and it is, therefore, valuable to monitor post-transfusion Hb levels to ensure attainment of the targeted Hb level. European standards require washed components to have a minimum of 40 g of Hb per unit, a hematocrit of 0.50 to 0.70, less than 0.8% hemolysis, and less than 0.5 g of supernatant protein per unit [1,3].

4. Red cells obtained by donor apheresis

It refers to the collection of two units of red cells from the same donor for the transfusion of one patient. The reduction of donor exposures may decrease the risk of transmission of infections and alloimmunization and other transfusion-related complications. However, this technique has many challenges in terms of logistics, regulatory requirements, and inventory management [1].

5. Irradiation

Transfusion-associated graft-versus-host disease (TA-GvHD) is a rare complication of transfusion of blood components containing lymphocytes and carries a significant risk of mortality [5]. TA-GvHD requires the infusion of allogeneic donor (non-self) lymphocytes, engraftment, and proliferation of "non-self" lymphocytes and subsequent attack on recipient ("self") tissues [6]. There may be many factors which may pose the risk of TA-GvHD in a recipient such as:

- Number of lymphocytes infused (leukoreduction alone may not be sufficient)
- Degree of HLA match between the donor and recipients
- Viability of the remaining transfused lymphocytes
- Degree of immunosuppression of the recipient

Irradiation is the main method of inactivating lymphocytes in the transfused components to prevent the risk of TA-GvHD [5]. Irradiation source includes gamma rays from either Cesium-137 blood component irradiators or Cobalt-60 source or X-ray produced from radiation therapy linear accelerators or stand-alone units. Irradiation causes damage to the nucleic acids of residual lymphocytes in blood components which prevents them from dividing and proliferating [3,5].

- Gamma- or X-ray irradiation of blood components is recommended to prevent TA-GvHD.
- The minimum dose achieved in the irradiation volume should be 25 Gy, with no part receiving >50 Gy. The dose of 25 Gy should be to the central area of the components with no portion receiving < 15 Gy.

- Red cells may be stored for a further 28 days from the date of irradiation or the product's original expiration, whichever comes first [7]. This is due to the release of excess potassium from the red cells in response to the irradiation.
- All granulocytes should be irradiated before issue and should be transfused with minimum delay.
- Bone marrow, peripheral blood stem cells, or donor lymphocytes as a part of hematopoietic stem cell transplantation (HSCT) should never be irradiated.
- All cellular blood products upon irradiation should be labelled with an irradiation-sensitive label which undergoes a visual change indicating irradiation status.
- The requirement of irradiated cellular blood components should be clearly requested by the treating clinician as well as irradiation of blood components should also be part of the bedside check prior to administration of these blood components to patients.
- Thalassemia patients on a routine basis, should not routinely receive irradiated blood components. Irradiated units are required only for those TDT patients intending for bone marrow transplantation.

Irradiated blood components for Allogenic HSCT [5]:

- All recipients of allogenic HSCT should receive irradiated cellular blood components from the time of initiation of the conditioning chemo/radiotherapy.
- Irradiated blood components should be continued until all the following criteria are met:
- More than 6 months since the transplant date
    - The lymphocyte count is $>1.0x10^9/L$
    - The patient is free of active chronic GvHD
    - The patient is off all immunosuppression
- If chronic GvHD is present or immunosuppressive treatment is required, continue irradiated blood components indefinitely.
- Bone marrow or peripheral blood stem cell donors (of all ages) should receive irradiated cellular blood components within 7 days prior to or during harvest.

6. Transfusion strategies in ABO-incompatible allogeneic HSCT for thalassemia patients

Patients undergoing HSCT require extensive transfusion support since they lack the ability to produce blood cells effectively. The transfusion support further becomes challenging when the patient receives an allogenic HSCT graft from a donor who has a different blood ABO type (Figure 1). ABO matching is not essential for selecting an allogenic HSCT transplant donor as ABO antigens are not expressed on hematopoietic progenitor cells but the selection of ABO type of blood component for transfusion support may be complicated in such scenarios [3]. In allogeneic HSCT, there can be the following categories of ABO incompatibilities (Table 2):

- **Identical:** The blood group of the donor and recipient are identical
- **Major Incompatibility:** Due to a naturally occurring antibody in the recipients. This scenario may pose some challenges such as
  - The potential of acute intravascular hemolysis
  - The ongoing production of ABO antibodies by recipient immune cells may be directed against the erythroid progenitor and mature red cells produced by the donor graft.
- **Minor Incompatibility:** Due to naturally occurring antibodies in the donor graft product. This scenario may pose some challenges such as
  - Hemolysis during infusion may be mild and self-limiting.
  - "Passenger Lymphocyte Syndrome": This condition typically presents after 5-16 days after infusion with acute, immune-mediated hemolysis. This hemolysis is due to the rapid generation of anti-A/ anti-B by the donor lymphocytes against the residual recipient red cells.
- **Bi-directional incompatibility:** Due to naturally occurring antibodies in both donor and recipient.
- **Rh incompatibilities:** If the recipient is Rh-negative and the donor is Rh-positive or vice versa Rh-negative red cells should be considered to prevent alloimmunization to D antigen. The selection of Rh-negative platelets is not mandatory.
- **Incompatibilities of non-ABO antigens:** Red cells antigens apart from ABO may also pose similar challenges but are often rarely encountered however they need to be screened.

Table 2. Categories of Allogenic HSCT based on ABO blood group of donors and recipient

Recipient ABO status	Donor ABO status O	A	B	AB
O	Identical	Major	Major	Major
A	Minor	Identical	Bi-directional	Major
B	Minor	Bi-directional	Identical	Major
AB	Minor	Minor	Minor	Identical

Selection of various type of blood components in an ABO incompatible HSCT requires careful evaluation and transfusion medicine specialist support since various factors such as stage of transplantation, original ABO group, type of product, date of infusion, ABO titer results, molecular engraftment results (if available) and previous transfusion histories may influence the blood group to be used.

Figure 1. ABO blood group type change during an ABO incompatible HSCT (HPC, hematopoietic progenitor cells) (10)

The recipient of ABO-incompatible HSCT can receive blood components based on the forward and reverse blood grouping, in both pre-transplantation and post-engraftment phases since the blood cells are derived from one progenitor source [8].

Patients in the immediate post-transplantation phase (between infusion and engraftment; Figure 1) require more careful assessment and selection of blood group of the blood components as they may present with shifting and mixed ABO type (Table 3 and Table 4). Samples in this phase may present with blood grouping discrepancies (which may cause delays) at blood centres hance all the requests should mention that the sample is post ABO-incompatible HSCT, days from infusion as well as recipients (original blood group) and donor blood group.

Post-transplant phase (pre-engraftment phase) should receive blood components with blood groups based on the ABO type of both the recipient and donor; ideally, the ABO type is compatible with both the recipient and the donor. See Table 4.

Table 3. Transfusion support for allogenic HSCT with ABO incompatibilities [3]

Type of Incompatibilities	Transplant Stage	ABO blood group selection		
		RBCs	Platelets	Plasma
Major Incompatibility	Preparative regimen	Recipient	Donor	Donor
	Transplantation	Recipient	Donor	Donor
	Recipient antibody detected	Recipient	Donor	Donor
	Recipient antibody NOT detected	Donor	Donor	Donor
Minor Incompatibility	Preparative regimen	Donor	Recipient	Recipient
	Transplantation	Donor	Recipient	Recipient
	Recipient antibody detected	Donor	Recipient	Recipient
	Recipient antibody NOT detected	Donor	Donor	Donor
Bi-Directional Incompatibility	Preparative regimen	Group O	Group AB	Group AB
	Transplantation	Group O	Group AB	Group AB
	Recipient antibody detected / recipient cells detected	Group O	Group AB	Group AB
	Recipient antibody NOT detected / recipient cells NO longer detected	Donor	Donor	Donor

Table 4. ABO incompatibilities during HSCT [10]

Category	ABO Group		Preferred ABO to Transfuse		Clinical Challenge	Possible Intervention
	Recipient ABO	Donor ABO	RBCs	Platelet/ Plasma		
ABO Compatibility	O	O	O	O, A, B, AB	None, due to ABO	None
	A	A	A, O	A, AB		
	B	B	B, O	B, AB		
	AB	AB	AB, A, B, O	AB		
Major ABO Incompatibility	O	A	O	A, AB	Acute hemolysis Delayed engraftment PRCA	Red cell depletion of HPC product/ plasmapheresis
	O	B	O	B, AB		
	O	AB	O	AB		
	A	AB	A, O	AB		
	B	AB	B, O	AB		
Minor ABO Incompatibility	A	O	O	A, AB	Acute hemolysis. Passenger lymphocyte syndrome	Plasma reduction of HPC product, monitoring HSCT patient for hemolysis
	B	O	O	B, AB		
	AB	O	O	AB		
	AB	A	A, O	AB		
	AB	B	B, O	AB		
Bidirectional ABO incompatibility	A	B	O	AB	Combination of major and minor ABO incompatibilities	
	B	A	O	AB		

Screening of Blood

Blood transfusion should always be provided by a licensed blood centre [2]. Any blood product transfused should be screened for transfusion-transmitted infections (TTI) as per the Government of India Regulations. In India, as per the Drugs and Cosmetics Act (1940 & amendments thereafter), each donated blood should be screened for five transfusion-transmitted infections (TTI) including Human Immunodeficiency Virus (HIV), Hepatitis B, Hepatitis C, Malaria and Syphilis [2]. See Table 5.

Table 5. Analyte and technology approved for serological screening of donated blood for transfusion-transmitted infections

S No	TTI Agent	Analyte	Technology
1	HIV	Antibody with/ without P24 Antigen	Rapid Tests/ ELISA/ CLIA
2	HBV	HBsAg (Antigen)	Rapid Tests/ ELISA/ CLIA
3	HCV	Anti-HCV (Antibody)	Rapid Tests/ ELISA/ CLIA
4	Malaria	Direct parasite/ Antibody/ Antigen	Thick Film/ Rapid Tests
5	Syphilis	Treponemal Antibodies	VDRL/ RPR/ TPHA

ELISA: Enzyme-linked immune-sorbent assay; CLIA: Chemiluminescence Immunoassay

For the screening of blood donations, both sensitivity and specificity should be the highest possible or available [9]. The main type of assays used for screening of blood products are immunoassays (IAs/ Serology), Enzyme immunoassays (EIAs), Chemiluminescent Immunoassays (CLIAs), Hemagglutination (HA)/ Particle Agglutination (PA) assays, Rapid/ Simple single-use assays (rapid tests), and Nucleic Acid Amplification Technology (NAAT) assay.

Nucleic acid amplification technology (NAT) is applied to screen the donated blood for viral nucleic acid (DNA or RNA). The concept is to detect low levels of virus in the sample by amplification. This is achieved by targeting the DNA/RNA segment (of the virus) and amplifying it by increasing the amount it can easily detectable. Establishing the presence of nucleic acid in the donor indicates that the donation is likely to be infectious. NAT can be based on either RT-PCR or TMA technology as well as performed on individual donations or on mini-pools to detect the nucleic acid of the infectious agents [9].

The major advantage of NAT in donor screening is to identify the donors during the early phase of their infections (window period) [10]. A multicentric study in India on ID-NAT, where blood samples (n=12,224) with their serology report were collected from eight blood banks from seven major cities reported a NAT

yield (NAAT reactive and serology non-reactive) of 1 in 1528 donors. [11]. The benefit of NAAT screening donated blood is based on the incidence of infections in the donor population, the effectiveness of the donor screening/ selection process, and the sensitivity of the serology screening method used [9].

In countries with a high incidence of infections, the introduction of NAAT is likely to detect a significant number of window period donations [11]. In India, NAAT screening of donated blood is still not mandatory, but it is applied as an additional layer of safety on existing serology-based tests used to screen donated blood, especially for multi-transfused patients.

Pathogen reduction/ Pathogen inactivation

Pathogen peduction (PR) or pathogen inactivation (PI) is a newer technology using chemical inactivation of the pathogens in the donated blood. This technology works by introducing heterocyclic chemical compounds in the blood components which interact with the base groups of the linked nucleotides of nucleic acids of the pathogens. Many PR/ PI agents are approved by FDA, but this technology is not yet approved to be used for TTI prevention in India.

National Hemovigilance Program of India (Prevention and Reporting transfusion reactions)

Transfusion is one of the most common life-saving interventions offered to patients with hemoglobinopathies. This intervention is often associated with many types of complications. These complications may be acute (within 24 hours) or delayed (after 24 hours) in nature with blood transfusions. Hemovigilance is a set of surveillance procedures covering the whole transfusion chain (from the collection of blood and its components to the follow-up of recipients), intended to collect and assess information on unexpected or undesirable effects resulting from the therapeutic use of labile blood products, and to prevent their occurrence or recurrence [1].

National Hemovigilance Programme of India (HVPI) was implemented in India in the year 2012 with the aim collect and analyze the data related to adverse reactions related to blood transfusion as well as providing recommendations to all stakeholders to make it safer for the recipients [12,13]. The silent features of HVPI are:

- The reporting is purely voluntary in nature and non-punitive.
- Centralized reporting and coordination (National coordinating centre is National Institute of Biologicals, Noida)
- Data is collected with indigenous software developed by NIB, Noida and is kept anonymous (Haemo-Vigil Software).

The recent 5 years HVPI data from India showed the overall incidence of adverse reactions as 8.4 per 10,000 blood products transfused with a rate of 8.5 in 2016

and 8.3 in 2017. FNHTRS and allergic reactions continue to remain the most frequently reported adverse transfusion reactions [13]. As per the HVPI there are a set of responsibilities of all the stakeholders in the chain of blood transfusion.

The responsibility of the clinical counterpart (bedside healthcare staff) of this chain is the following:

- Use or prescribe blood and blood components rationally.
- Document each episode of transfusion in patient files.
- Report all suspected transfusion reactions or adverse events related to transfusion.
- Document the details of the patients as well as the implicated units/ products.
- Send the details of the reaction to the transfusion medicine department or blood centres in standard format [12].

Transfusion medicine departments or blood centres have following responsibilities:

- To complete the workup of all transfusion reactions as per the "Transfusion Reaction Investigation Form" provided by HVPI.
- Reporting the details of the clinical and laboratory investigations to the respective medical department.
- To provide the details as per the Transfusion Reaction- Traceability Document.
- To assess the imputability levels of the adverse reactions in coordination with the attending physician.
- To report the details as per the Transfusion Reaction Reporting Form (TRRF) in the Haemo-Vigil Software

Recommendations

- The blood component should be screened for TTIs using highly sensitive and specific Anti-HIV-1 + Anti-HIV-2 immunoassay or HIV combination antigen-antibody immunoassay (EIA/ CLIA); HBsAg immunoassay (EIA/ CLIA); Anti-HCV immunoassay or HCV antigen-antibody combination immunoassay (EIA/ CLIA) (Level of Evidence: 1) .
- Rapid tests (highly sensitive and specific) may be used in blood banks with very low collections or in remote areas and should be replaced by ELISA (Level of Evidence: 1).
- Screening for Syphilis (treponemal antibodies) should be done using highly sensitive and specific tests such as treponema pallidum hemagglutination

assay (TPHA) or enzyme immunoassays. In populations with a high incidence of syphilis, screening can be performed using non-treponemal assays such as VDRL or RPR (Level of Evidence: 1).

- In malaria endemic countries, the donors should be screened for parasitemia using thick blood films or for evidence of malarial antigen using rapid tests or enzyme immunoassays (Level of Evidence: 1).
- NAT screening is an additional screening of donated blood apart from serology and may be used to provide an added layer of safety to further reduce the risk of TTI (Level of Evidence: 1).
- All patients undergoing HSCT should receive irradiated cellular blood components (Level of Evidence: 1).
- Thalassemia patients should receive leukodepleted red cell units for routine transfusions to avoid the risk of FNHTRs (Level of Evidence: 1).
- The transfusion medicine department or blood center supporting the transfusion for thalassemia patients should be enrolled and regularly report to the National Hemovigilance Programme of India (HVPI) (Level of Evidence: 1).
- Each clinician should report all the transfusion reactions to the transfusion medicine department for complete workup and diagnosis as well as for reporting to the HVPI portal. (Level of Evidence: 1).

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5c Extended Red Cell Phenotyping

Hem Chand Pandey, Nidhi Mehta

Patients and blood donors are routinely typed for the presence of ABO and RhD antigens on their red blood cells (RBCs) to ensure that appropriate units are selected for transfusion. ABO typing is done routinely due to the presence of pre-formed ABO antibodies in the plasma of the recipient with hemolytic potential. Similarly, typing for RhD antigen is performed in all as it is considered to be the most immunogenic antigen with >70-80% of patients who are RhD negative forming anti-D post-exposure to RhD-positive RBCs. Other blood group antigens are, however, not typed routinely in all the patients and donors as they are less immunogenic, no preformed antibodies exist in recipients and <1-2% of those who are exposed to foreign red cells develop alloantibodies [1].

Thalassemia patients are known to form alloantibodies to antigens from blood group systems other than ABO and RhD at a significantly higher rate (4 to 50%) when compared to the other patient groups [2]. This higher rate of alloimmunisation to other blood group antigens forms the basis of routinely typing thalassemia patients for blood group antigens other than ABO and RhD so that phenotype-matched blood could be transfused to these patients to prevent alloimmunization [3,4].

- **Antigen Typing:** Ideally thalassemia patients should be typed for red cell antigens at the first contact with the blood bank before transfusion therapy is initiated. Once they are on regular transfusion therapy, it is difficult to obtain a correct phenotype due to the presence of transfused cells.
- In a patient on regular transfusion therapy, it is necessary to have a three-month transfusion-free interval to obtain the correct red cell phenotype.

Types of antigen typing

- Limited antigen typing – In addition to ABO and RhD antigens, Rh C, c, E, e and K antigens are phenotyped. It is more practical to do this in resource-poor settings as >80% of alloantibodies are directed against these antigens [2-4].
- Extended antigen typing – In addition to the antigens typed in limited antigen typing, other blood group antigens including Jka, Jkb, Fya, Fyb and MNSs antigens are also phenotyped.

Extended phenotyping offers benefits over limited antigen typing as it prevents alloimmunization. Due to higher rates of alloimmunization in this group of

patients, it is recommended to transfuse RBC units, which are matched for those minor blood groups which are lacking in the patient, in addition to ABO and RhD antigen. It is also recommended for the management of alloimmunized patients as it helps in identifying additional antibody specificity and differentiating them from autoantibodies.

Limitations of serological assays for performing extended antigen typing

Serological assays are not useful to identify extended phenotypes in patients who were transfused in the past three months, due to the presence of transfused RBCs in circulation.

Evidence of benefit

Literature regarding the usefulness of providing red cells matching the extended antigen type of the thalassemia patient is limited. Retrospective data for the use of extended phenotyped blood and frequency of alloimmunization in general patients and multiply transfused patients is, however, suggestive of an overall decrease in alloimmunization frequency. One such study had shown a decrease in the frequency of alloimmunization from 0.51% to 0.32% in transfused patients and a decreased frequency from 33.9% to 17.5% in multiply transfused patients [5].

Pre-transfusion testing in thalassemia patients

At the first contact, one must perform ABO and RhD blood group along with phenotyping for other blood group antigens, if feasible. This should be repeated every time a sample has been received for crossmatch. ABO and RhD identical units should be selected for crossmatch. As thalassemia patients form alloantibodies at a higher rate, cross-match should be done using anti-human globulin after incubating at 37°C. Immediate spin crossmatch should be avoided for routine transfusions. All incompatible cross-match should be tested for red cell antibodies using an antibody screening panel. In patients with identified red cell antibodies, selected red cell units should be negative for corresponding antigens, in addition to being identical for ABO and RhD blood groups. For example, in a patient of B RhD positive blood group, who was identified to have anti-K, packed red cell units of B RhD positive, which is negative for K-antigen should be selected. In such cases, the turnaround time may be increased depending on the negative prevalence of the antigen and the expected delay should be informed to the treating clinician.

Since the incidence of risk of alloimmunization is as high as 4 to 5% in multi-transfused patients in India, allo antibody screening should be done as part of routine pre-transfusion compatibility testing for thalassemia [6]. The antibody screening assessment becomes more important in the following conditions:

- Incompatible cross-match on pre-transfusion testing
- Decrease in inter-transfusion interval/increase in transfusion requirements
- Laboratory features suggestive of hemolytic anemia

Antibody screens should ideally be done using a 2-cell or 3-cell panel from a commercial source or in-house prepared red-cell panels. Pooled red cell panels should be avoided for antibody screen in thalassemia patients.

- If the antibody screen is positive, antibody identification should be performed using an 11-cell or 14-cell panel depending on availability and the antibody should be identified.
- In the case of autoimmunization, it may not be possible to find a compatible unit for the patient. In such situations, least incompatible (best matched) but extended phenotype-matched units may be issued but such transfusions should be limited to the minimum.

Blood group genotyping in thalassemia patients

- Serological typing of red cell antigens is not reliable in multi-transfused thalassemia patients
- Genotyping is a complementary tool to overcome the limitations of serological assays. It is particularly useful in transfused patients with unidentified multiple alloantibodies to resolve undetermined blood group phenotype, where it is required to provide antigen-matched blood as well as in cases with antibodies to high-frequency antigens.
- Methods based on PCR-SSP, RFLP-PCR and DNA sequencing have been found to be adequate to perform DNA-based antigen typing for clinically significant minor blood groups such as RhD, C, c, E, e and K. [PCR-SSP: polymerase chain reaction- Sequence-specific amplification; RFLP-PCR: restriction fragment length polymorphism- polymerase chain reaction].
- It should however be kept in mind that genotyping analysis can only predict the blood group phenotype and in rare situations, genotype determination may not be correlated with antigen expression in the RBCs.

Recommendations

- Red cell phenotype for RhD, C, c, E, e and K antigens is recommended to be performed for thalassemia patients at the first contact (Level of Evidence:1).
- Pre-transfusion testing should include ABO and RhD testing with the selection of identical units for crossmatch. Crossmatch should be performed at Coombs phase (using AHG and incubation of 37o C) (Level of Evidence: 1).
- Antibody screening should always be performed as part of pre-transfusion testing (Level of Evidence: 1).
- Provision of phenotype-matched red cells for antigens which the patient lacks should be done for transfusion (Level of Evidence:1).

- Other clinically significant antigens (Jka, Jkb, Fya, Fyb and MNSs) may be phenotyped depending on the specificity of the alloantibody detected as well as the availability of reagents and feasibility (Level of Evidence:1).

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6a Chelation Therapy

Nupur Parakh, Sunil Gomber

Iron Overload: Pathophysiology

Regular blood transfusion remains the cornerstone of treatment in transfusion-dependent thalassemia (TDT) considering that the availability of hematopoietic stem cell transplant (HSCT) as a curative modality is still limited. However, repeated blood transfusions leadto its own complications, the most important of which is iron overload (IOL).

Assuming a transfusion frequency of 2–4 packed red blood cell (PRBC) units per month (200–250 mg of iron per unit), patients with TDT accumulate approximately 0.3–0.6 mg/kg/day of iron [1]. Additionally, when the transfused red blood cells (RBCs) senesce and get phagocytized by the reticuloendothelial macrophages, hemoglobin is metabolized and additional free iron is released into circulation. Another important factor contributing to IOL in patients with thalassemia (especially non-transfusion dependent thalassemia, NTDT) is the inhibition of hepcidin synthesis due to ineffective erythropoiesis, which promotes intestinal iron absorption despite systemic iron overload. Patients with TDT who are inadequately transfused also tend to accumulate greater iron (1-2 g of iron annually) through iron absorption compared to those who are regularly transfused. Various studies have concluded that compared to the hypertransfusion regimen, moderate transfusion (maintaining pre-transfusion hemoglobin between 9 and 10.5 g/dl) prevents excessive iron loading, with greater chances of spontaneous pubertal development and lesser extramedullary erythropoiesis [2].

Iron Toxicity

The iron in circulation binds to serum transferrin which gets saturated due to the continuous IOL. Once transferrin saturation (TSAT) exceeds 70%, the excess iron in plasma circulates as the non-transferrin bound iron (NTBI) and labile plasma iron (LPI) which are responsible for the cellular toxic effects of iron [3]. Reactive oxygen species (ROS) produced by the metabolism of NTBI plays a central role in inducing cellular dysfunction, apoptosis, autophagia, ferroptosis, genomic instability, and oncogenesis [4].

Different organs have a varied capacity to respond to iron-mediated toxicity, suggesting that toxicity thresholds are disease-specific, tissue-specific, and patient-dependent [4]. Some tissues like skeletal muscle are spared from iron

loading while others such as liver, myocardial muscle and endocrine tissue take up NTBI rapidly [4]. The liver is the first organ to be affected by IOL-mediated damage. Although, iron accumulation in the heart generally appears late (after > 70 units of PRBC transfusion), heart failure is a serious and potentially fatal complication of IOL [5]. The endocrine system, especially the pituitary, thyroid, and parathyroid glands, and the pancreas can be damaged by the accumulation of iron, with the consequent development of hypogonadism, infertility and diabetes mellitus [6].

Chelation therapy

In TDT, without effective iron chelation, death occurs from cardiac failure or arrhythmia, usually in late childhood or in the teenage years, with a median survival age between 12 and 17.1 years [7]. Iron chelators remove the excess iron and neutralize the NTBI. The ability of chelators to remove excess iron depends on the rate at which the chelator depletes storage iron and the rate of continued iron accumulation. An ideal chelator should have high affinity for ferric iron, low affinity for ferrous iron and other metals, high chelating efficiency, and the ability to attain negative iron balance with good tissue and cell penetration without redistribution of iron. It should have a slow rate of metabolism requiring once or twice daily doses. It should be cheap, easily available, have good tolerability and bioavailability [8].

Aims of Chelation Therapy

Chelation therapy can be instituted as a preventive therapy, rescue therapy, or emergency therapy [9].

Preventive Therapy	It is aimed at maintaining the iron balance in the body, i.e., iron intake from blood transfusion balances with iron excretion by chelation at all times.
Rescue Therapy	It aims at reversing the iron overload by increasing the rate of iron excretion, so that it exceeds the rate at which iron accumulates from transfusion.
Emergency Therapy	It warrants intensified chelation, usually given when the patient develops cardiac dysfunction and heart failure due to cardiac hemosiderosis.

Initiation of chelation

The usual recommendation is to start chelation therapy after 10-12 transfusions, or when serum ferritin (SF) levels are greater than 1000 ng/mL, or when liver iron concentration (LIC) is greater than 3 mg Fe/g [10]. A recent study suggested that the median (IQR) number of transfusions at which children with TDT have

SF >1000 ng/ml is 7.5 (2-24) [11]. Hence, iron overload in severe β-thalassemia patients might occur earlier even before they have received 10 transfusions.

Types of chelators

There are three chelating drugs that are commonly available and recommended for the treatment of iron overload in thalassemia: Desferrioxamine or deferoxamine (Desferal®, DFO); Deferiprone (Ferriprox®, Kelfer®, DFP) and Deferasirox (Asunra®, Exjade ®, Defrijet®, or Desirox®). Deferasirox and Deferiprone are used orally while Desferrioxamine is usually used by subcutaneous infusion or may be used intravenously in emergency conditions.

Deferasirox (DFX) is a tridentate iron chelator that binds iron in 2:1 ratio and iron is excreted by fecal route. As DFX has a long half-life (12-16 hours) in plasma, levels are maintained within the therapeutic range over a 24-hour period; it can therefore provide 24-hour chelation cover and binding of NTBI with only once-daily administration. It has become the most popular chelator among pediatric and adult TDT patients. Large clinical trials have shown its efficacy in controlling IOL in TDT patients [12,13]. Extensive Indian literature and studies also add to the efficacy and safety of DFX use in TDT patients [14-16]. DFX is the preferred initial chelator in children with a starting dose of 20 mg/kg body weight, given as oral suspension (tablet is dispersed in appropriate amount of apple/orange juice or water), 30 minutes before food. If the child tolerates the drug well, the dose can be increased subsequently at the rate of 3-5 mg/kg body weight up to 40 mg/kg, if the child has persistently high ferritin levels in follow-up visits (measured every 6mo) despite good compliance. In case of gastrointestinal intolerance, film coated tablet (FCT) of DFX can be used; the starting dose is 14 mg/kg body weight per day and dose should be calculated to the nearest whole tablet. To achieve negative iron balance in those with higher serum ferritin levels, the dose can be escalated to 30 to 40 mg/kg body weight per day for dispersible tablets and to 21 to 28 mg/kg body weight per day for FCTs. The dispersible tablet should not be chewed or swallowed as a whole, whereas the FCT tablet is to be swallowed whole before food or with a light meal (it can be crushed and sprinkled on soft food and consumed immediately in those children who cannot swallow the whole tablet). It is preferable to take it at the same time each day morning to improve adherence. The drug is approved for the treatment of chronic transfusion iron overload in children aged ≥2 years. The most common adverse events include gastrointestinal disturbances and skin rash. Mild, non-progressive increases in serum creatinine and transaminases have also been noted. If the child develops gastrointestinal intolerance, or persistent transaminitis, alternative drugs (DFO or DFP) can be tried.

Deferiprone (DFP) is a bidentate iron chelator that binds iron in 3:1 ratio, and it was the first orally active iron-chelating drug to be developed [17]. An advantage of this compound is that the iron (III) chelate of deferiprone carries no net charge and therefore can penetrate membranes easily, allowing the removal of potentially toxic iron from tissues through urinary route [18]. It has been approved in Europe, India, USA, and other countries for use. DFP has emerged as superior to DFO in reducing cardiac iron levels [19-21]. There are case series from India that report the efficacy of DFP in reducing IOL in TDT patients [22-24]. The recommended dosage for deferiprone is 75 mg/kg/d in 3 divided doses, up to 100 mg/kg daily. The common adverse events with DFP include arthralgia, arthropathy, gastrointestinal symptoms, and agranulocytosis. Reddish discolouration of urine due to excretion of the iron-deferiprone complex has been reported in DFP-treated patients and should be informed to parents while starting this medicine to prevent undue anxiety. DFP is commonly prescribed in children older than 6 years, although a few studies have established the safety and efficacy of DFP in infants and young children [24-27]. DFP is available as capsules (250 mg, 500 mg) although it is also available as oral solution (Ferriprox) in few countries. DFP is a safe and effective oral chelator in TDT and recommended to be administered orally in 3 to 4 divided doses of 75 to 100 mg/kg/day.

Deferoxamine mesylate (DFO), a hexadentate iron chelator, produced by Streptomyces pilosus, was first used for the treatment of transfusional hemosiderosis in 1962 [28]. DFO binds iron in a 1:1 ratio and has a high affinity for ferric iron as well as iron released from senescent RBCs. DFO is a large molecule that cannot be absorbed orally. It also has a very short half-life (20-30 minutes) and hence cannot be given by intramuscular or intravenous injection. Therefore, it is administered by subcutaneous infusion over 8-12 hours at a dose of 30–60 mg/kg/day on 5–7 days a week [29]. The chelated iron is excreted by fecal and urinary route. However, the cost of subcutaneous infusion pump is a major limiting factor for its routine use in TDT patients. It is the chelator of choice in emergency situations for patients presenting with severe cardiac iron overload on T2*Weighted MR, with arrhythmias and severe left ventricular failure due to its high affinity for ferric iron, high efficiency in attaining negative iron balance, and absence of iron redistribution. Twenty-four hours of continuous infusion of DFO at 50–60 mg/kg, via an indwelling intravenous catheter, is given over 6-12 weeks, which can improve survival in patients with heart failure and arrhythmias as has been shown in several studies [30]. For more details, refer to Chapter 13.

Intensive chelation is also given for children with TDT planned for hematopoietic stem cell transplantation. Acute pulmonary toxicity is reported with use of intensive intravenous chelation if infusion rate exceeds 10 mg/kg/h or where duration of infusion exceeds 24h.

The major dose-related adverse effects include local skin reactions, hypersensitivity, growth retardation, skeletal changes, infection with Yersinia enterocolitica, ophthalmic toxicity and ototoxicity. The rare side effects of this drug include renal impairment and pulmonary fibrosis [31-33]. Children receiving DFO should be monitored for physical growth every 3-6 months. Caution is advised when using DFO in children below 3y; careful monitoring of growth and bone development is recommended along with reduced dosage [34].

Practical aspects of DFO infusion: DFO is available as a lyophilized powder (500 mg per vial). It is dissolved in distilled water to make a 10% solution (500 mg dissolved in 5 ml of distilled water) and infused subcutaneously or intravenously, over 8-12 hours, 5-7 days a week, using an infusion pump. It is convenient to start the infusion in the afternoon after the child returns form school and continue till late night (over 8-10 hours) as a subcutaneous infusion using portable ambulatory pumps. The abdomen is considered the best site for subcutaneous administration; the skin over the deltoid or the lateral aspect of the thigh are the other alternative sites to administer DFO (See Figure 1). It is recommended to rotate the administration sites to minimise local reactions such as erythema, swelling, and induration. Infusion sets with needles to be placed at 90 degrees to the skin are available for subcutaneous infusion or butterfly needles of 25 gauge or smaller are generally used and are inserted at an angle of about 45 degrees to the skin surface. Most local reactions such as itching, erythema, induration, and mild to moderate discomfort are due to inadequate dilution of DFO. Ulceration at the site of a recent infusion results from an intradermal infusion of DFO and should be addressed by the deeper placement of the needle in subsequent infusions. Low-dose Vitamin C at a dose of 2-3 mg/kg/day is advocated during DFO infusion generally after 6-8 weeks of starting DFO therapy, as it increases iron excretion by increasing the availability of chelatable iron. Vitamin C supplements should not be given to patients with cardiac failure. As adverse effects related to DFO therapy are more at low ferritin levels, therefore the dose of DFO needs to be reduced to minimize DFO-related toxicities which can be guided by calculation of therapeutic index [= mean daily dose (mg/kg)/Serum Ferritin (ng/mL)] to keep this < 0.025 [32].

Combination Chelation Therapy

Patients having persistently high serum ferritin on monotherapy or who do not tolerate the maximum dose of one chelator or with moderate to severe iron overload on T2* MRI, are offered various combinations of the three available chelators. Different chelators can either be given on the same day (combined or associated chelation) or one after the other on different days (sequential or alternating chelation).

The success of combined chelation therapy has been demonstrated due to the synergistic effect of chelators due to 'iron shuttling', whereby a chelator with

rapid access to iron pools, donates iron to a chelator that has slower kinetic access and greater thermodynamic stability but can act as a 'sink', or stable acceptor for iron initially chelated by the shuttle molecule. In a systematic meta-analysis by Maggio, et al. the effectiveness and safety of combination therapy with DFO and DFP over DFO treatment alone has been documented in terms of decreasing LIC and SF [35]. Beneficial effects of combined chelation using DFO and DFP in improving cardiac T2* values have been also been shown in a meta-analysis [36]. Studies have also shown the beneficial effects of combined chelation using DFO and DFX in reducing SF and LIC and improving cardiac T2* values [37-39]. The result from these studies provided evidence for the use of combined DFX with DFO for the effects on SF, LIC and heart T2*. In the past decade the use of combined oral chelators has emerged as the most popular combination therapy with better compliance [40]. A large data is also available from India, showing added advantages of combination therapy with DFP and DFX over monotherapy without any adverse effects [41-43]. These findings provide evidence for the effects on SF, LIC and heart T2*. Alternating therapy with DFP and DFX has also been reported in two patients, who refused or had adverse effects with DFO, with improvement in LIC and SF [44].

Practical aspect of stepwise chelation

The choice of the most appropriate chelation regimen is based on the iron burden, patient preference, compliance with treatment, and toxicity of the iron chelator. The first-line chelator drug used in a particular country also depends upon the license for use of that particular drug. The various country-specific guidelines on iron chelation are as follows:

- The Canadian Guidelines recommend monotherapy with DFO or DFX, if SF is between 1,000 and 2,500 ng/mL or LIC is between 7 and 15 mg/g dry weight (dw), in the absence of cardiac dysfunction [45]. However, daily continuous DFO infusion at doses 50 mg/kg/day and combination therapy with DFO + DFX should be used if: SF > 2,500 ng/mL, LIC >15 mg/g dw, cardiac T2* <10 ms, or cardiac dysfunction is present. DFP is not licensed for use in North America.
- The UK Guidelines recommend using DFP as the first-line therapy if cardiac T2* <20 ms, LIC is between 2 and 7 mg/g dw, and SF is between 500 and 1,500 ng/mL [46]. DFO remains the recommended first-line treatment if cardiac T2* > 20 ms; DFX is reserved for patients who are non-adherent to DFO. DFP is used as a second-line agent in this context.
- The US Guidelines recommend maintaining existing iron chelation therapy as long as LIC is between 3 and 7 mg/g dw and SF is between 1,000 and 2,500 ng/mL. They endorse the use of DFX at maximum tolerated dose or DFO administered over 12 hours daily if cardiac T2* is between 10 ms and

20 ms in the absence of cardiac dysfunction [47]. In the context of cardiac dysfunction or a cardiac T2* <10 ms, the US Guidelines recommend the continuous use of DFO over 24 hours daily for upto 12 weeks duration.

- The Italian Guidelines recommend DFO for children younger than 6 years. For patients with severe iron overload, evidenced by a SF >3,000 ng/mL for 3 months, LIC >15 mg/g dw, cardiac T2* <12 ms, or cardiac dysfunction, intensive chelation with DFO or combination therapy with DFP + DFO is recommended [48]. Otherwise, for moderate iron overload, DFO remains the first-line agent, while DFX is used in patients with intolerance or non-compliance to DFO in the absence of severe iron overload. DFP is reserved for patients who are resistant or intolerant to DFX.
- According to the Australian Guidelines, the initial therapy depends on the age of the patient [49]. In the context of cardiac dysfunction, the Australian guidelines recommend the use of intravenous or subcutaneous DFO or combination therapy with DFO + DFP.

Targets of Chelation Therapy

Serum ferritin level is measured at least every 6 months with a target value of <1000 ng/ml. Utility of single SF value is limited and it must be interpreted with its trends and with other indicators of IO like T2* MRI and fibroscan.

Recommendations

- Chelation should be started once the serum ferritin is >1000 ng/ml (generally occurs after 10-12 transfusions) (Level of Evidence: 2).
- DFX is a safe and effective oral iron chelator which can be given orally in the morning as a single dose dispersible tablet (20-40 mg/kg/day) & film coated tablets (FCTs) in the dose of 14 to 28 mg/kg/day (Level of Evidence: 2).
- DFO is administered in a daily dose of 20-40 mg/kg in children and upto 50 mg/kg/day in adults, over 8-12-hour subcutaneous infusion, over 5-7 days a week (Level of Evidence: 2).
- DFO is recommended for intensive intravenous chelation if patient has symptomatic cardiac hemosiderosis (arrhythmia or cardiac failure) or severe cardiac iron overload detected on T2* MRI despite, using combination chelation in maximum tolerated doses over 6-12 months (Level of Evidence: 2).
- Avoid DFO as primary chelator in young children (<3 years) as their skeletal growth can be compromised (Level of Evidence:2).
- Intravenous DFO infusion is used as emergency therapy in patients with

cardiac arrhythmias or cardiac failure. Intensive chelation with DFO is also recommended prior to hematopoietic stem cell transplantation (HSCT) (Level of Evidence: 2).

- DFP is particularly useful in patients with cardiac IO (Level of Evidence: 1).
- The addition of a second chelator is recommended in case of:
  - ✓ Persistent (>6months) evidence of iron overload in the form of serum ferritin >2000 ng/mL or MRI evidence of IOL or both, or symptomatic iron overload, or suboptimal response on monotherapy, despite dose adjustment (Max tolerable dose) and good adherence (Level of Evidence: 2).
  - ✓ The use of DFX + DFP is the easiest and most cost-effective way to reduce iron burden (Level of Evidence: 2).
  - ✓ DFP + DFO combination therapy is more efficacious than DFP or DFO monotherapy in improving cardiac ejection fraction (Level of Evidence: 1).

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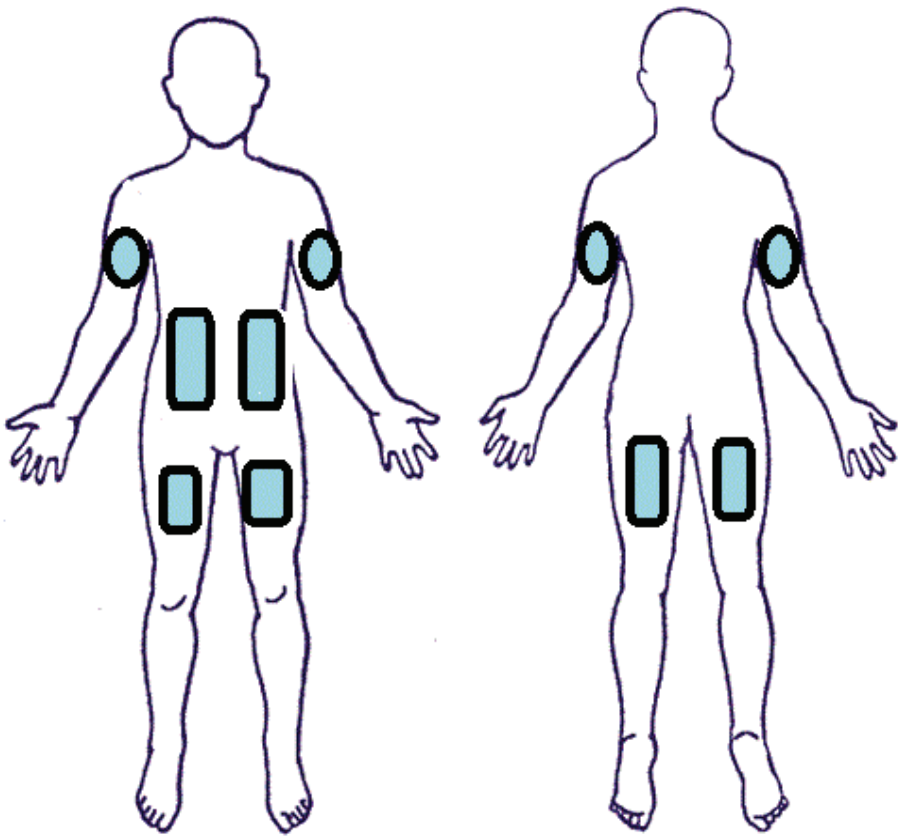
Figure 1. Sites of insertion of needle for subcutaneous administration of Desferrioxamine



6b Monitoring for Side Effects of Chelating Drugs

Manas Kalra, Rajiv Kumar Bansal

The commonly used chelating drugs in thalassemia include Desferrioxamine (DFO), Deferiprone (DFP) and Deferasirox (DFX). These drugs are generally well tolerated; side effects are more at high doses and if iron load is low. Adherence to chelation and regular monitoring of efficacy of chelation as well as side effects of these drugs enables optimising the treatment of thalassemia patients [1].

Deferasirox

Deferasirox is the first line chelator in thalassemia administered once a day by oral route. It is prescribed in doses of 20-40 mg/kg/d in TDT [1] although lower doses (5-10 mg/kg/d) are used in NTDT [2,3]. It is approved for patients with TDT aged ≥2 years and those with NTDT aged ≥10 years.

Deferasirox is a well-tolerated drug with a better safety profile compared to other chelators. Unlike DFO and DFP, DFX does not affect growth and bones or joints. Side effects are generally noted at higher doses (25-40 mg/kg/d) and can be reduced if doses are titrated according to iron burden. If serum ferritin falls below 1000 µg/L, the dose of DFX should be reduced and if serum ferritin falls below 500 µg/L, DFX may be continued in very low doses (3-5 mg/kg/d) [4,5]. The most common adverse events associated with the use of DFX include gastrointestinal disturbances, rash and renal side effects like non-progressive slight increase in serum creatinine and proteinuria [6]. These are related to the dose of DFX used.

The common side effects of DFX and their management are discussed below:

- Gastrointestinal side effects include diarrhea, abdominal pain, nausea, vomiting, and even gastrointestinal hemorrhage. These are seen in 15-25% patients and are generally mild to moderate in intensity [7-9]. Most of these adverse effects can be managed using aluminium free antacids, H2-blockers, and proton pump inhibitors or medications for symptomatic relief like anti-emetics for vomiting. One could also try using a different liquid like apple juice, orange juice or water for dispersing the DFX tablet. Film coated tablets are preferred in those who have intolerable gastrointestinal side effects. This has the convenience of administration with food and lowers the gastric mucosal irritation. In case diarrhea is suspected to be due to infectious etiology, appropriate anti-bacterial or anti-protozoal drugs are needed. In case of gastrointestinal hemorrhage, it is important to investigate

for gastric or duodenal ulcers as it has been reported rarely with the use of DFX [10]; this will warrant treatment with proton pump inhibitors and drugs for Helicobacter pylori eradication.

- Skin rashes are pruritic, maculopapular and diffuse. Approximately 5-10% patients develop skin rashes within first 2 weeks of initiating chelation with DFX [7-9]. Rash is mostly self-limiting or can be managed with anti-allergic medications or dose reduction of DFX. Very rarely patients may need systemic steroids or drug discontinuation due to severe or persistent rash. Once rash subsides, DFX can be resumed at 50% of the patient's last dose, with gradual increase in doses every 2 weeks if rash does not recur.
- Renal side effects: Non-progressive rise in creatinine is frequently seen with DFX [7,8,11]. An elevated serum creatinine should be confirmed by a repeat laboratory test performed soon after. The creatinine usually stabilizes, stays within normal range and almost never crosses 2 times the baseline value. Tubular dysfunction or Fanconi's syndrome, manifesting as proteinuria, urinary electrolyte wasting and glycosuria, has been described in multiple case reports. Tubular dysfunction is more common in pediatric patients with relatively low body iron. Rare reports of renal tubular acidosis and metabolic acidosis secondary to DFX are available [12]. Increase in serum creatinine by 33% from baseline leading to serum creatinine above the upper limit of normal for the patient may require reduction in dose of DFX by 10 mg/kg/day. In case of rising serum creatinine despite reduction in dose of DFX or persistent or significant proteinuria (urinary protein/creatinine ratio > 1.0 mg/mg), DFX should be stopped. If serum creatinine normalizes after stopping, DFX may be resumed at 50% of last dose with very gradual increase in doses. After starting DFX, serum creatinine should be monitored every 2 weeks for the first 3 months [5]. Thereafter, serum creatinine, serum creatinine clearance and urine for proteins should be monitored every 3 months. Additionally, urine protein/creatinine ratio and renal glucosuria should also be monitored.
- Visual and auditory disturbances are also reported with DFX use. Upto 0.3% patients using DFX develop lenticular opacities [13]. DFX-induced maculopathy has also been reported [14]. Deafness including sensorineural hearing loss has also been reported inconsistently [5]. Vision (slit lamp, fundus examination) and hearing assessments are recommended in patients prior to DFX and annually thereafter.
- Hepatic side effects in the form of transaminitis has been reported with the use of DFX in thalassemia [2,6,8]. However, it is important to consider that this may be because of other factors like hepatic iron overload, coinfection with hepatitis B or C or intensive chelation with DFO. DFX should be interrupted only if there is persistent unexplained rise in serum

transaminases. DFX should be reinitiated very cautiously at lower doses once liver enzymes normalize. In patients to be started on DFX, liver enzymes should be measured at baseline, every 2 weeks thereafter for next 1-3 months and every 3 months thereafter [5]. DFX is contraindicated in patients with severe liver disease and should be administered at a 50% reduced dose in those with moderate dysfunction.

- Cytopenias in the form of leukopenia and thrombocytopenia have been reported rarely with DFX [15]. However, cytopenia may be due to other causes like hypersplenism, use of deferiprone or viral infections. In case of unexplained cytopenia, DFX should be stopped. DFX is contraindicated in patients with platelet count < 50 x 109/L.
- Safety in pregnancy and lactation is not established. DFX should be stopped atleast 3 months before conception.

Contraindications

Deferasirox is contraindicated in patients with serum creatinine clearance < 60 ml/min, those with severe active hepatitis, pregnant women, those who develop anaphylaxis following DFX use and those with platelet count below 50 x 109/L [1,5].

Deferiprone

Deferiprone (DFP) is an oral chelator used in dose of 75-100 mg/kg/d in 3 divided doses in children ≥2 years and adults (although prescribed more commonly in children aged ≥6 years). Lower dose of 50 mg/kg/day may be adequate in some patients, however, doses of 75 mg/kg/d are most appropriate to achieve a negative iron balance. The use of DFP is associated with a wide spectrum of side effects which range from innocuous effects like chromaturia i.e., reddish/brown discoloration of the urine to life threatening adverse effects like agranulocytosis [5,16-18]. It is unclear if these side effects of DFP are dose related.

The common side effects of DFP and their management are discussed below:

- Cytopenias including agranulocytosis: One of the life-threatening side effects of DFP is severe neutropenia or agranulocytosis (absolute neutrophil count, ANC < 0.5 x 109/L) [5,16-19]. Agranulocytosis is reported in 1-2% of patients and less severe neutropenia in upto 5% of patients on DFP [19]. The incidence of cytopenias in patients on DFP are not linked to the dose of DFP, hence, it is important to diligently monitor the ANC; weekly for the initial 2-3 months after starting therapy and subsequently on every visit to the thalassemia centre, or earlier in case of any infection or fever [5]. If a child on DFP develops fever or neutropenia (ANC <1.5 x 109/L), DFP should be interrupted; neutropenia and agranulocytosis usually recover upon discontinuation of DFP. Severe neutropenia (ANC <

0.5 x 109/L) whilst on DFP which is unexplained by other causes like infection, hypersplenism etc, is an absolute contraindication to reuse DFP. Any child receiving DFP who develops fever and neutropenia, must be hospitalized and treated with intravenous broad-spectrum antibiotics. The treating doctor may consider the use of G-CSF in such situation although there isn't enough supportive evidence. Isolated fever without neutropenia is not a contraindication to use the drug, but generally it is discontinued till recovery from the febrile episode.

- Arthralgia and arthropathy: As high as 30% patients may complain of joint pains, especially in knees and therefore need analgesics and non-steroidal anti-inflammatory drugs. Arthropathy may benefit from stopping the drug transiently and restarting at a lower dose [20]. Erosive arthritis mandates cessation of drug completely. Ulnar deviation and joint deformities leading to functional problems may need surgery [20,21]. Routine radiographic imaging has no role in monitoring patients on DFP. Appropriate imaging and orthopedic consultation is needed if patient is symptomatic. If the patient develops deformity with the use of DFP, it is better to discontinue DFP.
- Gastrointestinal and hepatic side effects include nausea (12.6%), vomiting (9.8%), dyspepsia (2%), diarrhea (3%), abdominal pain (10.4%) and elevation of alanine aminotransferase (ALT) (7.5%) [16]. If the serum ALT exceeds 5 times the upper limit of normal and if no other cause for transaminitis is identified (like viral hepatitis, iron overload in liver), then DFP may be interrupted till ALT normalizes [1]. Estimation of serum transaminases must be done before starting a patient on DFP and every 3 months thereafter.
- Neurotoxicity and cerebellum syndrome (nystagmus, dystonia, axial hypotonia, ataxia and impaired motor coordination) have been linked to high doses of DFP [22]. Hearing and vestibular disturbances have also been reported with use of DFP [23].
- Cataracts have also been reported with the use of DFP [22].
- Zinc deficiency has been linked to the use of chelators especially DFP; the deficiency being more pronounced in those having diabetes [24]. Those with symptomatic zinc deficiency or having growth failure may be tested for serum zinc levels and thereafter be supplemented with a daily oral zinc supplementation of 40-50 mg for 1-2 months, ensuring that DFP and zinc are taken orally with a gap of 2-3 hours between the two (if taken together, DFP may bind to zinc and inhibit its absorption).
- Safety in pregnancy and lactation has not been established [16].

Contraindications

Deferiprone is contraindicated in women trying to conceive, pregnant women, those who develop severe neutropenia, those with significant joint deformities with use of DFP and those with hypersensitivity to the drug [5,16].

Desferrioxamine

Desferrioxamine (DFO) is the oldest iron chelator prescribed in thalassemia. It is used in doses of 20-60 mg/kg/d infused subcutaneously over 8-24 hours for transfusional hemosiderosis in children aged ≥3 years and intravenously as a continuous infusion for 6-12 weeks in those with severe cardiac siderosis on T2*MR [25]. The unwanted effects of DFO are seen especially at high doses or when the dose of DFO is not titrated despite a fall in iron load. It is important to titrate dose of DFO depending on iron load. If serum ferritin falls below 1000 µg/L, decrease the dose of DFO to keep the therapeutic index [mean daily DFO dose (mg/kg/d)/serum ferritin)] <0.025. Most of these effects occur on prolonged usage and typically take weeks or months to develop (over-chelation). Some effects like hypersensitivity and local reactions are largely independent of the dose given.

The common side effects of DFO and their management is discussed below:

- Local skin reactions like induration, edema, redness, itching and pain at injection site are seen in 17.8 – 85.7% patients [1,5]. It is more common if DFO is not diluted properly and used in higher concentrations. These can be managed by adding 5-10 mg of hydrocortisone to the DFO infusate or using a low potency topical steroid (hydrocortisone 1% or desonide 0.05% cream or ointment) at the affected site Alternately, heparin cream/spray/solution (1000 IU/ml) can be applied. To prevent these reactions, the concentration of the DFO solution should not exceed 10%; 500 mg of injection DFO should be diluted in at least 5 ml of water. Also, the needle should be inserted deep to avoid intradermal infusion and the site of administration should be rotated frequently. Topical anesthetics like lidocaine or prilocaine cream can be used prior to insertion of needle to minimise pain.
- Hypersensitivity reactions are more common with rapid intravenous infusion. DFO should be given by slow subcutaneous or intravenous infusion.
- Growth retardation and skeletal changes include platyspondyly (decreased vertebral height) due to metaphyseal changes and demineralization of vertebrae, rickets like changes like genu valgum [25-27]. Children receiving DFO and those getting higher doses over prolonged periods tend to develop disproportionate short stature with a short trunk [28]. DFO should not be used in doses exceeding 40 mg/kg/day in children to avoid growth

retardation. DFO should be preferably avoided in younger children until skeletal maturation is complete. The height of children receiving DFO should be recorded every 3 months and sitting height to be taken every 6 months to detect any growth disturbance, which can be confirmed using radiographs of the spine. It is important to pick up any change early as these are irreversible and not corrected by hormonal treatment.

- Visual disturbances include retinal changes such as electroretinography changes, retinitis pigmentosa, night-blindness, impaired colour vision, visual field defects, reduced visual acuity as well as cataracts. The predisposing factors include DFO dose >50 mg/kg/d [29], diabetes [30] and concomitant phenothiazine treatment [31]. In case of mild visual disturbances, interrupt treatment with DFO temporarily and restart later at lower doses once symptoms disappear. If changes are severe, avoid usage of DFO. Any patient receiving DFO should be assessed for any complaints related to vision at every visit. Annual examination by an ophthalmologist to assess retina, colour vision, visual acuity and visual field is recommended for patients receiving DFO; electroretinography, if available, should be performed annually if DFO is used in high dose.
- Hearing problems include high frequency sensorineural loss and tinnitus which tend to occur if DFO is used in high doses, in younger children and in those with low iron burden, therapeutic index >0.025 [32,33].
- Infections: Patients receiving DFO are prone to infections caused by siderophoric organisms like Yersinia enterocolitica, Vibrio vulnificus and mucormycosis [1,5,34]. Yersinia infections present with fever, enterocolitis, pharyngotonsillitis or polyarthritis; diagnosis is based on growth in culture or a 4-fold rise in IgG titres over a period of 15 days. Microbiology laboratory should be informed of suspected infection in a child with TDT receiving DFO to enable suitable conditions for growth of Yersinia (22ºC for 48 hours). It is important to assess every patient with fever for sepsis, wherein DFO should be stopped and empirical treatment started. Treatment comprises of IV trimethoprim sulphamethoxazole along with IV gentamicin. Alternative antibiotics are quinolones or ceftriaxone. Oral chelation with DFX can be continued in febrile thalassemia patients [5].
- Renal impairment in the form of acute renal failure, increased serum creatinine, and renal tubular disease has been reported in patients receiving DFO [5]. Serum creatinine should be monitored every 3 months in patients receiving DFO. DFO should be discontinued in patients with renal failure or severe renal impairment.
- Hypotension has been reported with rapid intravenous infusion of DFO. It can be treated by administering crystalloid fluids intravenously; avoid flushing IV line containing DFO [5].

- Pulmonary toxicity includes acute pulmonary syndrome and interstitial pneumonitis which have been associated with prolonged IV infusion (lasting more than 24h) and dose-dependent toxicity (>10 mg/kg/h) [35].
- Cardiac dysfunction with concomitant use of DFO and vitamin C has been reported. It is recommended to avoid coadministration in patients with cardiac failure. Vitamin C should be started atleast one month after starting DFO and in doses not exceeding 200 mg per day in adults and 2-3 mg/kg/day in children [25].
- Safety in pregnancy and lactation is not established and hence its use in first trimester is contraindicated. In special circumstances of cardiac dysfunction or arrhythmias, DFO may be used for intensive chelation in second and third trimester of pregnancy and during labour [25].

Contraindications

It is contraindicated in renal failure.

Table 1 depicts the parameters to be monitored in thalassemia patients for monitoring adverse effects of chelation therapy.

Table 1. Laboratory evaluation of thalassemia patients receiving chelating drugs

	Deferasirox	Deferiprone	Desferrioxamine
Complete blood count and differential cell count	Every visit	Every visit (weekly in the initial 2-3 months after starting drug); if ANC < 1.5 x 10^9/L monitor on alternate days or daily till counts normalize	Every visit
Serum creatinine and serum electrolytes	3 monthly (Every 2 weeks after initiation of DFX for 2-3 months)	-	3 monthly
Urine protein to creatinine ratio	3 monthly	-	3 monthly
Liver function tests	1-3 monthly (Every 2 weeks after initiation of DFX for 2-3 months)	3 monthly	3 monthly
Ophthalmic and auditory examination	Annual	Annual	Annual

Recommendations

- Deferasirox is preferred first line iron chelator in TDT especially in young children (Level of Evidence: 2).
- DFP is another oral chelator which is more convenient to use in older children (>6y) as it is available in capsule form (Level of Evidence: 2).
- DFO should be used cautiously and in lower doses in young children due to its effect on skeletal growth (Level Evidence: 2).
- Children on DFO need to be monitored for growth including sitting and standing height every 6 months (Level Evidence: 2).
- All children receiving DFO or DFP or DFX must undergo annual eye and hearing assessment (Level Evidence: 2).
- Use of DFP is associated with neutropenia; absolute neutrophil count should be measured before starting DFP therapy and every 2 weeks during initial months of treatment. If a child on DFP develops fever, stop DFP and estimated absolute neutrophil counts. Restart DFP only once fever subsides and ANC>1500/mm3. If a child on DFP develops agranulocytosis, admit the child and discontinue DFP. In such children DFP should not be used again (Level of Evidence: 2).
- If a child on DFO develops fever, stop DFO and assess for infections due to siderophoric organisms in addition to routine work up for fever (Level Evidence: 2).
- Use of DFX is associated with renal toxic effects and it is recommended to check the serum creatinine and urine for proteinuria every 3 months (Level Evidence: 2).
- Use of DFX is associated with transaminitis and if the transaminases exceed 5 times the upper normal limit, then DFX should be interrupted till serum transaminases normalize (Level of Evidence: 3).
- Dose of chelators especially DFO and DFX should be down titrated as serum ferritin falls to avoid toxic side effects (Level of Evidence: 3).
- Safety of iron chelators in pregnancy is not established. However, DFO has been used in some higher risk pregnancies, particularly in the final trimester (Level of Evidence: 4).

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7a Non-Transfusion Dependent Thalassemia

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Non-transfusion dependent thalassemia (NTDT) refers to a group of thalassemia patients who do not require regular blood transfusions for survival, although they may require occasional or even frequent blood transfusions in certain clinical situations and for certain periods of time [1]. Some patients may require more frequent transfusions later in life owing to complications of the disease, including splenomegaly [2]. NTDT comprises a heterogeneous group of anemias characterised by impaired production of β globin and/or α globin chains which include the following main diagnostic subgroups:

(i) β-thalassemia intermedia: The inheritance of 1 or 2 mild β-thalassemia alleles, or due to the co-inheritance of genetic modifiers which could mitigate the severity associated with inheritance of 2 severe β-thalassemia alleles

(ii) Hemoglobin E/β-thalassemia (mild and moderate forms): This condition is characterized by co-inheritance of a HbE gene and a β-thalassemia allele. This is common in South-East Asia and in Bihar, West Bengal and north-eastern states of India.

(iii) Hemoglobin S/β-thalassemia:This entity is most prevalent in parts of sub-Saharan Africa, Mediterranean region, Middle East, and parts of India (Central India especially Chhattisgarh, Bihar, Orissa, Uttar Pradesh, Maharashtra, North of Kerala and Tamil Nadu) where the sickle cell gene is common. This results from co-inheritance of sickle cell gene with 1 or 2 β-thalassemia alleles.

(iv) Hemoglobin C thalassemia:This is mostly observed in sub-Saharan and North Africa. This type of thalassemia results from co-inheritance of the structural variant known as HbC and many different β-thalassemia alleles.

(v) α-thalassemia intermedia (Hemoglobin H disease): This is the most common NTDT observed in South-East Asia. This results from deactivation of 3 α-globin genes[3].

Clinical Features

The clinical spectrum of NTDT ranges from mild to severe hemolyticanemia. Some patients have only mild anemia (Hb 7-11 g/dL) and remain transfusion independent till much later in life. Others may present early at 2-6 years of age with severe anemia, although they may not need repeated transfusions later on.

It is recommended to wait for 3-6 months before diagnosing such children as TDT or NTDT [4]. As anemia is allowed to persist, patients with NTDT tend to develop clinical manifestations like splenomegaly, skeletal deformities and growth retardation starting in childhood [4]. Complications tend to develop in later childhood and early adulthood like extramedullary hematopoiesis (EMH), hypercoagulable state, pulmonary hypertension (PHT), liver disease, endocrinal abnormalities and leg ulcers.

Clinical features suggestive of NTDT [5]

- Anemia observed usually after 2 years of age
- Anemia may affect quality of life or growth
- Hepatosplenomegaly of varying degree
- Skeletal changes suggestive of extramedullary hematopoiesis

Laboratory findings

The laboratory findings observed in patients with NTDT is listed in Table 1

Table 1: Laboratory observations in NTDT

Laboratory test	β-Thalassemia intermedia	HbE/ β+Thalassemia	Hb H disease	Hb S/β+ Thalassemia
Baseline hemoglobin	~7-10 g/dL	Mild: 9-11 g/dL Moderate: 6-7 g/dL Severe: 4-5 g/dL	2.6-13.3 g/dL	Hb can vary from 5g/dl to normal levels (severity varies depending on the type of β-thalassemia mutation that is co-inherited)
Blood peripheral smear	Microcytic hypochromic erythrocytes Target cells, nucleated RBC, increased reticulocyte count			Microcytic RBCs and target cells with occasionally sickled forms
			Inclusion bodies Numerous basophilic stippling	

Laboratory test	β-Thalassemia intermedia	HbE/ β+Thalassemia	Hb H disease	Hb S/β+ Thalassemia
Hemoglobin electrophoresis & HPLC	HbF (10-50%) HbA_2 > 4% or normal	Hb E (40-60%) Hb F (60-40%) +/-Hb A (with β-thalassemia) HbA2 increased	Variable Hb H (0.8-40%) HbA2 decreased	HbS (60–90%) HbA (0–30%) HbF (1–20%), Hb A_2 increased
DNA analysis	Commonly known mutations of both β^0 and β^+ thalassemia		Gap-PCR for common alpha thalassemia deletions	Common mutations for β^+ and Hb S thalassemia

Parental HPLC: One or both parents are heterozygous for beta-thalassemia or Hb S or Hb E trait or alpha thalassemia and their HPLC shows normal or borderline HbA2 or isolated increased HbF (usually up to 10%).

Genetic Testing and Molecular Analysis: NTDT arises due to gene mutations affecting beta-globin or alpha-globin production. The molecular defects in NTDT can be divided into four major groups: (a) Beta-globin gene mutations that lead to reduced beta-globin chain (b) Co-inheritance of alpha-thalassemia with beta-thalassemia can decrease severity of thalassemia by reducing alpha/beta-globin chain imbalance(c) Co-inheritance of gene defects like Xmn-1 polymorphism, some cases of delta/beta-thalassemia, and hereditary persistence of fetal hemoglobin lead to increased production of gamma-globin chains which results in an increased HbF levels and reduced alpha/beta-globin chain imbalance (d) Heterozygosity for beta-thalassemia and triplicated or quadruplicated alpha-gene locus and compound heterozygosity for beta- or delta/beta thalassemia.

Management of NTDT

Management of patients with NTDT is challenging in view of (a) heterogeneity of clinical features (b) irregular follow-up (c) lack of clear evidence to guide decision making (d) geographical variability in the patient characteristics.

Use of folic acid and Hb F inducers, transfusion therapy when required and iron chelation, are the mainstay of therapy in cases with NTDT. Regular follow ups are required to assess growth, monitoring of Hb and the need for transfusion, size of the spleen and any other complication.

Transfusions

Although repeated blood transfusionsare usually not needed in NTDT around the time of diagnosis, patients may need blood transfusions later on. Hemoglobin

level alone should not be a parameter to determine when to initiate transfusion therapy as evidenced by few studies where a difference of 1.8-2.6 g/dL in hemoglobin level was observed between the mildest and severe variety of NTDT [6,7]. It is also well known that many children with NTDT are well-adjusted while maintaining lower Hb levels. Transfusions should be considered when Hb is persistently <6-7 g/dL in association with growth failure [5-7]. Blood transfusions are often needed intermittently in NTDT or may also be offered more frequently in certain situations as shown in Table 2. Regular transfusions in most cases are usually not warranted in the long-term [2,3,5].

Table 2. Indications for transfusion in NTDT

Occasional transfusions	Frequent transfusions*
• Surgery: To maintain hemoglobin between 7-8 g/dL as in other children [8]. • Infections • Pregnancy: To maintain hemoglobin level > 10 g/dL which is needed forfetal growth and prevention of intrauterine growth restriction (IUGR), intrauterine death (IUD), or preterm delivery [9].	• Persistent hemoglobin levels < 7 g/dL despite being on maximum tolerated dose of hydroxyurea (HU) [10]. • Declining hemoglobin level with profound enlargement of spleen (rate >1 cm/year after 2 year of age) [2]. • Growth failure [4,11]. - Infants: failure to gain weight for 3 months - Children: height velocity < 3 cm/year - Older children: delay in puberty: > 13 years in females, > 14 years in males • Poor performance at school (not attributable to other neurodevelopmental disorder) • Progressive pathological fractures • Progressive skeletal facial changes: subjective, discuss with patient and family • Frequent hemolytic crisis (hemoglobin H disease)

*In all these settings, reassessment for tapering and withdrawing to be done when sustained clinical benefit is achieved.

It is a common practice to start regular transfusions once complications develop (Secondary Prevention) such as thrombotic complications or cerebrovascular disease, pulmonary hypertension, leg ulcers or pseudotumors. It may be more prudent to offer transfusions early in certain high-risk groups to prevent development of complications (Primary Prevention) as listed below in Table 3 [3,5,12]. It is, at times, difficult for patients and families to accept the regular transfusion therapy even for few months as it is feared that the patient has now become TDT. This necessitates appropriate counseling.

Table 3. Blood transfusions for preventing complications in NTDT

Primary Prevention	Secondary Prevention
• Splenectomised patients especially those who are: - Minimally transfused or transfusion naïve - Nucleated RBC count ≥300 × 106/L - Platelet counts ≥500 x 109/L • Family history of thrombosis • Conventional risk factors for thrombosis or cerebrovascular disease (silent infarcts, arterial narrowing, moyamoya disease) • Peri-pubertal (9 to19 y): Maintain Hb > 9 g/dL to allow optimum attainment of growth and sexual potential • Pregnancy	• History of thrombotic event or cerebrovascular event • Pulmonary hypertension • Leg ulcers • Extra-medullary hematopoiesis leading to symptomatic pseudotumors

Two different studies have shown benefit of regular transfusions in NTDT with variable age at which it would prevent complications in adult patients (age > 35 years) [13], (age >20 years) [14]. In such patients, there is evidence to suggest that transfusion therapy reduces the risk for thrombosis by 70% [13,15].

Adverse outcomes associated with transfusion therapy in children with NTDT include:

- Iron overload and subsequent endocrine complications
- Allo-immunization: the risk is highest in pregnant women, older, transfusion naïve or minimally transfused, and splenectomised patients.

Fetal Hemoglobin Inducers

Use of fetal hemoglobin inducers like hydroxyurea and thalidomide leads to increased production of γ-globin chains which decreases the imbalance in α/β-globin chain and leads to more effective erythropoiesis.

a) Hydroxyurea (HU)

Use of HU in NTDT can lead to an increase of 0.5-2.5 g/dL (average 1.5 g/L) in hemoglobin, with a significant decrease in transfusion needswith no serious adverse effects[16]. HU also decreases the complications of NTDT including extramedullary hematopoietic pseudotumor, thrombosis and cerebrovascular events, pulmonary hypertension, leg ulcers, hypothyroidism, and osteoporosis [13]. In splenectomised patients, there is also evidence that HU diminishes phosphatidylserine externalization in the red blood cell that can reduce thrombin generation and hypercoagulability. The indications for starting hydroxyurea [13] are as follows:

- Extramedullary hematopoietic pseudotumors
- Alloimmunized patients who require regular blood transfusions
- Pulmonary hypertension
- Thrombosis
- Leg ulcers
- NTDT patients homozygous for Xmn polymorphism, patients with Lepore or δB-thalassemia
- NTDT patients with Hb 7-10 g/dL

Initiating and monitoring a child on hydroxyurea therapy [3]:

- Hydroxyurea is initiated at a dose of 10 mg/kg/day; subsequently thedose may be increased by 3-5 mg/kg/day every 8 weeks to the maximal tolerated dose (not exceeding 20 mg/kg/day). It is available as a capsule, to be taken orally once a day with water.
- Concomitant folic acid supplementation is recommended
- While on HU, monitor the patient with complete blood counts, liver function and renal function tests, every 2 weeks for the initial 3 months, followed by monthly testing.
- Response to HU therapy is defined as a rise inhemoglobin level of >1 g/dl afteratleast 6 months of full tolerated dose. HU should be discontinued in patients not showing response
- In patients who have responded to initial HU therapy, the Hb levels and need for transfusions should be continued to be monitored atat 12, 18, and 24 months.

- Adverse effects of HU include reversible dose-dependent myelotoxicity (seen at doses > 20 mg/kg/d) and gastrointestinal adverse events in 1-30%. If there is myelosuppression (seen with higher doses of HU), HU needs to be stopped temporarily and restarted at lower doses once cytopenia recover. Dermatological adverse effects (hyperpigmentation, alopecia, maculopapular rash, or facial erythema) and neurological symptoms of headache and dizziness have been reported on long term use in a few studies. It should not be prescribed to pregnant or lactating women opting to breastfeed their babies.

b) Thalidomide:

Promising results have been shown with use of Thalidomide (50 mg/day) in NTDT in decreasing transfusion requirements and increasing the hemoglobin levels in a few studies [17,18]. However, due to lack of evidence on long-term safety, we do not recommend the use of thalidomide in NTDT. Thalidomide has also benefitted patients with NTDT who were alloimmunized and presented with severe hemolysis [19].

Chelation in NTDT

Annual iron overload in NTDT is estimated at 1.0 to 3.5 g compared to 2 to 12 g in patients with TDT on regular transfusions [20-22]. Figure 1 depicts the pathogenesis of iron overload in NTDT. All patients with NTDT aged ≥ 10 years [12] or after 10-12 transfusions [10] should be assessed for iron overload status.

a. T2* MRI to measure hepatic and cardiac iron overload should be done every 1-2 years starting from 10 years onwards. The time intervals for repeat T2* MRI can be earlier based on clinical indication or iron overload adjudged by T2* MRI (Every 2 years, if mild iron overload; annually if moderate iron overload and every 6 months, if severe iron overload).

b. Concomitant serial measurement of serum ferritin levels is recommended every 6 months

Starting Chelation in NTDT

- Chelation should be started when the serum ferritin is ≥ 800 ng/mL and/or the Liver Iron Concentration (LIC) is ≥ 5 mg Fe/g dry weight as interpreted onT2*MRI of liver.LIC supersedes serum ferritin level when both measurements are available.
- Start oral Deferasiroxat an initial dose of 10 mg/kg/day once evidence for iron overload is demonstrated [1,23,24].
- For patients initiated on chelation, therapy should be withheld when the serum ferritin drops below 300 ng/mL and/or the LIC falls in the normal range on the T2*MRI.

- If after starting DFX, LIC ≥ 7 mg Fe/g dry weight or serum ferritin > 1500 to 2000 ng/mL or reduction of serum ferritin < 15% from baseline, the dose of DFX should be increased to 20 mg/kg/day and LIC by T2*MRI should be repeated after 6 months and serum ferritin after 3 months.
- Safety considerations, monitoring, and related dose modifications for DFX in NTDT should follow standard guidelines used in patients with TDT.
- In case of intolerance to DFX, consider the use ofother drugs including Deferiprone and Desferrioxamine [25,26].
- Regular chelation akin to TDT patients is needed for patients with NTDT who require frequent blood transfusions for prolonged periods [3].

Figure 2 provides an algorithm for assessment and management of iron overload in NTDT.

Splenectomy

Splenectomy is no longer frontline mode of management due to the long-term complications following this procedure including an increased risk for venous thromboembolism (~5-fold), pulmonary hypertension (~4-fold), leg ulcers (~4-fold), silent cerebral infarction and infection (30-fold). Post-splenectomy thrombosis is encountered after a median duration of 8 years [27,28]. Splenectomy should be reserved for cases of NTDT who do not respond to HU therapy and develop

- Hypersplenism leading to worsening cytopenia despite blood transfusion program
- Splenomegaly accompanied by symptoms of left upper quadrant pain or early satiety
- Massive splenomegaly with concern about rupture

Vaccination against Streptococcus pneumoniae, Hemophilus influenzae, and Neisseria meningitidis may be required in such individuals.

Recommendations

- Diagnosis of NTDT should be made based on the clinical features including age of presentation beyond 2 years and non-requirement of regular transfusions (Level of Evidence: 2).
- Genetic testing and molecular analysis should be performed to ascertain genotypic profile of the patients (Level of Evidence: 2).
- Occasional transfusions are recommended to maintain Hb 7-8 g/dL for surgery or during infections as well as during pregnancy (Level of Evidence: 3).

- Frequent transfusions in NTDT are recommended for growth failure ascribed to anemia, Hb below 7 g/dL despite being on maximum tolerated dose of hydroxyurea, declining Hb with persistent splenomegaly, frequent hemolytic crisis and progressive skeletal changes resulting from anemia (Level of Evidence: 3).
- Transfusion therapy as primary prevention for complications may be recommended for nucleated RBC count ≥ 300 × 10^6/L and /or Platelet counts ≥ 500 x 10^9/L, family history of thrombosis, peripubertal period to allow attainment of growth and sexual potential (Level of Evidence: 3) and risk factors for thrombosis (Level of Evidence: 4).
- Transfusion therapy as secondary prevention is recommended for thrombotic events including cerebrovascular accidents and extramedullary hematopoiesis leading to symptomatic pseudotumours (Level of Evidence: 2) and leg ulcers (Level of Evidence: 3).
- In NTDT patients, iron overload status should be assessed using serum ferritin level every 3-6 months (and T2* MRI if feasible) starting at 10 years of age or when 10-12 transfusions have been given (Level of Evidence: 2).
- Iron chelation is started with Deferasirox in the dose of 10 mg/kg. Dose of Deferasirox is recommended to be increased if serum ferritin remains above 1500-2000 ng/mL or LIC ≥ 7 mg Fe/ gm dry weight (Level of Evidence: 2).

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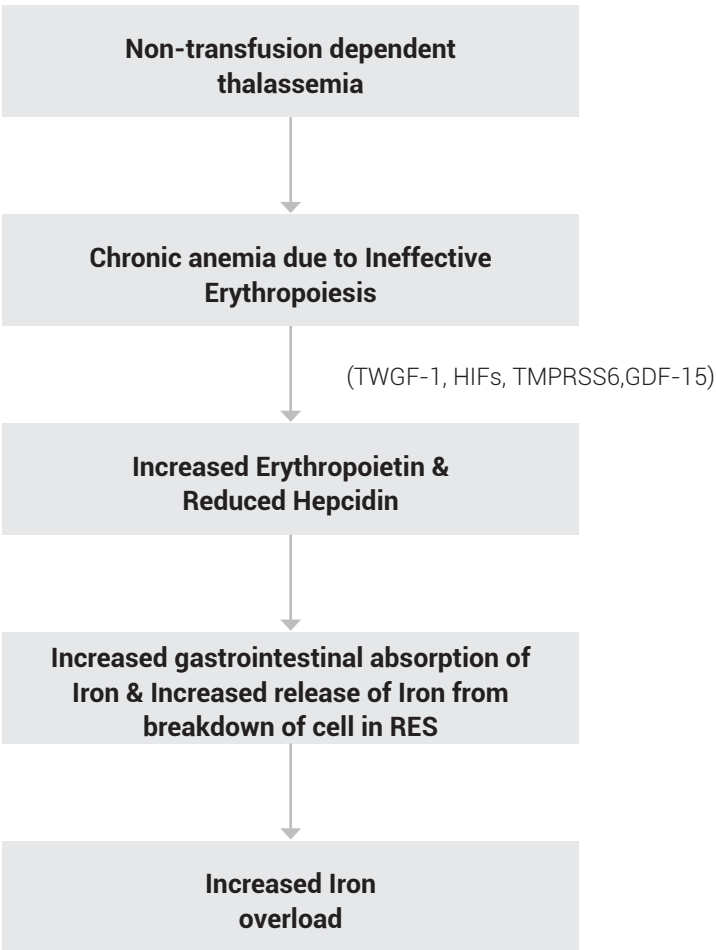
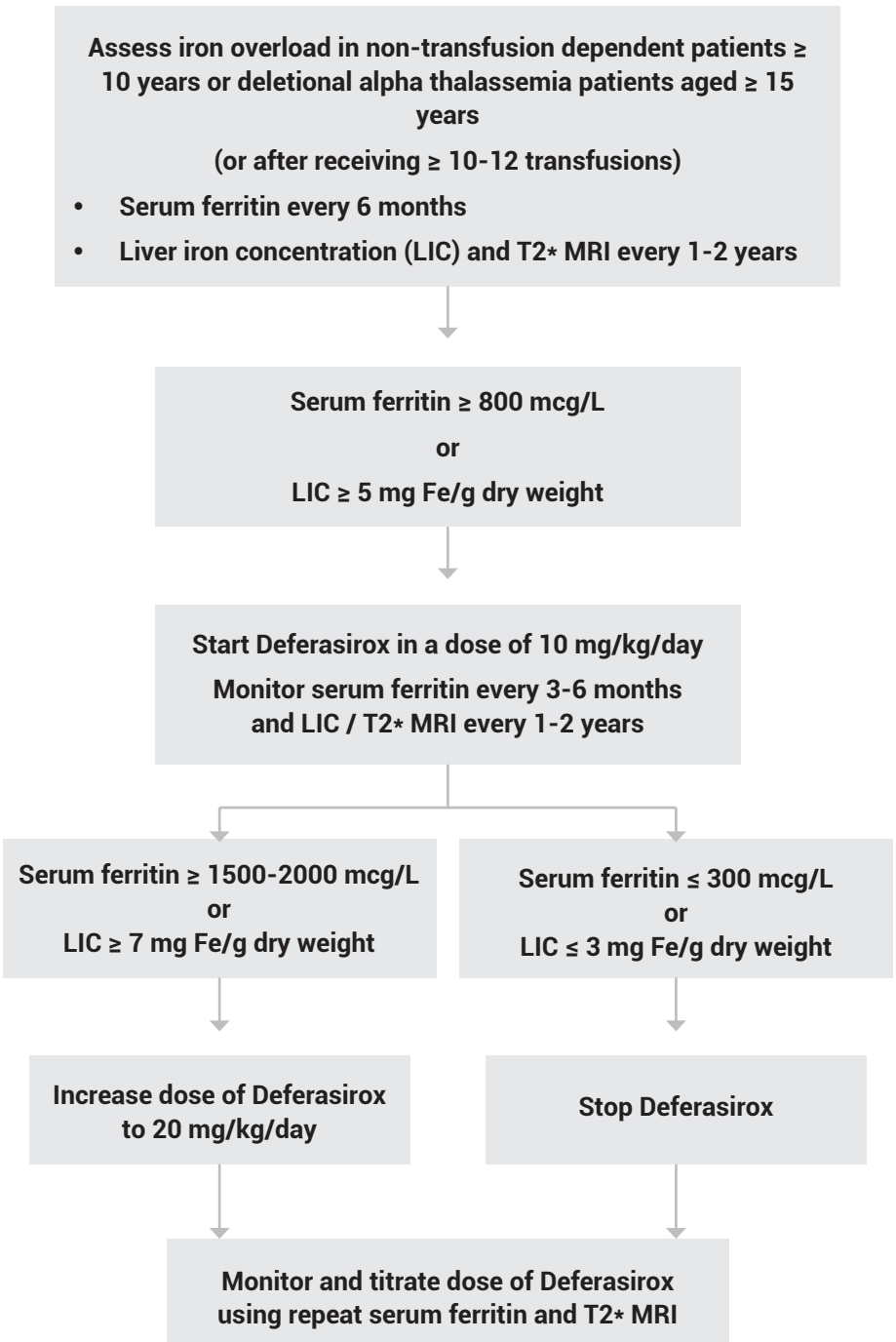
Figure 1. Pathogenesis of iron overload in NTDT



(TWGF-1: Twisted gastrulation factor-1, HIF- Hypoxia Inducible transcription factors, GDF-15 Growth differentiation factor 15, TMPRSS6- transmembrane protease serine- 6, RES- Reticuloendothelial system)

Adapted from Taher A, Vichinsky E, Musallam K, Cappellini MD, Viprakasit V. Guidelines for the Management of Non-Transfusion Dependent Thalassaemia (NTDT) [Internet]. Weatherall D, editor. Nicosia (Cyprus): Thalassaemia International Federation; 2013

Figure 2: Iron overload assessment and management algorithm for patients with NTDT



7b Complications in Non-Transfusion Dependent Thalassemia

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Non-transfusion dependent thalassemia (NTDT) is associated with significant complications including hypercoagulability, extra-medullary hematopoiesis (EMH), pulmonary hypertension (PHT), hepatic complications, cholelithiasis, leg ulcers, endocrine dysfunction, pregnancy and infertility, alloimmunization and hemolytic crisis.

Pathogenesis of Complications

The primary factors responsible for complications observed in NTDT include ineffective erythropoiesis, chronic anemia and hypoxia and iron overload. Chronic anemia is not only attributed to "ineffective erythropoiesis" but is also secondary to ongoing peripheral hemolysis of mature red blood cells (RBCs) as well as decreased hemoglobin production. Chronic anemia leads to a compensatory medullary and extramedullary hematopoiesis (EMH) which leads to skeletal deformities, osteopenia (risk for fractures), splenomegaly and hepatomegaly. Chronic anemia is associated with chronic hypoxia, generation of reactive oxygen species, and increased intestinal iron absorption due to decreased hepcidin levels. Iron overload is also attributed to hemolysis. There is an increase in the non-transferrin bound iron (NTBI) which leads to systemic inflammation and contributes to complications like the development of pulmonary hypertension (PHT), thrombotic complications, leg ulcers, cholelithiasis, organ dysfunction like cardiac failure and hepatic fibrosis and endocrinal problems like hypothyroidism, diabetes mellitus, and hypogonadism. Figure 1 depicts the pathogenesis of complications in NTDT.

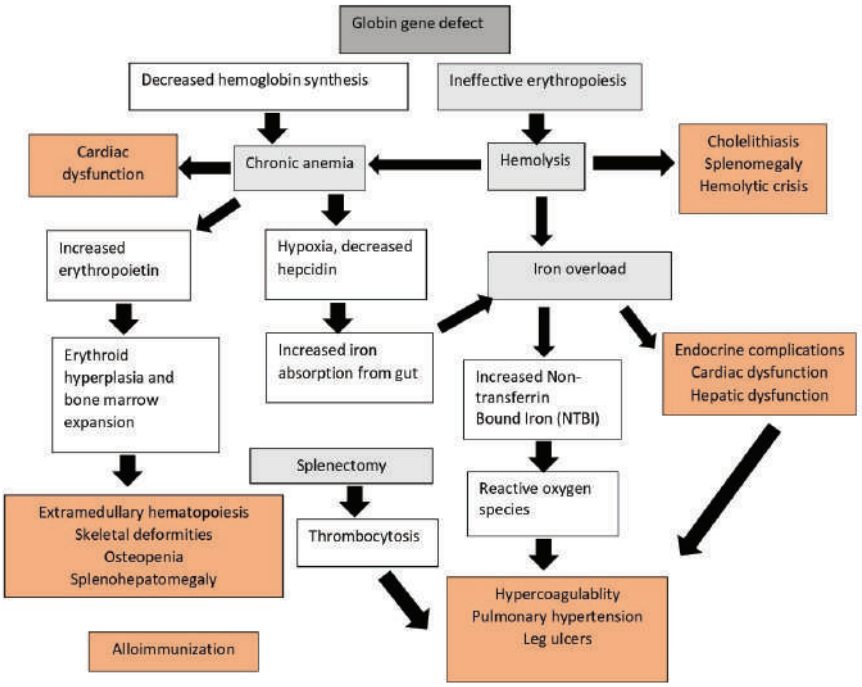


Figure 1. Pathogenesis of complications in non-transfusion dependent thalassemia

The various complications in NTDT include:

1. Hypercoagulability/ Thrombosis

Compared to transfusion dependent thalassemia (TDT), patients with NTDT are 4 times more prone to develop hypercoagulable states leading to thrombosis [1]. Prevalence of venous thromboembolism (VTE) in NTDT ranges from 3.9 to 29% [1,2]. In a systematic review, 29 to 83% of thalassemia intermedia (TI) patients were reported to have cerebral vascular involvement; majority being silent or asymptomatic [3]. Older age > 20 years, splenectomy, lack of regular blood transfusions, female sex, Hb < 9 g/dL, serum ferritin ≥ 1000 µg/L, nucleated RBC count ≥ 300 × 10^6/L, platelet count ≥ 500 × 10^9/L, and evidence of PHT are particularly associated with an increased the risk for VTE [1,4,5].

Evaluation

Cranial magnetic resonance imaging (MRI), especially diffusion weighted (DW) MRI, should be done starting at 20 years and every 1-3 years thereafter to detect asymptomatic vascular pathology in brain in adult NTDT patients with high-risk conditions (as above) [6].

Treatment

Thromboembolic events (TEE) should be managed using low molecular weight (LMW) heparin, viz, Enoxaparin 1 mg/kg (maximum 40 mg) subcutaneously twice daily for 5-10 days, along with oral warfarin (2.5 to 12.5 mg/day) which is continued over the next 3 months. Secondary prophylaxis with regular blood transfusions, aspirin (or clopidogrel) as well as hydroxyurea (HU) is recommended in patients with NTDT who have previously suffered from venous thromboembolism (VTE) and those with significant neurovascular lesions on imaging as it protects against development of newer brain lesions [7-9].

Aspirin prophylaxis (80 to 100 mg/d) and oral HU is recommended in splenectomized patients with platelet count >500,000/mL and/ or nRBCs ≥ 300 x 10^6/L, those with severe iron overload, older age and in those who had a history of (VTE) [7].

Low molecular weight heparin can be used for a period of 7–14 days postoperatively to prevent thrombosis and in pregnant women with NTDT.

Regular blood transfusions are recommended for patients with symptomatic CNS thrombosis, those with silent cerebrovascular changes and those with previous VTE [7, 11-13].

2. Pulmonary Hypertension

Pulmonary hypertension (PHT) leading to right-sided cardiac failure is a major complication in NTDT with an incidence ranging from 9.2% to and 37.3% [14,15]. Iron overload, previous splenectomy, platelet count ≥500 x 10^9/L, nRBC counts ≥ 300 x 10^6/L, minimally transfused patients, previous history of TE and advancing age have been shown to predispose to PHT [3,14-16].

Evaluation

Exertional dyspnea in the absence of cardiac failure should make the clinical suspicion of PHT. Decrease in distance in the 6-minute walk test can also aid clinical diagnosis. Right heart cardiac catheterization findings of elevated mean pulmonary artery pressure > 30-35mm Hg will confirm the diagnosis. Alternately, tricuspid regurgitation velocity (TRV) > 2.5-3 m/s on doppler echocardiography is a non-invasive method to detect PHT. Ventilation-perfusion scan is recommended to rule out pulmonary TEE. Patients with NTDT aged >20 years, especially those with high-risk conditions as outlined above, should be screened for PHT by annual echocardiography based on TRV.

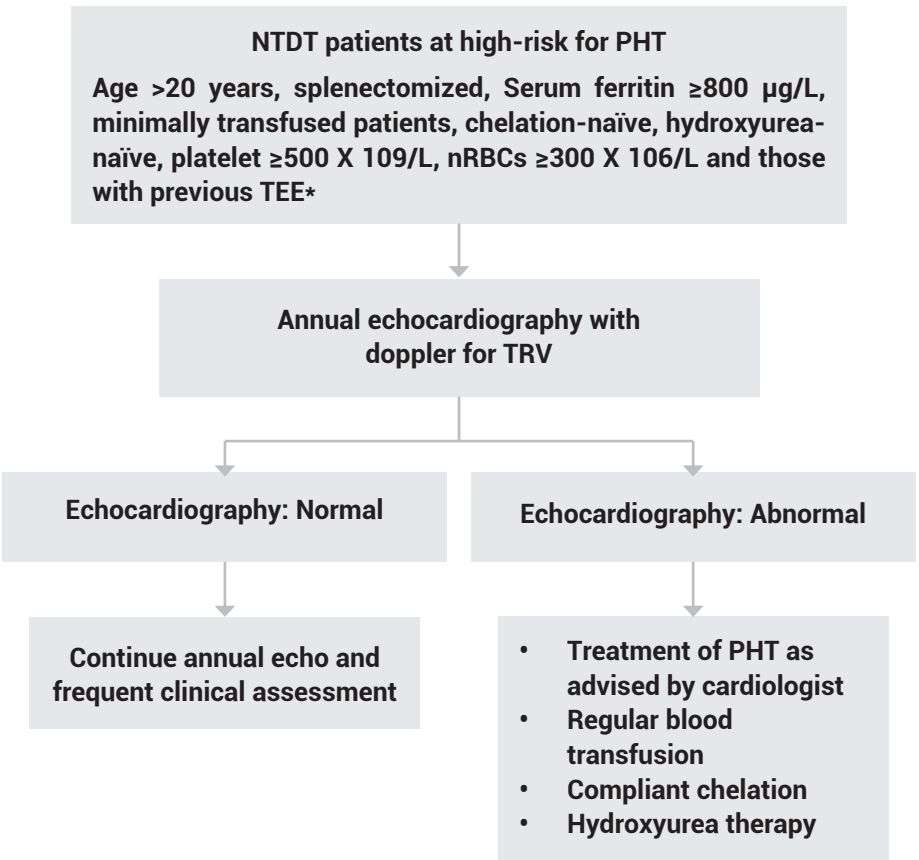
Treatment

NTDT patients who develop PHT should be treated in consultation with a cardiologist. Such patients should be put on a regular blood transfusion regimen, appropriate iron chelation, life-long aspirin prophylaxis (80-100 mg/day) and HU

therapy [4,7,17-19]. Drugs like sildenafil, bosentan and epoprostenol infusion have also been successfully used to treat PHT in NTDT [20-22].

Figure 2 provides an algorithmic approach to manage patients at high risk for PHT.

Figure 2. Algorithm for management of NTDT patient at high-risk cases for PHT



* Previous TEE: Continue regular blood transfusion, aspirin prophylaxis and hydroxyurea

3. Leg Ulcers

Patients with NTDT are predisposed to develop leg ulcers due to chronic hypoxia, increased thrombotic tendency, increased venous stasis (due to right heart failure and liver disease) and increased infections. With advancing age especially starting from second decade, nearly one-third of patients with NTDT develop leg ulcers especially around the ankles [23].

Evaluation

All older NTDT patients and those with risk factors for thrombosis should be examined routinely for evidence of ischemic skin changes and presence of ulcers in lower limbs.

Treatment

Leg ulcers should be managed by multi-disciplinary team comprising of surgeons, physicians, hematologists and dermatologists. The management includes occlusive dressing, topical antibiotics and leg elevation. Additionally, regular blood transfusions with compliant chelation and HU therapy may be beneficial [24,25]. Other modalities that have been tried with some success include oral pentoxyfilline, aspirin, vasodilator dialzep, topical sodium nitrite cream, topical G-CSF, exchange transfusion, oral vitamin C and skin grafting with variable benefits.

4. Extramedullary Hematopoiesis (EMH)

Ineffective erythropoiesis in NTDT leads to increased erythropoietic drive leading to marrow expansion as well as extramedullary hematopoiesis (EMH) in sites including liver, spleen, spinal canal and paraspinal area, peripheral and cranial nerves, brain, lymph nodes, thymus, heart, prostate, broad ligaments, kidneys, adrenal glands, pleura, retroperitoneal tissue and skin. While, most of the paraspinal pseudotumors are asymptomatic, those in the thoracic and lumbar region can lead to compressive neurological symptoms. The presentation is usually in the third or fourth decade of life but may be earlier. Incidence of EMH in NTDT is nearly 20% [4]. Risk factors include age > 35 years, splenectomy, poorly transfused patients and absence of HU therapy [4].

Evaluation

Detailed history and clinical examination are a part of assessment to exclude other causes like malignancies, abscess or hematoma. Imaging modalities will help delineate site of lesion. Radiographs may reveal thickened calvaria, wide ribs, and trabeculation suggestive of chronic anemia. Paraspinal pseudotumors can be detected using MRI. MRI is also useful to assess therapeutic response during follow up. Rarely, tissue diagnosis like biopsy may be needed in patients in whom clinical examination and investigations are equivocal and a possibility of underlying malignancy is very high; this entails risk of torrential bleeding and should be reserved for selected cases.

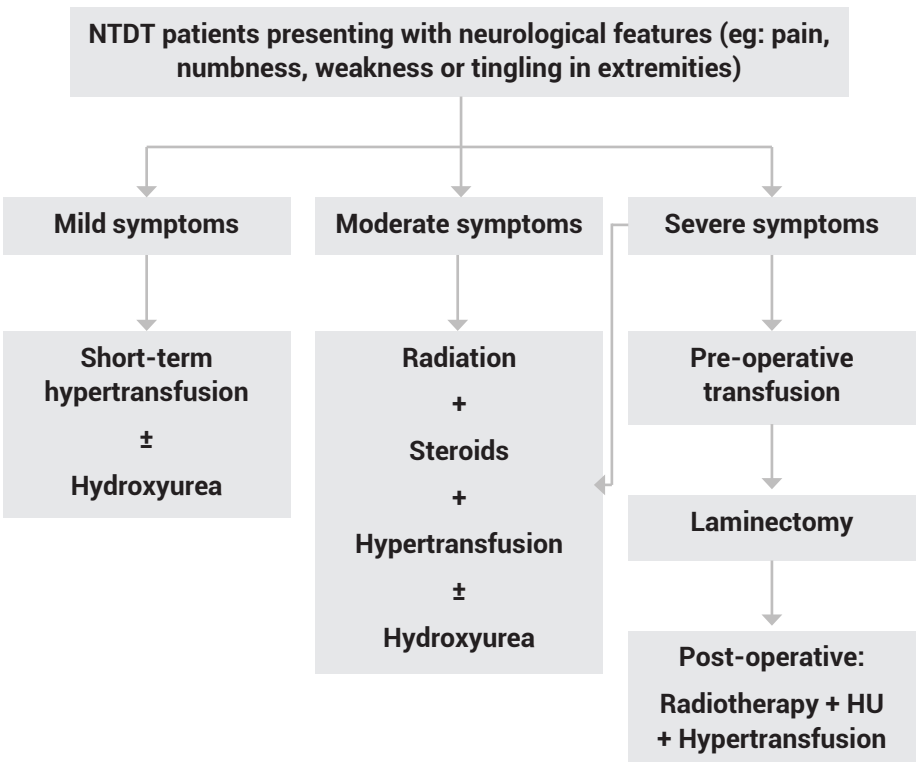
Prevention

There is insufficient evidence to routinely recommend blood transfusions and HU to prevent EMH.

Treatment

Treatment of symptomatic EMH involves hypertransfusion, oral HU (20-30 mg/kg/d), radiotherapy (RT) and surgery, alone or in combination. Mild neurological symptoms can be treated with hypertransfusion and HU. Low dose radiotherapy (900-3500 rads) with high dose steroids is recommended for moderate/severe neurological symptoms, along with hypertransfusion and oral HU [26,27]**.** Response is quick, seen in 3-7 days. Surgery is reserved for refractory cases as surgery may be associated with risk of excessive bleeding. Further, as these pseudotumors may be multiple or diffuse, operative procedure may not be feasible. A combination of two of the three modalities (low-dose radiation, blood transfusion, and hydroxyurea) is beneficial for treatment of any recurrence. Figure 3 depicts the therapeutic options for EMH with neurological symptoms

Figure 3. Management of Patient with Neurological Features due to Extramedullary hematopoiesis



5. Liver Disease

NTDT patients are prone to hepatic dysfunction due to multiple mechanisms: hepatocellular iron overloading which leads to transaminitis, hepatic fibrosis and cirrhosis; EMH leading to pseudotumors in liver; transfusion-transmitted infections and drug-induced hepatitis. Deferasirox (DFX) may also cause transaminitis which must be excluded by giving a patient a trial of stopping DFX and assessing improvement in the serum aspartate transaminase (AST) and alanine transaminase (ALT) levels over next 1-2 weeks. Prevalence of hepatic dysfunction varies with age from 3.3% in children ≤10y to 13.3% in patients older than 32y [28].

Evaluation

NTDT patients aged ≥ 10 years (maybe earlier than 10 years especially for children on deferasirox or HU) must be assessed for liver dysfunction by: 3 monthly liver function tests (LFT), annual abdominal ultrasound (to look for size and echotexture of liver, EMH), annual T2*MRI for liver (and elastography where available), and annual serological screening for hepatitis B and C especially if receiving regular transfusions. Hepatic dysfunction may be defined as AST >2 upper limit of normal, or evidence of Liver T2* MRI values ≤ 6.3 ms, or liver stiffness detected on elastography of liver, or evidence of hepatic iron overload on tissue biopsy (>5 mg iron/g dry weight of liver).

Patients with NTDT having hepatic dysfunction are predisposed to hepatocellular carcinoma (HCC) which may be attributed to toxic and mutagenic potential of NTBI as well as associated viral infections. HCC may be screened using alpha fetoprotein (AFP). An AFP level of 20 ng/mL is a commonly-used threshold to trigger evaluation for HCC in clinical practice [29]. However, elevated AFP levels are not specific for HCC and may reflect viral hepatitis or decompensated liver disease. Serum AFP levels >400 ng/mL in a high-risk patient are nearly diagnostic of HCC, with a specificity of >95%. Contrast enhanced ultrasound (CEUS) of abdomen is a good screening modality for detecting HCC which may be done annually in these patients in conjunction with AFP. NTDT patients ≥ 40y must be screened for HCC by annual alpha fetoprotein measurement along with contrast enhanced USG/ CT (low dose) abdomen. Liver biopsy may be considered by the gastroenterologist on a case-to-case basis.

Treatment

Management of liver disease in patients with NTDT should be done consultation with a hepatologist. Appropriate management of iron overload and TTIs is recommended.

Prevention

Chelation should be started in NTDT if serum ferritin ≥800 µg/L to prevent liver dysfunction [4,21]. Hepatitis B and A vaccination is recommended for all NTDT patients at diagnosis (definitely before first BT). Where-ever possible, seroprotection against hepatitis B should be monitored every 3-5 years. A booster dose of hepatitis B vaccine should be given if anti-HBsAg titres are <10U/L.

6. Cholelithiasis

The prevalence of cholelithiasis in NTDT has been reported as high as 68.4% in patients with Hb E/β-thalassemia and α-thalassemia (Hb H disease). Age >35 years, female gender and splenectomy were found to be significant risk factors for cholelithiasis while transfusion therapy and iron chelation were reported to be protective for cholelithiasis [4]. Patients with NTDT aged ≥10 years, those with serum ferritin > 800 µg/L and those who have undergone splenectomy should undergo annual ultrasonic evaluation for gall stones [4,28]. In case a patient with NTDT is scheduled for splenectomy, he should be screened for coexisting gall stones so that a cholecystectomy can be carried out in the same operative sitting. There is insufficient evidence to recommend regular transfusion therapy or HU in NTDT to prevent cholelithiasis.

7. Endocrine Complications Including Bone Health

Endocrine complications in NTDT patients include osteoporosis, hypogonadism, hypothyroidism, hypoparathyroidism, diabetes mellitus, and adrenal insufficiency (in decreasing order of prevalence). The prevalence of osteoporosis and hypothyroidism was reported as 30% and 16.3% in NTDT patients aged >32 years [28]. The poor bone health in NTDT is multifactorial in origin related to not only iron overload, but also chronic anemia leading to medullary expansion, hormonal imbalances and nutritional disturbances seen in this group.

Evaluation

Patients with NTDT aged ≥ 10 years, should undergo biannual assessment of weight, height, sitting height, height velocity as well as sexual maturity rating (SMR) to monitor growth. In case of faltering of growth or delayed puberty, evaluate the need for regular transfusions, hormonal and nutritional imbalance. In case of delayed puberty, bone age should also be assessed. Routine monitoring of serum calcium, phosphate, serum alkaline phosphatase (ALP), and serum 25 (OH) cholecalciferol, fasting blood sugar and T3, T4, TSH, should be performed annually after 10y of age. Bone Mineral Density (spine, hips, radius, ulna) by dual- energy X-ray absorptiometry (DEXA) can be considered annually in older patients (age ≥18y). In younger symptomatic patients, an endocrinologist must be consulted. In patients with diabetes mellitus, a consultation with a dietician is recommended.

Treatment

There are no clinical trials on use of bisphosphonates, teriparatide, calcium or vitamin D for treating osteoporosis in this group. Established endocrinopathies need to be managed as per standard treatment guidelines for TDT.

Prevention

Routine vitamin D (60,000 IU/month) and calcium (500 mg/d) supplements to be given to all NTDT patients. Transfusion therapy and chelation therapy must be followed as per indications in NTDT to prevent onset of endocrinopathies. Consider peri-pubertal transfusions with appropriate chelation to prevent delayed puberty and growth faltering. Regular exercise and healthy diet are recommended for all.

8. Alloimmunization

Risk of alloimmunization in NTDT is much higher than TDT patients [30]. Greater ineffective erythropoiesis in NTDT than TDT patients leads to increased immune dysregulation that predisposes them to alloimmunization. NTDT patients are more susceptible to alloimmunization as due to longer intervals between transfusions the alloantibodies may become undetectable prior to the next RBC antibody screening (delayed serological transfusion reaction, DSTR). Moreover, the transfusion of RBCs expressing the offending antigen could cause an anamnestic alloantibody response leading to a hemolytic transfusion reaction [31]. DSTR occur 2 days to 2 weeks after re-exposure to the implicated antigen and are associated with mild to moderate hemolysis and jaundice. Splenectomized patients are at increased risk for alloimmunization [31]. Alloimmunisation is also associated with increased prevalence of autoimmunisation.

Evaluation

NTDT patients should be screened for allo-antibodies by Coomb's testing. If found positive, identification of specific antibodies should be done using an 11-cell or 14-cell panel. Refer to Chapter 5 for details.

Treatment

Alloimmunized patients need treatment using oral steroids and if required, rituximab. Attempts to identify the alloantibody is worthwhile, as antigen negative blood can then be transfused, when required. Hydroxurea is useful in NTDT patients who develop alloimmunization but need regular transfusion [32].

Prevention

All NTDT patients should receive extended phenotypically matched blood, wherever possible. It is desirable to match for Rh C, c, E, e and K antigens also.

For previously alloimmunized NTDT patients, additionally screen for MNS, Fy^a, Fy^b, Jk^a and Jk^b antigens (Extended antigen typing) and give matched blood to prevent further alloimmunization.

9. Infertility and Pregnancy

Hypogonadism is not so common in NTDT and most women conceive spontaneously although they are prone to complications like spontaneous abortions, preterm labour, caesarean section, IUGR, TEE, splenectomy and alloimmunization [4,33]. If hypogonadism is present, patient may need assisted reproduction like induction of ovulation and spermatogenesis by a fertility expert. Additionally, evaluation by cardiologist is needed prior to conception. Medications that should be discontinued at least 6 months prior to conception include interferon, ribavirin, and HU [34]. Hypothyroid patients receiving thyroid-replacement therapy should receive increased doses to ensure they are euthyroid. Massive splenomegaly may be a hindrance to growing fetus and hence splenectomy can be considered for patients complicated with hypersplenism or massive splenomegaly before conception, and sometimes even post-partum considering there may be a pregnancy later.

Pre-conception counseling is advised to emphasise the risk of thalassemia in the offspring and offer prenatal testing and diagnosis. If a woman with NTDT wishes to conceive, pre-conceptional folic acid (1-5 mg/d) should be started and must be continued during pregnancy to decrease risk of neural tube defects in baby. All pregnant women with NTDT should receive regular transfusions during pregnancy to maintain pre-transfusion Hb > 10 g/dL. A good chelation regimen must be ensured prior to conception to optimise iron overload. However, stop chelation during pregnancy; if at all needed one can start Desferrioxamine in the second/third trimester. Additionally, stop HU, warfarin (switch to heparin), oral hypoglycemic drugs (switch to insulin), and anti-viral drugs like ribavirin at least 6 months before conception and during pregnancy. Intake of calcium and vitamin D must be continued during pregnancy. Pregnant woman with NTDT should be closely monitored for gestational diabetes by oral glucose tolerance test (16-28 weeks), hypothyroidism and cardiac and hepatic status during pregnancy. Close monitoring is also needed for TEE during pregnancy. Pregnant women with NTDT should receive prophylactic anticoagulant therapy (LMW heparin) in the peripartum period. Patients with a history of recurrent abortions and those who are splenectomized must be considered for prophylactic anti-coagulation with low-dose aspirin during pregnancy. Fetal growth during pregnancy should be monitored by ultrasound examination. Splenectomy should be considered for patients complicated with hypersplenism or massive splenomegaly before conception or postpartum. Contraception using barrier method is recommended. The use of oral contraceptive pills or intra-uterine devices is discouraged due to increased risk for TEE in these patients.

10. Hemolytic Crisis

It is more commonly seen in alpha thalassemia especially hemoglobin H disease [35]. It is triggered by infections (gram negative sepsis, gram positive infections, dengue), pregnancy and stress. Patients present with a sudden fall in hemoglobin, jaundice, fever, renal failure and shock. Fall in hemoglobin, leucocytosis, reticulocytosis, raised indirect bilirubin, raised LDH, raised SGOT, hemoglobinuria; raised serum creatinine and blood urea and metabolic acidosis may occur. Peripheral smear examination can help differentiate hemolytic crisis from aplastic crisis (reticulocytopenia, low platelet count and low total leucocyte count).

Evaluation

A hemolytic crisis should be suspected if patient presents with sudden anemia and jaundice. Patients with hemoglobin H disease should be monitored closely for severe anemia during infections or pregnancy.

Treatment

Blood transfusion followed by daily monitoring of hemoglobin is recommended. Intravenous hydration and alkalinization are to be started assuming 10-15% deficit in fluid volume. Oxygen, broad spectrum IV antibiotics and antipyretics are used for supportive treatment. Patient's urine output and urine for hemoglobinuria should be monitored.

11. Splenectomy

With the advent of better treatment, splenectomy is hardly indicated in the care of TDT now. NTDT also, is managed with early initiation of transfusion therapy in those with significant extramedullary disease such as massive splenomegaly, irrespective of the hemoglobin level as discussed earlier. Hence the indications for splenectomy continue to narrow down. In NTDT, despite appropriate management, splenectomy may be indicated if there is evidence of splenomegaly with at least two of the following: (1) Features of hypersplenism (manifesting as mono/multilineage cytopenias), (2) Symptomatic splenomegaly (Left upper quadrant abdominal pain, early satiety, massive splenomegaly with a risk of splenic rupture), (3) Splenic infarction.

Splenectomy should not be performed in children younger than 5 years as there are several risks associated with splenectomy and these include hypercoagulability and infections.

Complications of Splenectomy

Thrombosis: Post-splenectomy there is an increased risk (4 to 5 fold) for thrombotic complications like venous thromboembolism, pulmonary

hypertension, leg ulcers and silent cerebral infarction, compared to their non-splenectomized counterparts [2,4,5]. Spleen acts as a scavenger for pro-thrombotic platelets and red blood cells, as well as damaged erythroblasts and RBCs (defective β-chain production and a relative excess of α-chains) and micro-particles in circulation. Splenectomised NTDT patients who have high nRBC counts (≥300 x10^6/L) and platelet counts (≥500 x 10^9/L) are more likely to have TEE and PAH [4,4].

The risk is highest risk in the peri-operative period. A routine doppler ultrasound is recommended on post-operative day 7. Routine peri-operative thromboprophylaxis with LMW heparin is recommended until the patient is fully mobile. Also, routine low dose aspirin prophylaxis is given post-splenectomy to NTDT patients with additional high-risk conditions like presence of central venous catheter, previous/family history of thrombosis, silent brain infarcts, platelet count ≥ 500 x 10^9/L, nRBCs ≥ 300 x 10^6/L, major surgery, evidence of pulmonary hypertension.

Infections/sepsis: Splenectomized patients have an increased susceptibility to infections due to encapsulated organisms (Streptococcus pneumoniae, Hemophilus influenzae and Neisseria meningitides), Escherichia coli, Pseudomonas aeruginosa, Salmonella spp, and Klebsiella pneumoniae, malaria and babesia [36,37]. Patients with NTDT frequently have distorted sinuses due to EM erythropoiesis, and consequently develop sinusitis, which increases the risk of meningitis and brain abscess formation. The risk of infections is particularly higher in the initial 5 years after splenectomy (65% infections); 25% infections occur within first year and fulminant bacteremia has been reported even >25 years after splenectomy. Post-splenectomy sepsis is particularly more common in younger children and therefore splenectomy is avoided in children younger than 5 years. Overwhelming post-splenectomy infection (OPSI) syndrome is a well-known, often fatal condition which presents initially with flu-like symptoms (fever with chills, abdominal pain, headache, vomiting) but rapidly progresses to shock, DIC and death within 12-24 hours. Post-splenectomy sepsis has many of the features of adrenal hemorrhage (Waterhouse–Friederichsen syndrome) and has a 50% mortality despite intensive treatment. It is most important to recognize the infection at the earliest and start empirical broad-spectrum antibiotics at the earliest. Any sign of a systemic infection (eg, fever with a single oral temperature >38.3°C [101°F]) in an asplenic individual should be considered a medical emergency; most individuals presenting with a suspected bacterial infection should be admitted to the hospital, have blood cultures obtained, and receive broad-spectrum antibiotics pending the results.

Immunoprophylaxis

Vaccination against Streptococcus pneumoniae is a critical step in preventing post-splenectomy sepsis. Other vaccinations like those against H. influenzae,

N. meningitidis and Salmonella typhi are also important and should be administered. Table 2 highlights the immunoprophylaxis schedule for NTDT patients. Ideally, all patients should be vaccinated 4-6 weeks before splenectomy, else the child should be vaccinated after 2 weeks of splenectomy [37].

Table 1. Post-splenectomy immunoprophylaxis

Vaccine*	Schedule
Pneumococcal conjugate (PCV13) + Pneumococcal polysaccharide (23 valent)	1 dose 0.5ml IM of PCV 13 followed 8 weeks later by PPSV23 4-6 weeks (atleast 2 weeks) before splenectomy Booster every 5 years
Hemophilus influenzae type B	1 dose 0.5ml IM 4-6 weeks (atleast 2 weeks) before splenectomy
Meningococcal Group C conjugate vaccine: MenACWY	Two primary doses 8 weeks apart (Ideal) 4-6 weeks (atleast 2 weeks) before splenectomy Booster every 5 years
Influenza virus vaccination	Annual

* If previously vaccinated below 2y, then revaccinate

Post-splenectomy chemoprophylaxis

A broad-spectrum antibiotic should be given pre- and continued post-operatively as the risk of sepsis is highest in the peri-operative period. Penicillin prophylaxis is offered post-splenectomy up to the age of 16 years and over the age of 50 years. In addition, high-risk patients such as diabetics, should have life-long prophylaxis with penicillin [37]. Alternatives to Penicillin in case of allergy include erythromycin, amoxicillin, septran and cephalexin. The schedule for chemoprophylaxis after splenectomy is shown in Table 2.

Table 2. Post-splenectomy chemoprophylaxis

	Preferred drug	Alternate drugs
Daily prophylaxis	Penicillin V </= 3y: 125 mg BD >3y: 250 mg BD Amoxicillin 10 mg/kg twice daily (maximum 250 mg per dose)	Cephalexin 25 mg/kg BD (max: 250 mg BD) Azithromycin 5 mg/kg OD (max: 250 mg OD)
Emergency prophylaxis	Amoxicillin-clavulanate (14:1 formulation) 45 mg/kg (amoxicillin component) twice daily	Cefdinir 7 mg/kg BD (max 300 mg BD) Levofloxacin 10 mg/kg BD (max 375 mg BD)

Cholelithiasis in NTDT patients often warrants cholecystectomy and this should be considered if the patient is planned for a splenectomy.

With the availability of drugs like ruxolitinib and thalidomide, the need for splenectomy in thalassemia patients has decreased considerably.

Monitoring of NTDT Patients for Complications

Persons with NTDT should be monitored for disease and therapy-related complications, adequacy and safety of blood transfusions, iron overload, and side effects of iron chelators. Table 3 describes the routine monitoring for individuals with NTDT.

Table 3. Parameters for routine monitoring in patients with NTDT

S.No.	Monitoring Parameter	Monitoring Frequency
1.	Alpha and beta globin genotype	Time of diagnosis
2.	Anthropometry and growth velocity	Every 3-6 months#
3.	Complete blood count with differential	Every 6 months if no transfusion (monthly in infants to assess transfusion requirement)
4.	Liver function tests, renal function tests, serum calcium, serum phosphorus	Every 6 months
5.	Serum ferritin	Every 6 months starting at 10 years age (or after 10-12 transfusions)
6.	ECG and echocardiography	Annually from age 10 years

S.No.	Monitoring Parameter	Monitoring Frequency
7.	Tanner staging (for sexual maturity rating)	Every 6 months from 8-10 years
8.	Thyroid function test (Free T4 and TSH)	Annually from age 6 years
9.	FSH, LH, Estradiol, prolactin (females) Testosterone (males)	Annually from age 10 years
10.	Fasting Blood sugar, OGTT	Annually from age 10 years
11.	Serum 25 (OH) cholecalciferol levels	Annually
12.	Parathormone levels	Annually from age 10-12 years
13.	Bone densitometry (DEXA Scan)	Annually from age 18 years
14.	LIC on MRI	Annually starting at 10 years
15.	Cardiac T2* MRI	Start at 10 years; Every 2 years if T2* > 20 ms, annually if T2* 10 to 20 ms, and every 6 months if T2* <10 ms

Abbreviations: ECG: electrocardiogram, TSH: thyroxine stimulating hormone, FSH: follicle-stimulating hormone, LH: luteinizing hormone, OGTT: Oral glucose tolerance test, PTH: parathyroid hormone, DEXA: dual energy X-ray absorptiometry

Endocrine evaluation should be done if there is a fall on the growth curve or reduced height velocity

Adapted from: Khandros E, Kwiatkowski JL. Beta thalassemia: Monitoring and new treatment approaches. Hematol Oncol Clin North Am. 2019;33(3):339-353.

Recommendations

- Patients with NTDT aged ≥ 10 years should be regularly screened for complications like thrombosis, pulmonary artery hypertension, liver disease, extramedullary hematopoiesis, cholelithiasis, leg ulcers and alloimmunization, all of which are more commonly seen in NTDT compared to TDT (Level of Evidence: 3).
- Thromboembolic events (TEE) should be managed using low molecular weight (LMW) heparin and oral warfarin given for 3 months, regular blood transfusions with iron chelation and hydroxyurea (Level of Evidence: 3).
- Long-term prophylaxis with aspirin or clopidogrel is recommended in NTDT patients who develop thromboembolic episodes, those with significant neurovascular lesions on imaging, and splenectomized patients with thrombocytosis (platelet count >500,000/ml]. (Level of Evidence: 3)
- Cranial MRI (preferably DW MRI) should be done every 1-3 years starting at 20 years to detect asymptomatic vascular pathology in brain in NTDT patients at high-risk for thrombosis (previous TEE, family history of TEE, splenectomy, lack of regular blood transfusions, serum ferritin ≥ 1000 µg/L, nucleated RBC count ≥ 300 × 10^6/L, platelet count ≥ 500 × 10^9/L) (Level of Evidence: 3).
- NTDT patients who develop pulmonary hypertension should be referred to a cardiogist, in addition to providing him regular blood transfusion with iron chelation, oral hydoxyurea and life-long prophylaxis with aspirin (Level of Evidence: 3).
- Regular blood transfusions with iron chelation and hydroxyurea therapy should be started in NTDT patients with leg ulcers in addition to symptomatic measures like occlusive dressing, topical antibiotics and leg elevation. (Level of Evidence: 3)
- Treatment of symptomatic EMH involves hypertransfusion, oral HU (20-30 mg/kg/d), radiotherapy (RT) and surgery, alone or in combination. Low dose radiotherapy (900-3500 rads) with high dose steroids is recommended for moderate/severe neurological symptoms, along with hypertransfusion and oral HU (Level of Evidence: 3).
- NTDT patients aged ≥10 years must be assessed for liver dysfunction by 3 monthly liver function tests, annual abdominal ultrasound, annual T2*MRI for liver (and elastography where available), and annual serological screening for hepatitis B and C, especially if receiving regular transfusions (Level of Evidence: 3).
- NTDT patients ≥ 40y must be screened for HCC by annual alpha fetoprotein measurement along with contrast enhanced USG/ CT (low dose) abdomen (Level of Evidence: 3).

- Hepatitis B and A vaccination is recommended for all NTDT patients at diagnosis (definitely before first BT) (Level of Evidence: 3).
- All pregnant women with NTDT should receive regular transfusions during pregnancy to maintain pre-transfusion Hb > 10 g/dL and closely monitored for gestational diabetes by, hypothyroidism and cardiac and hepatic status during pregnancy. Prophylactic anticoagulant therapy (LMW heparin) is recommended in the peripartum period (Level of Evidence: 3).
- Splenectomy should be avoided as far as possible in NTDT patients, especially those younger than 5 years. If splenectomy cannot be avoided, ensure immunoprophylaxis and chemoprophylaxis and be watchful for sepsis and risk of thrombosis in splenectomized patients (Level of Evidence: 4).

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8 Monitoring of a Patient with Thalassemia

Tulika Seth, Anand Prakash

Monitoring of patients with Transfusion Dependent Thalassemia

Thalassemia is a chronic disease requiring lifelong transfusion and iron chelation; periodic monitoring helps optimize treatment and prevent potential complications [1-4]. The following aspects need regular monitoring:

1. Adequacy of transfusion
2. Transfusion-related complications
3. Adequacy of iron chelation
4. Iron chelator-related toxicity
5. Iron overload-related complications

I. Adequacy of transfusion

Pre-transfusion hemoglobin (Hb) and the volume of packed red cells transfused during every transfusion visit must be documented. The adequacy of transfusion is monitored by the following parameters:

a. Pre-transfusion hemoglobin

i. Target Pre-transfusion Hb to be maintained between 9.0 – 10.5 g%

ii. Increase in frequency/volume of transfusions to achieve appropriate pretransfusion Hb. The annual transfusion requirement is the total packed cell volume transfused during a calendar year divided by the patient's weight in June/July. An increase in annual transfusion requirements > 200 mL/kg/year points towards the development of antibodies/hypersplenism.

b. Anthropometry

Weight and height should be recorded on age-appropriate charts (WHO-IAP growth charts). Adequate growth is an indirect indicator of an optimal transfusion regimen (utpo a certain age only).

c. Degree of hepatosplenomegaly

Well-transfused children with adequate pre-transfusion Hb do not have significant organomegaly.

II. Blood transfusion related monitoring

Potential complications related to transfusion include:

a. Transfusion-transmitted infections (TTIs)

Nearly a third of patients with TDT develop TTIs secondary to blood transfusion with hepatitis C being the commonest. Many a time, these infections are chronic and indolent and picked up only on routine screening [5].

b. Transfusion reactions

Refer to Chapter 5 for details.

III. Adequacy of iron chelation [2]

a. Serum Ferritin

Serial monitoring of trends of ferritin can aid guide iron chelation therapy and should be done every 3-6 months. The aim is to attain a serum ferritin to less than 1000 ng/mL. Serum ferritin levels < 1000 ng/ml are shown to be associated with decreased incidence of endocrinopathies in the 2nd and 3rd decades. Values persistently above 2500 ng/mL are associated with an increased risk of mortality from cardiac iron overload. Serum ferritin may take a few months to show a declining trend The correlation of liver iron concentration with serum ferritin is poor when serum ferritin exceeds 3000 ng/mL.

Acute infections and hepatitis can erroneously elevate serum ferritin. Serum ferritin testing should be deferred for 3 weeks after an acute infection. A sudden rise in serum ferritin with elevation in serum transaminases points towards hepatitis. Refer to Chapter 6 for details.

b. Cardiac and Hepatic T2* MRI

Annual monitoring from the age of 8 years (to facilitate sedation) allows for a more accurate estimation of iron overload compared to S ferritin alone. The indications, methodology, interpretation, and utility of MRI T2* in managing iron overload have been discussed in detail in Chapter 9.

Adherence to iron chelation therapy should be monitored along with toxicities. Adherence is an important predictor of response to iron chelation therapy as well as complication-free survival in patients with thalassemia.

IV. Iron chelator-related toxicity [1,2,4]

The monitoring for toxicity of chelator depends on the chelator used. Refer to Chapter 6 for details.

V. Iron overload-related complications [2,4]

a. Monitoring of endocrine function is essential for early detection and correction of endocrinopathy due to iron overload. Refer to Chapter 10 for details.

b. Monitoring of cardiac complications

Symptoms of cardiac dysfunction due to iron overload occur many years following tissue iron overload in the myocardium. Annual cardiovascular assessment includes the following: history and physical examination for cardiac symptoms/ signs, ECG and echocardiography. Systolic dysfunction with dropping ejection fraction occurs late in the course of iron overload. Cardiac iron overload initially presents with diastolic dysfunction which is missed on routine echocardiographic evaluation. Hence, echocardiography is more useful only in settings of established symptomatic iron overload. Echocardiography can be used to assess improvement following intensifying iron chelation protocols. Refer to Chapter 13 for details.

The timing and frequency of the investigations recommended are given in Table 1.

Table 1. Monitoring a child with transfusion dependent thalassemia

	When to commence	Frequency	Target / Normal	Grade of recommendation
Adequacy of transfusion				
1. Pre-transfusion Hb (Post-transfusion Hb: NOT routinely required)	From the beginning	Before each transfusion	9.0 – 10.5g%	I
2. Weight/ height	From the beginning	3 monthly	Normal growth	I
3. Liver / Spleen size	From the beginning	3 monthly	No enlargement	
Adequacy of iron chelation				
1. Serum ferritin	After 10 transfusions	3 monthly	< 1000 ng/dL	II
2. 2* MRI	8 years	Annually	LIC < 7 mg/g dry wt Cardiac MRI T2* > 20 msec	II
Cardiac T2* > 20 ms 10-20 ms < 10 ms		2 yearly Annually 6 monthly		II
LIC 1.8 – 7 mg/g dry wt >7 - < 15 mg/g dry wt > 15 mg / g dry wt		1-2 yearly Annually 6-12 Monthly		II

	When to commence	Frequency	Target / Normal	Grade of recommendation
Toxicity of chelators				
1. AST, ALT, Creatinine, Urine routine and microscopy (On Deferasirox)		monthly	Transaminitis < 2 ULN Creatinine: WNL Urine R/M: No proteinuria	
2. Complete blood count (on Deferiprone)		monthly	No neutropenia	
3. Ophthalmology evaluation		Annual		
4. Hearing evaluation		Annual		
End organ toxicity due to iron overload				
1. ECG	10 years	Annual	Normal – no evidence of heart blocks (a late feature of iron overload)	
2. 2D ECHO	10 years	Annual	Normal – no cardiomyopathy (a late feature of iron overload)	II
3. Pubertal status (Sexual maturity rating)	10 years	Annual	Pubertal changes by 13 years – girls 14 years - boys	II
4. TSH	5 years	Annual		II
5. FBS	10 years	Annual		
6. LH, FSH, Estradiol, Testosterone	At puberty	Annual		II
7. Cortisol	10 years	Annual		II
8. Oral Glucose tolerance	10 years	Annual		II
9. IGF-1	10 years	Annual		
10. DXA scan	10 years	Annual		II
Transfusion transmitted infection / other complications monitoring				
1. HIV 1 & 2	From beginning	Annual	Negative	III
2. HBsAg	From beginning	Annual	Negative	III
3. HCV antibodies	From beginning	Annual	Negative	III
4. Transfusion reactions	From beginning	Every transfusion	Negative	II
5. Alloantibodies	From beginning	Every transfusion	Negative	II

LIC: Liver iron concentration.

Monitoring a Patient with Non-transfusion Dependent Thalassemia (NTDT)

The clinical manifestations of NTDT have mainly been attributed to chronic anemia and tissue hypoxia resulting in ineffective erythropoiesis, iron overload, and hypercoagulability. The complications that are seen in both TDT/NTDT include iron overload, endocrine disorders, liver dysfunction, bone disorders, cardiac dysfunction, and bone disease whereas pulmonary hypertension, thrombosis, and leg ulcers are specific to NTDT. [7,8] The guidelines suggested for TDT can be used for NTDT as well with a few differences as highlighted below.

I. Monitoring on Hydroxyurea

Hydroxyurea (HU) is initiated at 10-15mg/kg and the dose is incremented by 5 mg/kg every 2-3 months (usually not exceeding 20 mg/kg/d) till benefit is seen. The same dose with assessments every 3-6 months for dose adjustment to adjust for growth. Most NTDT patients do not tolerate more than 20 mg/kg of HU. In patients with NTDT on hydroxyurea, response assessment (rise in Hb > 1g/dL) is done every 3 months. Once stable then response re-evaluation is done every 6 months. Monitoring MCV rise from baseline is a useful marker to assess adherence to HU.

Monitoring for response is based on the following parameters:

1. Rise in Hb from baseline
2. Improvement in growth measures
3. Improvement in functional status and exercise tolerance
4. Improvement in quality of life
5. Improvement in clinical morbidities (pulmonary hypertension, extramedullary hematopoietic pseudotumor, leg ulcers)

Decrease / Withhold Hydroxyurea dose if:

- Cytopenia- absolute neutrophil count <1500/µL; platelet count <80K/µL.
- If febrile / suspected infection like pneumonia.

Monitoring on hydroxyurea (HU)

- Complete blood counts monthly for 6 months, then every 2-3 months. If fever or any crises or illness an urgent sample for CBC should be assessed.
- Liver function tests and serum creatinine needs to be monitored every 3-6 monthly when on HU.

II. Iron chelation

Children younger than 10 years should have serial monitoring of iron overload with a serum ferritin blood test every 6 months. Start serum ferritin testing by

3 years of age or whenever the diagnosis has been made. In NTDT even patients who have never received any blood transfusions can develop iron overload due to increased gut absorption. Iron chelation medicines are required to be initiated as soon as the serum ferritin ≥ 800 ng/mL [7]. For patients, more than 10 years of age, serum ferritin and T2* MRI Liver are used for monitoring iron load; serum ferritin should be performed every 3-6 months and T2* MRI Liver be performed every 1-2 years.

III. **Viral infections:** HIV, hepatitis B, and C annually if on blood transfusions, or have received blood transfusions in the past.

IV. **Pulmonary Hypertension (PHT):** An annual 2D ECHO monitoring is recommended for the assessment of tricuspid valve regurgitant jet velocity (TRV). A cut-off of 3.2 m/s has a positive predictive value of 93.3% in diagnosing PHT.

V. **Hepatocellular carcinoma (HCC):** Hepatocellular carcinoma is an emerging complication in thalassemia, however, it is much more common in NTDT than TDT [9]. Patients with severe iron overload (LIC ≥5 mg/g/dry wt in NTDT, ≥7 mg/g/dry wt in TDT), and chronic hepatitis B or C, and cirrhosis are at high risk of developing HCC. Refer to Chapter 7 for details.

The severity of manifestations and hence the frequency of monitoring varies significantly in patients with NTDT. The parameters and frequency of monitoring will need to be individualized based on the evolution of lineal symptoms in the patient.

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9 MRI Based Monitoring of Iron Overload

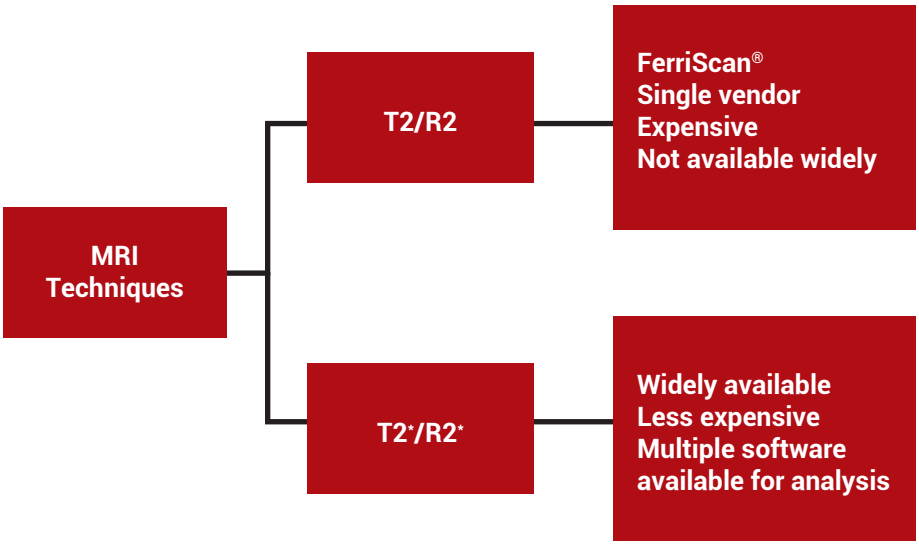
Kartik Ganga P, Bhavin Jhankaria, Tulika Seth

Serum ferritin and liver biopsy-derived liver iron concentration (LIC) have long been the standard methods for monitoring iron overload in patients with thalassemia. However, multiple studies have established that serum ferritin is a poor marker of absolute iron content in a patient and correlates weakly with the degree of cardiac siderosis [1]. Biopsy-derived liver iron concentration (LIC) correlates poorly with cardiac iron and therefore is a poor predictor of iron-related cardiac disease. Iron overload cardiomyopathy is a significant cause of morbidity and mortality with cardiac decompensation or arrhythmias causing sudden death. Optimization of chelation therapy can reverse cardiac iron loading and help improve life expectancy and overall quality of life. T2* magnetic resonance imaging (MRI) is the non-invasive gold standard for liver and cardiac iron estimation [2]. T2*MRI can predict cardiac failure up to one year before it occurs, giving time for chelation to work [3].

Detecting tissue iron using MRI

Iron deposited in tissues behaves like a tiny magnet when exposed to the MRI magnet, which causes the tissues loaded with iron to lose signal faster than tissues without iron. The rate of this signal loss can be measured and is called T2 or T2* (measured in ms) depending on the MRI technique used. The rate of signal loss can alternatively be represented as R2/R2* (measured in Hz) as the inverse of T2/T2* (i.e. R2=1/T2* and R2* =1/T2*). T2/T2* MRI, therefore, represents the physical property of that particular tissue which can be converted to LIC/MIC (which is a biological value) using algorithms that have been validated by comparison with tissue biopsies. Different MRI techniques are not equivalent. The two major techniques are the T2/R2 and the T2*/R2* methods as shown in Figure 1. T2*/R2* techniques are preferable over T2/R2 techniques due to their availability and validation with outcomes.

Figure 1. Types of MRI techniques that can be used for tissue iron assessment.



MRI sequences recommended for detection of iron overload

T2* MRI scan of the myocardium and liver is recommended for the detection of iron overload in tissues in patients with thalassemia. This is a non-contrast scan that does not need any special preparation and takes less than 10-15 minutes to perform. Apart from the T2*MRI sequence, a short-axis cine sequence to assess left and right ventricular ejection fractions should be performed as MRI is also the gold standard for the assessment of cardiac volumes and ejection fraction [4-6].

Optional MRI sequences

1. Newer sequences (if available) such as T1 mapping, which is a technique that may help identify iron overloading earlier than T2* [7]. This sequence needs further validation regarding utility prior to use in a daily clinical setting.
2. Long-axis cine sequences (two-chamber, four-chamber views)

3 Tesla Versus 1.5 Tesla Machine for T2* MRI

The strength of the magnetic field applied by the MRI machines is measured in Tesla (T) units. All clinical scanners are either 1.5 T or 3T. Traditionally 1.5T machines have been widely used for T2* estimation [8]. 3 T scanners can be used for T2* estimation. Typically R2*/2000 allows conversion from 3 T to 1.5 T value [9].

Grading cardiac & hepatic iron overload based on T2* MRI values

Classification of tissue iron overload using T2* has been depicted in Table 1 and Table 2 [10]. It is essential that a given center undertakes steps to validate and calibrate the method's measurements independently; otherwise, an inappropriate assessment of T2* may result**.** Centres must also have continuous quality assurance programmes such as regular phantom screening.

Table 1. Grades of cardiac iron overload

Myocardium at 1.5 T		Interpretation		Myocardium at 3T	
T2* (ms)	**R2***	**MIC**		**T2* (ms)**	**R2***
>20	<50	<1.16	Normal	>12.6	<79
10-20	51-100	>1.16-2.71	Mild to moderate	5.5-12.6	80-172
<10	>100	>2.71	Severe	<5.8	>172

Table 2. Grades of liver iron overload

Liver at 1.5 T		Interpretation		Liver at 3T	
T2* (ms)	**R2***	**LIC**		**T2* (ms)**	**R2***
>15.4	<65	<2.0	Normal	>8.4	<119
4.5-15.4	66-224	>2.0-7.0	Mild	2.3-8.4	120-435
2.1-4.5	225-475	>7.0-15	Moderate	1.05-2.3	>436-952
<2.1	>475	>15	Severe	<1.05	>952

Software/ analytical packages for calculating T2*

There are dedicated software packages from all MRI vendors apart from specialized cardiovascular software, which are validated methods of interpreting T2* data. Due to the cost consideration of most commercial software, open-source web-based tools (http://www.isodense.com/ic) and widely available excel-based analysis (initially devised by Dr. JL Fernandes) are commonly used in the subcontinent [11]. These need expertise and training though. Hence, commercially available approved software should preferably be used for T2* analysis.

A visual analysis must be performed of the T2* analysis to assess for artifacts and well as for the initial visual interpretation of the iron overload. Radiologists

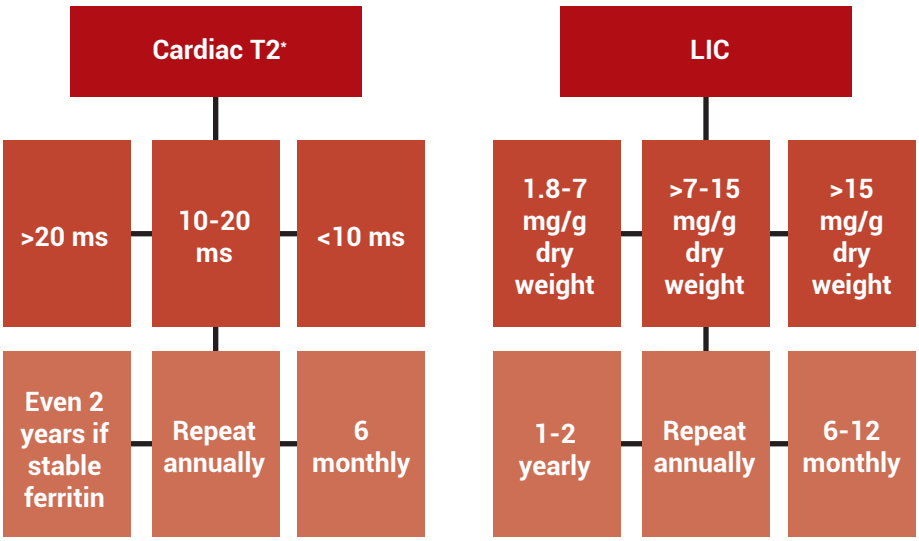
must pay special attention to the curve fitting for the T2* values and the fit error (especially for manual methods in excel based analysis) prior to classifying the iron overload.

Monitoring iron overload using T2* MRI

The first MRI T2* scan should ideally be performed at age of 8 years or older, or in younger patients once they can co-operate without the need for sedation [12]. This however is at the discretion of the referring doctor. In younger children, sedation may be required.

Repeat T2* MRI should be performed on transfused adults/children at time intervals determined by the degree of tissue iron overload as assessed on the baseline T2* values (Fig 4) [2]. Removal of cardiac iron in the heart is a slow process, and, especially in acute settings (i.e., acute heart failure), the clinical condition sometimes improves significantly, whereas T2* changes will not be proportional. Typically, if the baseline MRI T2* scan performed at 7-10 years suggests no cardiac iron overload, T2* MRI is repeated every two years thereafter. Figure 2 provides an algorithm for monitoring iron overload with repeated T2* MRI based on initial values.

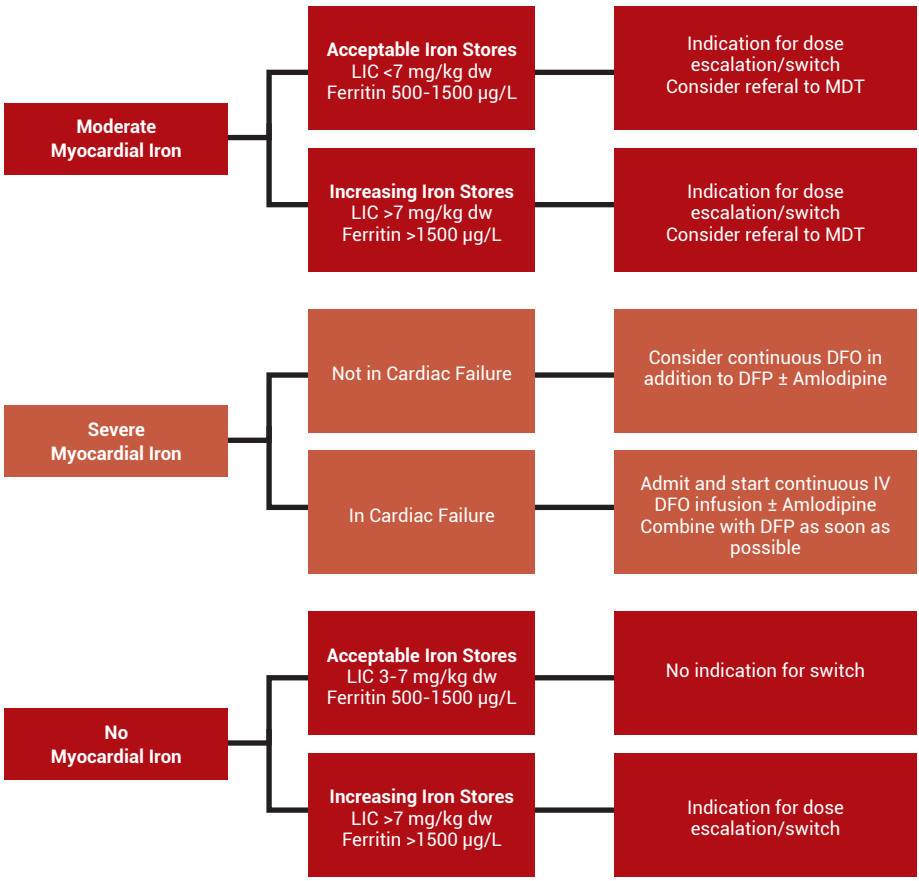
Figure 2. Algorithm for repeat T2* MRI assessment based on baseline T2* values



Using T2* results to guide therapy

The decision regarding modification of chelation therapy in the form of increasing dosage/ switching chelators can be guided by T2* MRI. One of the proposed algorithms adapted from the literature is given in Figure 3 [2,13].

Figure 3. Algorithm showing modifications in iron chelation therapy as per the MRI iron overload assessment



Role of MRI in non-transfusion dependant thalassemia (NTDT)

The initiation of T2* MRI for screening and monitoring in NTDT patients will depend on the clinical severity of the disease. Screening for liver and cardiac iron overload should be performed at intervals of 2-5 years or more frequently depending on whether the phenotype is mild, moderate, or severe and also if compliance with any chelation therapy (if needed) is poor [2,14,15]. Patients with NTDT at risk of iron overload should be considered for assessment with T2* MRI. Generally, T2* MRI should be started in those with serum ferritin ≥800 µg/L, however, it must be remembered that serum ferritin is unreliable in patients with NTDT with previous iron overload/ those already receiving chelation therapy. T2 * MRI must be performed regularly with the same intervals as recommended in Figure 2.

Additional information provided by MRI

MRI is the gold standard method for assessing left ventricular and right ventricular ejection fractions. This data can be acquired and performed simultaneously when the patient is scheduled for T2* MRI. Serial monitoring of LVEF can identify patients who might be at high risk of cardiac decompensation and require intensification of chelation therapy. When the EF starts falling below reference values, there is a 35-fold risk of cardiac failure [16]. Chronically transfused patients are in a high-output state produced by volume overload with the increased ejection fraction (normal LVEF of 60-65% and RVEF of 55-60%). The appropriate normal ranges for these patients are different from standard normal cohorts. Therefore, it is recommended that "normal for TM" reference ranges be used to enhance the identification of systolic dysfunction earlier [17,18]. Cardiac MRI can also help diagnose patients with pericarditis and myocarditis [19]. The data from the MRI scan can also provide liver and splenic sizes, obviating the need for an ultrasound which otherwise might be used to document these sizes. MRI can also provide information regarding extramedullary hematopoiesis, especially from the paravertebral regions and ribs, which might be missed on routine chest X-rays.

Monitoring of other organ functions and iron-mediated damage using MRI

Endocrine damage is an important clinical consequence of transfusion iron overload, MRI might be of value in assessing changes in endocrine tissue iron loading and identifying patients at risk of future endocrine deficiency. Studies show that pancreatic iron levels correlate with an increased risk of future myocardial iron loading. Increasing pancreatic T2* MRI is also a risk factor for diabetes and glucose intolerance development [20]. Increasing pituitary iron levels in a normal-sized pituitary gland suggests potentially reversible pituitary damage, with a shrunken gland representing irreversible damage [21]. However, the methodology and results need to be validated in more extensive studies before any recommendation can be made regarding clinical utility [13]. Since there are no there are established or accepted criteria available in the literature regarding normal and abnormal cut-offs of T2* MRI for pancreas or pituitary iron overload or their potential impact on management currently, no recommendations can be made regarding the routine use of T2* for pituitary or pancreas iron estimation. However, it can be considered as part of a research protocol assessing its potential impact.

Box 1 and Box 2 illustrate the important considerations for treating doctors and radiologists respectively.

Box 1– Key Points for Treating Doctors

1. T2* MRI of the heart and liver allows accurate assessment of the presence or absence of iron overload.
2. T2* < 20 ms suggests iron overload in the heart.
3. The scans can be performed on any 1.5 T or 3.0 T scanner, as long as the radiologist/center/hospital knows what to do, is routinely doing such scans, and knows how to interpret the results.
4. The ideal age at which to start referring patients be as soon as the child can cooperate.
5. The follow-up frequency depends on the results of the prior scan (as in Fig 4).
6. T2* of the pancreas and pituitary and T1 mapping is not necessary but may be asked if the radiologist/center/hospital is able to or willing to do it.

Box 2 – Key Referral Points for Radiologists

1. T2* MRI of the heart and liver have to be performed to assess iron overload.
2. A cine short axis sequence should always be performed to measure LV/RV function. LV/RV ejection fractions should be mentioned in the report.
3. Include the obtained T2* values and normal ranges in your report and mention whether there is iron overload or not.
4. Mention the analysis method (software name/excel based/open source) used to calculate the T2* values and the appropriate fit error values for the method used.
5. The formula used to calculate the LIC and MIC values should be mentioned in the report.

Recommendations

- T2* MRI is the method of choice for non-invasive tissue iron estimation as well as to monitor iron overload to titrate chelation therapy and to prevent iron overload cardiopathy (Level of Evidence:2).
- Initial T2* MRI should be performed as soon as the child can cooperate for the MRI scan usually at the age of 7-8 years (Level of Evidence: 3).

- Optimised and non-contrast T2* MRI protocols must be performed for routine monitoring of iron overload, which includes a minimum of T2* MRI for liver and myocardium and short-axis cine for assessment of cardiac ejection fraction (Level of Evidence: 2).
- T2* MRI has traditionally been done with 1.5 T scanners, but 3.0 T scanners may also be used as long as the values are expressed as 1.5 T values (Level of Evidence: 3).
- Liver assessment can be performed at the same time as cardiac assessment. Cardiac/Liver T2* MRI should be repeated every two years for no siderosis, annually for moderate siderosis, and half-yearly in case of severe siderosis (Level of Evidence: 2).
- LVEF should be assessed annually either by echocardiography or MRI in patients aged ≥8 years receiving transfusions (Level of Evidence: 2).
- It is recommended to use reference ranges that are specific to non-cardiac iron-loaded TM patients when assessing cardiac volumes and function as a surrogate for cardiac iron loading (Level of Evidence: 2).
- MRI assessment of iron overload in endocrine tissue is not recommended for routine clinical use (Level of Evidence: 2).

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10 Endocrine Evaluation and Monitoring

Anju Seth

Repeated blood transfusions and ineffective erythropoiesis result in progressive iron deposition in the endocrine organs including the pituitary, gonads, thyroid, parathyroid, pancreas, and adrenals in thalassemia. This translates into complications that emerge when children grow into adolescence. Endocrinopathies have emerged as an important cause of morbidity in children with transfusion-dependent thalassemia (TDT) and to a lesser extent in those with non-transfusion-dependent thalassemia (NTDT). Most children present with peri-pubertal growth failure or pubertal disturbances. Other endocrine complications also make their appearance commonly during the second decade of life like hypothyroidism, deranged glucose metabolism, hypoparathyroidism, and adrenal insufficiency [1-4].

Prevention is the best approach since the efficacy of intensive chelation in reversing established endocrinopathies is unknown [5,6]. An intensive and regular transfusion schedule, optimum chelation, maintaining an adequate nutritional status, and prompt recognition and treatment of comorbidities constitute the key strategies in the prevention of endocrinopathies**.** All patients should undergo a comprehensive endocrine evaluation annually, and regular screening for comorbidities like transfusion-transmitted infections, chronic liver disease, and cardiac dysfunction that can compound the endocrine dysfunction and affect the quality of life. These children and/or adults should be referred to an endocrinologist for opinion and further management as required [1,2].

Growth failure and pubertal disorders

Many factors contribute to growth failure in these children. Chronic anemia, hypoxia, and nutritional factors are responsible for growth failure before the age of five years; children who do not receive regular transfusions are especially prone to growth failure. After the age of 5 years, poor linear growth is likely to be due to adverse effects of iron overload on the growth hormone releasing hormone-growth hormone-insulin like growth factor-1 (GHRH–GH–IGF-1) axis. Beyond 10 years, absent or attenuated pubertal spurt contributes to growth failure because of the involvement of the hypothalamic–pituitary–gonadal (HPG) axis. At all stages, the presence of other comorbidities can further worsen the outcomes [7-9].

Disturbances in pubertal development

An entire spectrum of pubertal growth can be seen in thalassemia:

1. Normal onset and progression till completion of growth and development - These patients may still develop hypogonadism and secondary amenorrhea as an adult.
2. Delayed onset but normal progression once initiated - A girl with no onset of breast development by the age of 13 years and a boy with testicular size <4 ml at the age of 14 years is considered to have delayed puberty.
3. Normal/delayed onset with pubertal arrest after a variable duration - Pubertal arrest is considered if there is non-progress of pubertal stage over a period of 1 year, or if menarche does not follow thelarche within 5 years.
4. Failure of pubertal onset and primary amenorrhea - These patients may have primary or secondary amenorrhea.

Pubertal disorders are often associated with complications including short stature and poor growth spurt, poor bone mineral accretion, poor body image, and impaired fertility. The key factor implicated is hemosiderosis in the HPG axis, resulting in inadequate LH and FSH production, leading to hypogonadotropic hypogonadism (HH). Direct gonadal damage by iron overload is less likely [8-10]. It is recommended that all children with TDT should have regular (every 6 months) growth monitoring starting from early life. The recorded anthropometric parameters (both weight and height) should be serially plotted on the Indian Academy of Pediatrics (IAP) growth charts for Indian children [11]. A well-maintained growth chart is a sensitive indicator of the child's overall health. It helps detect growth faltering which indicates a possible systemic/endocrine disorder. For a more accurate assessment of a child's height, the genetic potential for height should be assessed by calculating the mid-parental height given by the following formula:

MPH for girls: (father's height + mother's height – 13)/2
MPH for boys: (father's height + mother's height + 13)/2

Growth failure

Assessment

The following children require a detailed evaluation [2,3,10]:

- Children manifesting growth faltering (progressive decline in height centile) on routine growth monitoring
- Height below 3rd centile on the IAP growth chart
- Expected height below the target range of + 6-7 cm below the mid-parental height

This includes

- A clinical/laboratory evaluation for any co-morbidities including endocrinopathies (especially hypothyroidism), chronic liver disease, and HIV.
- Bone age assessment: A lag of 2 years between chronological and bone age indicates delayed bone age.
- Assessment of pubertal development: delayed puberty usually co-exists and worsens growth failure.
- Assessment of GH secretion is performed in cases with height ≤ –3 SD and delayed bone age. This is done by measuring peak GH response to two different provocative stimuli. Baseline GH assessment does not serve any purpose and is not recommended.
- In cases with disproportionate short stature, radiographs of the tibia and spine should be done to look for platylospondylosis or changes secondary to metaphyseal cartilaginous dysplasia.

Management

- Correct nutritional deficiencies.
- Manage associated comorbidities including hypothyroidism, chronic liver disease, heart failure, etc.
- Ensure timely pubertal onset and progression, spontaneous, or with hormonal support as required in consultation with an endocrinologist.
- Children with proven growth hormone deficiency (GHD) benefit from GH therapy, though the dose required is often more than that needed for GHD due to other causes, because of the presence of concomitant GH resistance. For the same reason, the growth velocity attained after GH administration in these children is lower than that seen in children with primary GHD. The decision to use GH therapy in a patient with proven GHD would depend on the patient's age, pubertal status, comorbidities, cost-benefit ratio, and risk of adverse events. Certainly, in girls with a bone age of > 14 years, and boys with a bone age of > 16 years, when the long bone epiphysis is expected to be fused, GH therapy should not be started.
- Children with GH deficiency are likely to have other pituitary hormone deficiencies as well and should be screened for the same.
- Children started on GH therapy need to be monitored for response to therapy by assessing the growth velocity, change in predicted adult height as well as any adverse events of the therapy. They should therefore be under regular endocrine follow-up [12].

Pubertal disturbances

Regular clinical assessment of growth and sexual maturity rating (SMR) will identify children with any deviation from the normal course of puberty.

Assessment

- Documentation of pubertal staging.
- Rule out any systemic comorbidities which contribute to pubertal delay.
- Bone age assessment: usually delayed in adolescents with pubertal disturbances. It is also useful for the prediction of the remaining growth potential and final adult height of these patients.
- Pelvic ultrasound helps in assessing ovarian and uterine maturation.
- Hormonal evaluation:
  - LH, FSH, estradiol/testosterone (helps evaluate if puberty has started, and in adolescents with delayed puberty, differentiates hypogonadotropic hypogonadism from less frequently encountered hypergonadotrophic hypogonadism as a cause of pubertal disturbance).
  - Thyroid function test, to exclude associated hypothyroidism, a common cause of delayed puberty.
  - Evaluation of the GH-IGF-1 axis may be required in presence of significant short stature and delayed bone age.

Management

- Patients with normal onset of puberty just need regular monitoring to ascertain that the development continues at the usual pace.
- Some patients require priming with low-dose sex steroids for a period of a few months. This jump-starts puberty in these patients, which then progresses normally. The therapy comprises Ethinyl estradiol 2.5-5 µg daily for 4-6 months in girls. Boys are given 50-100 mg of testosterone esters intra-muscularly once a month for the same duration. The supplementation is then stopped and patients are observed over the next 6 months. Progressive breast enlargement in girls and attainment of testicular volume >8-10 ml indicates spontaneous progression of puberty. These patients are then just monitored for further progression.
- Adolescents with no spontaneous onset of pubertal development, those who fail to undergo spontaneous pubertal development after withdrawal of priming dose of sex steroids, or those with pubertal arrest at any stage after initiation of development, require incremental hormonal therapy mimicking normal physiology of puberty. This involves incremental administration of gonadotropins or/and sex steroids and is best carried out under the supervision of an endocrinologist [2,9,10].

Deranged carbohydrate metabolism

- Deranged carbohydrate metabolism manifesting as diabetes mellitus (fasting plasma glucose >126 mg/dl, or 2-hour post-prandial plasma glucose >200 mg/dl), impaired fasting glucose (IFG, fasting plasma glucose between 100-125 mg/dl), and impaired glucose tolerance (IGT, 2 hours post-prandial plasma glucose between 140-199 mg/dl). Patients have insulin resistance in the initial stages, followed by beta cell exhaustion and insulin depletion later on.
- Patients with impaired fasting glucose/impaired glucose tolerance need intensive iron chelation therapy with a combination of deferoxamine, deferiprone, and /or deferasirox. This may lead to an improvement in glucose intolerance.
- In these patients, dietary modification (judicious selection of complex carbohydrates and proteins with moderate restriction of fat) and exercise also play an important role.
- Oral hypoglycemic drugs including metformin, glibenclamide, and acarbose are used as the next step of management and help achieve glycemic control in children with established diabetes in addition to the above measures.
- Insulin therapy is required when all other measures fail and insulin deficiency develops.
- Patients with deranged carbohydrate metabolism need regular monitoring, responsive modification, and dose titration of their medications.
- HbA1c levels are unsuitable for monitoring long-term glycemic control. Patients with diabetes require self-monitoring of blood glucose along with dietary modifications, regular exercise, and regular follow-up with an endocrinologist [13,14].

Hypoparathyroidism

Hypoparathyroidism is detected either as a part of routine biochemical screening or by the presence of clinical features of hypocalcemia including paresthesias, tetany, seizures, or rarely, cardiac failure. Serum calcium, phosphate, and alkaline phosphatase (ALP) should be performed every 6 months after 10 years of age. Serum 25(OH) cholecalciferol (vitamin D) and parathormone (PTH) levels should be performed in the presence of hypocalcemia. Timely detection and management are important since apart from contributing to poor bone health, hypoparathyroidism-induced hypocalcemia can adversely impact cardiac functioning.

Assessment

- Hypoparathyroidism is suspected based on the presence of hypocalcemia and hyperphosphatemia on routine screening, or the occurrence of symptomatic hypocalcemia

- Measure Serum PTH levels in suspected patients. The presence of low/normal PTH in presence of hypocalcemia and hyperphosphatemia confirms hypoparathyroidism. Special care needs to be taken while collecting samples for PTH assessment. Collect blood in a cold heparinized vacutainer, cold centrifuge immediately, separate plasma, and keep in deep freeze till assessment. Alternatively, the sample collected in a cold vacutainer needs to be transported immediately to the laboratory over ice, where it is processed in an above-described way. Failure to use the correct procedure for sample collection can lead to an erroneous PTH result.

Management

- Active form of vitamin D (calcitriol) is administered in a dose of 15-20 ng/kg/day to a maximum of 1.5 µg/day, in two divided doses daily, to maintain serum calcium in the low normal range (7.2-8 mg/dl) and patient symptom-free. Intake of daily RDA of calcium (500 mg during childhood, 800-1000 mg during adolescence) should be ensured through diet or supplementation.
- Patients require regular monitoring of serum calcium and urinary calcium creatinine ratio for optimizing treatment outcomes and preventing hypercalciuria and resultant nephrocalcinosis [3,15].

Adrenal insufficiency

Clinical adrenal insufficiency is infrequently encountered, and evidence of "biochemical" deficiency is frequently obtained. Morning (8 AM) serum cortisol with ACTH stimulation test is performed annually beyond the age of 10 years. A morning (8 AM) cortisol level of less than 5 µg/dL is suggestive of adrenal insufficiency, while that above 18 µg/dL excludes it. Patients with levels between 5-18 µg/dL should be evaluated using the ACTH stimulation test. A post-ACTH cortisol level > 18-20 µg/dL excludes adrenal insufficiency.

Management

- Asymptomatic adolescents with biochemical evidence of adrenal insufficiency require only steroid supplementation during stressful episodes (the equivalent of 100 mg/m2/day of hydrocortisone during serious sickness and before surgery, 30-50 mg/m2/day during mild-moderate stress).
- The occasional patient with symptomatic adrenal insufficiency requires daily replacement with oral steroids [3,16].

Hypothyroidism

Hypothyroidism, is usually, primarily due to direct iron toxicity to the thyroid gland. It is often subclinical (normal fT4, mildly elevated TSH in the range of 5-10 mU/L) for a variable period before overt hypothyroidism (low fT4, elevated

TSH >10 mU/L) may set in some patients. Central hypothyroidism (low ft4, low/inappropriately normal TSH) is much less frequent.

Patients > 5 years of age should be screened by fT4/TSH annually.

Patients with subclinical hypothyroidism require intensified chelation therapy and 4-6 monthly follow-up. Those with overt hypothyroidism need treatment with an age-appropriate dose of L-thyroxine [2,3,17].

Table 1: Protocol to screen for endocrinopathies in children with TDT

Issues	Assessment	Age at which screening should commence	Frequency of testing
Poor growth	Height and weight monitoring 6-monthly throughout childhood	Since childhood	3 monthly
Delayed/arrested puberty	Assessment of linear growth and pubertal progress using Tanner Sexual Maturity Rating (SMR) staging 6-monthly.	10 years	3 monthly
Deranged carbohydrate metabolism	Annual oral glucose tolerance test (OGTT)	10 years	Annually
Poor bone health and Hypoparathyroidism	Serum calcium, phosphate, and alkaline phosphatase (ALP) Vitamin 25(OH)D and PTH levels in hypocalcemia Dual-energy X-ray absorptiometry (DXA) or quantitative computerized tomography (QCT) [18,19]	10 years	6-monthly Every 2 years#
Hypothyroidism	Free thyroxine (FT4)/ thyroid stimulating hormone (TSH) annually	5 years	Annually
Adrenal insufficiency	Morning (8 AM) serum cortisol with ACTH stimulation annually	10 years	Annually

* Any of these tests should be performed earlier also if there is evidence of growth faltering on a well-maintained growth chart or other clinical indications.

or in cases with fragility fractures

Recommendations

- Growth of all children with TDT should be monitored 6 monthly and plotted on the IAP growth chart (Level of Evidence: 2).
- Following children need evaluation for growth failure and should be referred for detailed evaluation: (Level of Evidence: 2):
- Children manifesting growth faltering (progressive decline in height centile) on routine growth monitoring
- Height below 3rd centile on IAP growth chart
- Expected height below the target range of 6-7 cm below the mid-parental height
- All children with TDT should undergo an annual endocrine screening starting at 10 years of age (Level of Evidence: 2).
- Children exhibiting pubertal delay/arrest, or derangements in one or more of the screening tests should be referred for endocrine evaluation (Level of Evidence: 5).
- Normalisation of total body iron load with very intensive combined chelation (Deferoxamine plus deferiprone) may reverse some endocrine complications of TM (Level of Evidence: 3).
- Patients with emerging/established endocrine involvement should be under regular care by an endocrinologist (Level of Evidence: 5).

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11 Bone Disease in Thalassemia

Sirisha Rani Siddaiahgari, Santanu Sen

With the advances in the medical management of thalassemia, newer complications are being recognized. Thalassemia bone disease (TBD) has become an important cause of morbidity in patients with thalassemia. Patients develop low bone mass and an increased risk of fractures despite adequate transfusions and chelation [1-3].

Spectrum of bone disease in thalassemia

Bone changes in thalassemia were first described by Cooley, et al. when they described characteristic facial changes of frontal and maxillary prominence, the depressed nasal bridge with mongoloid appearance. A wide array of bony changes are described in thalassemia including osteopenia, osteoporosis, fracture, pain, intervertebral disc disease, and bone deformities. Osteoporosis is seen in 40 to 50% of patients with transfusion dependent thalassemia (TDT) and osteopenia in another 45%, virtually affecting all patients [4]. Nearly one-fourth (22%) of patients with non-transfusion dependent thalassemia (NTDT) in the OPTIMAL CARE study reported osteoporosis [1].

Osteoporosis seen in thalassemia is unique, as it is seen at a younger age, with a higher frequency in males as compared to the general population. Male sex, hypogonadism, and chelation with desferrioxamine (DFO) were associated with a greater decline in BMD [4,5]. Fractures can be seen in approximately 40% of patients with TDT, usually in mid-adulthood. The upper limbs are the commonest site of fracture [6].

Pain has been documented at a higher frequency in patients with TDT as compared to the general population Nearly, one-third of patients complained of arthralgias probably secondary to iron overload and chelation. Low back pain is reported commonly as a result of osteoporosis, compression fractures, and/or intervertebral disc degeneration [7,8].

Definitions

Osteoporosis: As per the WHO international reference, standard diagnosis of osteoporosis can be made at bone mineral density (BMD) T-score < -2.5 or less at the femoral neck, lumbar spine, or total hip in postmenopausal women and

men ≥ 50 years of age. The reference standard from which the T-score is calculated is a Caucasian female between the age group of 20-29 years [9].

In the pediatric age group, osteoporosis cannot be defined based on densitometric data alone. The presence of one or more vertebral compression (crush) fractures in the absence of trauma or local disease is suggestive of osteoporosis. Osteoporosis can also be diagnosed in the presence of BMD Z-score ≤ -2.0 with a clinically significant fracture, such as ≥ 2 long bone fractures by age 10 years or ≥ 3 long bone fractures at any age up to age 19 years [10].

"Low bone mineral mass or bone mineral density" is preferred instead of osteopenia if BMD Z-scores are ≤ -2.0 SD. The preferred sites for pediatric dual-energy X-ray absorptiometry (DEXA) scans are the posterior-anterior (PA) spine and total body less head (TBLH) [9]. Femur is an unsuitable site for DEXA scan in children less than 15 years of age.

Pathophysiology

The pathophysiology of thalassemia bone disease has not been elucidated completely. It appears to be multifactorial (Figure 1) [7,11–13].

1. Chronic anemia and ineffective erythropoiesis lead to increased erythroid precursors in the marrow, leading to bone marrow expansion and mechanical interruption of bone formation, cortical thinning, and bone distortion resulting in increased fragility of bone. The trabecular bone in the lumbar spine is severely affected. Tissue hypoxia leads to increased erythropoietin and decreased levels of hepcidin leading to increased bone resorption.
2. Iron overload: Iron overload has a deleterious effect on bone formation by toxic effects on osteoblasts and decreased recruitment of mesenchymal stem cells. It further increases bone resorption by increasing intracellular oxidative stress in osteoclasts. Iron incorporation in the calcium hydroxyapatite also reduces the tensile strength of the bone.
3. Genetic polymorphisms in various genes such as Vitamin D receptors (VDR - BsmI and FokI polymorphisms), collagen type Ia1 (COLIa1), and transforming growth factor β (TGF-β) have been associated with an increased risk of osteoporosis.
4. Thalassemia-related comorbidities: Various co-morbidities associated with thalassemia like chronic hepatitis C, liver disease, and endocrinopathies like diabetes, hypothyroidism, hypoparathyroidism, are associated with lower BMD. Hypogonadotropic hypogonadism and delayed puberty result in poor bone mineralization and an inability to achieve peak bone mass. Adults with deficiency of GH/IGF-1 also have lower BMD at the lumbar spine.

5. Effect of iron chelators: Iron chelation may have a detrimental effect on the skeletal health of patients with TDT. DFO inhibits DNA synthesis, osteoblast and fibroblast proliferation, and collagen synthesis. Osteochondrodysplastic lesions in the long bones and platyspondylosis of the spine have been documented with high doses of DFO in young children [14,15]. This leads to shorter trunk length and hence upper to lower segment ratio is regularly monitored for patients while on DFO. Deferiprone has been associated with bony dysplasia and impaired growth of ulnar epiphysis, metaphysis, and diaphyses. Subchondral flattening of femoral and tibial condyles with irregular articular margins on knee radiographs have also been reported. [16,17]. Deferasirox has been associated with increased urine calcium creatinine ratio in a dose-dependent manner, hence, there is a need to monitor patients on DFX for urolithiasis, renal impairment and osteoporosis [18].
6. Nutritional status and physical activity: Deficiency of nutrients like Vitamin D, C, K, and zinc has been documented in patients with thalassemia. These may have an impact on BMD in association with disease-related morbidities. Reduced physical activity due to complications or parental anxiety increases bone resorption by enhancing the action of osteoclasts and reduction in osteoblast activity [4,7,11].

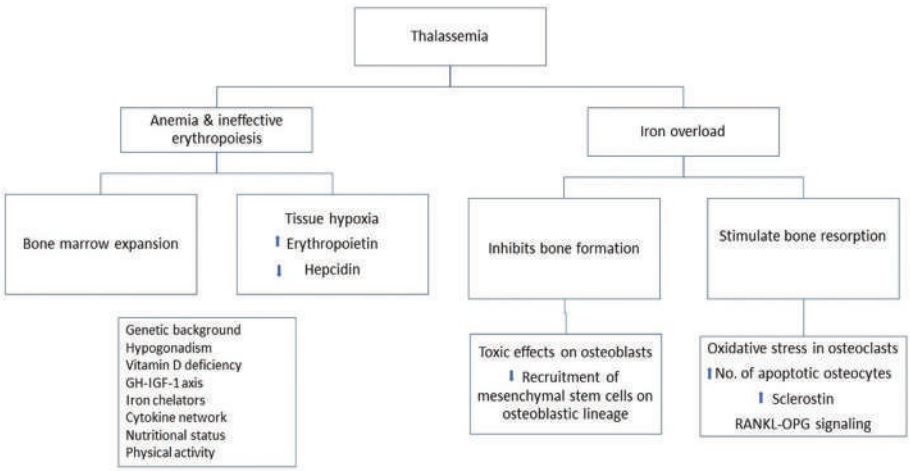
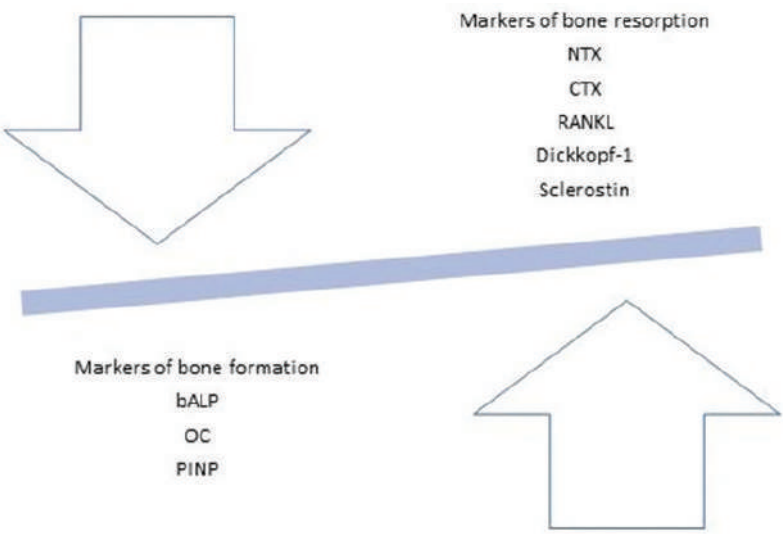


Figure 1. Pathophysiology of bone disease in thalassemia

Diagnosis

Biochemical

Patients with TBD have elevated levels of markers of bone resorption which correlate with the BMD in the lumbar spine. Increased levels of inhibitors of osteoblasts like Wnt signaling inhibitor dickkopf-1 and sclerostin correlate with BMD levels as well (Figure 2).



bALP: bone-specific alkaline phosphatase; OC: osteocalcin; PINP: Procollagen I Intact N-terminal; NTX: N-terminal cross-linking telopeptide of collagen type-I; CTX: C-terminal cross-linking telopeptide of collagen type-I; RANKL, receptor–activator of nuclear factor kappa B ligand.

Figure 2. Markers of bone turnover

Annual assessment of serum calcium, phosphorus, bALP (bone alkaline phosphatase), vitamin D levels, parathormone (PTH), and urinary excretion of calcium and phosphorus should begin from 10 years of age [19,20]. Markers of bone turnover that can be useful in clinical practice are shown in Figure 2. However, these are not easily available.

Radiological

a. A DEXA scan for assessment of bone mineral density is recommended for all patients with thalassemia every 2 years from the age of 10 years. DEXA is currently the gold standard investigation to assess bone health due to its low cost and low radiation exposure (2-5 mrem). DEXA is measured at the lumbar spine and femoral neck in adults, however, there is no consensus regarding which of these is a better site. The femur neck appears to be better as there is a lower degree of bone deformity at the femur neck and a lack of soft tissue interference. DEXA underestimates BMD in patients

with short stature and spinal abnormalities like scoliosis. For each 1 SD decrease in BMD Z-scores at the spine and femoral neck, the risk of fracture increases by 37% and 47% respectively [21].

b. Vertebral fracture assessment: A lateral spine X-ray is utilized for the diagnosis of vertebral fracture with the help of the Genant visual semiquantitative scale [10,11].

c. Trabecular bone score (TBS): TBS is a useful tool to assess bone quality, especially in a diabetic population. A higher proportion of patients with vertebral deformities have a lower TBS [22].

d. Peripheral quantitative computed tomography (pQCT): An axial QCT is a three-dimensional method that provides volumetric BMD of the spine and femur. There is emerging data that pQCT can predict bone disease and fracture risk better than DXA scans, however, the same does not apply to iron-overloaded patients [23,24].

Medical treatment of osteoporosis

Monitoring of serial BMD along with clinical assessment of fracture risk, and bone turnover markers can help determine the need for treatment. Management includes the following:

1. Management of thalassemia and its comorbidities

Regular adequate blood transfusions and optimal iron chelation can reduce the negative effect of anemia and iron overload on bone formation. Hypogonadotropic hypogonadism and delayed puberty lead to osteopenia and osteoporosis in thalassemia [4,5]. Continuous hormonal supplementation with transdermal oestrogenfor females and human chorionic gonadotrophin in males is recommended to prevent bone disease [11].

2. Vitamins and minerals

Regular calcium and vitamin D supplementation are recommended for all patients with thalassemia. A systematic review of the effect of vitamin D supplementation failed to show an improvement in BMD. Correction of zinc deficiency has shown improvement in BMD and glucose homeostasis in patients with thalassemia [7,25,26].

3. Agents reducing bone resorption

a. Bisphosphonates have been evaluated in patients with thalassemia extensively and have been shown to improve BMD substantially, however, their efficacy in preventing fractures has not been established. Hence, the

indication for using these drugs in thalassemia should be carefully evaluated in light of the need for lifelong treatment and adverse effects [12,25].

Bisphosphonates reduce bone resorption by inhibiting osteoclastic induction, proliferation, differentiation, and survival. The various doses and frequencies of bisphosphonates studied are mentioned in Table 1 [20,27,28].

All bisphosphonates except for clodronate improve BMD (nearly 50% at the lumbar spine and 40% at other sites). Zoledronate and alendronate have been more effective compared to neridronate in improving BMD in thalassemia [29]. A dose of 4 mg administered every 3 months has a higher incidence of adverse effects including flu-like reactions, bone pain, and hypocalcemic tetany in Indian patients [30]. Hence, it is recommended to optimise calcium levels before starting bisphosphonates. There are case reports of atypical hip fractures and osteonecrosis of the jaw with the use of bisphosphonates in patients with thalassemia. Patients should get a dental clearance before initiating bisphosphonates and no dental procedure should be attempted while on the drug to prevent osteonecrosis of the jaw. Bisphosphonates should be stopped at least 6 months prior, if pregnancy is being planned. There is no consensus regarding the adequate duration of treatment with bisphosphonates. As bisphosphonates lead to an improvement of BMD for up to 24 months post-discontinuation, it is recommended to stop the drug after 2 years of continuous use.

Table 1. Dose and frequency of administration of bisphosphonates in thalassemia

Bisphosphonate	Dose, Route	Frequency
Alendronate	70 mg, oral (sometimes intravenous)	Weekly
Pamidronate	60 mg, intravenous	Monthly
Zoledronic acid	1 mg, intravenous infusion	3 months/6 months/ annually
	4 mg, infusion infusion	3 months/6 months/ annually
Neridronate	100 mg, intravenous or intramuscular	3 months

b. **Denosumab:** An increase in RANKL/OPG ratio is implicated in the pathogenesis of TBD. Denosumab, an anti-RANKL antibody decreases bone resorption by inhibiting RANK binding to RANKL and decreasing noggin levels [31]. Noggin inhibits bone morphogenic proteins thus, decreasing bone formation. Denosumab has been approved for the management of osteoporosis in patients older than 18 years of age. Treatment with denosumab at a dose of 60 mg subcutaneously every 6 months increased BMD at the femur neck and lumbar spine by 6.0% and 9.2% respectively. The most common adverse effects were mild pain, nausea, and hypocalcemia. There is a possible relation between the use of denosumab and an increased risk of upper respiratory tract, and urinary and ear infections, which may be concerning in splenectomised patients. A "rebound phenomenon" with substantial BMD loss after treatment is described. Treatment with bisphosphonates in the follow-up period is recommended to prevent loss in BMD [32,33].

All patients on treatment with anti-bone resorptive drugs should receive regular calcium (1000 mg/day) and Vitamin D (800 IU) per day. Regular monitoring of urinary calcium is recommended to prevent nephrolithiasis [20].

4. Osteoanabolic drugs

a. Teriparatide, an osteoanabolic drug has been used in postmenopausal women, steroid-induced osteoporosis, and various inherited disorders associated with increased risk of fracture [25,27]. Teriparatide improves bone formation by direct action on osteoblasts, increasing sclerostin levels, and through the Wnt signaling pathway. In a case series of 11 patients with TDT, using teriparatide for over 18 months significantly improved BMD at LS and hip with no new fracture [34]. Nearly, two-third of these patients had previously been treated with bisphosphonates. Teriparatide has a superior anti-fracture efficacy as compared to bisphosphonates. Muscle and bone pain was experienced by 45% of patients and led to the discontinuation of the drug. It may worsen hypercalciuria in thalassemia.

b. Parathyroid hormone-related protein (PTHrP) analog (abaloparatide) and Sclerostin inhibitor (Romosozumab) are other osteoanabolic drugs that have been shown to improve BMD, however, data in patients with thalassemia are lacking and trials are needed [12,25,27].

Figure 3 outlines the management of osteoporosis in patients with thalassemia.

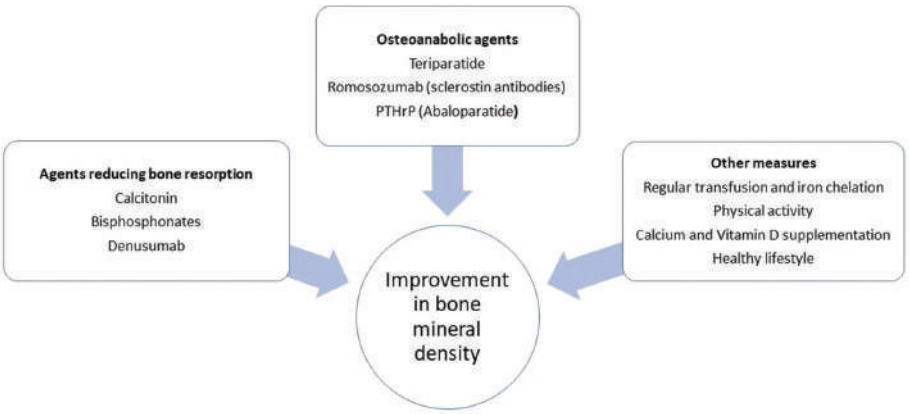


Figure 3. Management of osteoporosis in thalassemia

Prevention of early bone loss

Prevention of thalassemia bone disease begins early in childhood. Inhibition of ineffective erythropoiesis and consequent bone marrow hyperplasia by regular blood transfusion is important. Regular monitoring of iron overload along with iron chelator toxicity can prevent secondary bone changes. A healthy diet with regular physical activity, exposure to sunlight, calcium, and vitamin D supplementation, and avoidance of smoking and drinking alcohol is recommended. Early recognition and management of co-morbidities like hypogonadism, hypothyroidism, and diabetes are required due to the deleterious effects of these on BMD.

Recommendations

1. Patients with thalassemia suffer from low bone density and are at increased risk of fractures (Level of Evidence: 2).
2. Early detection and management of thalassemia-related complications can prevent the development of bone disease (Level of Evidence: 2).
3. Iron overload and iron chelation both contribute to skeletal disease. Regular monitoring of iron overload and toxicities of iron chelators is required (Level of Evidence: 2).
4. Annual assessment of bone health is recommended from the age of 10 years (Level of Evidence: 1).
5. Serum calcium, phosphorus, bALP, vitamin D, and PTH levels are recommended annually (Level of Evidence: 2).
6. Assessment of bone mineral density by DEXA scan is the gold standard for assessing bone health (Level of Evidence: 1).

7. Regular calcium and vitamin D supplementation is recommended for patients with thalassemia (Level of Evidence: 5).
8. Bisphosphonates are the preferred agents for the management of osteoporosis in thalassemia patients (Level of Evidence: 1).

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12 Fertility and Pregnancy in Thalassemia

Puneet R Arora, Avantika Sharma, Jyoti Pandey

Advances in the management of thalassemia syndromes have significantly improved quality of life and life expectancy in these patients. Fertility, a key aspect of quality of life is an important aspiration now for many. While fertility may be affected in many (50 to 80%), spontaneous pregnancies in well-transfused and well-chelated patients are known [1].

Subfertility in both genders is generally caused by hypogonadotrophic hypogonadism (HH), secondary to iron overload due to regular blood transfusions. Excessive iron deposits in the endocrine organs cause oxidative stress and dysfunction of the hypothalamic–pituitary-gonadal (HPG) axis leading to infertility/subfertility in those with transfusion-dependent thalassemia (TDT). Splenectomy further accelerates iron loading in the pituitary gland [2]. The onset of puberty and normal menstrual pattern in females and the onset of puberty in males is generally an indicator of least fertility affection. The management of subfertility in both TDT and non-transfusion dependent thalassemia (NTDT) is similar, with minor differences depending upon the degree of suppression of the hypothalamic-pituitary-gonadal (HPG) axis.

Patients who have undergone bone marrow transplantation for TDT may develop hypogonadism and infertility due to the myeloablative conditioning.

There are three broad reasons for fertility issues in thalassemia patients [3-5]:

1. Hypothalamic-pituitary-gonadal axis dysfunction.
2. Direct effect of iron on gonads leading to primary ovarian and testicular failure.
3. Effects of iron overload on organs such as the liver resulting in abnormal metabolism of steroid hormones.

Fertility in females with thalassemia

Women with TDT have a high chance of having a low ovarian reserve (leading to delayed puberty – no pubertal development by 13 years of age or hypogonadism – no breast development by 16 years of age), premature ovarian aging and hence leading to premature ovarian insufficiency (POI) and infertility.

Ovarian reserve is defined as the capacity of ovaries to produce eggs capable of fertilization, resulting in a healthy and successful pregnancy. Low ovarian

reserve is considered predictive of low chances of spontaneous pregnancy and poor ovarian response to ovarian stimulation [3,5].

Assessment of fertility in females with TDT can be done by [6-10]

- Measurement of anti-mullerian hormone (AMH). AMH is the earliest and most sensitive marker of change with age and has very little intercycle and intracycle variability. It has emerged as an important biomarker for the assessment of reproductive capacity.
- Measurement of pituitary hormones: Follicle-Stimulating hormone (FSH) and Luteinizing Hormone (LH). The levels of gonadotropins (LH and FSH) are inversely correlated with pituitary iron load, and directly correlated with anterior pituitary volume, especially in TDT.
- Ultrasound scan measures ovarian reserve indirectly by antral follicular count (AFC). The decline in ovarian reserve is directly related to the decline in the number of AFC. Low gonadotropin secretion in women with TDT results in the reduced ovarian antral follicular count and ovarian volume.

Management of subfertility in females

Nearly 80% of reproductive function impairment is due to hypothalamic-hypogonadism indicating that fertility is usually salvageable. In women with hypogonadism, oral ovulation induction medications (clomiphene, letrozole, etc) are of limited value. Pulsatile gonadotropin-releasing hormone (GnRH) infusion is only possible at the early stage of HPG-axis damage. Induction of folliculogenesis (ovulation induction) with gonadotropins (FSH, LH) directly is associated with successful pregnancy outcomes. Ovulation induction should be very carefully planned under the supervision of reproductive medicine specialists as the use of gonadotropins can often induce the growth of two or more follicles with the risk of twin or triplet pregnancy and/ or may result in ovarian hyperstimulation syndrome [5-7].

Serial follicular growth tracking ultrasound is usually commenced between days 12 and 14 of the menstrual cycle and is repeated as per women's response. When the dominant follicle is around 18 mm in diameter, an injection of 5000 IU of human chorionic gonadotropin (HCG) is given by subcutaneous/intramuscular route to trigger ovulation, and the couple is advised to have sexual intercourse within 36 hours or use assisted reproduction in the form of intra uterine insemination (IUI). If the couple fails to achieve pregnancy within 3-6 cycles of ovulation induction, additional factors responsible for infertility should be assessed such as male factors, tubal factors, or other ovarian factors such as endometriosis [3,7].

In TDT patients, associated complications such as diabetes mellitus and hypothyroidism can further contribute to infertility and complicate pregnancy

outcomes. Hence, reiteration of the role of pre-pregnancy counseling is very important, so that pregnancy is managed with standardized multi-specialty care, making it safe both for the mother and fetus [11,12].

Fertility in males with thalassemia

Adult males with TDT have a high chance of premature testicular failure [leading to delayed puberty defined as no pubertal development till 14 years of age or testicular volume of less than 4 mls at age of 16 years (absent puberty)] and infertility. Nearly 50% of patients may have oligospermia and asthenospermia along with abnormal sperm quality [13,14]. The major factors implicated in infertility include

- Pituitary iron overload leading to hypogonadotropic hypogonadism and subfertility
- Oxidative stress caused by labile plasma iron (LPI)

Iron deposition in the anterior pituitary is initially asymptomatic. Increasing iron overload leads to decreased gonadotropin reserve and decreases in spontaneous pulsatile gonadotropin activity. Low pituitary volumes have been associated with low LH levels and lower sperm content [14,15]. The increased generation of reactive oxygen species in presence of LPI results in damage to the sperm membrane, nucleus, and proteins thus impairing sperm quality. The normal defense mechanisms against oxidative stress like glutathione, carnitine, folate, zinc, and selenium may be decreased in patients with thalassemia. Studies on the biochemical parameters of seminal plasma have shown high iron content associated with low glutathione and zinc levels in seminal plasma despite normal plasma levels [16-19].

Adequate ways to measure testicular function are [14,20,21]

1. Assessment of semen parameters: sperm count, motility, and morphology
2. Evaluation of integrity of pituitary-gonadal axis: FSH, LH, Testosterone (Free).
3. Testicular volume assessment by ultrasonography.

Management of subfertility in males

Adult males desirous of having a child should have a detailed discussion with a fertility specialist regarding the fertility options available. Induction of spermatogenesis can be attempted under direct supervision; however, it is more challenging than inducing folliculogenesis in females with a success rate of 10-15%. The treatment process can be demanding and can sometimes take 2 years, and this should be emphasized before starting any such treatment [13,14,20,21]. The protocol for induction of spermatogenesis is shown in Figure 1.

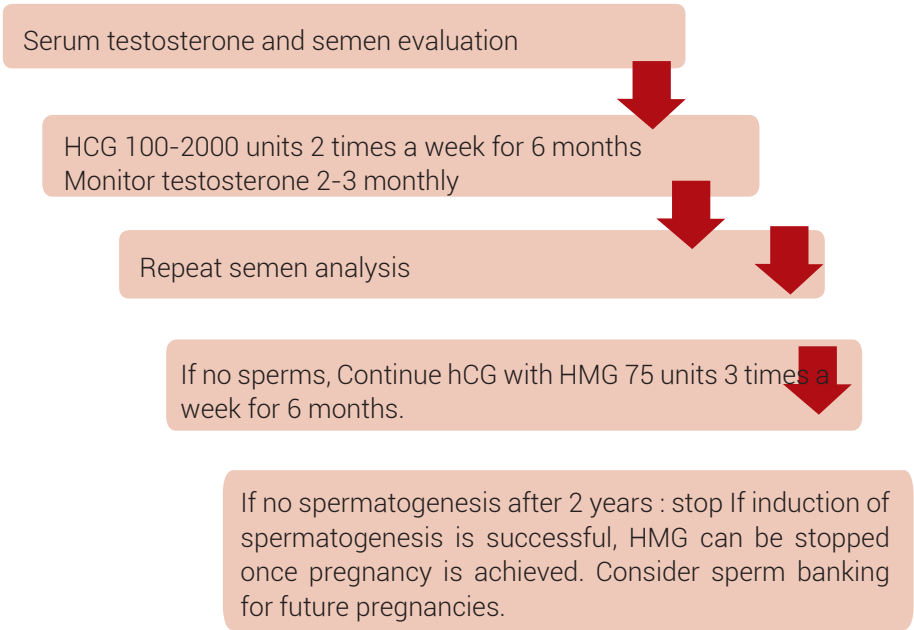

Figure 1. Induction of spermatogenesis

Assisted reproduction techniques like surgical extraction of sperms (Testicular sperm extraction, TESE) and intracytoplasmic sperm injection (ICSI) should be discussed as they have been associated with improved conception rates in such patients with a suboptimal semen analysis. Recent understanding of sperm DNA damage in males with thalassemia brings concerns about mutagenic risk in these individuals, especially after ICSI where natural protective barrier agents against gamete selection during fertilization are lost, but this is still under research [22].

Pre-Pregnancy Counseling and Management options

An important aspect of fertility management is to PLAN pregnancy after discussion with the treating doctor and a fertility specialist, whether spontaneous or assisted conception (ART) pregnancies. Pregnancies in patients with transfusion-dependent thalassemia can be high risk for both the mother and the baby. Proper pre-pregnancy counseling by a multidisciplinary team with a hematologist, reproductive medicine specialist, cardiologist, and obstetrician in conjunction with an endocrinologist should be a standard protocol. The counseling should involve both partners. It should include the following aspects:

1. Eligibility for planning a pregnancy (Table 1).
2. Reviewing the medications and planning a switch over to safer medication for pregnancy.
3. Discussing the risks associated with fertility treatment and pregnancy while optimizing complications.

Eligibility Evaluation for planning a pregnancy

Patients should be assessed specifically for cardiac function, and liver function and screened for infections to rule out the risk of vertical transmission to the fetus [5,23].

An increase in cardiac load is expected during normal pregnancy by at least 25 to 30% due to increased heart rate and stroke volume and this may put an extra burden on thalassemia patients. Cardiac assessment by echocardiography, electrocardiogram, 24-hour Holter monitoring to rule out any rhythm disorders, and T2* cardiac MRI to assess cardiac iron overload is important before planning pregnancy. Liver function should be evaluated for iron overload by biochemical tests and also by imaging techniques such as MRI [11,12,24].

All patients should be screened for viral infections like Human immunodeficiency virus (HIV), hepatitis B (HBV), hepatitis C (HCV), and rubella. Bone health dual-energy x-ray absorptiometry (DXA) scanning of the hip and spine should also be considered as a part of planning a pregnancy and should be done prior to conception.. All women should have folate, calcium and vitamin D supplementation before considering pregnancy. Screening should be performed for diabetes, thyroid dysfunction and alloimmunization (with regular monitoring of titres of acquired red-cell antibodies) [11,12,23,24].

Assessment of the partner for hemoglobinopathy, followed by genetic counseling is mandatory prior to starting any pregnancy treatment. Assessment of fertility as per approved guidelines should be done once pre-pregnancy checks are safe to proceed with the pregnancy.

Table 1: Eligibility evaluation test before planning pregnancy [11,12,23,24]

Parameter	Recommended investigations
Cardiac function	ECG, 24-hour holter monitoring, echocardiogram
Liver	Liver function tests and ultrasound to look for liver echotexture and gallstones, T2* MRI of the liver
Iron overload	Serum ferritin, T2* MRI for assessment of cardiac and liver iron overload

Parameter	Recommended investigations
Thyroid function	FT4/TSH
Pancreatic assessment	Glucose tolerance test and diabetic control, monitoring with serum fructosamine level, if feasible and available (to be maintained <300nmol/L)
Bone health	Serum Calcium, Phosphorus, Vitamin D, DXA scan
Infection Screening	HBV (Hepatitis B Vaccine in women with HbsAg negative status), HIV, Rubella. Consider screening for TORCH infections, if applicable
Assessment of Pre transfusion Haemoglobin	Hb to be maintained >10 g/dL
Assessment of clotting factors (only in patients with a history of thrombosis/ recurrent fetal loss)	Prothrombin time with INR, thrombophilia panel (if indicated), doppler studies, if applicable
Alloimmunization	Screening for acquired red-cell antibodies (rule out the risk of hemolytic disease of the fetus and newborn)
Risk of transmission of thalassemia to the fetus	Check partner for hemoglobinopathy Genetic counseling is to be provided in all cases
Vaccine prophylaxis	In women with history of splenectomy, ensure conjugated meningococcal C vaccine, H. influenza B vaccine and Pneumococcal vaccine have been given as per the recommendations
Antibiotic prophylaxis	In splenectomised patients, penicillin prophylaxis should be given before any surgical procedure, and erythromycin to be used if allergic to penicillin.

Review of medications

A review of medications is an important part of pre-pregnancy planning and counseling. The most important aspect is to check on iron overload status and chelation therapy. Patients on oral chelators Deferasirox or Deferiprone (DFX or DFP) should be advised to switch to Desferrioxamine (DFO) 3 months prior to conception. Bisphosphonates are absolutely contraindicated during pregnancy and if taken, should be stopped at least three months before planning pregnancy. Medications like interferon, ribavirin, and hydroxycarbamide (hydroxyurea) must be completely discontinued for at least six months before any pregnancy treatment. Supplementation with folic acid (5 mg daily), calcium, and vitamin D needs to be given regularly. Thyroid functions must be well controlled. Adequate thyroid supplementation and periodic monitoring of thyroid function tests is necessary [5,12,23].

The hemoglobinopathy status of the partner and, accordingly genetic counseling should be done. Pre-implantation genetic testing (PGT) and pre-implantation genetic diagnosis (PGD) should be discussed with the couple. If the partner is heterozygous, then molecular tests for hemoglobinopathy should be considered to screen embryos resulting from in-vitro fertilization (IVF) [25]. If both partners are homozygous for thalassemia, the use of donor gametes (either donor eggs or donor sperm) depending on consent from the couple should be preferred or adoption should be offered as an option.

Preservation of fertility in patients of thalassemia

Adequate transfusions and chelation remain the key to the preservation of fertility in individuals with thalassemia. Proper monitoring for early detection of the risk of losing fertility provides an opportunity to intensify treatment and improve the prospects of preserving fertility. In the case of minors, parents should be counselled and educated about the benefits and implications of fertility preservation strategies [1,13,14,21].

Various techniques available for fertility preservation are:

1. Sperm freezing (in affected males)
2. Oocyte freezing, Ovarian tissue cryopreservation (in affected females)
3. Embryo freezing (in case of married couples)

Multi-disciplinary approach with the involvement of a dedicated reproductive medicine specialist (fertility specialist) can result in good reproductive outcomes [26,27].

Management of pregnancy in thalassemia

Pregnancy in women with thalassemia is considered high risk, however, an increasing number of women with thalassemia are now giving birth to normal healthy babies. Fetal complications include Intrauterine growth retardation (IUGR), low birth weight (LBW), prematurity, and multiple gestations. The rate of multiple gestations is about 1.6 to 18.9% due to the use of assisted reproductive techniques [26,27].

There is an increase in metabolic demands and blood volume during pregnancy, resulting in changes in the cardiovascular system like myocardial hypertrophy, chamber enlargements, and multivalvular regurgitation. These physiological changes coupled with a low cardiac reserve and absence of chelation during early pregnancy can result in worsening cardiac function. Ideally, a pre-pregnancy T2*MRI > 20 ms and LIC < 7 mg/g/dry weight of liver should be achieved by intensive chelation in women desirous of childbirth [21]. Pregnancy is associated with a higher risk of thrombosis due to increased coagulation factors, reduced fibrinolytic activity, and protein S, along with reduced venous flow velocity. The risk of thrombosis is even higher in splenectomised patients with NTDT, necessitating anticoagulation therapy. Patients with diabetes should be monitored with serum fructosamine and levels < 300 nmol/L must be maintained for at least 3 months before conception. The folic acid should be administered in the dose of 5 mg/day to reduce the risk of neural tube defects [11,12,23,24].

Management during the antepartum period

Pregnant women with TDT/NTDT should undergo monthly antenatal check up till 28 weeks of gestation and then every 15 days till delivery. Adequate transfusions to maintain pretransfusion Hb >10 g/dL helps prevent fetal IUGR [28]. Women with NTDT may need transfusions during pregnancy if anemia develops (Hb < 8 g/dL) or fetal growth is compromised. If transfusions are initiated, a pre-transfusion Hb must be maintained > 10 g/dL. Before embarking on transfusions, a screen for antibodies should be performed and the patient should ideally receive Rh and Kell-matched transfusions to prevent the formation of alloantibodies [11,12,23,24,29,30].

The use of iron chelation in pregnancy remains controversial. Oral chelators are not considered safe in pregnancy due to the risk of teratogenicity. However, there are reports of women who have conceived while on DFX and delivered a healthy baby [31]. The drug was stopped as soon as the pregnancy was recognized. DFO as a subcutaneous infusion can be used in 2nd and 3rd trimesters, if the benefits outweigh the risks involved such as in patients with cardiac iron overload, cardiac dysfunction, or an increase in transfusion requirement with rising ferritin. DFO being a large molecule does not cross the placenta and has not been associated with any fetal anomalies. Cardiac evaluation should be done with ECG and echocardiogram during each trimester

[11,12,23,24]. Liver function tests and thyroid function tests should be performed in every trimester. Women with thalassemia are at a higher risk of developing gestational diabetes and should be screened with a glucose tolerance test at 16 weeks and again at 28 weeks, if evaluation at 16 weeks is negative. Patients with thalassemia are at high risk of thromboembolism. Patients with a history of splenectomy and a high platelet count should be given thromboprophylaxis with low-dose aspirin or low molecular weight heparin (LMWH) in consultation with experts. Splenectomised patients should continue penicillin prophylaxis due to the increased risk of infections. Erythromycin should be used in women allergic to penicillin. Fetal ultrasound should be performed between 7 & 9 weeks, and 11 & 14 weeks, and a second-trimester anomaly scan at 18 & 20 weeks. Fetal growth should be monitored with biometric scans every 4 weeks from 24 weeks onwards.

Management during labour

There are no specific guidelines for the timing of delivery in patients with TDT and it should be guided by the obstetric team, as per the national policy. Nearly 80% of women with TDT undergo caesarean section due to cephalopelvic disproportion with short stature and skeletal abnormalities in thalassemia. Active management of 3rd stage of labour is recommended in case a vaginal delivery is planned to reduce blood loss. Regional anesthesia is preferred over general anesthesia due to maxillofacial abnormalities resulting in difficult intubation in these patients. Patients may have reduced vertebral body height and scoliosis due to severe osteoporosis. The placement of the spinal/epidural catheter must be carefully planned. Continuous electronic intrapartum fetal monitoring is recommended to reduce the risk of fetal hypoxia. Intravenous DFO infusion 2 g/24 hours is recommended during the period of delivery to reduce the risk of cardiac decompensation and arrhythmias due to free radical damage in presence of high serum concentrations of non-transferrin-bound iron . Two units of cross-matched packed red blood cells must be arranged before delivery to avoid any delay in transfusion due to alloantibodies [11,12,23,24,29,30].

Management during the postpartum period

Women with thalassemia may be at high risk of thrombosis even during the postpartum period. There are no clear-cut guidelines regarding thromboprophylaxis currently [29,30]. Patients who are considered to be at high risk of thrombosis such as NTDT, splenectomised, or history of recurrent abortions should be administered LMWH for 7 days, in case of vaginal delivery and for 6 weeks after a caesarean section. Antibiotic prophylaxis should be initiated in splenectomised patients [11,12,23,24].

Mothers should be encouraged to breastfeed their babies. They should be counselled regarding the risk of transmission of infections in case of HIV, Hepatitis C, and Hepatitis B. Women who wish to breastfeed their babies should

continue DFO infusion. DFO is secreted in the breastmilk but not absorbed by the baby, hence, considered safe during breastfeeding. The safety of oral iron chelators during breastfeeding has not been established [11,12,23,24]. Intake of bisphosphonates during breastfeeding is not recommended.

A pre-discharge cardiology review must be obtained due to the risk of postpartum cardiac complications [29,32]. Discharge advice must also include contraception advice. Intrauterine devices and estrogen-containing pills are not recommended due to the risk of infections and thrombosis, respectively. Barrier contraception and progestin-only pills are preferred in this subset of patients [12,23,24].

Recommendations

1. The testicular function in patients with TDT/NTDT can be tested by assessment of semen parameters, testicular size and hormonal evaluation (Level of Evidence 1).
2. Ovarian reserve can be tested by measurement of the anti-mullerian hormone, pituitary hormones, and antral follicle count in adult females with TDT/NTDT (Level of Evidence 1).
3. Patients with thalassemia should undergo a thorough evaluation and counseling before embarking on any fertility treatment (Level of Evidence: 1).
4. Gonadotropins can be used for the induction of spermatogenesis or folliculogenesis under the supervision of a fertility specialist (Level of Evidence: 1).
5. Pregnancy in thalassemia is considered high-risk and should be managed in consultation with a hematologist, preferably, at a center that has experience in managing such pregnancies (Level of Evidence: 2).
6. Pregnancy in women with thalassemia can be safe for mothers and babies, provided that intensive treatment has been started well in time with adequate transfusions and chelation, women are receiving adequate folic acid (5mg/d) in the periconceptional period and during pregnancy, if the women have a normal resting cardiac performance and if her blood sugar and thyroid functions are controlled (Level of Evidence: 2).
7. Pregnant women with thalassemia (TDT or NTDT) should undergo monthly antenatal check up till 28 weeks of gestation and then every 15 days till delivery (Level of Evidence: 4).
8. Fetal growth should be monitored with biometric scans every 4 weeks from 24 weeks onwards (Level of Evidence: 3).
9. During pregnancy, pre-transfusion Hb must be maintained >10g/dl (Level of Evidence: 4).

10. Pregnant women with thalassemia have increased predisposition to cardiac decompensation and cardiac evaluation should be done with ECG and echocardiogram during each trimester (Level of Evidence: 2).
11. If chelation is needed during pregnancy in view of cardiac iron overload, cardiac dysfunction, or an increase in transfusion requirement with rising ferritin, Desferrioxamine may be used as a subcutaneous infusion in the second or third trimester, if the benefits outweigh the risks involved (Level of Evidence: 2).
12. If chelation is needed during pregnancy or lactation, desferrioxamine may be used as safety of oral chelators has not been established (Level of Evidence: 4). Intravenous desferrioxamine infusion 2 g/24 hours is recommended during delivery to reduce the risk of cardiac decompensation and arrhythmias due to free radical damage in presence of high serum concentrations of non-transferrin-bound iron (Level of Evidence: 4).
13. Pregnant women with thalassemia with a history of splenectomy and a high platelet count should be given thromboprophylaxis with low-dose aspirin/ LMWH in consultation with experts (Level of Evidence: 4).
14. Barrier contraception and progestin-only pills are recommended for contraception considering that these do not have prothrombotic effect (Level of Evidence: 4).

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13 Cardiac Complications and their Monitoring in Thalassemia

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Cardiac complications are the leading cause of morbidity and mortality in patients with transfusion dependent thalassemia (TDT). A recent meta-analysis showed cardiovascular disease has an overall prevalence of 42% and accounts for 71% of deaths in β-thalassemia major [1]. Cardiovascular complications can be broadly grouped into two main clinical categories [2-4]:

Iron overload complications

(which may be reversible with intensive chelation)

A) **Cardiomyopathy:** Either left ventricular (LV) systolic dysfunction or LV diastolic dysfunction (restrictive cardiomyopathy)

B) **Arrhythmias** include bradyarrhythmia such as heart block and tachyarrhythmia such as atrial fibrillation, flutter or ventricular arrhythmias

C) **Arterial changes:** Loss of vascular compliance

Non-iron overload complications

A) **High output failure** due to chronic anemia

B) **Pulmonary hypertension** especially in cases of non-transfusion dependent thalassemia (NTDT)

C) **Arrhythmias** especially atrial arrhythmias later in life

D) **Thromboembolic complications** are higher in splenectomized patients and those with non-transfusion dependent thalassemia (NTDT). Deep vein thrombosis, pulmonary embolism, portal vein thrombosis and stroke (also linked to atrial fibrillation) have been reported in these patients.

E) **Cardiac dysfunction** due to co-morbidities like endocrinopathies

F) **Arterial changes** due to loss of vascular compliance

G) **Pericarditis/myocarditis** also have been reported rarely

The clinical significance of having these categories lies in the fact that iron overload complications even if severe, may be reversible to a great extent with intensive chelation therapy, before symptomatic heart failure develops; once symptomatic heart failure develops, there is a high immediate risk of death. Prevention of iron overload should be the primary aim. Prevention of iron overload in early life also has been shown to prevent some of the "non-iron

overload" complications in later life such as atrial fibrillation.

Associated factors to be considered in cardiac complications

A wide spectrum of nutritional deficiencies are common in thalassemia and cardiac function has been shown to worsen in presence of deficiencies of carnitine, thiamine, vitamin D and selenium [5]. Endocrinopathy associated with iron overload like hypothyroidism, hypoparathyroidism, growth hormone deficiency, adrenal insufficiency and hypogonadism can also exacerbate cardiac dysfunction [6].

Clinical clues for cardiac involvement

Patients with significant iron overload may remain symptom-free initially. Early detection of cardiac iron overload on regular monitoring with cardiac T2* MRI, is thus of paramount importance. All patients should be evaluated clinically for symptoms and signs of cardiac iron overload and dysfunction. Subtle early signs may be confused with symptoms of anemia such as exercise intolerance, breathlessness and fatigue. This may make the clinical diagnosis of iron overload difficult in patients with TDT. Special attention should be paid to the following:

1. Any difficulty in breathing such as dyspnea on exertion/orthopnea/pedal oedema should be considered a red flag sign for underlying cardiac dysfunction.
2. Tender hepatomegaly: If the patient presents with pain in the right hypochondrium with tender hepatomegaly due to liver congestion, evaluation for cardiac failure is necessary.
3. Anyone with difficulty in tolerating regular transfusion (usual volume and speed of transfusion) should also undergo evaluation for heart failure.
4. Patients with arrhythmias may present with palpitations, giddiness or syncope.
5. Chest pain may be a presentation of pericarditis /myocarditis.

In advanced stages, patient may present with classical signs and symptoms of heart failure. Clinical features of left heart failure include dyspnea on exertion, orthopnea, and crepitations on auscultation, while right heart failure would present with neck vein distension, hepatomegaly and peripheral edema. The development of these classical signs and symptoms of heart failure denotes poor prognosis.

Cardiac Evaluation and Frequency of Monitoring

Cardiac evaluation should include an electrocardiogram (ECG), echocardiography and cardiac T2* MRI.

1. ECG helps in identifying arrhythmias. Morphology of P wave and QRS complexes, QT intervals, QT dispersion (to pick up early predisposition for sudden cardiac death) should be noted. Any abnormality in ECG requires referral to a cardiologist.
2. Echocardiography is the most practical way of regular long-term monitoring of chamber size, systolic and diastolic function as well as pulmonary artery hypertension (PAH). However, there can be an inter-observer variability in measurements.

   Worsening of diastolic or systolic function on serial echocardiography could indicate possible cardiac iron overload. "Stress echocardiography" is helpful to unmask subclinical disease where the ejection fraction fails to rise, or even falls, in response to exertion or simulated exercise using intravenous dobutamine [7].

   Strain echocardiography is useful for quantifying myocardial deformation and should be used wherever available. This detects subclinical myocardial damage (but is not specific to iron overload). Global longitudinal strain (GLS) is reduced in clinically silent thalassemia patients at the initial stages. However, there is no correlation between cardiac T2*MRI and GLS assessed by echocardiography. It may be suggested that intensive correction of anemia and strict treatment of viral infections should be done along with close follow-up in clinically silent thalassemia patients with reduced GLS and normal cardiac T2*MRI [3,8].

   An echocardiographic evaluation should include the following parameters as shown in Figure 1. A separate record book to be maintained for thalassemia echocardiography records [4]. Basal ECHO dimensions in thalassemia are different than normal population; e.g. The lower limit and the mean for left ventricular ejection fraction (LVEF) are higher in TDT patients compared to normal population [6].

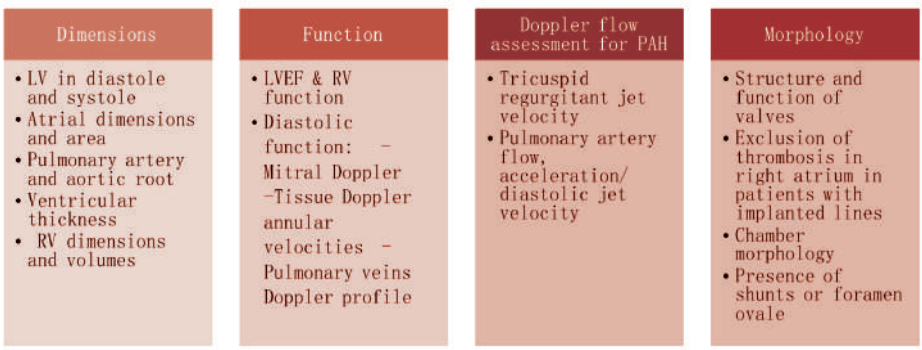

Figure 1. Echocardiographic parameters to be recorded in thalassemia patients

Echocardiography is recommended annually after 8 years of age and 6 to 12 monthly thereafter, if any of the following is present [2,6]:

- Cardiac T2*MRI <20 ms (millisecond)
- New onset symptoms / abnormal ECG finding / Abnormal Echocardiogram

In the presence of cardiac disease, the frequency and type of assessment should be tailored according to the patient's needs (e.g. 3 or 6 monthly). Refer to Chapter 9 for details.

Besides diagnosing cardiac dysfunction or failure, echocardiography may be suggestive of pulmonary arterial hypertension (PAH). In this case, the further plan should be made in consultation with the cardiologist and would include:

- Right heart catheterization for evaluation of pulmonary artery hypertension when tricuspid regurgitation (TR) velocity is high (>3 m/s, despite optimal transfusion therapy).
- Pulmonary function tests, CT pulmonary angiography or lung perfusion scanning for complete diagnostic evaluation of PAH.

3. Cardiac T2*MRI (CMR T2*): CMR is the best modality to quantitatively measure myocardial iron with good reproducibility [2-4,6,8]. CMR T2* assesses the time taken for the decay of the myocardial signal by 63% and is measured in milliseconds (ms). CMR T2* value in the interventricular septum has been shown to be highly representative of the mean total cardiac iron concentration. It should be performed on 1.5 Tesla MRI machines as T2* values on 3 Tesla machines are shorter and chances for artefacts are greater [6]. The lower the T2*value, the more is the myocardial iron. CMR T2* is recommended after the age of 8 years and repeated every 2 years if T2* >20 ms, annually if T2* 10 – 20 ms and every 6 months if T2* <10 ms [2,6].

   The probability of reduced LVEF is higher as the cardiac T2* value falls. It is considered a reliable predictor of heart failure. Patients with CMR T2* value < 10 ms are at high risk of developing cardiac decompensation and those with cardiac T2*< 6 ms have a 50% chance of developing heart failure within 1 year. This warrants aggressive chelation therapy.

4. Holter (24 hours ECG) recording: Additional testing includes Holter, which is a standard method for detecting and investigating cardiac arrhythmias. It is indicated in patients with a history of giddiness, syncope, palpitations, new onset ECG changes in P wave morphology etc.

5. Cardiac biomarkers including cardiac troponins (e.g., in suspected myocarditis) or natriuretic peptides like pro-BNP (Brain Natriuretic Peptide) are also useful for the evaluation of patients with known or suspected heart failure.

Management of Cardiac Complications in Thalassemia

As discussed in the previous section, the factors associated with heart failure in TDT include:

- High serum ferritin (>2500 ng/ml); although a lowered ferritin level does not guarantee freedom from heart failure [1-3]
- High iron overload in liver [4]
- Shorter cardiac T2*MRI value
- Reduced LVEF: The restrictive LV filling pattern may predict death from heart failure.

There are differences in the treatment of heart failure between those with and without underlying thalassemia. In the case of reversible toxic cardiomyopathy, the treatment is mainly directed at the removal of iron, rather than improvement in myocardial performance. Additionally, important co-morbidities need to be considered in the case of thalassemia. Hypoparathyoidism, hypothyroidism and growth hormone deficiency exacerbate iron overload cardiomyopathy [8]. Besides, all patients in heart failure with endocrinopathies should be assumed to have adrenal insufficiency and administered hydrocortisone. Hypogonadotropic hypogonadism a common endocrinopathy associated with low sex steroids may exacerbate symptoms of heart failure. Diabetes mellitus needs to be strictly controlled in all those with acute and chronic heart failure. Levels of thiamine, vitamin B6, folate, fat-soluble vitamins and trace elements zinc, copper, and selenium should be looked for in cases with cardiac failure. Carnitine replacement therapy should be considered as this has been associated with clinical improvement.

There is considerable overlap in the presentation of heart failure and myocarditis. Typical changes of myocarditis like chest pain, diffuse ST-T–wave changes or increased cardiac enzyme levels are not commonly seen with iron-induced cardiomyopathy.

Treatment of acute decompensated heart failure with a reduced ejection fraction

The aim of treatment is to support the patient till intensified chelation therapy can reduce cardiac iron overload. Bedside echocardiography and hemodynamic monitoring are a must in the management of decompensated heart failure. Immediate commencement of 24-hour continuous (uninterrupted) intravenous iron chelation treatment with desferrioxamine and deferiprone as indicated should be started. For further details refer to chapter 6.

Supportive hemodynamic therapy and management of cardiac arrhythmias should be done in consultation with the cardiologist in an ICU setting. Blood pressure is typically low in thalassemia patients and should not guide specific therapy. Maintain cerebral and renal perfusion; avoid aggressive inotropic

therapy, which can be detrimental [1,2]. Only minimum diuretic treatment (mostly infusions rather than bolus) should be used because of the importance of maintaining preload [1].

In case of arrhythmias, maintain electrolytes, especially magnesium to stabilize ventricular arrhythmias. Specific antiarrhythmics are to be decided in consultation with a cardiologist.

Supportive therapy in acute heart failure includes

- Give hydrocortisone on the presumption of inadequate adrenal response to stress [6].
- Check thyroid, liver, renal function and other metabolites like glucose, calcium, magnesium, vitamin D and carnitine and correct these when necessary [1].
- Maintain Hb between 10 and 12 g/dL (frequent small-volume transfusions) [1-3].
- Search for precipitating conditions such as infections.

Specific anti-failure medications like ACE inhibitors or β-blockers to be started in consultation with a cardiologist. As cardiac storage iron is removed very slowly even with intensive iron chelation, treatment will be needed for many years. The treatment is monitored by clinical status, LVEF, T2*MRI and serum ferritin trends.

Treatment of myocardial iron overload without cardiac decompensation

The strategy for managing iron overload without cardiac decompensation is as follows

1. T2*MRI <6 ms: Chelation strategy would be similar to overt heart failure (maximal chelation). IV desferrioxamine given as continuous drip along with deferiprone and amlodipine, if there are no contraindications [1-3].
2. T2*MRI between 6 to 10 ms: Chelation therapy to be intensified, but not necessarily to maximal chelation. The dose of present chelators may be increased, new chelators added if possible and add desferrioxamine as subcutaneous infusion, if the patient is already on maximum dose of the oral chelators [1-3]. Subclinical LV dysfunction or mild LV dysfunction may also need intensive chelation.
3. T2*MRI between 10-20 ms: Ensure compliance, check the dose or change in chelator.

   Longitudinal trends in T2*MRI are important as mentioned previously and medicines are to be titrated as per the values and clinical features of the patient. Ensure compliance with medications at every stage.

Cardiac iron chelation strategies

Desferrioxamine (DFO), deferasirox (DFX) and deferiprone (DFP) all are known to remove cardiac iron. DFP monotherapy offers superior cardiac protection and improves survival compared with routine DFO therapy [9,10]. DFO monotherapy either as a subcutaneous infusion or continuous intravenous infusion is helpful. Intermittent subcutaneous infusions have been known to reduce cardiac iron at a rate of 1.1% to 2.2% per month [1,6], whereas continuous DFO clears cardiac iron at nearly 5% per month. In the case of continuous iv infusion, poor compliance and risks related to IV access like thrombophlebitis and sepsis need to be addressed. DFX monotherapy can be used successfully in patients with detectable cardiac iron and normal cardiac function. It should be cautiously used to treat cardiac siderosis in patients with high liver iron loading. Combination chelation therapy is recommended in moderate to severe cardiac iron overload or when LVEF is impaired as it offers improved outcomes without additional toxicity issues in severe cardiac iron loading compared with the use of DFO alone. Intensified chelation is recommended in patients with myocardial iron loading who have reduced LVEF or a consistent trend over time with several measurements toward abnormality by CMR T2* or echocardiography [4]. DFP combined with DFO (both given daily together) is commonly prescribed and is recommended in severe cardiac siderosis.

Treatment of arrhythmias

These patients are divided into 2 groups based on hemodynamic compromise

1. Treatment of arrhythmias with hemodynamic compromise

In patients with arrhythmias and cardiac decompensation, continuous IV DFO infusion is recommended. For specific antiarrhythmics, a cardiologist should be consulted. Electrolyte deficiencies should be identified. Serum potassium should be maintained at >4.5 mmol/L and normal magnesium level ensured. Associated endocrine abnormalities, such as diabetes mellitus, hypoparathyroidism, and thyroid disorders should be identified and treated appropriately. In hemodynamically compromised patients, defibrillators may be required.

2. Treatment of arrhythmias in ambulatory patients – mostly atrial fibrillation

The same principles as in non-thalassemic patients are followed and treatment should be guided in consultation with a cardiologist. The risk of stroke is higher in thalassemic patients by virtue of the documented prothrombotic tendencies (as compared to non-thalassemic patients). Permanent anticoagulation would need to be considered at an early stage for all but the mildly affected group, who experience infrequent, short-lasting bouts of AF.

Recommendations

- Cardiac health should be monitored with annual ECG, echocardiography and T2* MRI in TDT patients starting from 8 years onwards (Level of Evidence: 2).
- In TDT patients with cardiac dysfunction, hemoglobin should be maintained at 10-12 g/dL (Level of Evidence: 2).
- Deferiprone monotherapy offers superior cardiac protection and improves survival compared with routine desferrioxamine therapy (Level of Evidence: 1).
- Deferasirox monotherapy can be used successfully in patients with detectable cardiac iron and normal cardiac function. It should be cautiously used to treat cardiac siderosis in patients with high liver iron loading. (Level of Evidence: 2)
- It is recommended to intensify chelation in patients with myocardial iron loading who have reduced LVEF or a consistent trend over time with several measurements toward abnormality by CMR T2* or echocardiography (Level of Evidence:1).
- TDT patients with decompensated acute heart failure with reduced ejection fraction should be managed in consultation with a cardiologist using beta-blockers and ACE inhibitors (Level of Evidence: 2).
- TDT patients with decompensated acute heart failure should be given intravenous hydrocortisone on the presumption of inadequate adrenal response to stress and should also be evaluated for other endocrinopathies like hypothyroidism, hypoparathyroidism (Level of Evidence: 4).
- TDT patients with decompensated acute heart failure should receive intensified chelation therapy with 24-hour continuous (uninterrupted) intravenous iron chelation treatment with desferrioxamine and deferiprone (Level of Evidence: 2).

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14 Liver in Thalassemia

Purva Kanvinde, ATK Rau

Thalassemia syndrome is the most frequent hemoglobinopathy encountered in pediatric clinical practice in India. Besides the various deleterious clinical manifestations of chronic anemia that are invariably associated, the disorder also affects the functioning of many organ systems in the body which in turn contribute to the significant morbidity and occasional mortality. The liver is one of the common target organs affected in thalassemia and while it is compromised very early in the course of the disease, its large size and significant reserves preclude the appearance of the signs and symptoms of its dysfunction usually until the second decade of life. Symptomatic liver disease in thalassemia can however occur earlier if there is underlying chronic liver infection and other co-morbidities [1].

Pathogenesis of hepatic injury in thalassemia

Chronic liver disease has now emerged as an entity of immense clinical significance when managing a child with thalassemia. Figure 1 depicts the mechanisms of liver damage in thalassemia. Of these, iron overload is a major contributor. Pathophysiology of iron overload is shown in Figure 2.

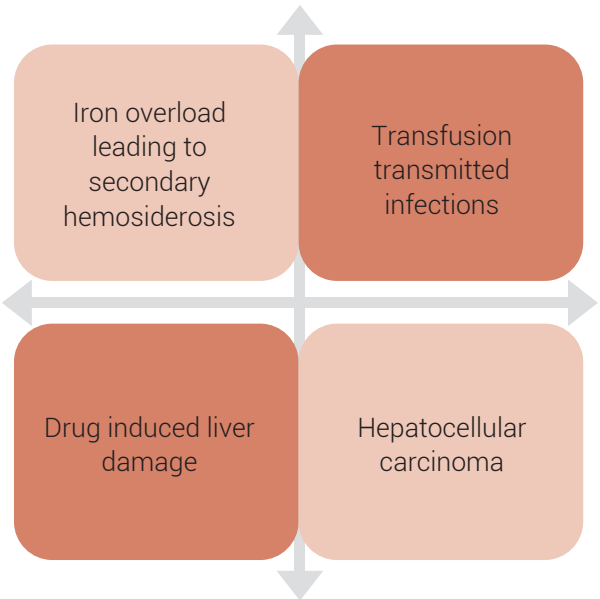

Fig. 1: Various mechanisms for liver damage in thalassemia

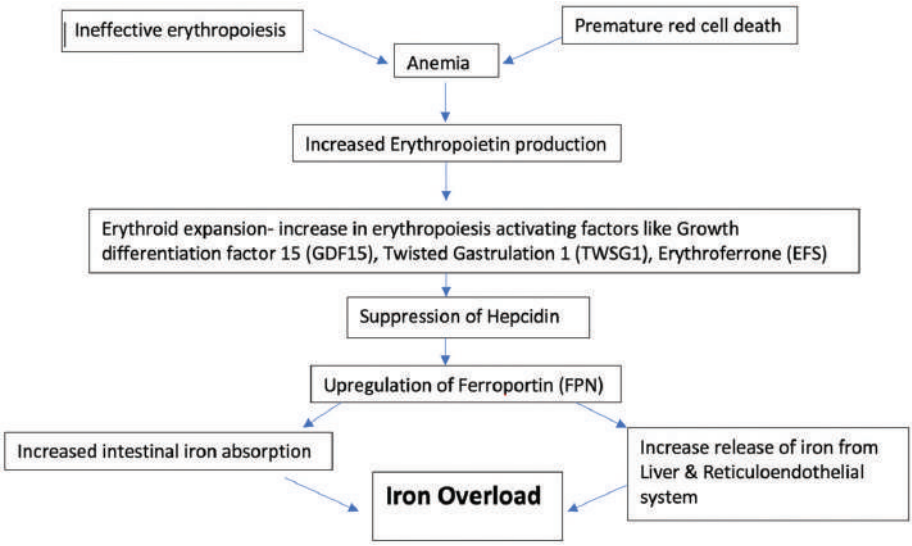

Fig. 2: Pathophysiology of iron overload

Adapted from Maria Sposi N. Oxidative Stress and Iron Overload in β-Thalassemia: An Overview. Beta Thalassemia [Internet]. 2020 Sep 23; Available from: http://dx.doi.org/10.5772/intechopen.90492.

Iron exists in nature (in plants) in the ferrous (Fe2+) form. On its ingestion, it is converted by the loss of one electron into the ferric (Fe3+) form by the sustained action of gastric acid in the stomach. Thereafter, it is absorbed into the gut mucosal cell (enterocytes) in the first part of the duodenum, with the aid of the divalent metal transporter (DMT) proteins under the influence of hepcidin a hormone produced by the liver, which regulates the transport of iron into the extra- cellular space by acting through the stimulation of ferroportin (a transmembrane protein). The absorbed iron is then transported by transferrin (transport protein) and stored mostly in the liver and partly in the reticuloendothelial cells as inert ferritin. Iron overload occurs in thalassemia due to (a) frequent transfusions, with each ml of packed RBC delivering 1 mg of iron into the body (b) ineffective erythropoiesis leading to early RBC senescence, chronic anemia and tissue hypoxia. This causes an increased production of erythropoietin and suppression of hepcidin which stimulates the upregulation of ferroportin thereby increasing intestinal absorption of iron and c) the non-existence of a natural and efficient Iron removal mechanism in the body.

When the iron binding capacity of plasma transferrin is exceeded, non-transferrin bound iron (NTBI) spills over into the blood. NTBI (free iron) a highly toxic iron species, under normal circumstances is rapidly cleared by the liver through DMT and incorporated into ferritin. When the storage capacity of ferritin is exceeded, NTBI accumulates in the hepatocytes causing severe oxidative stress and the generation of toxic reactive oxygen species which causes intracellular peroxidation of lipids and severe cell protein damage. The damaged liver responds excess NTBI by fibrosis and collagen formation. Progressive deterioration of functional capacity follows which ultimately culminates in cirrhosis. With adequate chelation, clinical symptomatology hardly ever manifests before the second decade of life but once it does, deterioration is rapid. If complicated by transfusion-transmitted chronic hepatic infections, deterioration is further accelerated. In addition, there is a constant risk of developing hepatocellular carcinoma that increases greatly in iron-overloaded patients with concomitant Hepatitis C (HCV) and Hepatitis B Viral (HBV) infections [2].

Liver iron estimation

As this aspect is dealt with in detail in other sections of these guidelines, the following is a brief outline of the matter. Liver iron status in the body is estimated by the following:

1. Blood markers like serum ferritin, serum transferrin and the total iron binding capacity (TIBC)
2. Liver tissue markers like Liver Iron Concentration (LIC) by biopsy, T2* MRI, SQUID
3. Toxicity markers like NTBI or the labile plasma iron (LPI) estimation and liver fibrosis score in seriously compromised patients

Liver biopsy and LIC estimation per gram of dry liver weight is the gold standard for the assessment of iron overload in the liver. However, being an invasive procedure, it is often associated with the risk of serious injury and bleeding. Additionally, a representative sample for analysis may not be forthcoming as liver iron is distributed unevenly in the liver. Further, though the heart is another organ significantly at risk for severe dysfunction in overload states, liver iron levels are noted to have minimal correlation with cardiac iron overload and hence may not reflect total body iron excess. Magnetic resonance imaging (MRI) R2 or R2* assessment of LIC (mg of iron/g dry weight) is the method of choice presently for the monitoring of the iron load in patients with thalassaemia. A 6-month to 2-yearly T2* MRI assessment should be performed in all patients to monitor the effectiveness of chelation therapy [2]. The severity of hepatic iron overload is measured by the hepatic T2* MRI values in Table 1.

Table 1. Grading of hepatic iron overload as assessed by liver biopsy and T2*MRI.

Severity	Dry weight (mg of iron/g dry weight)	Hepatic T2* (ms)
Mild	2-7	4.5-15.4
Moderate	7-15	2.1-4.5
Severe	>15	<2.

Promising results were seen in a few studies using transient elastography (TEG) for the assessment of liver fibrosis in thalassemia but the results of ongoing studies focusing on its relevance in children are still awaited to make it the standard of care at present [2].

Treatment of hepatic iron overload

The aim of chelation therapy is to maintain the serum ferritin levels at around 1000 µg/L, thereby delaying the progression towards cirrhosis. Deferasirox (DFX) is the chelator of choice for the management of the mild to moderately iron-overloaded liver. With a half-life of over 12 - 18 hours, it is bioavailable in the body for extended periods of time. It chelates both cardiac and hepatic iron and is also effective in extreme levels of overload in emergent situations. DFX treatment for extended periods (more than 3 years) has also been found to stabilize and in some instances, reverse hepatic fibrosis as seen by serially improving Ishak inflammatory scores. Desferrioxamine (DFO), an extremely effective iron chelator is administered by slow subcutaneous infusion over a protracted period of time as its bio-availability in plasma is limited. However, the need to infuse it with an infusion pump throughout the day in an active school going child is an obvious disadvantage. Deferiprone (DFP), the earliest oral iron chelator, is more effective in reducing cardiac iron than liver iron overload but has a short half–life thereby rendering it inefficient compared to later chelators [2]. Readers are directed to Chapter 6 for a more detailed review on chelation therapy.

Transfusion-transmitted infections (TTIs)

Hepatitis C virus (HCV)

The use of repeated packed cell transfusions as the mainstay of treatment for many decades has resulted in a markedly high incidence of transfusion-transmitted infections in children with thalassemia especially in resource limited countries. HCV was rampant in children with thalassemia in the West till 1992 and in India till 2002 when serological screening of all blood products was made mandatory [3]. Today, most infections occur as a result of transmission during the window period (the duration between contracting the infection by the donor and its detection by standard tests). However, recently, this has been

shortened by the introduction of the Nucleic Acid Amplification Test (NAAT) which is able to identify minute quantities of viral DNA early in the infection. HCV virus exists in six distinct genotypes (1-8) with 86 different subtypes. These genotypes are unequally distributed across the globe and have variable response to different therapeutic regimens. Genotype 3 predominates in India. The overall prevalence of HCV antibodies in thalassemia patients across India is reported as between 5–25 % [3-5].

Laboratory diagnosis of Hepatitis C infection

Serum anti-HCV antibodies by enzyme immunoassay should be done annually in all children with thalassemia receiving therapy. If positive, HCV-RNA by polymerase chain reaction (PCR) testing is carried out to identify patients with heavy viral loads. Genotype determination if available, helps to tailor treatment. In case, it is not accessible or affordable, a pan-genotypic therapeutic regimen can also be initiated with a significant chance of success.

Indications for therapy for HCV infection

All children and adolescents with thalassemia and HCV infection aged 3 years or more, should be administered antiviral therapy, regardless of disease severity.

Endpoint of therapy

Sustained virological response (SVR) is defined as undetectable HCV RNA titres in plasma 12 weeks (SVR12) after completion of treatment. A further assessment at the end 24 weeks (SVR 24) may be needed to confirm the remission [6,7].

Pre-treatment assessment in an HCV positive child

These are mainly tests to assess the severity of liver function compromise and the presence or absence of fibrosis/ cirrhosis which also impacts therapy [8]. These include:

i) Serial liver function tests including serum transaminases.

ii) Assessment of non-invasive biomarkers like serum aspartate transaminase (AST) to platelet ratio index (APRI) and FIB 4 index (calculated as age in years x AST x Platelet count X ALT / 100) which are easily available and simple to use but require further validation in children.

iii) Liver biopsy is the gold standard for the grade and extent of fibrosis and inflammation, however, being invasive, it should be reserved for situations where the aetiology is unclear.

iv) Fibro scan (ultrasound-based liver elastography) is a non-invasive and promising modality to determine the presence or absence of cirrhosis but requires specialised probes to assess children and is not easily available.

v) Hepatitis B screening (HBs Ag) to detect HBV co-infection

Treatment of HCV infection

Directly acting antivirals (DAAs) have an SVR of more than 95% and have now replaced interferons as the mainstay of therapy in children with HCV infection [8]. Co-administration of DAAs with chelation therapy is not contraindicated and does not require any dose adjustments. Prior to starting DAAs, a complete drug history should be taken to check for drug interactions (mainly with anticonvulsants and anti-arrhythmic drugs). The treatment protocol depends on genotype, presence or absence of liver injury (fibrosis/cirrhosis) and prior treatment for HCV, if any. Pan-genotypic combinations also have high SVR rates and can be used when genotyping is unavailable.

Table 2 depicts the current therapeutic options for HCV infection.

Table 2. Recommended direct antiviral agents with dosing in treatment-naïve or interferon exposed children >3 years and adolescents with cirrhosis

Drugs	Duration
Sofosbuvir/Velpatasvir (Weight based dosing)	12 weeks
Glecaprevir/Pibrentasvir	8 weeks
Sofosbuvir/Daclatasvir	12 weeks
Genotype 1,4,5, 6	
Sofosbuvir/ Ledipasvir (Weight based dosing)	12 weeks
Genotype 2,3 with/without cirrhosis	
Sofosbuvir/ Ribavarin	12 weeks (genotype 2)
	24 weeks (genotype 3)

Prevention: Immunization

Vaccination against hepatitis A and B is recommended for all children with thalassemia.

Post-treatment monitoring

1. Estimate HCV RNA levels between 12 and 24 weeks after completion of treatment to establish SVR.
2. Assess serum transaminase levels annually for liver function status.
3. Ultrasound abdomen annually for assessment of liver echotexture and HCC screening.

Table 3 depicts the doses of different DAAs in children and adults with HCV infection.

Table 3. Dosages of Direct Antiviral Agents in children and adults with HCV infection

Body weight	**Dose of Sofosbuvir/Velpatasvir (once daily, oral)**
<17 kg	150 mg/37.5 mg
17- 30 kg	200 mg/50 mg
>30 kg	400 mg/100 mg
Body weight	Dose of Ledipasvir/Sofosbuvir (once daily, oral)
<17 kg	33.75 mg/ 150 mg
17- 35 kg	45 mg/200 mg
>35 kg	90mg/400 mg
Body weight	**Dose of Glecaprevir/Pibrentasvir (once daily, oral)**
<20 kg	150 mg/ 60 mg
20- 30 kg	200 mg/ 80 mg
30- 45 kg	250 mg/120 mg
>45 kg	300 mg/120 mg
Body weight	**Dose of Ribavarin (twice a day)**
<47 kg	15 mg/kg/day orally per day in 2 divided doses
47- 49 kg	600 mg/day
50- 65 kg	800 mg/day
66-80 kg	1000 mg/day

Complications of HCV infection

HCV infection in thalassemia, on a background of iron overload, increases the chances of cirrhosis and is a significant risk factor for the development of hepatocellular carcinoma and fulminant hepatic failure [9].

Hepatitis B

The incidence of HBV infection in thalassemia in India ranges from 0.7 to 1.5 % in various studies. The introduction of the HBV vaccine in the universal

immunization programs of many countries has led to a marked decrease in transmission rates all over the globe but it still remains a major challenge in healthcare programs in low and middle-income countries. The disease occurs in phases as determined by the seropositivity status and chronicity of each stage as shown below in Table 4.

Table 4. Phases of Hepatitis B based on seropositivity and chronicity

	Phases	Characteristics
1.	HbeAg positive chronic infection (Immune tolerant)	Minimal disease
2.	HbeAg positive chronic hepatitis (Immune reactive)	Active inflammation of the liver and markedly elevated liver enzymes
3.	HbeAg negative chronic infection (Immune control)	Natural antibodies clear the virus while the hepatic enzymes return to the baseline
4.	HbeAg negative chronic hepatitis (Immune reactive)	Elevated liver enzymes and chronic inflammation
5.	HbsAg negative disease (Immune control)	Carrier state

Diagnosis of HBV infection [9]

Diagnosis is usually by serological assessment as below:

a) HBV Markers to confirm the presence of infection and its phase

HBs Ag should be done annually in all children with thalassemia. If HBsAg is positive, HBV DNA analysis should be carried out to help decide about the initiation as well as subsequent monitoring of therapy. HBe Ag and Anti HBe antibody titres are useful in determining the phase of the disease. HBV genotype analysis is however not needed during initial evaluation as treatment of all genotypes is uniform in this infection.

b) To evaluate the extent of liver involvement:

Biochemical parameters including increases in serum transaminases (more than four times the upper limit of normal), serum albumin, bilirubin, gamma-glutamyl transpeptidase (GGT) and the coagulation parameters (prothrombin time and INR) above the standard reference range for the particular laboratory must be estimated to assess the extent of liver involvement. Liver biopsy is the gold standard for determining disease activity but being invasive, should be reserved only for situations when the biochemical and HBV markers are inconclusive. Other non-invasive biomarkers such as Transient Elastography need further validation in children.

Indications for starting treatment

a) Increasing viral load as determined by serial quantitative HBV DNA tests or a single HBV DNA viral load of >20,000 IU/ml.

b) Significant inflammation as reflected by rising levels of liver enzymes - more than double the upper limit of normal irrespective of liver histology.

c) Children with cirrhosis irrespective of the presence of HBV DNA copies and ALT.

d) HBe Ag positive patients or HBe Ag negative children with chronic hepatitis B which is suggested by values of HBV DNA more than 2,000 IU/ml, ALT more than 4 times the upper limit of normal and/or at least moderate liver necro-inflammation with or without fibrosis.

Figure 3 depicts the indications for starting treatment for HBV infection.

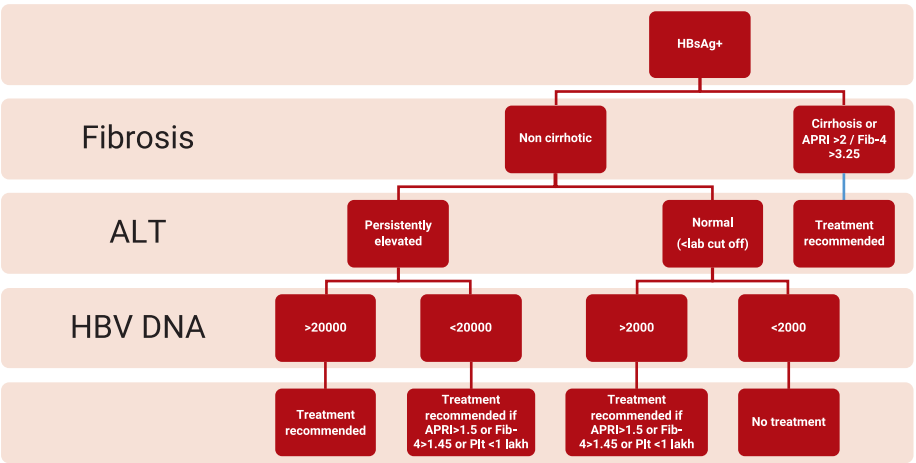

Figure 3. Indications for treatment for hepatitis B

Therapy of HBV infection [10]

The goal of therapy is to reduce the viral load and infectivity as well as prevent further liver injury. The three main classes of antivirals presently in use for the treatment of HBV infection are:

a) Nucleoside analogues like Lamivudine, Telbivudine and Entecavir

b) Nucleotide analogues like Adefovir and Tenefovir

c) Alpha Interferon

In view of their efficacy and minimal side effects, the drugs of choice in the paediatric population for chronic Hepatitis B currently are Entecavir and Tenofovir. Tenofovir disoproxil fumarate (TDF) – (Tenvir®) is approved for children > 2 years by EMA and > 12 years by US FDA. In India, TDF is recommended in children only above 12 years of age while Entecavir may be used in children above 2 years of age. There is no obvious cost advantage with either medication in patients above the age of 12 years. Tenofovir alafenamide (TAF) is approved only in children > 12 years of age. Table 5 shows drugs and their doses for pediatric HBV infection.

Table 5. Drug dosages in pediatric patients

Drug	Pediatric dose	Strength and formulation available
Entecavir	0.015 mg/kg/day once a day	0.5 mg tablet
	Weight-based dosing	Dose
	10-11 kg	0.15 mg
	11-14 kg	0.2 mg
	14-17 kg	0.25 mg
	17- 20 kg	0.3 mg
	20-23 kg	0.35 mg
	23- 26 kg	0.4 mg
	26-30 kg	0.45 mg
	>30 kg	0.5 mg
Tenofovir disporoxil fumarate	10- 35 kg	8 mg/kg/day (max 300 mg)
	≥35 kg	300 mg/day

Treatment of chronic Hepatitis B in the presence of concomitant Hepatitis C infection

Patients fulfilling the standard criteria for HBV treatment should receive treatment with either nucleoside analogues or nucleotide analogues based on availability and cost. Reactivation of HBV may occur while receiving DAAs for HCV treatment therefore HBs Ag positive patients undergoing DAA therapy should be considered for concomitant nucleoside / nucleotide analogue prophylaxis until 12 weeks after completion of DAA treatment. These patients need to be monitored closely for side effects / toxicity of the two-drug combination therapy.

Endpoint of therapy

Long term suppression of HBV DNA titres (HBV DNA titres <10 IU/ml on treatment) is the goal. A sustained response is identified as HBV DNA of less than 2,000 IU/ml for more than 12 months after the end of therapy. Biochemical response in the form of ALT normalisation is an additional endpoint (monitored by ALT every 3 months for at least 1-year post-treatment).

Serological response: The disappearance of HBe Ag and the development of anti-HBe antibodies is the ideal endpoint but this may not happen for a prolonged period of time despite adequate treatment The loss of HBs Ag and development of anti-HBs antibody is also a desirable endpoint which may occur variably with treatment.

Monitoring of disease status

Children with chronic hepatitis B, not meeting the initiation of treatment criteria, should be monitored with ALT and HBV DNA levels every six months. In children undergoing treatment, liver function tests should be performed every 3 months during the first year and every 6 months thereafter. Serum HBV DNA should be determined every 3–4 months during the first year and every 6–12 months thereafter. As with HCV infection, a yearly ultrasound liver scan is recommended for monitoring course of the HBV infection [10].

Immunization

Universal active immunization before exposure to the virus is the most effective way to prevent HBV infection. Gomber, et al. had assessed the anti HBs titres in a study on 85 children with thalassemia major over a defined period [11]. The seroprotection rates in the HBV immunized children after an average duration of 10.8 years of primary immunization was significantly higher when compared with non-immunized healthy controls (72.9% vs 52.9%, P=0.07). Further, the few seronegative HBV immunized children also achieved full sero-protection (anti HBs titre >10 IU/ml) after a single booster dose of HBV vaccine, establishing

that a single booster dose of HBV vaccine 5 years after primary vaccination can provide adequate sero-protection for almost all thalassemia patients. Vaccination against Hepatitis A is also recommended for all children with chronic HBV infection.

Complications of HBV infection in children with thalassemia

HBV infection in thalassemia on a background of iron overload, like HCV, increases the chances of cirrhosis and acts as a significant risk factor for development of Hepatocellular carcinoma and fulminant liver failure in future. Those with persistent active hepatic disease (about 8-10% of all those infected) have an even higher risk of developing cirrhosis and HCC (2-5%) later [9].

Drug induced liver damage

Glucuronidation is the main metabolic pathway for DFX with subsequent biliary excretion. DFX can cause a rise in ALT which commonly occurs within a month of its initiation. Elevations of liver transaminases more than 4 times the upper limit of normal was noted in as much as 6% of patients, leading to its discontinuation in 1-2% of patients on treatment. Cases of severe liver injury and a few fatalities have been reported post initiation of DFX [12,13]. However rapid recovery from liver compromise is also seen on cessation of therapy. The exact mechanism for DFX induced rise in transaminases is as yet unknown. Therefore, it is important to monitor LFT every 2 weeks for the first month of therapy and thereafter once every three months. In case of abnormal liver function tests (LFT) results, DFX should be stopped forthwith and reintroduced later at lower doses and escalated slowly once the LFT returns to normal limits [12].

Hepatocellular carcinoma

The markedly increased survival in children with thalassemia has led to a significant increase in the incidence of HCC as death due to complications from organ involvement and infection have now largely been overcome. Iron overload and viral hepatitis are important risk factors for the development of HCC [9]. Thalassemia patients with high risk for development of HCC include those with:

1. Concurrent HCV and/or HBV infection,
2. LIC of ≥ 5 mg Fe/g dry weight (DW) in NTDT,
3. LIC of ≥ 7 mg Fe/g DW in TDT
4. Serum ferritin of ≥1000 ng/mL, or
5. Advanced cirrhosis.

Management

Screening for HCC

All patients with thalassemia and cirrhosis with other high-risk factors should be screened annually by contrast-enhanced abdominal ultrasound to identify the early changes of HCC. Alpha fetoprotein level estimation is an unreliable test for screening as well as diagnosis in HCC in thalassemia and is used currently only to monitor the course of the illness.

Prevention of HCC

Effective and timely chelation therapy to prevent the development of iron overload and serial screening with timely and appropriate treatment for viral infections (HCV & HBV) are the mainstays of HCC prevention in these children.

Treatment of HCC

Treatment of HCC in thalassemia is similar to that in non-thalassemia patients. Treatment includes surgical resection, chemo-embolization including trans-arterial chemo-embolization (TACE) and percutaneous radiofrequency ablation. Liver transplant should be offered only in selected cases.

Recommendations

1. Magnetic resonance imaging (MRI) R2 or R2* using liver iron content (LIC) (mg of iron/g dry weight) is the method of choice for iron load monitoring in thalassemia (Level of Evidence: 1).
2. Deferasirox is today the chelator of choice for the iron overloaded liver (Level of Evidence: 1).
3. Serum anti-HCV antibodies by enzyme immunoassay need to be done annually (Level of Evidence: 1).
4. All children with HCV aged more than 3 years need to receive DAAs irrespective of the severity of liver involvement (Level of Evidence: 1).
5. HBs Ag should be done annually in all children with thalassemia (Level of Evidence: 1).
6. The drugs of choice for chronic hepatitis B in the pediatric population are currently Entecavir and Tenofovir, depending on the age of the patient (Level of Evidence: 1).
7. Vaccination with hepatitis B vaccine is recommended for all children with thalassemia and negative for HBV infection (Level of Evidence: 1). Vaccination against Hepatitis A is also recommended for children with thalassemia who are seronegative to Hepatitis A (Level of Evidence: 1).
8. Annual ultrasound screening for HCC should be done for all children with thalassemia (Level of Evidence: 1).

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15a Hematopoietic Stem Cell Transplantation for Thalassemia – Part I

Sunil Bhat, Revathi Raj

Referral for HSCT

Recent advances in hematopoietic stem cell transplantation (HSCT) for patients with transfusion-dependent thalassemia (TDT) have improved the outcomes significantly, making it an important curative option for thousands of patients across the globe, including in the developing countries like ours. All families with a child diagnosed with TDT should be offered the option of HSCT. This chapter attempts to address the aspects of HSCT, which should be known to a referring pediatrician/physician, when preparing the family for HSCT. Shared care after HSCT is equally important and the referring pediatrician/physician should be acquainted with the protocol for long-term follow up, including immunizations. The referring pediatrician/physician must identify the right patient for undergoing HSCT. A suitable donor is equally crucial for the optimum outcome in a given patient. Once, these steps are accomplished, the patient and/or family should be explained the steps of HSCT including pre-transplant interventions, if any, pre-transplant work-up, conditioning regimens, infusion of stem cells, immediate post-transplant 2-week phase of supportive care before engraftment, engraftment and immunosuppression to prevent rejection and graft versus host disease (G*v*HD). After the procedure of HSCT, as soon as the patient is stable and discharged, the immediate and long-term follow up ensues.

Identifying the right patient

Age at referral

Hematopoietic stem cell transplantation for TDT can be performed at any age, though the outcomes are significantly better at a younger age (preferably < 7 years) The option of HSCT should be given to every family at the time of initial diagnosis. With increasing age, comes the risk of tissue iron overload, and the liver is the crucial organ in HSCT for thalassemia. The liver processes all the chemotherapy used to prepare the patient to receive new stem cells. Hence, iron overload in the liver can potentially increase the chance of transplant-related morbidity and mortality. The best outcomes from published data are in children aged below 7 years [1]. Infants are more prone to immediate toxicity in the form of mucositis. Hence, they require more supportive care and older patients are more prone to liver toxicity in the form of sinusoidal obstruction syndrome [2]. The challenges increase with advancing age as organ damage is

higher in older children and the mortality rates are higher. Peripubertal children must be referred to with careful consideration as the intensive chemotherapy given for conditioning will have an impact on the progression of puberty.

Chelation history

Since the 1990s, the impact of chelation on HSCT outcomes has been documented. Lucarelli, et al. published the outcomes based on chelation history, and the patients were divided into Classes 1, 2, and 3 based on the impact of chelation [1]. The overall survival and thalassemia-free survivals were 94% and 87%, 84% and 81%, and 50% and 47% in Class 1, 2 and 3, respectively. Potentially hepatotoxic drugs like Busulfan have now been replaced with Treosulfan with limited hepatotoxicity, making HSCT relatively safer for Class 3 patients except for those with a. CMC, Vellore High-Risk Class 3, i.e. children aged > 7 years with hepatomegaly > 5 cm. These children's families need to be counselled about a significantly higher risk during HSCT [3].

Risk Stratification: A Prognostic Scheme Developed by Pesaro Group

The factors that impact prognosis and outcomes in HSCT in thalassemia include hepatomegaly >2 cm, portal fibrosis, and irregular chelation history [1]. On the basis of these risk factors, a prognostic scheme was developed by the Pesaro group, which categorized patients into three risk classes influencing the probability of survival as follows:

1) Class 1: None of these factors
2) Class 2: 1 or 2 of these factors
3) Class 3: All the 3 above factors

Liver and spleen size

The liver reflects the burden of iron overload in a patient with TDT, and hepatomegaly predicts a higher risk of a specific complication called ***sinusoidal obstruction syndrome***. The size of the spleen reflects the inadequacy of transfusion, thereby causing hypersplenism and pancytopenia. In most cases, the size of the spleen is reduced with aggressive chelation and hyper-transfusion before HSCT. The referring physician must counsel families that 6 to 8 weeks of preparation is required for these children before embarking on HSCT. Splenectomy is not a contraindication to HSCT.

Alloimmunization

Red cell alloimmunization is a particular problem during HSCT, and it is advisable to screen children who have issues with cross-matching or are found to be Coomb's test positive. This has to be highlighted during a referral to help the transplant team ensure adequate precautions are taken to keep 4 to 6 units of compatible red cell units ready for HSCT. These children might also have platelet refractoriness and therefore, the hospital needs to arrange additional donors for platelet transfusions during HSCT.

Co-morbidities

Children with iron overload tend to have insulin-dependent diabetes mellitus, hypothyroidism, or subclinical hypoadrenalism. Splenectomy per se is not a contraindication for HSCT, but these children must be monitored more vigilantly for sepsis during HSCT [4]. In children over eight years, referral with a cardiac MRI and ECHO to rule out cardiac and liver iron would help the transplant team decide on the conditioning regimen.

Viral infections

Transfusion-transmitted infections such as hepatitis B, C, or HIV are known to impact HSCT as they affect the liver or increase the risk of infections. However, the newer antiviral agents are safe during HSCT, and these viral infections are not a contraindication for HSCT.

Finding a suitable donor

Donor

HLA typing forms the basis of donor selection. HLA typing of the child, sibling, and parents must be done at the time of initial counseling. Early HLA typing has advantages as it helps the family focus on a potentially curative option. A matched family donor is available only in 30%, and including parents in the typing is essential as about 5% of parents could be fully matched. HLA typing in the laboratory typically can be done as – low resolution, intermediate resolution, and high resolution for various alleles representing the HLA. These include Class I – A, B, and C, and Class II – DRB1 and DQB1 antigens - a total of 10 antigens. The child has one antigen from each parent for A, B, C, DRB1and DQB1. In the case of a sibling donor, low-resolution typing suffices (Example shown in Table 1). For alternate donor transplantation, high-resolution typing to choose the optimal donor is ideal (Example shown in Table 2).

Table 1. Example of low-resolution HLA typing of a fully matched family donor

	HLA-A	HLA-B	HLA-DRB1
Patient	A* 01, A* 02	B* 01, B* 02	BRB1* 01, DRB1* 02
Donor	A* 01, A* 02	B* 01, B* 02	BRB1* 01, DRB1* 02

Table 2. Example of high-resolution HLA typing of a fully matched unrelated donor

	A*	A*	B*	B*	C*	C*	DRB1*	DRB1*	DQB1*	DQB1*
Patient	0101	0202	0101	0202	0101	0202	0101	0202	0101	0202
Donor	0101	0202	0101	0202	0101	0202	0101	0202	0101	0202

An unrelated donor search worldwide in Bone Marrow Donors Worldwide (BMDW) or Indian donor registries (e.g. DATRI) is initiated for patients with no family donor. From identifying a donor to obtaining donor clearance (takes about 6 weeks) to finally performing HSCT, can take variable time. For children with a haploidentical donor, it is essential to perform a test called Donor Specific Antibody screen as antibodies against a half-matched donor result in a high rejection rate during HSCT [5,6].

The donor's age is essential as donor safety requires a minimum weight of over 8 kg for safe anesthesia for harvesting the stem cells. If there is a choice of donors, it is preferable to avoid female donors for male recipients as this mismatch results in a higher incidence of G*v*HD. The blood group difference between the patient and the donor is not a contraindication. In case of blood group incompatibility, the stem cells need to be processed adequately to remove red cells, and plasma and transfusion support during HSCT will need to be planned as per guidelines for ABO-incompatible transplantation. Eventually, the recipient will change to the donor's blood group.

Social aspects

HSCT for thalassemia major is an elective procedure that needs to be planned according to the family's commitments [7,8]. Adequate financial resources, if family opts for private sector hospital, need to be arranged for hospitalization and the prolonged stay of the family near the transplant center. It is best to avoid the rainy season or the peak of the flu season, as viral infections result in significant morbidity and mortality. The donor's requirements also need to be considered, and both parents need to be available.

Counseling Before Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplants have become a widely accepted curative option for children and families with TDT. This brings the challenge and responsibility of providing comprehensive information to the patient and/or the family so that they can make an informed decision. Indian Council for Medical Research (ICMR) has issued recommendations for all donor types in allogeneic HSCT in patients with TDT in India [9].

The important points that should be discussed at length with the patient &/or family are as follows:

Age and status of chelation at the time of transplant: Age and status of chelation along with hepatomegaly primarily decides the risk category (class 1, 2 and 3) before transplant and thereby the outcome [10]. As a simple rule, the lesser the risk category, better is the outcome. HSCT is an elective treatment option, yet it is not advisable during the first two years of life as chemotherapy, radiation, and other drugs will adversely affect the growing brain. There is evidence to show that patients <7 years with a healthy liver have the best outcomes [11,12]. With advancing age comes organ damage, which increases the risk category and, ultimately leads to poorer transplant outcomes.

An allogeneic HSCT is not 100% successful in each patient. Newer literature reports a success rate of around 90%, which is very encouraging [14]. It should also be made very clear that, at present, an allogeneic HSCT is the only available curative option for children with thalassemia in India. This could change in the coming future [13].

The timeline of a HSCT should be explained well to the patient and the family. Counseling should include the landmarks, namely the pretransplant hyper-transfusion with aggressive chelation, to improve the class of the patient; pre-transplant immunosuppressive therapy, if indicated; conditioning, stem cell infusion, engraftment, discharge, first 100 days post-transplant, chimerism, and care during 365 days. The need for revaccination after stopping immunosuppression in all children should be emphasized [15].

Transplant-related mortality (TRM) means mortality during the first 100 days of a transplant. The family needs to be informed about the possibility of complications leading to morbidity and/or mortality in the peri-transplant period. The outcomes are improving with advancing technologies and better supportive care, but TRM is a reality and should be accepted by the family at the outset. It should be quoted between 10-20% for patients undergoing hematopoietic stem cell transplants [14].

Rejection: The rates of rejection in a transplant for thalassemia were reported earlier as about 10%, being higher (around 30-35%) in class III patients [10]. However, with advances in pre-transplant interventions as well as improved conditioning regimens, has decreased these rates significantly (5 to 10%) in matched related transplants [16]. However, the rejection rates are higher in the alternative donor (matched unrelated or haploidentical donors) transplants [14] as well as in sex-mismatched HSCT across all subtypes. In matched unrelated donor transplants, a suggestion of autologous stem cell harvest and preservation should be discussed with the family, underlining its need in case of rejection or if the unrelated donor declines to donate.

Graft-versus-host-disease (GvHD): This is one of the most dreaded complications of an allogeneic hematopoietic stem cell transplant and is seen with all subtypes of transplant including matched sibling to alternative donor

stem cell transplants. GvHD means a fight between the donor cells and the recipient's tissues, broadly divided into two categories, acute (in the first 100 days after transplant) and chronic GvHD (after 100 days of transplant). Acute GvHD may involve skin, liver, and gut, and occurs in 20-35% of allogeneic HSCT patients. Chronic GVHD may involve any organ of the body (skin, eyes, liver, bones, brain, bone marrow, etc.) and may be life-threatening in some instances [17]. Chronic GvHD occurs across all subtypes and is reported to occur in 10-15% of patients [14].

Infections during HSCT are an important cause of morbidity and mortality and should be informed to the family before they consent for transplant. These include bacteria, viruses, and fungal and protozoal infections. The viral pathogens include Cytomegalovirus, Herpes virus, Adenovirus, Herpes Zoster virus, Epstein Barr virus, BK virus, slow viral infections of the brain and fungal pathogens include Candida, Aspergillus and Mucormycosis. Despite using different antibiotics, antifungal and antiviral agents, it is not always possible to eradicate them and family should be counseled that while staying in a high-efficiency particulate air (HEPA)-filtered room, if an infection happens, most of the time, these infections reactivate from within the patient's body (gastrointestinal tract commonly), rather than from outside. Whatever the cause of infection, peri-transplant infections can be life-threatening and this must be emphasized to the family of the recipient.

Long-term complications of HSCT

Hormonal imbalance and bone health*:* Since various chemotherapeutic agents (busulfan, cyclophosphamide, irradiation, fludarabine, thiotepa, and treosulfan, etc.) and or irradiation are used in the conditioning of HSCT, it is logical that it will have long-lasting effects on the growth and endocrinal organs of the body (thyroid, testes and ovary, pancreas, etc.) and thereby the quality of life [18]. This would need close follow-up with the primary paediatrician or the endocrinologist. Infertility can also occur post-transplant, and methods for pre-transplant fertility preservation are are seldom available in our country. Although the newer drugs (treosulfan and fludarabine) are comparatively less toxic than the earlier ones (cyclophosphamide and busulfan), the menace of growth and endocrinal abnormality exists even today.

Regimen-related toxicity: It is defined as the toxicity associated with the chemotherapy or irradiation used during the patient's conditioning. These agents can damage any organ of the body and induce short- or long-term toxicities. A few of these can be life-long and may endanger the life of the patient. The common organs affected are skin, brain, heart, lungs, liver, gut, bones, and kidneys. Conditioning regimens and allogeneic graft can also impact a child's cognitive and mathematical skills in the years to come [18]. While it may be milder toxicity in most patients, it can be severe in a few unfortunate patients.

Second malignancy is one of the rarer side effects of HSCT. Literature reports around 2-3% estimated long-term risk of second malignancy. Still, it can vary depending upon the conditioning used and undefined factors of pharmacodynamics or pharmacokinetics in the patient. A disciplined, stricter yearly follow-up for life-long should be required so that an early diagnosis can be made and help offered in a timely and viable manner in these patients [18].

Counseling about marriage and progeny: Refer to Chapters 12 and 17.

Recommendations

- HSCT counseling must begin at the time of diagnosis as early HSCT results in better survival (Level of Evidence: 3).
- Liver size over 5 cm results in higher morbidity and mortality in HSCT and is a reflection of the body's iron (Level of Evidence: 2).
- Splenomegaly indicates the quality of transfusion, and patients with hypersplenism will require more transfusion support, and splenectomy status is not a contraindication to HSCT referral (Level of Evidence: 2).
- Adult thalassemia HSCT carries a higher risk of mortality (Level of Evidence: 3).
- Alternate donor HSCT – matched unrelated donor (MUD) or haploidentical HSCT is now a realistic option in thalassemia (Level of Evidence: 4).
- The best age for a hematopoietic stem cell transplant for children with thalassemia major is between 2 to 7 years. However, if the patient and/or the family has missed that age bracket and still wants the transplant, it can be done, but the odds change toward worse outcomes (Level of Evidence: 2).
- Hematopoietic stem cell transplant for thalassemia is an advanced curative treatment option that is not free from risk of mortality (Level of Evidence: 2).
- Complications of HSCT especially peri-transplant infections and long-term endocrinal and growth disturbances must be emphasized to the family of the recipient (Level of Evidence: 2).

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15b | Hematopoietic Stem Cell Transplantation for Thalassemia – Part II

Satyendra Katewa, Gaurav Kharya

Stem Cell Transplant Registry

For a successful Hematopoietic Stem **Cell Transplant (HSCT)**, the patient's genetic typing needs to match that of the donor. Every patient has a 25% chance of finding an HLA identical donor in each of his siblings [1]. This probability increases with an increasing number of siblings. In communities with a close genetic pool and shared haplotypes, either of the parents can also be an HLA identical match for the child, the probability of which is close to 5-10% [2]. In the absence of a suitable donor within the family, there is a need to find an unrelated hematopoietic stem cell donor. Donor registries work towards creating a diverse database, where the search for a life-saving donor meets a match.

The first successful matched unrelated donor transplant took place in 1973, when a patient with inherited immunodeficiency received an allogeneic HSCT from a donor identified as a match through a blood bank in Denmark [3]. The United Kingdom started the world's first bone marrow donor registry, established by the Anthony Nolan Trust in 1974 [4]. As of August 2019, there are more than 35 million donors and cords listed at the World Marrow Donors Association (WMDA), a network of unrelated donor registries including 83 stem cell donor registries, 30 cord blood banks, and 4 donor centres (DC). The representation of Indians, however, in any of these registries is very poor. India is in dire need of a functional registry with donors belonging to diverse ethnic backgrounds [5]. In India, there are five organizations listing donors for unrelated donor recipients:

- Datri Blood Stem Cells Registry (>450000 donors) (www.datri.org)
- Marrow Donor Registry India (MDRI) (35,768 donors) (www.mdrindia.org)
- Bangalore Medical Services Trust (BMST) India as an intermediary of Deutsche Knochenmarkspenderdatei (DKMS) Registry with approximately 21,695 donors being listed in its database (www.dkms-bmst.org).
- GeneBandhu (7,991 donors) (www.genebandhu.org)
- Be The Cure Registry - Jeevan Foundation (6449 donors) (www.jeevan.org)

Becoming a HSCT donor

Figure 1 outlines the process of becoming a donor for HSCT.

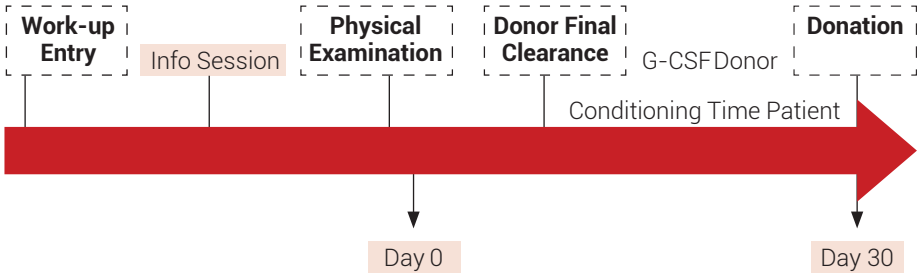

Figure 1.Timeline of events in case of matched unrelated donors

Confidentiality: Protecting patient and donor confidentiality through all stages of the transplantation process are of paramount importance. The WMDA states that "donor and patient identity must remain confidential during the search process so that only appropriate registry personnel have access to these data". Under no circumstances shall the personal information of either the donor or the recipient be disclosed.

Conditioning Protocols for HSCT in Thalassemia

Preparatory regimens for HSCT of patients with Thalassemia must achieve two objectives.

- Elimination of the hematopoietic marrow (Myeloablation)
- Creating a tolerant environment to allow the transplanted marrow to survive and thrive (Immunosuppression)

Evolution of Conditioning regimen

Initially Busulfan (Bu) and Cyclophosphamide (Cy) were the two most commonly used drugs in myeloablative conditioning regimens [6]. The choice of two strong alkylating agents was associated with the specific characteristics of β-thalassemia: hyperplastic bone marrow and frequent alloimmunization from repeated transfusions that require a strong approach to minimize graft failure risk. Amendment in the dosage of these agents improved outcomes in patients with advanced disease [7]. However, differences between patient groups have been reported since the early 1990s, with Pesaro-risk Class 3 and HR Class 3 (CMC, Vellore Classification for children aged >7 years and with a hepatomegaly of >5 cms) receiving more attention [8,9]. Since then, several protocols have been developed to minimize graft failure and GvHD, including early debulking of bone marrow hyperplasia and exploiting the highly immunosuppressive action of Fludarabine (Flu) [10,11]. Likewise, the new agent Treosulfan (Treo) was

Author	Year	Country	N	Median age	Conditioning regimen	GVHD prophylaxis	Graft source	Donor type	aGVHD I–II/ III–IV	cGVHD	TRM	GF	OS	EFS	FU
Lucarelli, et al. [6]	1990	Italy	99		Bu 3.5 mg/kg/dx4, Cy 50 mg/kg/dx4	Csa, Mtx	BM	MFD	4.3	0.8					12
			39	6.5							5.1	0	94	94	
			36	10.5							19.4	8.3	80	77	
			24	12							37.5	16	61	53	
Lucarelli, et al. [7]	1998	Italy	125	<17	Bu 3.5 mg/kg/dx4, Cy 30–40 mg/kg/dx4	Csa, Mtx	BM	MFD	NA	NA	19	33	78	54	NA
Sodani, et al. [8]	2004	Italy	33	16	Protocol 26	Csa, Mtx	BM	MFD	9/0	NA	6	6	93	85	36
La Nasa, et al. [9]	2005	Italy	68	15	Bu 3.5 mg/kg/dx4 -TT 10 mg/kgCy 30–40 mg/kg/dx4	CSA-Mtx ± ATG	BM	MUD	37	26	30	3.7	70	70	43
Bernardo, et al. [10]	2008	Italy	20	13	Treo 14 g/m2/dx3, TTP 8 mg/kg, Flu 40 mg/m2/dx4, ATG 10 mg/kg/dx3	Csa, Mtx	BM	MFD/ MUD	10/5	5	5	10	95	85	20
Anurathapan, et al. [11]	2014	Thailand	22	17	PTISx2, Flu 35 mg/m2/dx6, Bu 130 mg/m2/dx4, ATG 1.5 mg/kg/dx3	Csa/Tac/ MMF	BM/ PBSC	MFD/ MUD	9/14	18		0	93	90	36
Anurathapan, et al. [12]	2016	Thailand	31	10.1	PTISx2, Flu 35 mg/m2/dx6, Bu 130 mg/m2/dx4, ATG 1.5 mg/kg/dx3	Cy, Tac, Siro, MMF	TCD PBSC	Haplo	29/.3.2	16.1	3.2	3.2	65	64	12

Table 1. Shows evolution of various conditioning regimens used in HSCT for thalassemia and the outcomes

Keys – aGVHD: Acute Graft versus Host Disease, ATG: Anti-thymocyte Globulin, BM: Bone Marrow, Bu: Busulphan, cGVHD: Chronic Graft versus Host Disease, Csa: Cyclosporine, Cy: Cyclophosphamide, EFS: Event Free Survival, Flu: Fludarabine, FU: Follow Up, GF: Graft Failure, MFD: Matched Family Donor, MMF: Mycophenolate Mofetil, Mtx: Methotrexate, MUD: Matched Unrelated Donor, OS: Overall Survival, PBSC: Peripheral Blood Stem Cell, PTIS: Pre-Transplant Immunosuppression, Siro: Sirolimus, Tac: Tacrolimus, TCD: T-Cell Depletion, Treo: Treosulphan, TRM: Transplant Related Mortality, TTP: Thiotepa

adopted because of its lower toxicity profile and sinusoidal obstruction syndrome (SOS) risk, with multiple studies demonstrating the drug's ability to reduce the likelihood of GvHD [10].

Recent studies have reported improvements in Overall Survival (OS) and Thalassemia Free Survival (TDS) after myeloablative or Reduced Intensity Conditioning (RIC) regimens were supplemented with Peripheral Blood Stem Cell – Haploidentical – HSCT plus T-cell depletion and Pre-Transplant Immune Suppression (PTIS) [12]. Further advances are required in PBSC-HSCT and novel approaches with Post-transplant Cyclophosphamide (PTCY) or ex-vivo graft manipulation strategies to further improve outcomes.

Recommendations

- Myeloablative Conditioning (MAC) has been replaced by Treosulfan-based Reduced Intensity Conditioning (RIC) for HLA identical donor settings (Level of Evidence: 2).
- PTIS by the virtue of reducing the burden of alloreactive T-cells not only helps in reducing the risk of graft failure but also helps in reducing the risk of GvHD. This strategy might be more beneficial in Indian settings where majority of patients are allo- sensitized due to poor transfusion practices (Level of Evidence: 2).
- The inclusion of Fludarabine and ATG reduces the risk of GvHD and improves outcomes in all cases of HLA disparity and unrelated donors (Level of Evidence: 1).
- The best results were obtained using BM stem cells from an MFD, with OS and TFS probabilities of over 90% and 80%, respectively (Level of Evidence: 1).

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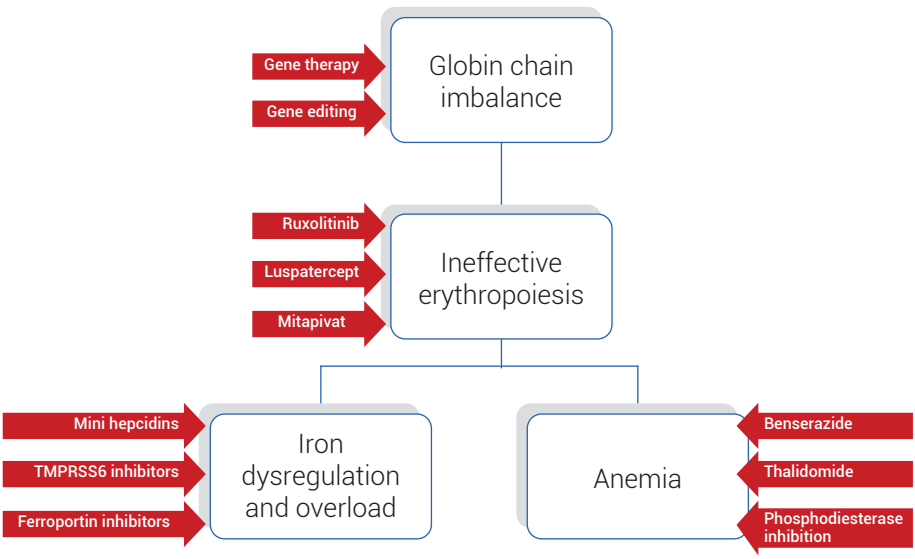
16 Newer Therapies in Thalassemia

Mamta Manglani, Jagdish Chandra, Pranoti Kini, Praveen Sobti

Ideal comprehensive management has improved the life span to at least 50 years in patients with transfusion dependent thalassemia (TDT) [1]. However, co-morbidities unrelated to thalassemia are now unfolding in this group. Also, the quality of life (QoL) is not as good [1]. Additionally, ideal care is available to less than 15 to 20% of patients with thalassemia in India. Besides, hematopoietic stem cell transplantation (HSCT), the only curative option for thalassemia, is currently available in India to only a select group of patients with hardly any centers in the public sector. Even, if available, not all are fortunate to find a suitable donor. Hence, the need for novel therapies in thalassemia which would circumvent the problems associated with repeated packed red cell transfusions and iron overload. The future holds an array of drugs, which are currently in various phases of development and have the potential of achieving good results in TDT and non transfusion dependent thalassemia (NTDT). Figure 1 highlights the targets for development of new drug [2,3]

Figure 1. Targets for Newer Drugs for Thalassemia



The various mechanisms of action for newer therapies are:

1. **Reducing anemia through HbF induction**

a. **Thalidomide:** Thalidomide (TLD), a drug known for its immunomodulating and anti-angiogenic properties, has recently been demonstrated to increase HbF production by promoting the expression of globin gene [4]. In-vitro studies have shown that thalidomide slows erythroid maturation, increases proliferation of immature erythroid cells [4]. Data regarding the efficacy and safety of TLD use in NTDT and TDT is now emerging with enthusiastic results in various recent studies published recently as shown in Table 1. The dose of TLD used was 2-3 mg/kg/day in most studies. The common adverse effects of TLD are sedation, neutropenia and constipation. Currently a multicentric randomized control trial is underway in India which is comparing low dose vs standard dose Thalidomide in 226 patients of TDT over a period of 18 months.

Relatively low cost and easy availability of TLD makes it a good candidate for use in TDT too as it has the potential to decrease the need for repeated and frequent blood transfusions. However, the limited but encouraging body of literature available is based on use of a range of drug dose and the optimal dose of TLD for its use in thalassemia is still not clear.

Table 1. Studies assessing efficacy of Thalidomide in thalassemia

Study group	Participants	Outcomes	Results
Burani, et al. [4]	140 subjects, Median follow up: 22 months	Complete response (CR) defined as Hb ≥ 9 g/dl without transfusion, Partial response (PR) defined as >50% reduction in transfusion requirement	57.2% CR, 14.6% PR
Shah, et al. [5]	25 subjects, Follow up: 3 months	Major response defined as > 50% reduction in transfusion requirement Minor response defined as 25-50% reduction in transfusion requirement	68.2% major + minor response
Chandra, et al. [6]	37 subjects, Follow up: 6 months	Major response - > 50% reduction in transfusion requirement Moderate response defined as 25-50% reduction in transfusion requirement	Major response: 51.3%, moderate response: 32.4%, no response: 16.2%

b. **Benserazide:** Benserazide has been found to relatively safe with not too many toxic effects and been approved for over 50 years in Europe and Canada for Parkinson's disease treatment. Benserazide activates HBG gene transcription and subsequent studies have shown that it induces fetal

hemoglobin (HbF) in erythroid progenitors from hemoglobinopathy patients, transgenic mice containing the entire human β-globin gene (β-YAC) and anemic baboons [2]. Orally administered escalating doses of benserazide in an anemic baboon induced γ-globin mRNA up to 13-fold and establish an intermittent dose regimen for clinical studies as a therapeutic candidate for potential treatment of β-hemoglobinopathies [2].

c. **Phosphodiesterase 9 inhibitors:** The induction of HbF with the phosphodiesterase 9 inhibitor IMR-687, which increases cyclic guanosine monophosphate, is currently being tested. IMR-687 has already completed studies in Sickle Cell Disease and has shown good results with increase in HbF and hemoglobin with improvement in hemolysisparamters. A similar Phase 2 study to determine the safety and tolerability of IMR-687 given orally once a day for 36 weeks, in adults with TDT and NTDT is underway [7].

2. Ineffective Erythropoiesis (Erythroid Maturation Agents)

a. Luspatercept / Sotatercept

Luspatercept and Sotatercept are recombinant protein which bind to transforming growth factor β (TGF-β) superfamily ligands, including growth differentiation factors and activins, thereby ameliorating their inhibitory effects on late-stage erythropoiesis. Activin receptor ligand traps, namely sotatercept and Luspatercept, reduce SMAD2/3 signaling causing increase in maturation of the erythroid cells and thereby improve anemia in β-thalassemia [7,8].

BELIEVE trial in adults with TDT and BEYOND trial in adults with NTDT are the main trials which have studied Luspatercept [9,10]. Results are encouraging and US FDA and EMA have approved the use of Luspatercept in TDT >18 years of age at the recommended starting dose of 1 mg/kg once every 3 weeks by subcutaneous injection [2,8]. Pediatric trials on Luspatercept are still ongoing. Although, Luspatercept is now available in India, it is quite expensive and therefore, will be affordable to only a few patients. Central Drugs Standard Control Organization (CDSCO) of India has recently accorded approval for phase IV study of Luspatercept in adult patients with TDT.

Sotatercept is another activin II receptor ligand trap that showed promising results in adults with TDT in phase 2 trials. Unlike Luspatercept, Sotatercept has not been permitted for further phase 3 studies as it has been found have lesser specificity for activin receptor-II ligand trap and reported to have lesser efficacy with greater off-target effects [2,3,8].

b. Ruxolitinib

Ruxolitinib, a JAK-2 inhibitor, has been tried in patients of TDT with splenomegaly [2,3,8,11]. The data on Ruxolitinib is however sparse and has shown conflicting results.

c. Mitapivat

Mitapivat, a pyruvate kinase (PK) inhibitor, has shown promise in reducing the ineffective erythropoiesis as well as increase in hepcidin with reduction of iron in liver in patients with PK deficiency, who are similar to NTDT. Trials were then conducted in 13 patients with NTDT given orally for 6 weeks in doses of 50 mg twice daily, escalated to 100 mg in 6th week and results were encouraging. A phase 3 study in adults with TDT and NTDT is planned [2,7].

3. Iron Dysregulation and Overload

a. Mini Hepcidins

Mini Hepcidins have shown to reduce ineffective erythropoiesis as well as splenomegaly in a TDT mouse model. They also reduce the iron overload and transfusion requirements. Studies are underway to look at their potential for future use in NTDT patients [7,12].

b. TMPRSS6 Inhibitors

TMPRSS6 gene encodes matriptase-2 (MT-2) which regulates hepcidin synthesis. In mouse models with NTDT, inhibition of TMPRSS6 caused an increase in hepcidin & improvement in hemoglobin & reduction in iron overload. Phase II clinical trials are now being conducted for patients with NTDT [3].

c. Ferroportin Inhibitors

Inhibiting ferroportin restricts availability of iron, targeting both the iron overload as well as ineffective erythropoiesis. After successful results in NTDT mouse model, phase II studies are recruiting participants with NTDT [3,12].

4. Globin Gene Imbalance

a. Gene Therapy

In gene therapy, also called gene addition, a retroviral or lentiviral vector enclosing the normal β / γ genes is inserted into previously collected autologous CD34+ erythroid progenitor cells ex vivo. These are then infused into the patient after myeloablative conditioning. This restores the normal β / γ chain production, thus reducing the α/β imbalance [2]. The results of the initial trials (HGB 204/HGB 205) were promising for patients with Non β0/β0 types, but not for β0/β0 types or IVS 1-110 homozygous patients. This was improved upon by enhancing the vector copy number in the ensuing trials (HGB 207/HGB 212) to give better results in the severe types of mutations too [2].

Gene addition is now commercially available (EMA approved) for patients > 12 years of age with Non β0/β0 genotype, who are eligible for stem cell transplant, but do not have a matched related donor. [2] It is not yet available in India. Presently, it is prohibitively expensive to use it as an imported product.

Another Globe lentiviral gene insertion with intrabone administration has shown very promising outcomes in children (3/6 achieved transfusion independence) as well as reduced requirement of transfusions in the 3 adults [2,13].

Insertion of a hairpin RNA sequence which represses the Bcl11a gene into the erythroid progenitors using a lentivirus, is also being tried presently in sickle cell patients [2,8].

b. Gene Editing

The Bcl11a gene located on chromosome 2 is a promising target for gene editing. Deletions in the enhancer region of Bcl11 a gene leads to unhindered production of γ globin chains while not altering the HBB gene. Trials using Zinc Finger Nuclease (ZFN) and CRISPR-Cas9 techniques in TDT patients are ongoing, with encouraging results [2,8].

Other potential targets that may help in increasing hemoglobin levels in thalassemia are:

1. Macrophage manipulation

Clodronate loaded liposomes have been used to deplete macrophages in a mouse model of thalassemia. It improved anemia, iron overload, spleen size and a decrease in α chains. This mechanism can be explored in human trials [3,14].

2. Apotransferrin

The use of Apotranferrin (Apo-TF) in thalassemia mouse model improved maturation of erythroid precursors. This has not been tried yet in human trials, but has the potential [3].

3. Heat shock proteins

Heat Shock Proteins (HSP 70) are required for terminal erythroid differentiation by nuclear accumulation, but are unavailable in thalassemics due to excess α chains, which trap them in the cytoplasm. Exportin (XPO1) is responsible for this sequestration of HSP 70 in the cytoplasm. Inhibiting XPO1 can improve the nuclear HSP70 and thus reduce ineffective erythropoiesis in thalassemia. This is yet another unexplored target for TDT and NTDT [3,14].

In conclusion, it is important to note that both TDT/NTDT might see a sea change in management in the near future. Gene therapy and Luspatercept are already in the fray and India is looking forward to these! Thalidomide might prove to be a boon and a substitute for expensive drugs and treatments, with ongoing trials to determine the right tolerable doses. Overall, TDT and NTDT is awaiting exciting times ahead, with many potential options in the pipeline!

Recommendations

1. Due to insufficient data on long term safety, thalidomide is not recommended, at present, routinely in thalassemia (Level of Evidence: 3).
2. Luspatercept has been approved for phase 4 studies in India and can be used in adults with TDT in a starting dose of 1 mg/kg subcutaneous injection every 3 weeks (Level of Evidence: 3).

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17a Psychosocial Counseling

Meghna Madnani, JS Arora

Thalassemia is a chronic condition where psychosocial concerns including financial and emotional problems affect the patients and family members [1]. Thalassemia affects the emotional status, daily activities, family practices and occupational capabilities of patients and their parents due to the complicated and expensive lifelong treatment [2]. This leads to significant psychosocial morbidity in those with thalassemia. Addressing these issues through counseling is a crucial component of the comprehensive care provided to these patients to ensure they achieve their full life potential. Persons living with Thalassemia are vulnerable to anxiety and depression due to limitation in school and social activities, facial and skeletal deformities, organ damage and fear of death [2]. This may lead to aggression, communication problems, loneliness, and despair [3].

As per WHO definition, health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity [4]. Hence, healthcare professionals, besides focussing on the clinical welfare, should also address the patients' emotional and social issues. To improve the quality of life of thalassemia patients and their families, the clinician should plan to include as a unit, thalassemia patients, siblings, and parents, to identify psychological problems and accordingly administer therapy, including physical therapy, counseling and vocational rehabilitation [5,6].

Challenges to be addressed through psychosocial counseling can be categorized as (1) Psychological challenges and (2) Social issues.

Psychological challenges

There is lack of published evidence for psychological support interventions in thalassemia [7]. The following are practical recommendations for psychological support. Psychological problems are different at diverse phases of life. Variables include literacy, economic status and domiciliary zone of the family. Broadly psychological problems can be discussed in various stages of their lives.

At the time of diagnosis

Concerns: At the time of diagnosis the family is broken to hear the news of chronic disease to be managed by life-long repeated blood transfusions. They are in shock, state of denial, anger, guilt. They are further shattered once they understand the genetic basis and possibility of antenatal diagnosis. Therefore, at diagnosis, maximum psychological support is needed.

Interventions: (a) The family is informed that they are not alone it's common in their community, though many people may not be aware of it. (b) Information about genetics of thalassemia and the need for cascade screening to check carrier status of siblings and extended family and the option of antenatal diagnosis is provided. (c) Patient is advised to register with a nearest established day care thalassemia centre for regular packed red cell transfusions, and chelation therapy. Need for regular transfusions is stressed. (d) Patients are counselled regarding the various support groups and facilities available for patients with thalassemia. Refer to Chapter 21 for details.

At first transfusion

Concerns: Parents are worried about the pain during the procedure of venous access, the transfusion, and the side effects of transfusion. They have several queries regarding the process of blood transfusion.

Intervention: (a) Patient/family to be explained the technique to be followed for transfusion through visual aids such as pictures, videos etc. (b) Accurate information about the blood transfusion and procedure helps build trust and should be encouraged [7]. For older children, they should be taken into confidence for procedures, blood transfusion by reducing uncertainties, correcting misconceptions, positive reinforcement about their own ability to cope [7]. (c) Counseling regarding complications of transfusions, frequency, duration and amount to be transfused should be provided. Parents are encouraged to interact with other parents of older children to know the first-hand experience from them. They are also encouraged to register with local parents' organizations/societies for thalassemia. Encourage the child and parents to ask their queries. Literature on general management should be provided to the family. Basic information about HSCT should also be provided in the counseling sessions. Using effective coping strategies to allay the child's anxiety includes distracting the child by some play or cartoon visuals.

Initiation of iron chelation (2 to 4 years of age)

Concern: Need for initiation of iron chelation and adherence to the same.

Intervention: Appropriate iron chelation with good adherence from the time of initiation will prevent the complications of iron overload. Parents should be counselled regarding the dose, method of administering the medication, side effects, monitoring etc. and an information leaflet preferably should be given to parents [9-11].

During adolescence

Concern: Body image issues due to short stature as well as lack of pubertal development are often the cause for psychological and psychiatric problems in this age group. They may have typical hemolytic facies and therefore perceive themselves as different from peers and fear being shunned by peers. They also develop doubts about getting attention from opposite sex.

Intervention: Timely referral to a psychiatrist as well as enhancing their self-image by discussing their positives – related to education, intelligence, sense of humour, confidence and smartness, caring behaviour is more significant in developing longer lasting relationship than just physical appearance. Encourage them to seek appropriate guidance for medical issues related to short stature and attaining pubertal changes [8]. During adolescence adherence to treatment is often low, as patients want to take independent decisions while they are not mature enough. Parents need to continue to play an active role in monitoring adolescents self-care. Psychological support helps to convince the patients to be compliant with adequate treatment for better outcome [9-12]. Psychological intervention is necessary for those who may require life-long hormone replacement therapy.

During adulthood (20 to 45 years)

Concern: Higher education, skill development, employment and business opportunities are the main concerns once a person with thalassemia reaches adulthood. After optimum education and financial security, they are worried about marriage prospects and family (children).

Intervention: In villages and smaller towns with no access to comprehensive care, persons with thalassemia are more likely to drop out from school because of health issues, financial constraints or fear of shortened life span. Patients and parents should be exposed to successful stories of achievers among their peers. Information related to privileges available for higher education should be imparted and they should be encouraged to pursue careers of their interest. Career counseling workshops should be organized from time to time for the patients as well as their parents to enable them to select the right careers as per their passion and capability. Though, persons with thalassemia are not covered under RPWD Act 2016 for the purpose of reservation in employment, they have the advantage of non-discrimination on the ground of their disorder. They cannot be refused job or promotion on the pretext of being medically unfit. Refer to Chapter 21 for details.

Marriage Counseling: Besides medical treatment, all persons with thalassemia should be encouraged to develop relationships and plan for a successful married life [12]. They should be offered genetic counseling and prenatal diagnosis [13]. If the partner is a thalassemia carrier or minor, there is a 50% chance of having a child with thalassemia major in each pregnancy and a 50% chance of a child with thalassemia trait. If the partner is non-thalassemic, all children will be thalassemia carrier (non-affected). The above probability would apply even if the person with thalassemia major has been cured by Hematopoietic Stem Cell Transplantation (HSCT). If both the partners are thalassemia major, they should be counselled to meet a fertility specialist and consider options of either donor sperm/ovum or adoption. Refer to Chapter 12 for details.

Middle age (45+ years of age)

Concern: There may be wide range of issues at this stage of life. As in non-thalassemia persons, several serious health problems can occur such as hypertension, diabetes mellitus, bone disease etc. On the other hand, few may be well settled, financially independent, and married with normal children, in good health but still harbouring a fear of shortened life span. Some may be financially stable but single, with no one in their family to give moral support. Some may have lost one or both parents, making it hard to manage basic needs.

Intervention: All aspects of counseling need to be reinforced during this phase of their life. Most of those who are financially secure and have family need to be reassured regarding staying healthy with proper medical advice and regular adherent treatment for thalassemia as well as for any other medical conditions they may be additionally suffering from. Those requiring financial support and/or moral support should be guided to the Thalassemia Societies for financial and peer help.

Throughout the treatment

Age-appropriate counseling regarding the disease should be done for all persons with thalassemia. Patients should be encouraged to accept the challenges by highlighting the positive aspects such as blood transfusion is just once or twice in a month and at other times, s/he can enjoy her/his life fully without many restrictions. Encourage the persons living with thalassemia as well as the family members to not hesitate to disclose the disease status during admissions to school or college or for employment and this can be achieved by their complete acceptance of the diagnosis without feeling stigmatization. This would be possible only if public awareness is improved and there is an acceptance by the society with inclusiveness in all walks of life.

Social issues: Isolation and stigmatization

Social integration, connecting and interacting with people and contributing to society can go a long way in preventing social isolation and stigmatization [14]. Awareness about the disease among common public helps accepting the disease better and reduces the probability of stigmatization. Similarly, having the patient accept the various aspects of the disease as well as treatment as a part of life, through understanding of the disease, helps to reduce self-stigmatization.

Appropriate counseling to improve their self-esteem, which includes positive self-talking on a daily for at least 10 minutes and family members being supportive towards the patient helps to reveal their strengths. Assisting them in mainstreaming through better opportunities in society for education and jobs, with privileges would also help reduce social isolation [14,15]. Counseling and guiding them with available privileges and opportunities helps significantly.

Recommendations

- Children with thalassemia and their parents should have access to psychosocial counseling (Level of Evidence: 3)
- Aspects covered in counseling will vary based on the age of the patient; it begins at the time of diagnosis and continues during transfusion initiation, initiation of iron chelation, childhood, adolescence and adulthood (Level of Evidence: 3).
- Counseling will support integration into the mainstream and help prevent social isolation (Level of Evidence: 3).

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17b Diet in Thalassemia

Jasmine Kaur Ahuja, J S Arora, Nisha Iyer

Nutrition is the fundamental part of development and health of children. Improved nutrition shapes a healthy infant, child and adult with robust immune systems [1]. Patients with thalassemia are at an increased risk for multiple nutritional deficiencies [2,3]. Increased iron overload, poor dietary intake, metabolic complications, and endocrinological disturbances are among the factors which contribute to the nutritional depletion in these patients [4]. Hence, the role of diet in thalassemia needs to be reinstated.

A detailed assessment and evaluation of nutrition and growth, followed by dietary intervention must be planned at diagnosis to sensitize the family about the importance of a balanced and nutritious diet for the child. Growth deficits in thalassemia are multifactorial. Failure to thrive and "falling off" the growth curve are signs to be watched by the pediatrician.

Growth failure in thalassemia is divided into two phases:

1. The first phase occurs when the child is below 10 years of age and is associated with growth deficits primarily due to anemia [3]. Optimum transfusions supplemented with a balanced diet containing adequate amounts of nutrients, vitamins and minerals is recommended [4]. The food should be a balanced diet consisting of various (as diverse as possible) food groups/components in different combinations. Exclusive breastfeeding should be practiced for the first six months of life and thereafter optimal complementary feeding should be started. Energy and nutrient-dense thick homogenous and locally available foods such as staple cereals, whole grains, broken wheat, millets, pulses, rice, khichdi, vegetable stuffed paratha, atta or besan halwa, porridge, dhokla, besan or dal chilla, dosa, soyabean, moong sprouts, seasonal fruits& vegetables, peanuts, nuts, egg, paneer, ghee and oil should be consumed. Nutritious snacks between the three main meals are recommended to ensure adequate energy intake. Avoid empty calories and unhealthy foods such as juices, chips, candies and processed foods.
2. In the second phase (11 to 18 years of age), growth deficiency is mainly due to iron overload affecting the growth hormone–IGF axis which leads to bone mineralization disorders, growth failure, and endocrinopathies [1]. Intensive iron chelation with adequate transfusion and healthy balanced diet rich in energy, protein, calcium and micro-nutrients is essential for optimum growth and puberty [3-6].

Role of Various Nutrients

Iron: There is a dilemma about the role of dietary iron in thalassemia. Though dietary iron is not the real villain in transfused patients, reduction of dietary iron has been the focus of nutrition intervention for decades [4]. Instead, the focus needs to shift to a well-balanced diet rich in antioxidants and minerals. With optimal chelation therapy, the excess iron is removed from the body and a low iron diet is not an absolute necessity. Dietary iron has minimal contribution to iron overload compared to the iron accumulated from transfused red cells. The iron obtained from one unit of packed red cells is about 200 mg which amounts to 4800–12,000 mg of iron per year, which is much higher than the iron that comes from 80-100 g of meat (5 mg), amounting to 400–700 mg of iron absorbed from the diet per year [5,7-9]. Adding iron inhibitors in the diet and avoiding iron enhancers might be useful [8,9]. Inhibitors such as tannin in tea and coffee, as well as milk (also a source of calcium), should be encouraged along with meals. Calcium supplements (if advised) can be taken along with meals to inhibit iron absorption. Iron enhancers such as vitamin C rich foods should be avoided along with meals and instead be consumed two hours apart from meals. Cast iron utensils should preferably not be used for cooking. However, iron supplements have no place in thalassemia in all age groups including pregnancy. All fruits including pomegranate (anaar), apple, grapes, and watermelon should be consumed in normal amounts, preferably in between meals. Avoid any meal supplements, protein powders and nutrition energy bars that may contain excess iron.

Zinc: Zinc deficiency is prevalent in thalassemia [6]. Zinc deficiency enhances certain manifestations seen in thalassemia such as low bone mass, hypogonadism, growth hormone deficiency, diabetes and poor immune function. Zinc deficiency occurs primarily due to urinary losses with Deferiprone or Kelfer (oral iron chelator) coupled with inadequate dietary intake [7,10]. All patients receiving Deferiprone (Kelfer), having growth failure, documented zinc deficiency or low bone mass must be supplemented with zinc (15-40 mg/day) [11]. Many patients with thalassemia and iron overload also have diabetes mellitus, which further adds to increased zinc losses. Patients should be encouraged to consume recommended dietary allowances of zinc in diet. The dietary sources of zinc include whole grains, breakfast cereals fortified with zinc, dairy products such as milk, yoghurt and cheese, chickpeas, peas, kidney beans, pumpkin seeds, nuts like almonds and cashews, meats, poultry seafoods (crab and lobster). Sprouted pulses, grains and seeds have better bioavailability of zinc because of reduction in phytates [11]. However, routine supplementation of zinc in patients with thalassemia is not recommended.

Vitamin C: Vitamin C is an important antioxidant and has a crucial role in thalassemia [12]. It is a water-soluble vitamin which cannot be synthesised in

the body on its own [13,14]. Irreversible oxidation of ferric iron deposits leads to the destruction of dietary vitamin C, thus leading to its deficiency causing scurvy. Children with transfusion-dependent thalassemia are deficient in vitamin C and are more likely to develop scurvy, besides posing a risk of oxidative stress [12]. Vitamin C supplementation is recommended in patients on deferoxamine chelation. It is given at the dose of 2-3 mg/ kg/ day [15]. Vitamin C should not be used in the early stages of intensive (intravenous deferoxamine) chelation therapy for patients with cardiac failure or with severe iron overload on myocardial T2*MR [8]. Vitamin C rich foods can be included in the diet, but should be consumed 1 to 2 hours apart from meals, as vitamin C increases the gastrointestinal absorption of non-heme iron [16].

Calcium: Calcium is a very important mineral that plays a role in bone structure. The causes of hypocalcemia in thalassemia include hypoparathyroidism and vitamin D deficiency. Prevention of bone disease needs focus on lifestyle measures such as good calcium intake, weight bearing exercise and adequate levels of vitamin D at all ages. Adequate calcium, vitamin D and zinc intake during skeletal development can increase bone mass in adult life, thus preventing bone loss and fractures [17]. Calcium-containing diets include milk and milk products, cereals, pulses and legumes - soyabean, tur dal and black gram dal, millets (pearl millet i.e. bajra), finger millet (ragi), sorghum (jowar), sesame seeds, green leafy vegetables such as curry leaves, amaranth leaves, drumstick leaves, lotus leaves and cauliflower leaves, eggs, mutton and fish. In adolescents, besides the aforementioned routine measures, smoking and excess alcohol consumption should be avoided.

Vitamin D: It is required for bone health at all ages even in normal individuals. Persons with thalassemia are known to have vitamin D deficiency due to inadequate intake or availability [18]. Hence, adequate intake through dietary sources of vitamin D is important [17]. Since vitamin D is a fat-soluble vitamin, WHO recommends consuming fats amounting to 15-30% of total calories. Vitamin D levels in thalassemia are also dependent upon the dietary intake of energy, protein and fat as these macronutrients play a crucial role in vitamin D metabolism [19]. A balanced intake of all macronutrients along with vitamin D and calcium are therefore required for optimal bone health. Vitamin D levels are often found to be low despite routine daily supplementation of 400–1,000 IU vitamin D. A variety of doses have been used previously [4,7,18]. The intermittent high-dose supplementation with oral 50,000 IU vitamin D2 every 3 weeks proved to be an effective and safe strategy for increasing 25-OH D levels. Alternatively, a 50,000 IU supplement be provided every other week for 8 weeks for subjects with 25-OH vitamin D levels less than 15 ng/mL or 50,000 IU every 4 weeks for those with levels <30 ng/m. Vitamin D 50,000 IU of ergocalciferol (D2) orally at time of transfusion for six to eight transfusion cycles or 18–24 weeks has also been used. This dosage translates to approximately 1700–2300 IU per day

depending upon the frequency of transfusion. Sources of vitamin D include natural sources like sunlight–Ultraviolet B radiation (290-315 nm) wherein vitamin D can be obtained through exposure to sunlight for 5 to 15 minutes between 10.00 am to 15.00 pm [20]. Dietary sources of vitamin D include cod liver oil, salmon fish, mackerel, sardines, tuna, egg yolk, mushrooms, and fortified foods like milk, butter and cheese [19].

Monitoring for bone disease is recommended after the age of 10 years: an annual bone mineral density (BMD) assessment is crucial [21]. All infants should receive 400 IU of vitamin D as supplementation as per standard protocol. In older children, 1000-2000 IU vitamin D supplementation daily is recommended. Monitoring vitamin D levels will help guide dosing [22]. In vitamin D deficiency, daily oral vitamin D (2000-4000 IU) along with daily oral calcium supplementation (500-1000 mg; 50-75mg/kg/day) should be given for 12 weeks. To decrease the risk of kidney stones in thalassemia patients, it is recommended to use calcium citrate instead of calcium carbonate [23]. In osteopenic patients, non-pharmacologic interventions should be optimised along with therapeutic doses of vitamin D and calcium [18].

Folic acid: Folic acid is an essential nutrient for the synthesis of nucleoproteins. Food sources of folate should be added in the daily diet [24]. Dietary sources of folic acid include dark green leafy vegetables (turnip greens, spinach, asparagus, broccoli), beans, peanuts, sunflower seeds, fresh fruits, fruit juices, whole grains, liver, and sea foods. Folate deficiency is more common in non-transfusion dependent thalassemia (NTDT) patients or those on low transfusion regimens compared to optimally transfused patients with transfusion dependent thalassemia (TDT) as ineffective erythropoiesis is significant in NTDT patients with a higher turnover of red cell production, leading to depletion of folic acid [24]. Folate deficiency enhances the prothrombotic state and hence, folate supplementation helps in reducing risks of thrombosis related to high homocysteine levels and atherosclerosis [25]. Folic acid supplements at 1-5 mg/day especially to thalassemia should be considered, more so if patients is on low transfusions [24]. A recent study has shown that 5 mg/week folic acid supplementation in TDT resulted in adequate serum FA levels in 5 to 18-year-old children [26]. Folic acid in the dose of 1 mg per day is recommended in thalassemia trait [27]. Concomitant folic acid supplementation is recommended for patients on Hydroxyurea [28]. Folate demand in pregnancy is normally increased. All pregnant women should be supplemented with folic acid 5 mg/day. Folic acid 5 mg/day should be started 3 months prior to conception in adult female patients planning for pregnancy to prevent fetal neural tube defects [29,30]. Folic acid supplementation is essential specially in ageing thalassemia population, who are more prone to thrombotic complications and atherosclerosis.

Vitamin E: Vitamin E is present widely in vegetable oils and plant foods. Dietary deficiency of this nutrient is not normally encountered in general population

[30]. However, in children and adults with thalassemia, there is enhanced oxidative stress and significantly lower superoxide dismutase (SOD) enzymes which leads to reduced levels of vitamin E. Dietary sources of vitamin E include vegetable oils (e.g. olive oil, corn oil, safflower and sunflower oil), invisible fats in cereals and nuts. It is available easily from Indian diets [31]. The need of vitamin E supplementation in thalassemia is not yet clear. Therefore, its routine use is not recommended. It may be given as clinically indicated [24].

Diet in Diabetes Mellitus (DM): DM in thalassemia patients may be due to a combination of insulin resistance and insulin deficiency [32]. Patients with impaired glucose tolerance should be managed with a strict diabetic diet as well as weight reduction, where applicable along with intensive iron chelation. Foods with low glycemic index (e.g. green leafy vegetables, most fruits, raw carrots, kidney beans, chickpeas and lentils) should be preferred to foods with high glycemic index (e.g.: white rice, white bread and potatoes). Lifestyle modifications should be implemented in thalassemia patients which include a regular physical activity, high fibre diet, moderate fat consumption and restriction of refined carbohydrates. Referral to a dietitian/ diabetes educator for education on hypoglycemia, insulin injection education and carbohydrate counting should be recommended to adjust diet for optimum glycemic control. It is essential to maintain normal levels of vitamin D before pregnancy to prevent gestational diabetes. Optimum vitamin D levels also lowers the risk of gestational diabetes [33]. Dietary modifications for gestational diabetes as for DM should be followed [33].

Recommendations

1. It is recommended to have a normal balanced diet rather than focussing on special dietary iron restrictions (Level of Evidence: 2).
2. All patients receiving Deferiprone (Kelfer), having growth failure, documented zinc deficiency or low bone mass must be supplemented with zinc (Level of Evidence: 2).
3. Vitamin C supplementation (2-3 mg/ kg/ day) is recommended in patients on desferrioxamine chelation. (Level of Evidence: 2).
4. Vitamin C should not be used in the early stages of intensive chelation with intravenous desferrioxamine for patients with cardiac failure or with severe cardiac iron overload on T2*MRI (Level of Evidence: 3).
5. Adequate dietary intake of calcium and vitamin D should be ensured by all age groups (Level of Evidence: 2).
6. All infants should receive 400 IU of vitamin D as supplementation as per standard protocol. In older children, 1000-2000 IU vitamin D supplementation daily is recommended. Monitoring levels will help guide dosing [22] (Level of Evidence: 2).

7. In vitamin D deficiencies, oral vitamin D of 2000-4000 IU along with calcium supplementation of 500-1000 mg (50-75mg/kg/day) should be given daily for 12 weeks (Level of Evidence: 2).
8. Folic acid supplements at 1-5 mg/day especially to thalassemia patients on low transfusions, should be considered (Level of Evidence: 3).
9. Folic acid in the dose of 5 mg weekly is recommended in regularly transfused TDT patients (Level of Evidence: 2)
10. Concomitant folic acid supplementation is recommended for patients on hydroxyurea, pregnant women and aging thalassemia patients. (Level of Evidence: 2).
11. For thalassemia patients with diabetes mellitus, along with pharmacological measures, lifestyle modifications like consuming a diet high in fibre content, moderate fat content and low on refined carbohydrates, and regular physical activity is recommended (Level of Evidence: 2).

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18 Antenatal Screening for Thalassemia

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Thalassemia is one of the commonest monogenic disorders, associated with a notable financial burden on healthcare services. Affected families go through immense physiological, emotional, and social stress. As per the National Family Health Survey (NFHS)-4, conducted in 2015-16, the overall prevalence of consanguineous marriages is approximately 9.9% [1]. This along with the vast practice of inbreeding in Indian communities is responsible for high carrier frequency rates.

The aim of screening (carrier testing) is to identify carriers such that adequate information may be given to the carriers prior to the birth of a child with thalassemia. Planning a strategy for screening requires knowledge of the prevalence in the community, genotype-phenotype correlations and resources available. Screening is useful both at the level of individual families and at the level of the community. Screening strategies limited to beta thalassemia are detailed here:

The various aspects related to screening include the following:

- Public education and awareness
- Carrier screening
- Genetic counseling of individuals and at-risk couples
- Access to prenatal diagnosis
- Dialogue with religious and community leaders to ensure screening strategies are acceptable based on the cultural practices of the region.

The two types of screening are:

a) Mass screening – Provided to all individuals before childbearing age. It is performed at the community level and requires larger resources and has logistic challenges.

b) Targeted screening – Cascade, pre-marital, pre-conceptional or antenatal

Strategies used in mass/ community screening

Raising public awareness regarding beta-thalassemia is required for screening programmes to be successful either at the community or at the individual level. Public awareness needs to be raised in both urban and rural communities using the display of posters, handing out of pamphlets, radio and television programs

and audio-visual displays in prominent places. Social media platforms, street plays and the involvement of primary healthcare workers (ASHA workers) in information, education and communication (IEC) activities is important.

Laboratory tests

1. **Red cell indices**

   Low MCV (<80 fL) and MCH (<27 pg) with a relatively high RBC count could guide which individuals need further testing [2].

2. **Naked Eye Single Tube Red Cell Osmotic Fragility Test** (NESTROFT) is of historic importance as it is technically cumbersome and has poor standardisation [3]. Various discrimination indices like the Mentzer index, Shine and Lal index and England and Fraser index, popularly used for population screening, also have suboptimal diagnostic reliability [4].

Table 1 summarises the interpretation of the common screening tests discussed above which are useful for both community and individual patient screening [5]. At the community level for mass screening, readily available tests like CBC, red cell indices and HPLC are adequate and molecular genetic tests are not required.

Table 1. Interpretation of RBC indices and HPLC reports to aid screening of thalassemia trait

Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)	RBC Count	Red Cell Distribution Width (%)	High-performance Liquid Chromatography	Possible Diagnosis
>80	>27	Normal	12-15	HbA2 < 3.5% HbF <2%	Normal
<80	<27	Raised/ High normal	12-15	HbA2 > 3.5% HbF < 2%	β-thalassemia trait
<80	<27	Raised/ High normal	12-15	HbA2 < 3.5% HbF <2%	Silent β-thalassemia
<80	<27	Raised/ High normal	12-15	HbA2 <2% ± HbH, Hb Bart's	Alpha thalassemia trait*
<80	<27	Raised/ High normal	12-15	HbA Decreased HbA2 Decreased HbF Increased	δβ-thalassemia
<80	<27	Normal/ Low	>15	HbA2 < 3.5% HbF <2%	Iron deficiency anemia
<80	<27	Raised/ High normal	12-15	HbS, HbE, HbD, etc	Other hemoglobinopathies

*Molecular tests needed for confirmation

Strategies used for targeted screening

High-performance liquid chromatography (HPLC) provides both qualitative and quantitative estimation of different haemoglobin fractions. It is automated, high-throughput, and needs small sample volumes. The results are precise and reproducible. HPLC is the recommended method to detect beta-thalassemia carriers. The interpretation of HPLC report to detect carriers and the limitations (false negative tests) is described in Chapter 4 on diagnosis.

Targeted screening can be performed in the following settings:

a. **Pre-marital screening:** This allows carriers to make informed decisions when planning a family. It is mandated by law in a few countries (Iran, Saudi Arabia, Palestinian Territories, and Cyprus) [6-9]; However, it is voluntary in most others.

b. **Cascade screening:** It is the screening the extended family of an index case of beta-thalassemia major is a proven strategy that should be recommended to all families at diagnosis [10].

c. **Antenatal screening** as part of routine first trimester screening tests in pregnancy include evaluation of complete blood counts (CBC) including red blood cell indices. HPLC should be interpreted in conjunction. This is recommended in all pregnancies irrespective of the presence or absence of a family history of beta-thalassemia, given the high prevalence of carriers in various communities in India.

d. **Prenatal diagnosis:** Genetic diagnosis using next-generation sequencing platforms for beta-globin gene mutations by chorionic villus sampling or amniocentesis should be offered. This is described in detail in Chapter 19.

Thalassemia screening, akin to genetic testing, should be conducted after due informed consent and pre-test and post-test counseling. Hence, robust counseling services should be available in all programmes of carrier screening, either at the community level or targeted screening.

Recommendations

1. Antenatal screening for thalassemia carrier status with blood counts and HPLC should be actively recommended as part of routine antenatal care early in pregnancy. Screening should be done at the first antenatal visit and integrated with other standard-of-care tests. Screening with HPLC should be voluntary but universally applicable with an "opt-out" policy (Level of Evidence: 2).
2. Paramedical and ASHA workers should be educated and involved in universal antenatal screening with HPLC (Level of Evidence: 3).
3. Novel and simplistic IEC (Information, Education and Communication) materials should be developed to generate maximum public awareness.

Education of college students with social media platforms will help educate the community regarding the need for screening for carrier status (Level of Evidence: 3).

4. Genetic counseling centres should be developed at all district-level hospitals for the proper communication of screening protocol and counseling of high-risk couples (Level of Evidence: 3).
5. Option of screening for carrier status based on individual needs of the family i.e. pre-marital, pre-conceptional and antenatal should be offered (Level of Evidence: 2).

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19 Prenatal Diagnosis of Hemoglobinopathies

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The important strategies for prevention of hemoglobinopathies are

1. Identification of couples-at-risk (both partners: thalassemia carrier/minor/trait) which includes couples with previously affected children
2. Genetic counseling regarding the risk of having child homozygous for thalassemia
3. Prenatal testing to identify affected fetus

At present, majority of referrals for prenatal diagnosis for thalassemia are among the couples who have already have a previously affected child with thalassemia (secondary prevention). However, the goal should be to offer primary prevention i.e., couples who have undergone voluntary screening before conception and are confirmed to be thalassemia carriers/minor/trait. They are counseled regarding preventing the birth of a thalassemia homozygous child, through prenatal testing of the fetus in the first trimester in the first pregnancy itself. With increasing awareness, there are couples-at-risk who are approaching for prenatal diagnosis with the aim of primary prevention.

Two individuals, who have thalassemia minor/trait, should not be dissuaded from getting married, as they can avail the option of prenatal testing of the unborn fetus, to prevent birth of a thalassemic child. Additionally, they can opt for other methods of conception (Assisted Reproductive Techniques, ART) – using donor sperm/ovum or adoption of a child, to fulfil their parenting instinct.

The following steps are needed for an effective prevention program:

- Mass screening to identify thalassemia carriers among youth, followed by genetic counseling with an advice to test the partner in the future, if not tested earlier.
- If both partners are identified as thalassemia carriers, they should be counseled and referred for prenatal testing when the woman conceives.
- If not tested earlier, each pregnant woman should be offered thalassemia carrier screening and, if she is a carrier, her partner also should be advised testing immediately.
- The molecular testing to find the mutations in both partners should be done before they plan their pregnancy, in case of primary prevention. This should be available to the laboratory analyzing the blood obtained by chorionic villus sampling (CVS) or amniocentesis.

- This needs to be done preferably before 10 to 12 weeks of pregnancy, so that CVS can be performed between 11 and 15 weeks of pregnancy. If the pregnant woman presents later than 15 weeks for prenatal diagnosis, an amniocentesis can be done upto 18 weeks of gestation.

To achieve the above goals, the following services are mandatory [1]:

- Carrier screening by a complete blood count (CBC with MCV <27 Fl, MCH <27 pg and high RBC counts - erythrocytosis) and high-performance liquid chromatography (HPLC) with adequate access to the laboratory, also during pregnancy, if not tested earlier.
- Training of healthcare professionals in genetic counseling.
- Facilities for prenatal diagnosis (PND) include training of personnel in sampling techniques and access to a molecular testing laboratory for DNA analysis with a reasonable turnaround time.

It is important to keep in mind that prenatal testing is voluntary, based on informed consent given by the couple.

With so many hemoglobin variants known till date, it is important to understand, in which situations homozygous or compound heterozygous state will lead to a symptomatic disease and warrants prenatal testing [2]. Table 1 shows various genotype combinations and the corresponding phenotype, which would be helpful for counseling and taking decisions regarding the need for prenatal testing [1,3].

Table 1. Combination of different hemoglobinopathies and likely phenotype*

Symptomatic thalassemia syndromes	Sickle cell disease syndromes	Usually asymptomatic/ occasionally very mild symptomatic states
1. βT / βT (includes β^0 / β^0, β^0 / β^+ and β^+ / β^+ genotypes) 2. βT / Hb Lepore 3. βT / δβ 4. βT / HPFH 5. βT /βE (Hb E)	1. βS / βS 2. βS / βT 3. βS / βD (Hb D)	1. Compound heterozygote for Hb D and Hb E (βD / βE) 2. Compound heterozygote for Hb D and β-thalassemia (βD/βT) 3. Compound heterozygote for Hb S and Hb E (βS / βE) 4. Compound heterozygote for Hb S and HPFH (βS / HPFH) 5. Hb D (βD / βD) 6. Hb E (βE / βE) 7. Homozygous β^{++} thalassemia (silent) 8. Compound heterozygous ααα / β^+ thalassemia

Antenatal testing strategy

Antenatal testing for hemoglobinopathies relies on invasive procedures which include CVS (more commonly) and amniocentesis. These involve a very small risk of fetal loss. However, the first step of antenatal screening is genetic counseling, which should include the following aspects:

- Explaining about the nature of disease, its treatment and prognosis.
- The couple should be explained that there is a 25% chance in each pregnancy of having a thalassemia homozygous or symptomatic disease in the unborn child, in view of most hemoglobinopathies being autosomal recessive.
- Risk of the invasive procedure: Currently the rate of procedure-related pregnancy loss for CVS and amniocentesis are similar and is estimated to be very low (0.1–0.3%) in the hands of experienced health care providers, which needs to be explained to the couple [4]. The obstetrician conducting the procedure needs to explain the procedure-related risk in the context of the patient's background risk as well.
- Accuracy of the PND tests: Despite all due precautions (as explained below), there remains about a small chance of about 1-2% that the test results could be erroneous [3]. This emphasizes the need for improved quality control in laboratories to ensure that accuracy of PND is ensured [5-7].
- The procedures for PND should be done as early as possible, given that termination of pregnancy can be performed only till 24 weeks gestation, provided appropriate certification from doctors is obtained.

It is important to carefully evaluate the HPLC report of the couple-at-risk and get molecular testing done, depending upon the hemoglobinopathy diagnosed as per the HPLC report.

Mutation analysis is performed for the couple and/or their previous affected child, There are more than 350 mutations known in the β-globin gene [2]. For β-thalassemia, most laboratories test initially for the common five mutations seen (in 90% of patients) in our country [IVS 1-5 (G→C) (HBB: c.92+5 G>C), IVS 1 -1 (G→T) (HBB: c.92+1 G>T), Codon 41/42 (- TCTT) (HBB: c.124_127delTTCT), Codon 8/9 (HBB: c.27_28insG) and the 619 bp deletion] using ARMS PCR [8]. This is followed by HBB gene sequencing for families negative for above mentioned common mutations. Some laboratories in the present era resort to direct sequencing of the HBB gene by next generation sequencing (NGS). Though most mutations are picked up by Sanger sequencing, a small proportion may be missed as β-thalassemia encompass a large spectrum of mutations including large deletions, limiting the utility of sanger sequencing as a diagnostic method [9-11]. Large deletions can be picked up by multiplex ligation probe amplification (MLPA). Molecular confirmation and PND in such families can be

offered either using indirect methods like Restriction Fragment Length Polymorphism (RFLP) (if the DNA sample of the previous affected child is available) or cord blood HPLC analysis if the diagnosis in the affected child is beyond doubt. These two techniques are very rarely used presently.

Chorionic Villus Sampling (CVS) is the preferred procedure for PND for thalassemia and other hemoglobinopathies. It can be done from 11 to 12 weeks of pregnancy, till as late as 16 weeks. Those pregnant women, who report later than 15 weeks of gestation for PND can be offered amniocentesis. This can be performed at 16 to 18 weeks of pregnancy. Maternal cell contamination (MCC) can be a source of misdiagnosis particularly in CVS samples, so a careful microscopic dissection to remove contaminating maternal decidua is performed prior to the DNA extraction and analysis [12]. Ruling out maternal contamination before running the mutation studies is additionally done using Variable Number Tandem Repeat (VNTR) markers, such as for ApoB, IgJH and D1S80 [12]. Recently, techniques like QF PCR are also being used [13].

Based on the mutations in the couple and/or affected child, the CVS is screened for those mutations using ARMS/HBB gene sequencing. It is preferable to confirm CVS DNA tested by ARMS technique by HBB gene sequencing, if possible. Using two techniques ensures consistency and accuracy.

Rarely, cordocentesis can be done if the gestation age is 18 to 20 weeks and amniocentesis is not feasible. HPLC on the blood thus collected is performed and the proportion of adult hemoglobin to fetal hemoglobin is used to determine the diagnosis.

Recommendations

1. Antenatal screening is the best way to identify couples at immediate risk of having a child affected with thalassemia, preferably before eight weeks of gestation (Level of Evidence: 3).
2. Screening test recommended for detection of carrier state of beta-thalassemia is high-performance liquid chromatography to quantify HbA2, HbF and other variants such as HbS and HbD (Level of Evidence: 2).
3. Prenatal diagnosis involves identification of genetic mutations in the couple before testing the fetal DNA. Fetal diagnosis involves molecular testing in the first trimester using CVS in the 11^{th} to 15^{th} week of gestation or amniocentesis in the 16^{th} to 18^{th} week of gestation (Level of Evidence: 2).
4. While testing the fetal DNA, parental DNA samples and appropriate positive and negative control DNA's (preferably as duplicates) should be amplified in parallel in the same set of PCRs, when using ARMS as a diagnostic tool for mutation analysis. It is recommended to use a limited number of amplification cycles to minimize co-amplification of any maternal DNA (Level of Evidence: 2).

5. CVS should be carefully dissected to remove maternal decidua under the microscope to minimize maternal contamination (Level of Evidence: 2).
6. To maintain accuracy, results need to be confirmed by two different techniques, especially if ARMS PCR has been used as a first choice for mutation detection (Level of Evidence: 2).
7. Bi-directional HBB gene sequencing is the preferred technique in the present era (Level of Evidence: 2).
8. Any diagnostic test using genetic testing must be done after obtaining appropriate parental consent in writing detailing the type of tests to be undertaking, procedural risks entailed, etc. The fetal reports must clearly indicate the type of test undertaken explaining the chances of misdiagnosis based on technique used (Level of Evidence: 4).

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20 Establishing Thalassemia Day Care Center

Amita Mahajan, JS Arora, Vinita Srivastava

It has been recognized for some time now, that in areas of high prevalence, dedicated day care units are the most effective way to deliver optimal multidisciplinary healthcare for patients with thalassemia [1,2]. An ideal thalassemia day care (TDC) center is patient-centered and enables safe transfusions, monitoring and treatment of patients with thalassemia in a patient-friendly environment. This chapter summarizes the organization of thalassemia day care centers as the fulcrum around which multidisciplinary care for thalassemia is delivered.

Benefits of a dedicated Thalassemia Day Care Center include:

- Planned appointments for red cell transfusions can be given, leading to minimal disruption of school/office hours of patients and accompanying persons
- Patients can have easy access to trained personnel
- Monitoring of patients can be better
- Provides a platform for distribution of government support for medications including iron chelators
- Peer-support is available to all patients and parents
- Facilitates organization of group activities (medical and recreational) for patients/parents and formation of support groups
- Cost effective for the patient and institution (both private and governmental)

The National Health Mission (NHM) guidelines also clearly state the benefits of TDC Centers at District hospital level and have laid down the guidelines for the same [2].

Location of TDC

The NHM guidelines suggest that all districts in areas of high prevalence should have dedicated TDC units supported by Referral Centers. Having to travel long distances to a TDC is a major reason for treatment abandonment in India [2,3]. The units must be dedicated but not isolated. TDC may either be standalone centers (especially in districts) or located within a multidisciplinary hospital. A hub and spoke model where standalone centers can refer patients to a TDC in a multispecialty hospital ensures that patients can access all allied specialties

and services under one roof. It may share space and services with other red cell disorders in view of their common needs. The center must offer a welcoming, comfortable, age-appropriate environment. At large centers that cater to both children and adults, it is ideal, if feasible, to have separate areas for children and adults preferably in proximity to each other. Table 1 outlines the amenities required for an optimally functional TDC.

Table 1. Amenities required for an optimally functional Thalassemia Day Care Center

Recliners/Beds: 5-10 as per patient load
Working area for Doctors/ Nurses documentation
Emergency trolley with medicines/ Access to Oxygen supply
Procedure area – for fixing IV lines and collection of blood samples.
Washroom and wash-basin
Drinking-water dispenser
Telephone & Intercom line, Computer with printer
IV stands, Weighing/Height measuring equipment
Sitting area for accompanying persons Play/recreational area where feasible Storage cupboards Television Air-conditioning
Requisite stationary: Patient files with monitoring proformas, Blood bank forms, Standard operating procedures (SOPs) for transfusion, management of reactions, patient files, consent form, adverse event notification forms.

Key services

The most important function of TDC is to deliver safe transfusion in a patient-friendly environment. Appointments for transfusions can be streamlined through TDC with communication with the blood center/ bank. Close coordination with a blood center with the mandate for optimal screening and delivering safe blood (preferably NAAT-tested) without delay is a pre-requisite. The blood center should preferably have the capability for leucodepletion, extended red-cell typing, antibody screening and be able to generate adequate supply from voluntary blood donors.

To deliver a better service, the TDC needs to closely collaborate with hospital administration to arrange afternoon, evening or weekend transfusions.

The key personnel include a trained doctor for prescribing packed red cell units, managing adverse reactions, monitoring, prescribing chelation and coordinating referral to other specialties as indicated. The specialist hemoglobinopathy nurse

plays a crucial role in the management of patients with thalassemia. Her responsibilities include the supervision of packed red cell transfusions, patient support and communication, and to encourage compliance [4,5,6]. To develop this expertise and continuity of care, it is ideal that dedicated staff is assigned to this unit and not rotated [3]. The specialist nurse is the biggest asset of this unit with the closest contact to the patient, and acting as liaison between the patient and medical team. The medical personnel should receive ongoing education regarding optimal management of thalassemia. A dedicated "Medical Social Worker" is needed to guide patients/parents regarding available welfare programs through governments as well as available support from NGOs. Counselors should be available to counsel patients and parents regarding the disease, management, prenatal diagnosis for the next pregnancy as well as cascade screening. Auxiliary staff is required for taking blood samples/ bringing blood bags from the blood center.

Clinical information systems to organise data and to develop patient registries are strongly recommended. It is strongly recommended that patient monitoring sheets are filled on every visit as per the format in the NHM Guidelines Section C, Annexure C-1 [2]. This ensures adequate management and serves as a baseline of minimum monitoring that is needed for care. Ideally, each patient's record file is maintained in physical format along with a computerized record file by the specialist nurse. Digital data capture of patient records is made available in some states in India and is strongly encouraged.

Standalone Day Care Centers without access to hematologist and allied specialists should be linked to a referral center for comprehensive care and organizations working for the cause of thalassemia in the region/state.

Chelation

The personnel at Day Care Unit should be trained to prescribe chelation drugs in appropriate doses and coordinate smooth delivery of free medications in government supported units.

Monitoring

Optimal monitoring is a pre-requisite to prevent organ dysfunction in patients with transfusion dependent thalassemia. It is ideal that the routine monitoring of patients is done in a systematic manner at the facility, thereby ensuring compliance with monitoring and ensuring real-time response to any changes in the patient's condition [7].

Coordination with allied specialties

Patients with thalassemia require inputs from a number of allied specialties especially gastroenterology & hepatology, cardiology, endocrinology and fertility specialists [8-10]. It is therefore, imperative that the day care facility either

organizes multi-specialty clinics at regular intervals or arranges referral to these specialists in a thalassemia referral unit in a coordinated manner.

Stand-alone day care units and day care units in referral hospitals both play an important role in the care of patients with thalassemia. Coordinated care in a hub and spoke model ensures comprehensive care.

Recommendations

1. Delivery of thalassemia care should be through day care centers (Level of Evidence: 3).
2. Medical and nursing personnel should coordinate education, transfusions, chelation and record maintenance (Level of Evidence: 3).
3. Access to allied specialties ensures comprehensive care (Level of Evidence: 3).

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21 Aids for Persons with Thalassemia

Amita Mahajan, JS Arora, Shobha Tuli, Vinay Shetty

Optimal management in the current era can lead to a significantly prolonged life span of patients with thalassemia with good health related quality of life in the absence of organ dysfunction. Further, curative treatment i.e., allogeneic Bone Marrow Transplant (BMT), a curative option, is now feasible for many more patients. However, both, optimal supportive management and BMT have significant financial implications for families. Increased awareness about the challenges faced by these patients and families and strong advocacy by various support groups has led to a number of initiatives that can help to mitigate the financial burden on the families. This chapter summarizes some of the key aids that the patients/ families can access.

Government Aids

Statutory recognition of thalassemia as a disability

1. The Rights of Persons with Disabilities (RPwD) Act, 2016, enacted on 28.12.2016 (enforced w.e.f. 19.04.2017) recognizes persons with blood disorders (Thalassemia, Hemophilia and Sickle Cell Disease) as 'persons with disabilities'(PwD) eligible for a Unique Disability Identity Card (UDID) [1]. Those certified as having "percentage of disability" equal to or more than 40% (as certified by a medical board of designated hospitals) are termed as persons with benchmark disabilities. Patients with TDT are included in this category (Level 2- Level 9 disability), although those who are thalassemia traits or non-transfusion dependent thalassemia (Level 1 disability), do not qualify as benchmark disabilities. Once a diagnosis of TDT is established by appropriate clinical and laboratory criteria and the patient has a Hb which remains persistently below < 7g/dL, and he/she needs a regular transfusion to maintain Hb > 10 g/DL, he/she qualifies to be eligible for getting benefits due to a person with benchmark disability. With passage of time, as and when new complications develop, the level of disability needs to be reassessed and higher scores should be awarded. The process of evaluation is dynamic and should be reviewed periodically every 3 years. However, in patients with severe disability score above 80%, permanent certificate can be issued subject to proof of survival [2].
2. As per the RPwD Act, 2016 and rules made under the same, competent medical authorities notified by the States or Union Territories (UTs) are

empowered to issue Certificate of Disability to patients with Thalassemia. The panel that certifies to the presence of and the degree of disability in patients with thalassemia comprises of the following:

a. Chairperson-Chief District Medical officer or the Chief Medical officer of the hospital

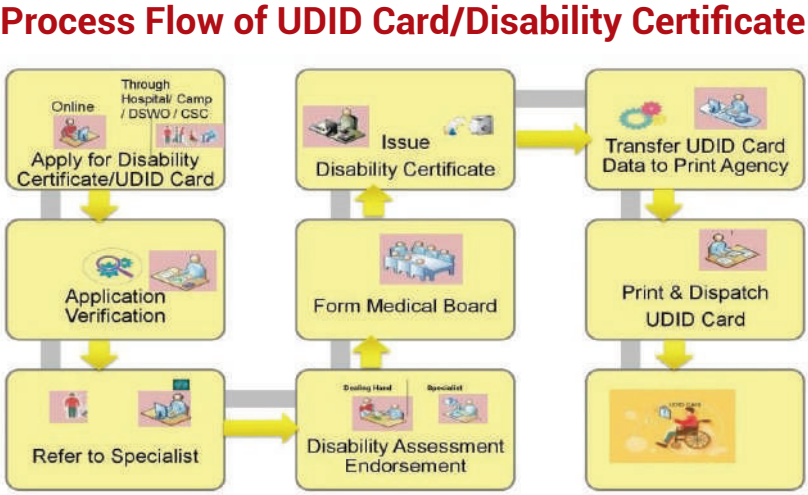
b. General physician or paediatrician as the case may be

c. Sub-specialists such as cardiologist, orthopaedic surgeon etc if there is specific organ dysfunction.

3. Patients can apply online on the UDID web portal with documentation confirming the diagnosis and of receiving appropriate medical care on a consistent basis. They need to be examined at designated government hospitals for the above procedure.

4. The patients are graded for their level of disability. All transfusion-dependent patients are eligible for certification for 40% disability. The process is briefly summarised in Figure 1.

Figure 1. Process flow of UDID Card/Disability Certificate



Benefits of UDID card

Patients can apply online on the UDID web portal with documentation confirming the diagnosis and of receiving appropriate medical care on a consistent basis and need to be examined at designated government hospitals [3]. The UDID Card is issued to every patient with TDT who applies for the same and it entitles him/her to certain benefits as follows:

- Every child with benchmark disability can access to free education from six to eighteen years of age including books and other learning materials.

- All government and government-aided institutions of higher education have to reserve not less 5% seats for persons with benchmark disabilities and an upper age relaxation of five years for admission.
- Scholarships in appropriate cases to students with benchmark disability
- Extra time for completion of examination papers
- Non-discrimination for education and employment: The Act also qualifies denying reasonable accommodation (adjustment) as discrimination which implies that organizations are expected to accommodate PwD with certain allowances and exemptions taking into account their specific disability.
- The right to equality, life with dignity, and respect for his/her own integrity equally with others
- A number of states/ UTs also offer compassionate allowance or pensions to patients. The quantum varies from State to State.

Union Government

The National Blood Transfusion Council has mandated that blood should be provided free of cost to patients with TDT [4,5]. According to Rule No. (4.4) of the "Revised Guidelines - 2022 for Recovery of Processing Charges for Blood and Blood Components - reg." issued by the Directorate General of Health Services dated 14 June 2022, it is mandatory for blood centres (Government supported and non-Government supported) to provide blood/ blood components free of cost to the following patients: thalassemia, hemophilia and sickle cell anemia. It is permissible for the institutions to levy processing charges as per the NACO guidelines. For more information: http://nbtc.naco.gov.in/

Prime Minister Relief Fund (PMRF)

Patients/families can apply for a onetime assistance for medical treatment at an empaneled hospital from PMRF especially for curative treatment such as BMT. Applicants can apply through an application form available on the website with:

- Copy of residence proof
- Income certificate
- Original medical certificate mentioning the diagnosis & estimated expenses
- Two passport size photographs
- Letter of recommendation from a member of parliament.
- The maximum support that can be granted is INR 300,000.
  For more information: https://pmnrf.gov.in

Rashtriya Arogya Nidhi (RAN) Scheme

This is a financial assistance scheme for patients below threshold poverty line suffering from specified rare diseases including thalassemia receiving treatment at government hospitals/institutes having super specialty facilities. The financial assistance is in the form of 'one-time grant'" with no reimbursement of expenditure already incurred. Families of government employees or those covered under Ayushman Bharat - Pradhan Mantri Jan Arogya Yojna (PMJAY) are not eligible.

Documents required:

- Application form in prescribed proforma duly signed by the treating doctor and countersigned by the Medical Superintendent of the Government hospital/institute
- Copy of the income certificate.
- Copy of the ration card.

For more information: https://main.mohfw.gov.in/major-programmes/poor-patients-financial-assistance/rashtriya-arogya-nidhi

Ayushmaan Bharat – Pradhan Mantri Jan Arogya Yojana

This centrally sponsored flagship scheme aims to provide an annual health cover of up to Rs. 5 lac per annum per family to those belonging to socio-economically backward classes at empanelled hospitals. As thalassemia is covered under the National Health Mission (NHM), the routine care for e.g. transfusion and chelation are not covered under this scheme. The patients, however, can access support for related or unrelated medical issues including for surgical procedures [6].

Income-tax rebate

The Central Board of Direct Taxes as per rule 80DDB allows tax rebate on income of up to INR 40,000 to patients and parents of patients with thalassemia. As per amendment 11DD, this certificate can now be procured from any specialist and not necessarily a government specialist doctor.

For more information: https://cleartax.in/s/section-80u-deduction

Fare Concession for Thalassemia Patients

Patients and one escort accompanying them can avail concessions in fare of up to 75% while traveling by train. The concession is available for reaching to the place of treatment and for returning back to home after diagnosis or regular check-up.

For more information: https://railmitraindia.medium.com

State Governments

The National Health Mission recommends that the following facilities should be provided by individual state governments:

- Physical infrastructure for setting up Thalassemia Day Care Centres
- Iron chelators – Deferoxamine, Deferiprone and Deferasirox
- Leuco-depletion blood bags (with in-line filters) & bed-side leucocyte filters
- Laboratory investigations at civil hospital
- Pre-natal diagnosis
- Screening facilities

For more information: https://nhm.gov.in

Chief Minister Relief Fund (CMRF)

All states have provision for financial assistance from the CMRF. The documents required are similar to that for PMRF but a letter of support is needed from the local MLA instead of an MP. The quantum of support varies from state to state.

State Specific Programs

- Different states have insurance-based schemes for persons with TDT receiving treatment in hospitals which are registered under the scheme. In some cases, the support is given to the registered hospital by way of reimbursement. The financial limit and the range of support varies from state to state. Some provide support for HSCT, with the extent of support and the conditions for eligibility varying from state to state.
- Schedule of Municipal Corporation Hospitals: Municipal Corporation Hospitals have included chelators and leucocyte filters in their "Schedule of Items" which is required to provided free of cost to all persons receiving treatment in their hospital.
- Many states offer free bus services and a few offer a 50% concession on bus fares to patients with thalassemia.
- Employee State Insurance Scheme (ESIC): Children of employees working in organizations registered under the scheme, drawing a salary of less than INR 21000/- per month are eligible for benefits.

For more information: https://www.esic.nic.in/information-benefits

Support from Parent's Employers

Parents of TDT patients who are employed in public sector undertakings are eligible for reimbursement of expenses incurred for management of TDT, if prescriptions are given by doctors attached to government hospitals. Reimbursement of expenses incurred for management of TDT is also provided by some private sector organizations for their employees who have children with TDT or themselves have TDT.

Corporate Social Responsibility (CSR) Initiatives

Many corporate organizations and public sector undertakings, offer support for patients with thalassemia from their CSR funds. An example of this is the "Thalassemia Baal Sewa Yojana", funded by Coal India and launched by the Ministry of Health and Family Welfare, Govt. of India for BMT for thalassemia patients < 12 years with matched family donors or unrelated donors and family income < Rs. 5 lacs per annum, can avail financial assistance up to Rs 10 lacs for BMT at designated hospitals if they met the set criteria [7].

Non-Governmental support

A number of support groups assist patients with:

- Access to subsidized medicines
- Blood collection
- Free medicines & investigations to needy patients
- Access to advanced testing
- Counseling
- IEC & educational material

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22 Transition of Care to Physician

Ritika Sud, Jagdish Chandra

The last few decades have seen strides in the care of thalassemia in our country, thus leading to an increase in lifespan of these patients. But with this, we are facing newer challenges in the management of thalassemia including the major challenge of transition of care (TOC) from pediatric care to adult care settings – a rather unchartered territory in preceding years when patients' survival into adulthood was rarely seen. Fortunately, increasing number of patients with transfusion-dependent thalassemia (TDT) are living to become adults, thus prompting the need for adult care physicians to be initiated in the care of these patients.

Definition and purpose of transition

The American Society for Adolescent Health and Medicine describes transition as an active medical process, ''the purposeful, planned movement of adolescents and young adults with chronic physical and medical conditions from child-centred to adult-oriented health care systems'' [1]. Transition is a change taking place in a continuum, and not a single administrative event in which the individual graduates from the pediatric to the adult setting (transfer is the end result of a process of transition). The goal of transition is to maximise lifelong functioning through the provision of developmentally appropriate healthcare services that continue uninterrupted as one moves from adolescence to adulthood.

Need for Transition: Pediatric Vs Adult settings

In most states, pediatric hospitals continue to serve as the primary centre for the treatment of adult patients with TDT. As transition is a relatively new concept for thalassemia patients, it often remains within the walls of pediatric hospitals, with pediatricians continuing to provide care to adult patients as well. Although there are some advantages to adults being treated in pediatric settings where they began treatment, it has its pitfalls too. The pediatric setting provides interdisciplinary, comprehensive speciality care in a family-inclusive patient-centred setting. The approach is psychosocial and supportive, with a developmental focus, and long-established relationships with care providers. Patients have more access to research and new treatments.

Despite these benefits, there are several reasons to move TDT patients to adult care once they turn adults, the most crucial being that caregivers are trained in

internal (adult) medicine and are better equipped to deal with adult health issues [2]. They communicate directly with patients, making way for privacy and self-reliance. In-patient admissions are easier and care more centralized. Though the adult care givers may appear less familiar with thalassemia management at first and care may seem more disease-centred than patient-centred, in the larger interest of the patient, transition is definitely the way forward. Additionally, caring for sick patients of the adult age group by the pediatrician may invite legal issues in case of adverse events/outcomes.

Barriers to transition

Most barriers to transition are due to the novelty of transition and are likely to reduce as transition to adult care settings becomes more commonplace. Nevertheless, barriers remain in place for the present and it is only through dialogue and implementation of ideas that these barriers will eventually fall [3]. Not surprisingly, the most challenging issue in transition at present is the difficulty in identifying adult primary care providers, as few are willing to equip themselves with necessary knowledge, training, and experience to treat pediatric-onset diseases. This is largely on account of lack of time, as they are busy with their commitment to other adult diseases. Often there is a lack of institutional support, even if the physician is willing or able to devote time to planning and addressing transition issues. At times patients and their families too are resistant due to fear of the unknown. Medical insurance also creates barriers as patients try to switch their care providers. Lack of communication between pediatric and adult care facilities can be a problem and can lead to further communication gaps between patients and their adult care providers. Transitions based on age, and not maturity, add to the list of reluctant patients who are not prepared to move to adult care settings. Abruptly ending pediatric care services without a period of transition during movement from one care provider to another creates further impedes transition. Lastly, differences in basic orientation and practice between pediatric and adult care centres makes transition a tedious process.

Unmet needs in transition care

Most of the challenges faced during transition are similar across various childhood-onset diseases including thalassemia. Three categories of unmet needs seem particularly prominent around the time of transition:

1. Need to improve self-management of chronic medical conditions
2. Need to enhance capacity of the adult healthcare system to accommodate young adults with chronic medical needs
3. Need to reduce lapses in care during the transition period

Taking cognizance of these, guidance and recommendations can be formulated to ease the challenges of transition.

Guidance for facilitating transition

A successful transition is critical to ensure patient longevity. In due course of time, a larger number of patients will face the challenge of transition. Transition is not limited to an individual but rather involves many people, which includes the patient and the caregiver, nursing personnel, social workers, pharmacists, physicians and many other providers. Hence, there is a need for a well-planned and adequately timed transition. The transition process comes with concerns and expectations and, is a stressful time for patients and their families alike due to movement to a new environment. Keeping the various stages of physical and mental development of an individual in mind, the following are some suggestions for a smooth transition:

1. **Develop chronic disease self-management skills**

   Unlike several other pediatric-onset diseases, patients with TDT are fortunately mentally normal and if managed properly enjoy good general physical health. Hence, they can easily be empowered for self-management with minimal counseling [4]. These interventions should be done in an age-appropriate manner as described below.

   First decade

   Personal as well as medical independence and problem-solving must be inculcated in the child at the earliest. This can be ensured by making the child an integral member of the treatment team early in the course of the disease. The child's understanding of his or her condition must be assessed and built upon from time to time. Offering choices to the child during decision-making helps build the child's confidence and relations with their health providers. Discussing long-term goals and plans helps children develop a perspective of the various stages of life. The children should be made to understand the need for regular blood transfusions, proper chelation and follow-up for monitoring.

   Adolescents

   This is a sensitive period and the young and vulnerable thalassemics may seem rather demanding. However, emotional support and encouragement to accept rather than begrudge their illness, takes them a long way. Their involvement in care must increase at this time; and they should be taught to balance their lives and understand that despite the illness, they can lead near normal, long and productive lives. The concept of transition in various aspects (health care, moving to college) of their lives must be planned with the help of an "interdisciplinary team."

2. **Identify and support the receiving care team**

   Differences in practice, knowledge and culture exist between pediatric and

adult healthcare systems. Engaging and educating providers who will assume responsibility for a patient's care may help allay fears associated with transition for patients, families and healthcare professionals alike. Communication between the present and the future care team can minimize lapses in care, resulting in improved health outcomes and experiences for patients.

The transition care team must include a coordinator, a counselor, a trained nurse, and pediatric and interdisciplinary adult caregivers. Primary care physicians in most situations will be required to work as co-ordinators as well, with the help of counselors and/ or medical social workers. With a transition care team in place, it would be easier to approach new beginnings and bring the sensitive matter of pediatric care to a smooth end. Adding a comprehensive clinic that includes all specialities at the adult care setting, on a weekly or fortnightly basis would ease management issues. Adolescents moving to adult care settings can start interacting with the adult care team at these clinics at least one year in advance. Nurses, medical social workers and counselors should be adequately trained for the care of thalassemia patients. The pediatrician should take responsibility of such trainings.

3. Provide guidance to patients and families as they move between health care systems

During transition, individuals are required to move between two healthcare environments, a task that may be difficult for them and providing support will make the change easier. A team-based approach is essential to ensure transition in an efficient manner. Active involvement of peer mentors and good counsellors would help in smoothening the ridges. Generating documents, sharing information, and creating a personal health record (PHR) must be done. An up-to-date PHR is an important tool to ensure that necessary information remains with the patient, and is shared with the adult care team.

4. Institutional policy

Having a hospital/institutional policy in addition to a departmental protocol would further ease administrative concerns [5]. The hospital policy should address issues such as the age of transition and could vary in different settings. In some countries, 12 years is the age cut-off while others have allowed pediatric teams to continue managing till 21 years of age. Since adolescence is an integral part of the pediatric age group, patients with TDT should be looked after by pediatric services till 18 years of age. However, the patient may remain under pediatric care till 21 years of age in situations, when transition may not be feasible due to academic and career related milestones and/or ill health.

When the process of transition is begun, the family needs to be involved and informed, in order to familiarise them with the new set up and one

needs to ensure adequate resources (human and technical). A transition registry with line-listing of all patients due to be transitioned should be maintained to avoid loss of patients in transition. A checklist should be in place for pediatric and adult care providers for data transfer.

5. **Joint Clinics**

   Clinics should be held with both, pediatric and adult care teams available, for sorting out patients' concerns [6]. This joint clinic should include counselors and/ or medical social workers as well as nurses. The periodicity of such meetings can be decided based on the number of patients. The meetings should address the medical issues that the patient may be having including general health, transfusion frequency, iron overload status and its complications. Apprehensions of patients and family regarding care in adult settings should be addressed. The completeness of the records should also be looked into.

6. **Transfer of patients**

   The actual transfer of the patients should be done at the appropriate age, based on the institutional policy. However, patients should not be transferred to the adult care setting during any acute medical problems. Patients should carry with them the relevant records and specific plans, if any, for the next few months.

Recommendations

1. Patients with thalassemia should be managed in pediatric settings at least till the age of 18 years. After 18 years, TOC to the adult care team is recommended (Level of Evidence: 4).
2. The process of TOC should begin one year before the actual transfer (Level of Evidence: 4).
3. Patients/ care givers should be need educated in self- management of disease (Level of Evidence: 4).
4. It is recommended to have a hospital policy stating age of TOC, the process of TOC etc. (Level of Evidence: 4).

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23 Alpha Thalassemia

Pranoti Kini, Nisha Iyer, Sujata Sharma

Alpha thalassemia is widely prevalent and one of the commonest autosomal recessive disorders worldwide. The estimated prevalence of alpha gene carriers (deletion of one of the pairs of genes) globally is about 5%, with a higher prevalence in the Middle East, Mediterranean region and Southeast Asia. The prevalence in Southeast Asia is as high as 22.6% [1]. Hemoglobin Bart's disease is seen in 0.5-5 in 1000 births in Southeast Asia and HbH disease in approximately 4-20 per 1000 births [2].

Alpha Thalassemia is caused by deletion or non-deletion changes in the alpha genes, which are present on chromosome 16 (HBA1 and HBA2). The most common cause is deletions of large DNA fragments that involve the alpha genes.

Types of α-Thalassemia

Normally all individuals inherit four (2 copies each of HBA1 & HBA2) alpha globin genes, two from each parent. The normal genotype, therefore, is represented as αα/αα. Depending on the number of genes affected, alpha thalassemia is divided into the following types:

1. **α-Thalassemia Silent Carrier (αα/α-)**

   It occurs when one alpha gene is deleted. The mutation is benign and the individual is a silent carrier. There is generally sufficient production of alpha genes to ensue enough normal haemoglobin. The individuals are mostly asymptomatic or sometimes may present with mild anemia during periods of stress and pregnancy.

2. **α-Thalassemia Minor (αα/–) or (α-/α-)**

   a. **α-globin gene missing on each chromosome (α-/α-)**

      This is called the trans form of alpha thalassemia trait. The trans form of alpha thalassemia trait is common in African-Americans (20–30 percent) and people of African descent.

   b. **α-globin genes missing on the same chromosome (αα/--)**

      This is called the cis form of alpha thalassemia trait. They have mild microcytic hypochromic anemia (MCV <80 fl, MCH <27 pg) [3].

3. **Hemoglobin H Disease (α-/- -)**

   It is caused due to defect of three alpha genes by compound heterogeneity for α-thalassemia silent carrier and α-thalassemia minor. These can be deletional or non-deletional abnormalities of three globin genes and the affected individual thus has only one functional gene. This hereditary disorder is known as HbH disease and has a wide phenotypic spectrum from mild anemia to hemolytic anemia requiring red blood cell transfusions. Those with mild anemia need to be managed as Non-Transfusion Dependent Thalassemia (NTDT), while the severe form needs regular packed red cell transfusions like Transfusion-Dependent Thalassemia (TDT).

4. **Non-deletional α-thalassaemia (αTα/ or ααT/)**

   The rare forms of non-deletional α-thalassemia, Hb Constant spring and Hb Paksé are characterized by elongated alpha chains that result from mutation of termination codon in alpha-2 globin gene. Non deletional types are more severe than the deletional types. The excess of beta globin chains precipitate within RBCs causing membrane damage which results into hemolytic anemia. Some patients may not be diagnosed until adulthood while some may have severe NTDT phenotype. Heterozygotes of non-deletional alpha thalassemia have borderline MCV and MCH and may be missed to be picked up in most screening programmes that use RBC indices as screening tool [3].

5. **Alpha Thalassemia major /Hb Bart's hydrops fetalis (--/--)**

   This is the most severe and fatal form of thalassemia caused by affection of all four alpha genes. In newborns, there occurs an excess production of γ globin chains. These γ globin chains form tetramers resulting in hemoglobin Bart (Hb Bart). In Hb Bart's hydrops fetalis, there is severe fetal anemia and death in utero. Hb Bart has increased oxygen affinity and is inefficient in oxygen unloading at the tissue level of the developing fetus, resulting in hydrops fetalis, rendering the fetus non-viable. The clinical characteristics seen here are the prenatal onset of generalized edema accompanied by severe pleural and pericardial effusions. These are a result of congestive cardiac failure due to severe anemia. Extramedullary erythropoiesis, hepatosplenomegaly, and a large placenta are also seen.

Pathophysiology

Reduction in the production of alpha chains causes decreased production of HbA ($\alpha_2\beta_2$) and decreases the overall hemoglobin content. Excess unpaired β-globin chains form tetramers on the surface of RBC membrane and cause damage. This causes instability and hemolysis. In deletional HbH, the levels of HbH increase with rise in body temperature whereas in non-deletional type,

HbH directly precipitate at the membrane surface and generate reactive oxygen species even in the steady state. This explains why the non-deletional HbH is more severe than deletional HbH [4].

Clinical Features

The silent carriers and minor/traits for alpha thalassemia are usually asymptomatic, or may have mild anemia. Deletional forms of HbH have a wide spectrum of manifestations ranging from mild anemia to severe hemolysis, requiring transfusions intermittently like the NTDTs. They may have complications of iron overload, depending on the degree of anemia and transfusions required.

Non-deletional forms of HbH disease are characterized by severe anemia, often manifesting early in perinatal period or infancy, requiring regular blood transfusions [4]. They are associated with increasing splenomegaly, iron overload, and a variety of other clinical complications, including infections, leg ulcers, gallstones, and folic acid deficiency if not given comprehensive care. It is associated with a particularly high rate of thrombotic complications [4].

Hb Bart's most commonly presents in fetal life with severe anemia leading to hydrops fetalis and thus is incompatible with life. Those newborns with some modifiers, who are born with Hb Bart's present with severe anemia since neonatal life and need lifelong regular packed red cell transfusions [5].

Diagnosis

Complete blood count (CBC) with peripheral smear

Low hemoglobin (~9 and 11 g/dL in deletional and 8 and 9 g/dL in non-deletional HbH), elevated RBC count with low MCV and MCHC are typical findings on CBC [6]. Elevated RBC count may be the differentiating parameter from iron deficiency anemia which has a low RBC count. Peripheral smear shows microcytic hypochromic red cells. There may be signs of hemolysis, with nucleated red cells in severe forms of alpha thalassemia. Detection of HbH as HbH inclusion bodies seen as "golf-ball-like appearance of red cells" in a peripheral blood film when stained with supravital stain like brilliant cresyl blue is the hallmark of this disease [6].

Qualitative and quantitative hemoglobin analysis

Both high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) can be used for hemoglobin analysis. HPLC reports the presence/absence of HbH, Hb Bart's and HbCS (or HbPS), whereas Capillary electrophoresis also quantifies the amount. The characteristic finding in alpha thalassemia syndromes is the identification of fast-moving haemoglobin species (HbH β^4) or (Hb Bart, s ^{4}). Also, the HbA2 (α^2,δ^2) may be low due to reduced production of alpha globin chains [7].

Molecular diagnosis

Molecular testing methods include targeted deletion analysis for common deletions, sequence analysis, and deletion analysis of HBA1 and HBA2 alpha globin genes and the HS-40 regulatory region. Targeted deletion analysis using GAP-PCR can be performed initially [8]. The commonly used methods for non-deletional mutations are reverse dot blot analysis, primer specific amplification or PCR following enzymatic digestion. Unknown or rare deletions may be identified using quantitative PCR, long range PCR and multiplex ligation-dependent probe amplification (MLPA). The target NGS approach can be used to analyse entire globin genes coding regions, the key regulatory regions, and modifier genes [5,7].

Management of α-Thalassemia

Most patients with deletions/non-deletional mutations of 1 or 2 out of the 4 genes are asymptomatic or may have mild anemia that requires no specific management. Carriers or traits, if diagnosed, will require pre-marital or antenatal counseling to potentially prevent the possibility of a child being born with a more severe disease form.

Managing HbH disease

This disease has a wide phenotypic spectrum from individuals having mild anemia to having severe hemolytic anemia requiring red blood cell transfusions. Those with mild anemia can be treated as NTDT, while the severe forms can be treated as TDT and put on regular transfusion program [9]. Splenectomy is found to be beneficial in older children >6 years of age. Splenectomy for the treatment of transfusion-dependent HbH disease has the potential to convert a TDT to become NTDT. But splenectomy comes with its own complications of overwhelming post-splenectomy sepsis and must be advised only after taking into consideration the risk versus benefits in an individual patient [6,10].

Managing α Thalassemia Major and HbH with hydrops fetalis

Termination of pregnancy: Ideally irrespective of the gestational age of the diagnosis of Hydrops fetalis due to α-thalassemia major or HbH with hydrops, termination of pregnancy must be offered [11]. If MTP is not an option, the pregnancy must be referred to a center that offers comprehensive care for mother as well as in utero therapy for the fetus.

Care of the mother antenatally: Fetuses with hydrops fetalis due to α-thalassemia have an increased risk of a number of pregnancy related complications like antepartum hemorrhage, severe pregnancy induced hypertension, pre-eclampsia, eclampsia, polyhydramnios etc. The pregnancy is high risk for the mother and the fetus and maternal monitoring of all the complications and close follow up is essential [11].

In utero transfusions (IUT) can be offered for fetuses with hydrops fetalis with α-thalassemia [12]. Those fetuses, diagnosed antenatally and treated with 2 or more transfusions, result in a better resolution of the hydrops, delivery closer to term, better APGAR scores, lesser mechanical ventilation, shorter post-delivery hospital stay and better neurodevelopmental outcomes. In utero transfusions at earlier gestational age is associated with better neurodevelopmental outcomes as well [13]. There is inadequate data to recommend volume of transfusion and frequency at present. There are studies underway trying to understand the impact of in-utero therapies and their standardization in various hemoglobinopathies leading to hydrops fetalis.

In utero hematopoietic stem cell transplantation (IU-HSCT): Based on the concept of feto-maternal immune tolerance during the pregnancy, there have been a small number of cases who have undergone IU-HSCT. Most have been in the second trimester. There has been an increasing amount of success with groups trying increasing doses of CD34+ stem cells without conditioning regimen (chemotherapeutic drugs can be potentially feto-toxic) to attain a mixed chimerism status, which is consolidated postnatally, with a reduced intensity regimen conditioning and a HSCT with the maternal stem cells [14].

The neonates that survive need to be put on a regular transfusion protocol similar to TDT, due to defects in the beta globin chain, with close monitoring and follow up of complications that occur due to chronic iron overload and infections.

Gene therapy (in utero as well as post-natal) is another potential area that is being explored to treat α-Thalassemia Major.

Recommendations

1. A complete blood count and peripheral smear to look for hypochromic and microcytic red cell indices, along with quantification of hemoglobin variants using automated HPLC, are recommended for diagnosis of Alpha Thalassemia (Level of Evidence: 1).
2. GAP-PCR or reverse dot blot methods should be used as first-level genetic screening to diagnose alpha thalassemia heterozygous/homozygous state (Level of Evidence: 1).
3. Termination of pregnancy should be offered irrespective of gestational age, if hydrops fetalis develops, or in a confirmed molecular diagnosis of α-Thalassemia Major antenatally (Level of Evidence: 3).
4. Antenatal care of the mother should be offered at a centre equipped to handle high risk pregnancies with fetal medicine services (Level of Evidence: 5).
5. Earlier initiation as well as more frequent in utero transfusions improve outcomes with respect to postnatal survival as well as long term neurodevelopmental scores (Level of Evidence: 3).
6. In utero HSCT may be considered in a clinical trial setting (Level of Evidence: 4).

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Appendix

Appendix 1
Sample Data Set

Name: _____

Date of Birth: _____

Age at 1st Transfusion: _____

Patient Record Book

Thalassemia Centre
Patient Regn. No.

_____

_____

Thalassemia Day Care Centre

_____

_____

_____

Thalassemia Diagnosis and Treatment Register

Name		Surname	
Age		DOB (DD/MM/YYYY)	
Sex	Male/ Female	Thal Clinic No:	
CR NO		UHID No	
Father's Name		Mother's Name	
Address			
House No	Street Name	Village	
District	State	Pin	
Contact Details			
Phone	Number 1		
	Number 2 (Landline)		
Aadhar Card	Father	Mother	Child

Diagnosis and Treatment Summary

ANEMIA	
Age of onset of anemia	
Age of Diagnosis	
Complaints other than anemia if any	
TRANSFUSION HISTORY	
Age at first transfusion (years)	
Hemoglobin prior to first transfusion (gms/dl)	
Extended red cell phenotyping (DATE)	ABO Rh D C c E e Kell
CHELATION HISTORY	
Age of starting chelation (years)	
S. Ferritin at start of chelation (ng/ml)	
Chelation (starting drug/dose)	
Chelation (present dose)	
FAMILY HISTORY	
NO OF SIBLINGS	
THALASSEMIA STATUS OF SIBLINGS Screened/not	
VACCINATION STATUS	**DATE/ DOSES**
Hepatitis B Hepatitis A	
Viral markers (date/report)	HIV antibody HBsAg Anti HCV antibody
DIAGNOSIS	
CBC (Date)	Hemoglobin _____(gm/dl) TLC _____ Differential P L M E Platelet count _____ MCV _____(fl)

Diagnosis and Treatment Summary

HPLC (Date)	
Molecular diagnosis	Y/N If Y: details
Parental HPLC	Mother Father
HLA typing (date/ report)	
Donor search status Last search done on	Result
Genetic counseling	Y/ N Date
Counseled for BMT	Y/ N Date

Transfusion Monitoring Chart (At every transfusion)

Date (dd/mm/yyyy)	Weight	Pre transfusion Hb	Duration from last transfusion (days)	PRBC volume transfused (ml)	Leukodepletion (Y/N) (Source/Bedside)	Transfusion related issues if any	Post Transfusion Hb (If applicable)

Chelation Monitoring Chart (3 Monthly)

Date (dd/mm/yyyy)	Weight	Drug (s) Dose	S. Ferritin	CBC	Urine protein	SGPT/ Cr	Any adverse events

Annual Monitoring Chart
(To be done as applicable for the patient)

	Date	Date	Date	Date	Date
Weight (kg)					
Height (cms)					
Sitting height (cms)					
Growth velocity					
Bone age					
Liver size Spleen size					
CVS exam					
Facial changes (Y/N)					
Tanner stage					
Annual PRBC loading					
HIV antibody HBsAg Anti HCV antibody					
Alloantibody screen					
LFT (enter abnormal value)					
RFT (enter abnormal value)					
iCa/P/ALP (enter abnormal value)					

Annual Monitoring Chart
(to be done as applicable for the patient)

	Date	Date	Date	Date	Date
25 OH Vit D3					
S. Ferritin (enter all values)					
FBS/PPBS GTT					
T3/T4/TSH					
S. PTH					
MRI Cardiac/ Liver					
Fibroscan					
Bone Mineral Density					
S. FSH/LH					
S.Testosterone S.Oestradiol					
IGF-1/ IGF-BP3					
ECG					
ECHO					
Cardiac MRI					
Audiology evaluation					
Vision evaluation					
Fertility assessment					

Appendix 2
Standard operating practices for arranging and transfusing blood in a thalassemic patient

Arranging blood unit

1. All thalassemia patients should be registered with the transfusion centre as well as at the blood centre. It would be preferable if the blood centre is aware of the proposed transfusion schedule to ensure the availability of blood.
2. The sample for cross-matching is 2 ml EDTA and plain sample.
3. The collection, labelling of the sample, and filling of the blood request form will be done by the same nurse. Do not leave samples unlabeled as this can result in errors. The samples should be labelled with name (first, middle, last), age, gender and unique hospital ID number. It is ideal to use barcoded labels.
4. Labelling should be done in legible handwriting. All forms have to be initialled and dated and signed by the staff nurse collecting the sample.
5. Blood group, volume to be transfused and product manipulation (leukodepletion/ irradiation) should be mentioned in the blood request form.
6. Maintain transfusion records (Appendix 2)

Checking blood unit

1. Once blood component is received from the blood centre, it should be checked by a doctor and a nurse ideally to prevent errors. Patient details (Name, Hospital ID number, blood group) on the bag should be counter-checked with the hospital file and donor details on the bag (donor number and blood group) should be checked against the compatibility report and cross-match label from the blood centre.
2. Check the expiry date of the product
3. If the bag appears distended, or there is bubbling inside the bag, speak to your blood centre team
4. In case of any documentation errors, send the unit back to the blood centre for verification.
5. If 2 units are required, issue them one after the other. Do not store blood in domestic refrigerators.
6. Blood transfusion notes should be documented in the admission sheet. Mention the date, time, weight of the child and duration of transfusion. Sign the transfusion notes with your name mentioned below.

   e.g., For a 12 kg child with thalassemia and hemoglobin of 9 gm/dl:

   "1-unit PRBC checked. Transfuse 200 ml over 4 hours. In case of transfusion reaction, stop immediately and inform the duty doctor"

7. In severe anemia with CCF or impending CCF, a smaller volume of 5-10ml/kg is transfused over 6 hours with cardiac monitoring and midway frusemide.
8. Blood need not be warmed to room temperature prior to each transfusion. Blood is transfused using a blood warmer if the patient is suffering from cold antibody-mediated autoimmune hemolytic anemia.
9. Informed consent prior to each transfusion and record it in the file/ transfusion release notes from the blood centre (Sample consent form provided as Appendix A4).
10. A larger size (20G and above) cannula is preferred for blood transfusion as a smaller caliber can lead to mechanical damage and hemolysis. However, this may not be practical in children. Hence, the largest possible cannula may be used in the given circumstance. Avoid 24 and 26 G cannulas for blood transfusion.
11. All blood transfusions should be through the standard BT or leukodepletion filter. The staff nurse handling the product should be trained in using blood sets and leukodepletion filters as per need.
12. Do not administer any drug through the same BT set.
13. The first 25-30 ml should be administered over 10-15 min and patients should be observed closely for any adverse effects. Parents should be counseled to watch for any adverse reactions.
14. Monitor vitals every 15 minutes for the first 30 min and then once in 30 min till the end of transfusion.
15. Discard used blood bags and blood sets as per biomedical waste management rules followed in your hospital/ transfusion centre. Discard in yellow bin.

Appendix 3
Informed Consent for Blood Transfusion

I, Mr./Mrs./Miss.....son/daughter/wife of.....admitted under (Physician/ surgeon's name) ..... in the department of ..... for..... disease. I have been explained in a language that I understand that

1. I/ My patients condition/ surgery/ therapeutic procedure requires the possibility of blood/ blood component transfusion.
2. I / My patient understand that the blood / blood component have been prepared and tested in accordance with rules established under the national regulations.
3. I / My patient have been explained the benefits as well as risks (including transmission of HIV, Hepatitis B, Hepatitis C, syphilis / malaria and other transfusion related adverse events) of such transfusion. I understand that these risks remain despite the testing mentioned in point no 2
4. I/ my patient have also been explained the alternatives to transfusion and their benefits and limitations.
5. I / My patient had the opportunity to ask any questions / clarifications related to the need / benefits / risks / alternatives to transfusion.
6. I / My patient believe that i/we have been sufficiently informed to decide to give a consent for transfusion of blood or blood component.

I / My patient give consent to the transfusion of blood and blood component as deemed necessary by the treating physician / surgeon.

(Signature/left thumb impression) Patient / Pt. Representative	(Signature/left thumb impression)	(Signature/left thumb impression)
Name: _____	Witness name: _____	Doctor name: _____
Relation to patient: _____	_____	_____
Date:	Date:	Date:
Place:	Place:	Place:

Appendix 4

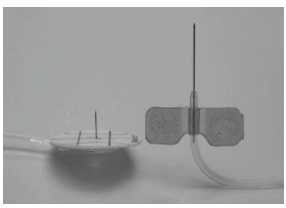
How to Administer Chelation therapy

1. Deferoxamine mesylate:

Available as 500 mg dry powder.

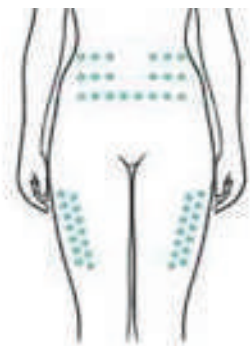
A. Subcutaneous route

Requirements – Portable infusion pump, 25-27 gauge straight butterfly (right) or a perpendicular angled needle (ThalaSet) (left), 10-20 ml syringe.



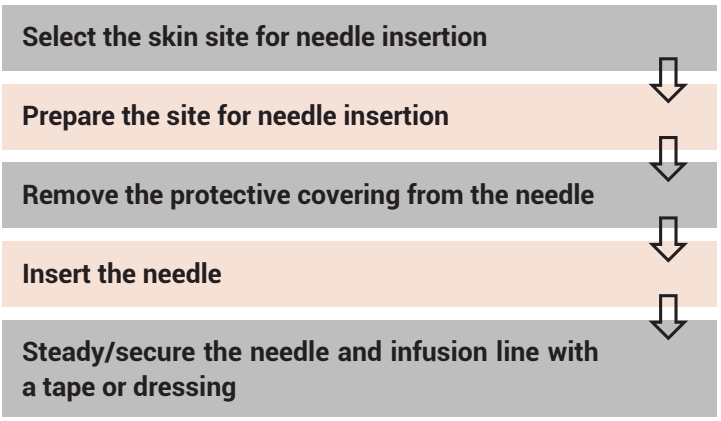
Reconstitution – Each vial is to be reconstituted in 5 ml sterile water infusion (SWI).

Site – Preferred site is the abdomen (as it is safest and to avoid vessels and nerves) & thigh.



Duration of administration - The reconstituted solution is administered subcutaneously over 8-12 hours via pump (may also be given for 24 hours). The drug should be preferably given daily or at least 5 days a week.

Steps to secure the needle –



B. Intravenous route

Requirement – Indwelling catheter.

Reconstitution – Each vial is to be reconstituted in 5 ml SWI.

It requires further dilution with either 150 ml 0.9% Sodium Chloride / 0.45% Sodium Chloride / 5% Dextrose / Lactated Ringers.

The final concentration for intravenous administration should not be more than 3.5 mg/ml.

The rate of infusion should not exceed 10mg/kg/hour and it should be administered under cardiac monitoring.

2. Deferasirox

A. Dispersible tablet

Available as 100 mg, 250 mg, 400 mg or 500 mg tablets.

To be consumed empty stomach (at least 30 minutes before food), once a day and preferably at the same time every day.

Method of oral administration – Completely disperse the tablet in non-metallic glass in water, orange juice, or apple juice using a non-metallic spoon until a fine suspension is obtained.

Disperse doses of <1000 mg in 100 ml of liquid and doses of ≥1000 mg in 200 ml of liquid.

Patients should be told not to chew tablets or crush the tablet.

Film-coated tablet (FCT)

Available as 180 mg & 360 mg tablet.

Method of oral administration – The tablet is to be swallowed whole before food or with a light meal.

3. Deferiprone

Available as a 250 mg or 500 mg capsule.

To be taken orally in three divided doses.

Keep at least an interval of 4 hours between Deferiprone and other medications or supplements containing polyvalent cations such as aluminium and zinc.

Appendix 5
Thalassemia: What Parents Need to Know? Guidelines for Parents

Indian Academy of Pediatrics (IAP)

GUIDELINES FOR PARENTS

Thalassemia: What Parents Need to Know?

Convener: **Mamta Manglani**
Members: **Santanu Deb, Rajiv Kumar Bansal, K Muthukumar**
Reviewer: **Pooja Dewan**

10 FAQs on THALASSEMIA: WHAT PARENTS NEED TO KNOW?

1. What do we mean by thalassemia?
2. How is thalassemia classified?
3. How did my child get it?
4. What are the signs and symptoms of thalassemia major (TDT)? What should I look for in my child?
5. What is the treatment of TDT? Is there a cure?
6. What are some of the complications of red blood cell transfusions, and what should I do to prevent or treat them?
7. Can my child go to regular schools, get regular vaccines, play with other kids, etc.?
8. Will my child be able to marry and have children?
9. Are our relatives at risk of getting a child with thalassemia? How can we identify it?
10. Does the government and the society offer any support to my child due to her/his special needs?

Under the Auspices of the IAP Action Plan 2020–2021

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Q1

What do we mean by thalassemia?

Thalassemia is a single gene (inherited from parents) blood disorder caused by reduced production of a protein called globin chain which is required in the right amount to form a normal, stable hemoglobin, an important part of red blood cells. These red cells with such hemoglobin cannot function properly, and they last shorter periods of time in the blood circulation, causing anemia due to defective circulating red cells.

Red blood cells carry oxygen to all the cells of the body. Oxygen is required for all the cells of the body, without which they cannot function well. Anemia due to thalassemia causes easy fatiguability, feeling of tiredness, weakness, or shortness of breath. Anemia can also affect organs adversely and cause death.

Q2

How is thalassemia classified?

In intrauterine life, the hemoglobin (the protein inside the red cells in the human body) is composed of mainly fetal hemoglobin, which is made up of 2 α (alpha) and 2 γ (gamma) chains, and this constitutes 70% of the hemoglobin at birth. After birth, the fetal hemoglobin slowly transitions into adult hemoglobin (HbA) which constitutes approximately 95% of the total hemoglobin by 6 months of age, and this is composed of 2 α (alpha) and 2 β (beta) chains. These chains are produced through signals by normal genes in the cells of the bone marrow that produce the red cells. For α (alpha) chains, there are a total of four genes responsible, two inherited from each parent. Whereas for β (beta) chains, there are two genes, one each from the mother and the father.

Reduced or absent synthesis of α-globin chains and β-globin chains cause *α-thalassemia* and *β-thalassemia,* respectively. In India, our children suffer more commonly from β-thalassemia and, therefore, when we refer to thalassemia in India, we mean β-thalassemia.

Based on the number of genes that are abnormal or defective, thalassemia is termed as "thalassemia minor/carrier/trait" and "thalassemia major" or "thalassemia intermedia". When one gene is defective and the other gene is normal, we term the state as "thalassemia minor/carrier/trait". Whereas, when both genes are defective, it is called as "thalassemia homozygous"—which could be *clinically mild (not requiring regular transfusions) and called as thalassemia intermedia, now referred to as nontransfusion dependent thalassemia (NTDT) or clinically severe, i.e., thalassemia major (requiring regular frequent transfusions) now known as "transfusion dependent thalassemia (TDT)".*

What is Thalassemia Minor/Carrier/Trait?

As mentioned earlier, every person has two genes which control formation of β-chains, which are part of hemoglobin in our body. Thalassemia trait/minor/carrier is a person with one normal gene and one defective gene. Such persons have mildly decreased β-chain production and, therefore, a slightly lower than normal hemoglobin. However, they are like any normal person and do not require any treatment. They are usually unaware about their carrier state, unless they undergo a blood test called "HPLC". Once you are aware of your thalassemia minor status, please inform your doctor about it and do not take iron supplements unless you have proven iron deficiency anemia by doing some blood tests for estimating the iron in your body.

How do I know that I am a Thalassemia Minor/Carrier/Trait?

A simple blood test called *high performance liquid chromatography (HPLC) or hemoglobin electrophoresis for hemoglobin variants* can detect thalassemia carrier. This is required to be done only once in lifetime.

Everyone should be aware about their thalassemia status before planning to have children to avoid thalassemia major in their child.

What is β-thalassemia Major or Transfusion Dependent Thalassemia?

Beta-thalassemia major is a genetic (or "inherited") blood disorder that is also called Cooley's or Mediterranean anemia or sometimes simply called "thalassemia". β-thalassemia major, the most severe form of the disorder, prevents or greatly reduces the body's ability to produce "adult" hemoglobin (Hb) and causes severe anemia requiring blood transfusions.

Q3

How did my child get it?

Beta-thalassemia major is an inherited disease. When *both parents are thalassemia carriers or minor or traits*, their child could be born with thalassemia homozygous, i.e., thalassemia major/intermedia or TDT/NTDT. There is a *1-in-4 (25%) chance during each pregnancy that the child will be born with a severe form of the disease*, i.e., thalassemia major or intermedia (both defective genes), *2-in-4* (50%) will have thalassemia carrier status (one normal gene and one defective gene) and *1-in-4* (25%) will be unaffected or normal (both normal genes), when both husband and wife are thalassemia carrier or traits or minor **(Fig. 1)**.

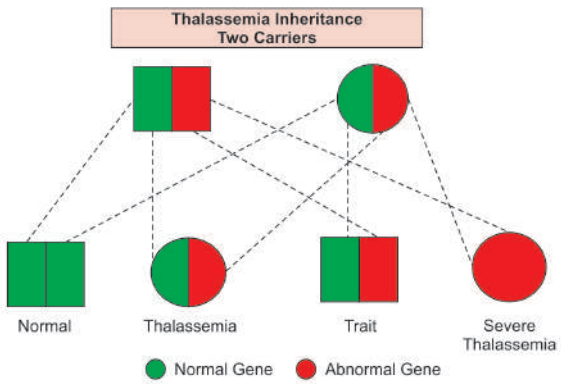

Fig. 1: The chances of having a child with thalassemia in each pregnancy when both parents are carriers.

Is it my fault, if my child is born with thalassemia major (TDT)?

No. Just as you cannot control what color eyes your child will inherit, you cannot control whether your child will inherit thalassemia. However, getting yourself tested for thalassemia carrier/minor/trait status prior to pregnancy enables you to discuss options with the doctor to know what you could do so that you have a normal child.

Q4

What are the signs and symptoms of thalassemia major (TDT)? What should I look for in my child?

Babies who are born with transfusion-dependent thalassemia are perfectly normal till about first 3–6 months of life. After which they start looking pale, are more irritable, do not feed well, do not grow well, and develop enlarged liver and spleen as they grow. Unless diagnosed in time and treated adequately, they may develop hemolytic or chipmunk facies, i.e., prominence of certain facial bones—fronto-parietal prominence, malar prominence, malocclusion of teeth, etc. **(Fig. 2)**.

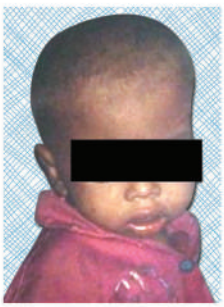

Fig. 2: Hemolytic facies in a child with transfusion-dependent thalassemia.

The most important things to look for include:

- Is your child looking pale?
- Is your child growing well or gaining weight adequately?
- Is your child's appetite decreasing?
- Does his or her belly look bigger?
- Is your child crying a lot or is irritable?
- Does your child feel excessively sleepy/tired?
- Does your child suffer from repeated infections?
- Anything else that is out of the ordinary.

What will Happen to my child now that he has Thalassemia Major or Transfusion-dependent Thalassemia?

Medical treatments have improved greatly over the years; there is reason to believe that your child, taking advantage of the therapies available now and in the future, will live a long and full life.

Q5

What is the treatment of TDT? Is there a cure?

Red Blood Cell Transfusions

Regular red blood cell transfusions **(Fig. 3)** are the lifeline of a child with thalassemia major or TDT. The hemoglobin should be maintained at 9–10.5 gm% before transfusion. They should receive packed red cell transfusions, preferably without white cells (leukodepleted, **Fig. 4**) every 3–4 weeks, and this frequency might increase as the child grows.

Fig. 3: Transfusions in a "daycare center".

Fig. 4: Bedside leukocyte filter.

Chelation Therapy

Repeated red cell transfusions lead to iron overload in these children. This iron gets deposited in various organs of the body including hormone-producing endocrine glands (such as thyroid gland, parathyroid glands, pituitary gland, and pancreas), heart, bones, liver, and spleen.

Certain medicines called iron chelators are given to remove excess iron from the body. This would reduce the damage that it causes to the organs. However, they need to be taken in the correct doses as well as regularly for good effect. These are injectable and oral. The injectable medicine, deferoxamine, also known as desferrioxamine, is generally reserved for use in those children who cannot tolerate oral medications due to their side effects. The oral medications include deferiprone and deferasirox. Both are effective and should be consumed regularly as per your hematologist's prescription.

You will be advised to perform certain blood and other tests regularly to look for any adverse events due to these medications. If you are taking deferiprone, report to your doctor if your child develops joint pains or joint swellings. Additionally, a complete blood count, liver, and kidney functions are required at least on monthly basis to monitor the side effects of the oral chelators. With deferasirox and desferrioxamine, an annual ophthalmology evaluation and audiometry also needs to be done.

Curative Treatment

The curative treatment for thalassemia major (TDT) is hematopoietic stem cell transplantation (HSCT) popularly known as "bone marrow transplant (BMT)". It can be done with a full HLA-matched sibling/family donor or an matched unrelated donor (MUD) for a successful outcome. The treatment center will recommend that your family should be tested to look for a "match" in case you are interested in this option. If there is a matched donor available, you and the treatment center staff will review the options and make decision for transplant or medical treatment. As the best results are obtained in children younger than 8–10 years of age, this option should be considered at an early age of the child.

Q6

What are some of the complications of red blood cell transfusions, and what should I do to prevent or treat them?

The most common complications of transfusions are fever and allergic reactions. Leukocyte filters **(Fig. 4)** help to significantly reduce the non-hemolytic febrile transfusion reactions (fever with chills after transfusions). *Allergic reactions* are due to proteins in the blood plasma and can be managed by certain simple medications which will be prescribed by your treating doctor. If these occur recurrently, triple saline washed red cells can be given.

Report to your doctor immediately, if your child develops fever, breathlessness, palpitations, excessive pain in abdomen, jaundice, skin rashes or dark or red-colored urine, following transfusion. This could suggest a transfusion-related complication.

There is a *risk of viral infections* with transfusions, but the likelihood of transmission is *exceedingly small* because the donated blood is tested for hepatitis B, hepatitis C and HIV, syphilis, and malaria. Nucleic acid amplification

tests (NAAT) tested red cell transfusions can reduce the risk of viral infections further but are presently not a standard of care in the country.

Finally, there will be *iron overload* after a couple of years of transfusions. This requires removal of the iron with medication as mentioned earlier.

To monitor for complications of *iron overload,* follow your doctor's advice. Certain tests need to be done annually to diagnose these complications and treat them. Five years onward, a blood sugar and thyroid profile, 7 years onward, a DEXA (dual emission X-ray absorptiometry) scan and a T2-weighted MRI for iron overload in the heart, liver, pituitary, and pancreas is indicated. At 10 years onward, growth evaluation and hormonal tests as indicated. Starting at the age of 12 years in girls and 14 years in boys, annual evaluation for attainment of puberty and accordingly, related tests might be required.

Q7

Can my child go to regular schools, get regular vaccines, play with other kids, etc.?

Yes, your child is like any other child. With proper treatment, your child will grow like any other child. S/he can get regular vaccines—ensure that hepatitis B as well as hepatitis A vaccines have been administered to your child as these will protect the liver from these viruses. S/he can attend regular school, play with other children, etc. The disease is genetic and does not get transmitted from one to another.

Does my child need any specific diet or nutritional supplements?

Nutrition of your child is particularly important. You are advised to encourage your child to eat a balanced diet consisting of dairy products, grains, fruits, and vegetables. Avoid excessive intake of iron rich foods in diet such as red meats, beans, raisins, dates, jaggery, almonds, and green leafy vegetables. Also avoid cooking in cast iron cookware. Drinking tea or coffee along with meals can be helpful as tannin can impair iron absorption. Supplements including vitamin D, calcium, and zinc (especially for those on deferiprone) are advised.

Q8

Will my child be able to marry and have children?

Yes, your child will be able to marry. If appropriate and regular treatment is taken, people living with thalassemia achieve puberty and can also conceive. Discuss this with your doctor since the age of puberty evaluation. However, the rule of inheritance based on the partner's status for thalassemia would decide the status of the offspring. If your child is undergoing BMT, please discuss the fertility status and outcomes with the treating BMT physician about the same.

BMT cures the disease in the index case, but the thalassemia genes can still be passed on to their progeny. This should be discussed with your doctor.

Can this disease affect our future pregnancies? If so, can it be prevented?

Yes, as mentioned earlier, the chances of having a thalassemia major or TDT child is *25% in each pregnancy*. Genetic studies in the child with TDT as well as the parents are essential for diagnosing the fetus in the next pregnancy. This should be done before planning the next pregnancy. Prenatal diagnosis in the first trimester of pregnancy (between 10 and 15 weeks) by chorionic villus sampling (CVS) can be done to determine the thalassemia status of the fetus and decision to continue or not should be taken in consultation with the treating doctor.

Q9

Are our relatives at risk of getting a child with thalassemia? How can we identify it?

Yes, your relatives are at a higher risk of being thalassemia trait or carriers and having a child with thalassemia major (TDT) as the gene is more common in certain communities. Your extended family, especially children and young persons in reproductive age groups, must be encouraged to undergo testing with HPLC or hemoglobin electrophoresis to know their status. They should undergo counseling, if both partners in a marriage are thalassemia carriers or trait.

Q10

Does the government and the society offer any support to my child due to her/his special needs?

Thalassemia has been certified as a disability by the Government of India in 2016. Your child is eligible for a disability certificate, which can be obtained from a government hospital. This will provide her/him access to **inclusive education in government/government-recognized educational institutes, reservation in government jobs, and social welfare schemes such as** reservation in allocation of land and poverty alleviation schemes.

Groups like Red Cross Society, Thalassemia International Federation, Thalassemia Welfare Society and *Thalassemia* Patient Advocacy Groups (PAGs), and various other local thalassemia societies formed by parents' groups offer considerable support to children with thalassemia and their families. As parents of a child with thalassemia, it is also your duty to spread awareness about voluntary blood donation among friends, relatives, and colleagues as well as encouraging healthy young volunteers to enrol themselves for bone marrow donor registries too.

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Manglani M, Deb S, Bansal RK, Muthukumar K. Thalassemia: What Parents Need to Know? In: Indian Academy of Pediatrics (IAP). Guidelines for Parents. Accessed on May 5, 2023.

Appendix 6
List of Abbreviations

ACTH	Adrenocorticotropic hormone
AFC	Antral follicular count
AFP	Alpha fetoprotein
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ARMS PCR	Amplification refractory mutation system polymerase chain reaction
ART	Assisted reproductive technology
AST	Aspartate transaminase
ATMDS	Alpha thalassemia myelodysplastic syndrome
BM	Bone marrow
BMD	Bone mineral density
BMDW	Bone Marrow Donors Worldwide
BMT	Bone marrow transplantation
BNP	B-type natriuretic peptide
2,3 BPG	2,3 Biphosphoglycerate
CBC	Complete blood count
CE	Capillary electrophoresis
CEUS	Contrast-enhanced ultrasound
CLIA	Chemiluminescent immunoassays
CNS	Central nervous system
CPDA	Citrate-phosphate-dextrose-adenine
CVS	Chorionic villus sampling
DAAs	Directly acting antivirals
DEXA	Dual-energy X-ray absorptiometry
DFP	Deferiprone
DFO	Desferrioxamine
DFX	Deferasirox
DMT	Divalent metal transporter
DSTR	Delayed serological transfusion reaction
DVT	Deep vein thrombosis
ECG	Electrocardiogram
ECHO	Echocardiogram
EIA	Enzyme Immunoassay
ELISA	Enzyme-linked immune-sorbent assay
EMH	Extra-medullary hematopoiesis
FNHTR	Febrile non-hemolytic transfusion reactions
FSH	Follicle-stimulating hormone
FT4	Free thyroxine
G6PD	Glucose-6-phosphate dehydrogenase

GH	Growth hormone
GHD	Growth hormone deficiency
GHRH	Growth hormone releasing hormone
GLS	Global longitudinal strain
GnRH	Gonadotropin-releasing hormone
GSTM1	Glutathione S-transferase M1
GVHD	Graft versus host disease
HA	Hemagglutination
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HH	Hypogonadotropic hypogonadism
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
HPG	Hypothalamic–pituitary–gonadal
HSCT	Hematopoietic stem cell transplantation
HPFH	Hereditary persistence of fetal hemoglobin
HPG	Hypothalamic-pituitary-gonadal
HU	Hydroxyurea
HVPI	Hemovigilance Programme of India
IAs	Immunoassays
IAP	Indian Academy of Pediatrics
ICMR	Indian Council for Medical Research
ICSI	Intracytoplasmic sperm injection
IGF1	Insulin-like growth factor 1
IEC	Information Education Communication
IGT	Impaired glucose tolerance
IFG	Impaired fasting glucose
IOL	Iron overload
IUGR	Intrauterine growth retardation
IUT	In utero transfusion
IVF	In-vitro fertilization
KLF1	Krüppel-like factor-1
LBW	Low birth weight
LDH	Lactate dehydrogenase
LH	Luteinising hormone
LIC	Liver iron concentration
LMIC	Low and middle income economies
LMWH	Low molecular weight heparin
LPI	Labile plasma iron

LVEF	Left ventricular ejection fraction
NAAT	Nucleic acid amplification technology
NFHS	National Family Health Survey
NHM	National Health Mission
NRBC	Nucleated Red Blood Cells
MCC	Maternal cell contamination
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MLPA	Multiplex ligation-dependent probe amplification
MRI	Magnetic resonance imaging
MTHFR	Methylenetetrahydrofolate reductase
NFHS	National family health survey
NHM	National Health Mission
NTBI	Non-transferrin-bound iron
NTDT	Non-transfusion dependent thalassemia
OCEBM	Oxford Center for Evidence-Based Medicine
OS	Overall survival
PAH	Pulmonary artery hypertension
PBSC	Peripheral blood stem cell
PCR-SSP	Polymerase chain reaction –Sequence-Specific Primer
PE	Pulmonary embolism
PHR	Personal health record
PHT	Pulmonary hypertension
PI	Pathogen inactivation
PND	Pre-natal diagnosis
PR	Pathogen reduction
PRBC	Packed red blood cells
PTCY	Post transplant cyclophosphamide
PTH	Parathormone
PTIS	Pre transplant immune suppression
pQTC	Peripheral quantitative computerized tomography
PwD	Person with disability
QF PCR	Quantitative fluorescent polymerase chain reaction
QTL	Quantitative trait loci
RCT	Randomized controlled trials
RFLP	Restriction fragment length polymorphism
RFID	Radio Frequency Identification
RIC	Reduced intensity conditioning
ROS	Reactive oxygen species

RPWD	Rights of Persons with Disabilities
RVEF	Right ventricular ejection fraction
SAGM	Saline, Adenine, Glucose and Mannitol
SMR	Sexual maturity rating
SNP	Single nucleotide polymorphisms
SOP	Standard operating procedure
SOS	Sinusoidal obstruction syndrome
SVR	Sustained virological response
TACE	Trans-arterial chemo-embolization
TACO	Transfusion-associated circulatory overload
TA-GVHD	Transfusion-associated graft-versus-host disease
TBLH	Total body less head
TBD	Thalassemia bone disease
TBS	Trabecular bone score
TDC	Thalassemia Day care
TDT	Transfusion dependent thalassemia
TEE	Thromboembolic events
TEG	Transient Elastography
TESE	Testicular sperm extraction
TFIIH	Transcription factor II human
TFS	Thalassemia free survival
TGFβ1	Transforming growth factor β1
TI	Thalassemia intermedia
TIBC	Total iron binding capacity
TM	Thalassemia major
TOC	Transition of care
TPHA	Treponema pallidum hemagglutination assay
TRALI	Transfusion-related acute lung injury
TRM	Transplant related mortality
TRRF	Transfusion reaction reporting form
TSAT	Transferrin saturation
TSH	Thyroid stimulating hormone
TTIs	Transfusion-transmitted infections
UDP	Uridine diphosphate
VDR	Vitamin D receptors
VNTR	Variable number tandem repeat
VTE	Venous thromboembolism

Notes

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