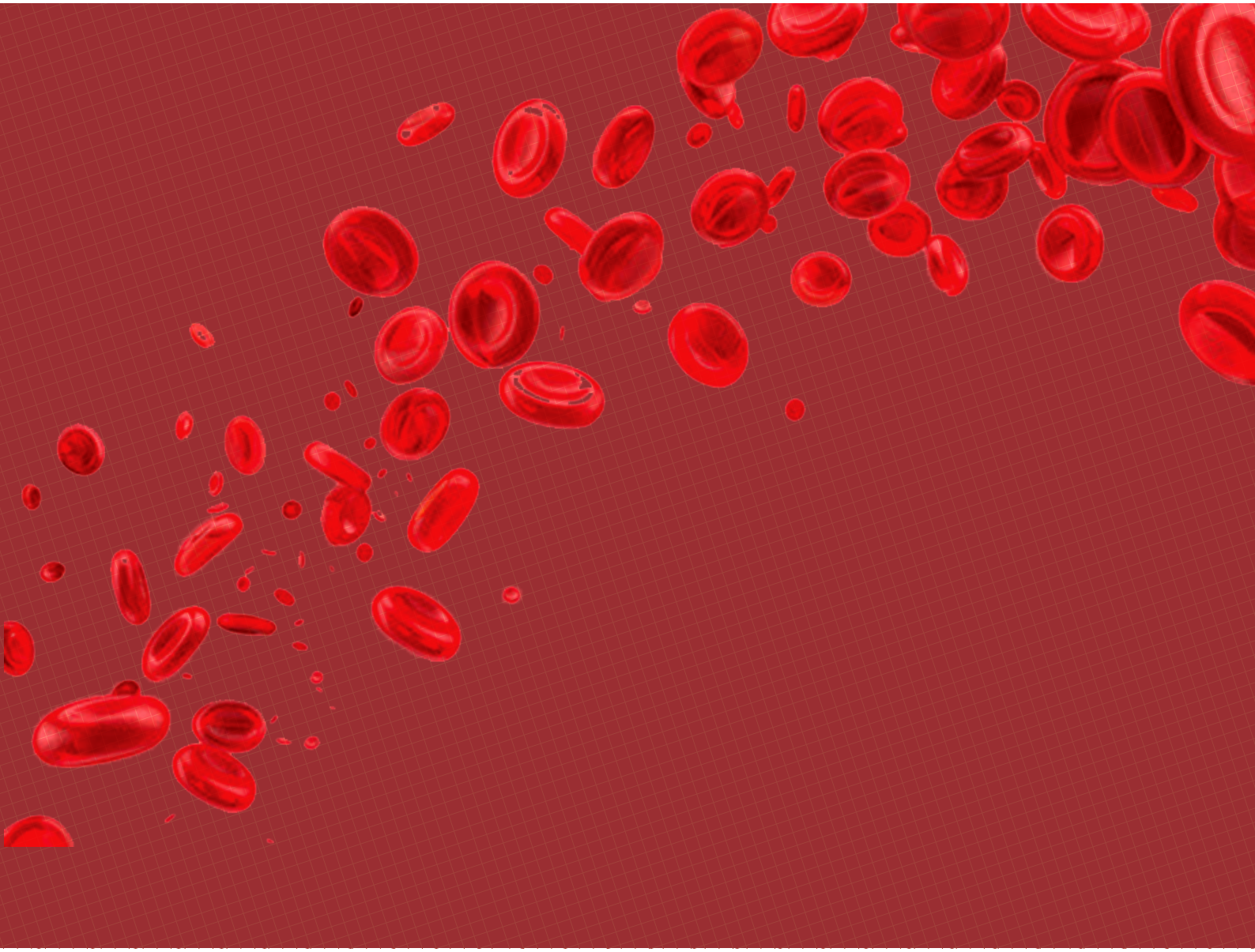
2081

NATIONAL GUIDELINE FOR HEMOGLOBINOPATHIES



Government of Nepal
Ministry of Health and Population
Department of Health Services
Epidemiology and Disease Control Division
Teku, Kathmandu

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FOREWORD

Hemoglobinopathies such as Thalassemia and Sickle-cell anemia are significant public health problem globally. The severe consequences ranging from physical, psychological to social effects, these genetic blood disorders definitely need to be addressed.

The national burden of hemoglobin disorder is currently being estimated by the Nepal Health Research Council with collaboration with the Ministry of Health and Population. Some of the studies that have been conducted are based on hospital and laboratory based data. Although these conditions may not be cured, we must ensure affordable, simple interventions those are available to people suffering from these diseases to increase quality of life and ultimately life expectancy.

Nepal is adopting cost-effective strategies to reduce the burden of hemoglobinopathies by complementing disease management with the prevention program such as early screening and genetic counselling. This guideline is expected to serve as a reference for the effective primary, secondary as well as tertiary preventions of hemoglobinopathies.

I would like to thank all the contributors for their invaluable input during preparation of this national guideline. I believe this updated guideline will be more useful and relevant to all those concerned stakeholders. So, with the implementation of this guideline, I anticipate the decline in the prevalence of hemoglobinopathies in the future.

Dr. Bikash Devkota
Secretary
Ministry of Health and Population

FOREWORD

The inherited disorders of hemoglobin represent the most common monogenic diseases. They are an important public health problem globally with severe physical, psychological and social consequences for those affected and their families. The resolution on sickle-cell disease from the 59th World Health Assembly in May 2006 and the resolution on thalassemia from the 118th meeting of the WHO Executive Board call upon affected countries and the Secretariat of WHO to strengthen their response to these conditions. In addition, a resolution on the prevention and management of birth defects, including sickle-cell disease and thalassemia, was adopted by the 63rd World Health Assembly in May 2010.

Despite efforts, the management of these disorders are not satisfactory. There are still policy level issues in the management of the disease. The inadequacy of our health facilities in treating the disease is still a big challenge. Besides this, adequate and timely unavailability of medicines, blood transfusion facilities and laboratory services have made it a bigger challenge in treatment of these disorders.

Several reports of hospital and Laboratory based studies have identified hemoglobinopathies as the major disease among the blood related hereditary disease in Nepal.

The National Guideline for Hemoglobinopathies revised and published under the initiative of the Epidemiology and Disease Control Division is an important step in the direction of Hemoglobinopathies management. Owing to the many changes that has taken place beyond the Guidelines publication, the National Guidelines for the Sickle Cell Disease and Thalassemia Management has been updated with a

I would like to thank all the contributors including team of a dedicated subject experts for their hard work, dedication in helping to bring this updated Guideline. I am sure this Guideline will help health service providers at every level in pursuing the management of these disorders.

Dr. Tanka Prasad Barakoti
Director General
Department of Health service

FOREWORD

Hemoglobinopathies are the most common inherited diseases around the world. In Nepal, various studies have also shown its burden in specific province and in ethnic group as well.

In National health Policy 2019, in clause 6.12.4 mentioned that "Proper system shall be developed to prevent and treat hereditary diseases." So, in view of inherited disorder, we all the concerned stakeholder should also focus on preventive measures to decrease the burden of hemoglobinopathies and for the management of existing diseased population, there is also need to strengthen our health facilities to diagnose and treat this disorder .

A gap of knowledge of the health care professionals at a larger scale is one of the major issues in the management of the disease. So, this updated guiding document would be helpful in management of hemoglobinopathies including its diagnosis and in taking required preventive measure.

I would like to thank Dr. Pomawati Thapa, Section Chief, NCD & MH Section, lead Consultant Dr. Amit Shrestha and contributors from all the subject experts in updating this guideline. I hope this guideline will be proven as an invaluable guiding document in the management of hemoglobinopathies at all level of the country.

Dr. Chandra Bhal Jha
Director
Epidemiology and Disease Control Division

ABBREVIATIONS

ABG	Arterial Blood Gas
ACS	Acute Chest Syndrome
BMT	Bone Marrow Transplant
CBC	Complete Blood Count
CVA	Cerebrovascular Accident
CXR	Chest X-ray
DHTR	Delayed Hemolytic Transfusion Reaction
EDCD	Epidemiology and Disease Control Division
EMEA	European Medicines Agency
FDA	Food and Drug Administration
GH	Growth Hormone
GIT	Gastrointestinal Tract
GoN	Government of Nepal
Hb A	Adult Hemoglobin
Hb F	Foetal Hemoglobin
Hb	Hemoglobin
HCT	Hematocrit
HPLC	High Performance Liquid Chromatography
HSC	Hematopoietic stem cells
HU	Hydroxyurea
ICU	Intensive Care Unit
LDH	Lactate Dehydrogenase
LFT	Liver Function Test
LVEF	Left Ventricular Ejection Fraction
MoHP	Ministry of Health and Population
MTD	Maximum Tolerated Dose
NTDT	Non-Transfusion Dependent Thalassemia
OOS	Osteopenia Osteoporosis Syndrome
PRBC	Packed Red Blood Cell
PTH	Parathyroid hormone
RBC	Red Blood Cell
RFT	Renal Function Test
SCA	Sickle Cell Anemia
SCD	Sickle Cell Disease
SCT	Sickle Cell Trait
TDT	Transfusion dependent Thalassemia
TIA	Transient Ischemic Attack
TLC	Total Leucocyte Count
UTI	Urinary Tract Infection
WHO	World Health Organization

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I

INTRODUCTION

A. Background

Hemoglobinopathy is a hereditary genetic disease consisting mainly of sickle cell disease and thalassemias, which account for a great proportion of births affected by a genetic disorder. The thalassemias are a heterogeneous group of hemoglobin (Hb) disorders in which there is excessive destruction of red blood cells (RBC) as a result of the defective synthesis of one or more globin chains. It is inherited in autosomal recessive pattern. Several types of thalassemia have been described and named according to the affected globin chain, the most common types of clinical importance being α, βδ, and β thalassemia. Thalassemia is more prevalent in the Mediterranean Basin, the Middle East, Southern and Eastern Asia, the South Pacific and South China with reported carrier rates ranging from 2% to 25%.

Sickle Cell Disease (SCD) is another hemoglobin disorder that requires lifelong management and contributes to infant and childhood morbidity and mortality. SCD is caused by inheritance of two abnormal HbS genes, which is also inherited in autosomal recessive pattern. Sickle cell syndromes include Sickle Cell Disease (SCD, HbSS), also called Sickle Cell Anemia (SCA), as well as disorders due to sickle cell gene combined with another hemoglobinopathy such as Hb C, E, or β thalassemia.

Although reliable data are still lacking for many regions of the world, recent data indicate that about 7% of the world's population is a carrier of a hemoglobin disorder and that 300,000–500,000 children are born each year with the severe homozygous states of these diseases (World Bank 2006, report of a joint WHO-March of Dime meeting 2006). Sickle cell syndromes are more frequent and constitute 70% of affected births world- wide, the rest (30%) are due to thalassemias.

In Nepal, Sickle cell disease is more commonly found in western region of Nepal. Though it is seen as a public health problem in Tharus of western (mid-west and far-west) Nepal, it is found sporadically in non-Tharus also. A case series reports done from Bheri hospital in mid-west of Nepal from 2068 BS had drawn attention to the magnitude of sickle cell disease, especially in the Tharu community of Nepal. A population-based study done by Nepal Health Research Council in Bardiya Municipality of Bardiya District had shown a prevalence of sickle cell trait in about 10.5% and sickle cell disease in 1% of Tharus of age range 1-29 yrs. A retrospective study done by National Public Health Laboratory, at five different sites of Nepal Government that uses capillary electrophoresis for screening of hemoglobin disorders from January 2019 to March 2019, identified 1,470 hemoglobinopathy cases, with sickle cell disorders being most common in Provinces 5, 6, and 7, while beta-thalassemia was more prevalent in the other provinces. This study also showed a concentration of cases within the Tharu community.

In Nepal, β Thalassemia is more commonly seen. It is found mostly in the low-land Terai region and some in the mid-hill region, and unlike sickle cell disease, it is seen prevalent in all ethnic communities. A retrospective study conducted by National Public Health Laboratory in 2019 showed that HbE is found to be more prevalent in Rajbanshi community of eastern Nepal. According to the data, the incidence of thalassemia in Nepal is found to be in the range of 5-10%. The number of patients with thalassemia is estimated to be more, but because of various factors, many patients are still underdiagnosed. Thus, there is an urgent need to come up with a policy that every patient in every part of Nepal has equal access to quality medical care. Health authorities need to recognize Hemoglobin disorders as a significant threat to public health.

The main goal of this guideline is to provide optimal treatment to those affected and prevent the birth of children with disease through carrier screening, genetic counseling and prenatal diagnosis.

B. Rationale for guideline

Hemoglobin disorders are one of the significant public health problems in Nepal. The government of Nepal has been trying to reduce the disease burden by two significant interventions: a] effective case management and b] preventive measures. However, we must strengthen our health system's technical and managerial capacity to implement these interventions effectively. For this, we do not have any guiding documents. Therefore, a national guideline is required. This guideline is expected to fullfill the gap.

C. Objectives of the Guidelines

This guideline's general objectives are to serve as a reference for health managers, doctors, nurses, laboratory professionals, and paramedics for case management and implementing preventive measures.

Specific Objectives

- To provide basic information about hemoglobin disorders
- To provide guidelines for diagnosis, treatment, and management of sickle cell diseases and thalassemia.
- To control the number of hemoglobinopathies by taking preventive measures.
- To make health care professionals be able to cope with problems of hemoglobin disorders.
- To improve health status and quality of life to increase the life expectancy of patients with hemoglobin disorders.
- To create awareness of hemoglobin disorders in the community.

D. Sickle cell disease and thalassemia services in Nepal

The Government of Nepal Ministry of Health and population have been providing financial aid for those patients who are impoverished. As of guidelines for the Medical Treatment of Deprived Citizens 2080, the following disease and conditions are included in this provision.

1. Kidney Disease
2. Heart Disease
3. Cancer
4. Parkinson Disease
5. Alzheimer
6. Spinal Injury
7. Head Injury
8. Sickle cell Anemia

E. Designated treatment centers for Sickle Cell Disease

As per guidelines for the Medical Treatment of Deprived Citizens 2080

1. Bir Hospital, Kantipath, Kathmandu
2. Kanti Children's Hospital, Maharajgunj.
3. Civil Service Hospital, Min-Bhawan, Kathmandu
4. Rapti Academy of Health Sciences, Ghorahi, Dang

5. Mahakali Provincial Hospital, Mahendranagar, Kanchanpur
6. Seti Provincial Hospital, Dhangadi, Kailali
7. Bheri Hospital, Nepalgunj, Banke
8. Lumbini Provincial Hospital, Butwal, Rupandehi
9. District Hospital, Kapilvastu
10. District Hospital, Bardiya
11. District Hospital, Nawalparasi (Susta Pachhim)
12. Ghodaghodhi Hospital, Ghodaghodhi Kailali
13. R.C Memorial hospital, Barabardiya municipality, Bardiya
14. Patan Academy of Health Science, Lalitpur
15. Dhulikhel Hospital, Kavrepalanchowk
16. Ashwin Medical College and Hospital Pvt. Ltd, Bhaisepati, Lalitpur
17. RC Tharu Memorial Municipality Hospital, Bardiya
18. Karnali Academy of Health Sciences, Jumla

F. Public Health Interventions for Hemoglobinopathies

In hemoglobinopathies, the balance between clinical case management and preventive measures is the basis for public health intervention. Therefore, prevention at a different level should be considered.

Level of Prevention	Definition	Interventions
Primordial	Inhibit risk factors	Awareness to avoid risk factor
	Halt onset of disease	Screening
Secondary	Halt the progression of a disease	▪ Early diagnosis ▪ Treatment & care
Tertiary	Reduce or limit impairments and disabilities and promote the patients adjustment to irremediable conditions	▪ Regular follow-up ▪ Treatment and care, ▪ Lifestyle adjustment to the disease

Public health interventions can be implemented to reduce disease burden and prevent morbidity and mortality. However, different activities should be designed based on local context, feasibility, and acceptability.

Public Messages on Sickle Cell Disease (SCD)

Message 1: A person with SCD can live a long, high-quality life. People with SCD can lower their chances of difficulties from the disease and enjoy many normal activities by

- Getting regular check-ups with their doctor.
- Following treatments prescribed by their doctor, such as taking a medication called hydroxyurea.
- Preventing infections by taking simple steps, including washing their hands.
- Practicing healthy habits like drinking 8 to 10 glasses of water per day and eating healthy food.

Message 2: A child gets sickle cell disease (SCD) when receiving two sickle cell genes one from each parent

- A child who inherits only one sickle cell gene has sickle cell trait (SCT).
- If both parents have either SCD or SCT, they need to discuss this information with each other and with a doctor when making decisions about family planning.

Message 3: Anemia is a common effect of SCD, but it can be treated

- In SCD, red blood cells die early causing anemia, and not enough red cells are left to carry oxygen throughout the body.
- Infection or enlargement of the spleen, may worsen anemia.
- Blood transfusions are used to treat severe anemia.

Message 4: SCD can be cured for certain patients.

A bone marrow transplant, which involves collecting healthy cells from a donor's bone marrow and transferring them to a patient, can cure SCD. However, a bone marrow transplant may not be the best choice for all patients because it comes with serious risks.

Public Messages on Thalassemia

Message 1: Thalassemia is a group of inherited blood disorders. It is passed on to you through the genes of your parents in an autosomal recessive pattern.

Message 2: In Thalassemia red blood cells die early causing anemia, and not enough red cells are left to carry oxygen throughout the body.

G. Guideline for Management

Based on rights of patients for access to care.

- Provide optimal care to all patients of thalassemia and sickle cell disease by establishing day care facilities for transfusion and monitoring as per Swasthya Sanstha Sanchalan Mapdanda 2077 Clause no.59 .
- Ensure availability of safe blood to thalassemic patient by strengthening existing Blood Banks to provide facilities for component separation and leuco-depletion with the promotion of voluntary non-remunerated blood donation to fulfill the blood requirements.
- Ensure availability of iron chelation and hydroxyurea with the inclusion of such medicine in National List of Essential Medicines Nepal.
- Developing and implementing protocols for early diagnosis and intervention in cases of Sickle Cell Disease (SCD) and Thalassemia.
- Developing and implementing protocols for Newborn screening of SCD for timely intervention with prophylactic penicillin and vaccinations.
- Facilitate establishment of Bone Marrow Transplant (BMT) centers.

CHAPTER

II

PREVENTION OF HEMOGLOBINOPATHIES

A. Background

Hemoglobinopathies are a major public health challenge worldwide. Management of thalassemia major requires regular transfusions of blood that has to be free of infectious agents and mitigation of complications of iron overload by expensive chelation therapy which is demanding and expensive for the family as well as the government. In Nepal, because of cost and inadequate health infrastructure, optimal management is available to only a fraction of those affected with thalassemia major. Majority of patients have a poor quality of life with a high incidence of premature death. According to the Nepal Thalassemia Society, the minimal annual cost for the treatment is estimated to be about 2,37,830 per patients, which is a social and economic burden for patient family as well as to the government.

Sickle Cell Disease, also has a significant effect on the economy of the family, given the severe complications of the disease, and the need for routine and lifelong care.

World Health Organization (WHO) has clearly outlined the goals for control of hemoglobinopathies - provide affordable and adequate therapy for those affected, while at the same time reduce the number of births of children with the disease through strong political, administrative and financial support.

The epidemiology of thalassemia has changed in some countries like Cyprus and Greece in the past decade, due to the successful implementation of prevention programs. This has led to a marked reduction of births with thalassemia major, and improved management and quality of life for those with the disease. Thus, prevention strategies are important and an extremely cost- effective public health measure.

Preventing hemoglobinopathy disorders is based on identifying individuals at risk through carrier screening programs or family history and providing adequate information on risk and possibilities to reduce that risk. There are several possible strategies for screening, depending on factors such as the frequency of the disease, heterogeneity of the genetic defects, resources available, and social, cultural and religious factors.

Screening and counseling programs can lead to a significant reduction in affected births. Currently, newborn screening is only recommended for sickle cell disease, since early recognition of the disease can prevent mortality and morbidity caused by bacterial sepsis or sequestration crisis in the first months of life. Newborn screening for β-thalassemia is less frequent, as it requires expensive DNA analysis. Advances in molecular biology and biotechnology may in the future provide faster and cheaper methods (e.g. microchips) for newborn screening of the hemoglobinopathies.

B. Inheritance Pattern in Hemoglobinopathy disorders:

Both Sickle cell Disease and Thalassemia are inherited in autosomal recessive pattern.

Inheritance of Thalassemia:

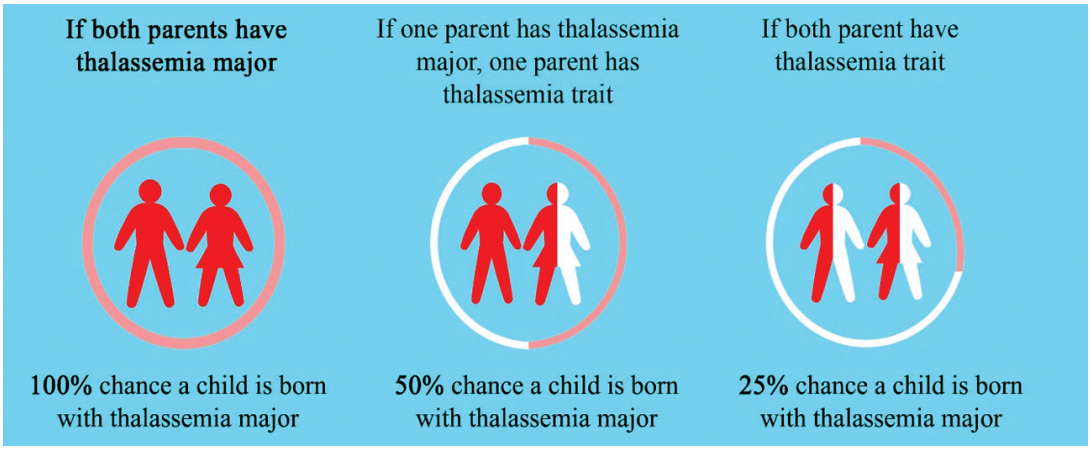
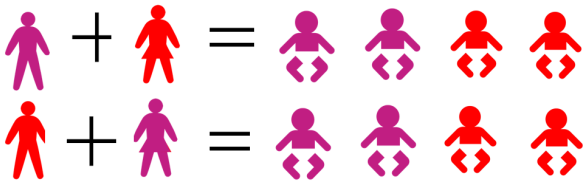


Fig.1 Inheritance pattern in Thalassemia

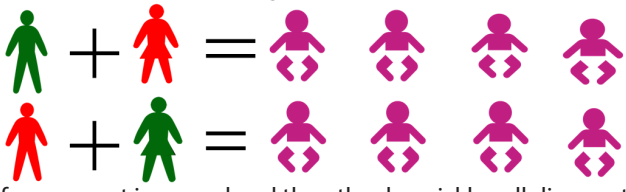
C. Inheritance Pattern of Sickle Cell Disease:

An illustration of different combinations of parents genetic status and the probability of the children being affected with sickle cell disease is as follows:

If both parents have sickle cell disease, there is **100%** chance a child is born with sickle cell disease

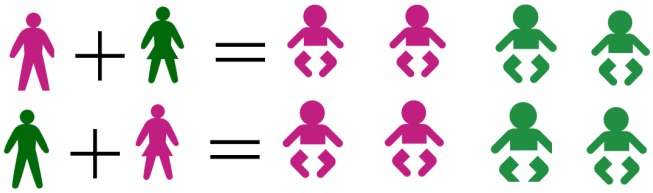


If one parent has sickle cell trait and the other has sickle cell disease, then children have **50%** chance of being diseased and **50%** of being carriers.



If one parent is normal and the other has sickle cell disease, then children have **100%** chance of being carriers

If both parent have sickle cell trait, their children have **25%** chance of being diseased, **25%** chance of being normal, and **50%** chance of being carriers.



If one parent has sickle cell trait and the other is normal, then children have a **50%** chance of being normal and **50%** chance of being carriers.

Fig.2 Inheritance pattern in Sickle cell disease

D. Preventive measures for Hemoglobinopathies

1. Adequate financial and/or technical support from all the three level of government is necessary to facilitate prevention programs. If possible non-governmental organizations (NGOs), and many other organizations, and private sectors can be involved in supporting these efforts.
2. Community Education and Awareness:
   - Address misconceptions about hemoglobinopathies, including transmission, gender bias, and stigma related to the disease and carrier states.
   - Provide information about prevention options and their availability through public health programs.
   - Effective communication strategies are critical in raising awareness about hemoglobinopathies. This can be achieved through:
     - Mass Communication: Use media campaigns (TV, radio, and social media) to spread information about hemoglobinopathies.
     - School Programs: Include hemoglobinopathy education in school curricula and organize awareness activities.
     - Community Outreach: Organize health talks, awareness campaigns, and distribute educational materials to the general public.
3. Training and Capacity Building: Train healthcare professionals, including doctors, nurses, technical laboratory staff, and social workers.
   - Training and Capacity Building, Cost-effective screening programs for adolescents supported by pre-screening educational programs with integrating into school level educational curriculum.
   - Establish pre-marital and pre-conception screening services at the community level.
   - Incorporating with the Health Insurance program for easy access and affordable care.
4. Family Screening: Offer extended family screening for known carriers and patients to identify other at-risk individuals.
5. Prenatal Diagnosis:
   - To carrier couples to prevent the birth of affected children.
   - Screening of all pregnant women in hemoglobinopathies affected geographies and population.
6. Integration into existing public health programs
   - WHO PEN and PEN-Plus
   - Sexual and reproductive health
   - Newborn screening into immunization programs
   - HIV and malaria programs especially focusing on screening for pregnant women, stigma, and counseling.
7. Research and Evaluation: Conduct research, collect data and evaluate prevention program outcomes to refine strategies over time.
8. Surveillance and National Registry: A separate National registry to collect data on the number of hemoglobinopathy cases, carrier status, patient demographics, and treatment outcomes and for further tracking mechanism.
9. Technological innovation for diagnosis, treatment, referral and care coordination
   - Electronic medical health or health information systems.
   - Tele-medicine and mobile technology health platform for virtual consultations.
   - Digital registries with other existing Non Communicable Diseases.

CHAPTER

III

GUIDELINE FOR MANAGEMENT OF SICKLE CELL DISEASE

A. Background

Sickle Cell Disease (SCD) is a hemoglobin disorder that requires lifelong management and contributes to infant, childhood as well as adult morbidity and mortality. A person can be a sickle cell carrier or have sickle cell disease if normal hemoglobin (HbA) is replaced by faulty sickle hemoglobin. Sickle hemoglobin (HbS) is a result of a point mutation in the beta-globin chain where valine is substituted for glutamic acid at the sixth position of it. If only one subunit of beta globin is affected, the person has a trait, and if both are affected, the person has sickle cell disease. Patients with sickle cell trait inherit HbS from one parent and HbA from the other, making them heterozygous and carriers of disease. Patients with sickle cell disease inherit two genes that code for HbS from both parents, making them homozygous. At times a patient may inherit the beta thalassemia gene from one parent and the sickle cell gene from another parent. The most common types of Sickle cell hemoglobinopathies are – HbSS, HbS-beta thalassemia, and SCT.

HbSS: People who have the HbSS form of SCD, inherit sickle cell genes, from both parents. This is commonly called sickle cell anemia / disease and is usually the most severe form of the disease.

HbS-beta thalassemia: People who have this form of SCD, inherit one sickle cell gene, from one parent and one gene for beta thalassemia, another type of hemoglobinopathy from the other parent. Those with HbS-beta thalassemia usually have a severe form of SCD.

Sickle cell trait : A child who inherits only one sickle cell gene from one parent has sickle cell trait . People with Sickle cell traits are usually less severe and live a normal everyday life.

There are five haplotypes of sickle cell disease and two clinical sub-phenotypes. Five haplotypes are Senegal, Benin, Bantu, Cameroon, and Indo-Arab. Among them, features in Nepalese population with SCD are similar to the Indo-Arab haplotype. Clinical features are relatively mild in the Indo-Arab type. Among the two distinct clinical sub-phenotypes, i.e hemolysis-associated endothelial dysfunction and viscosity- vaso-occlusive sub-phenotypes. Hemolysis- endothelial dysfunction sub-phenotypes have predominant features of stroke, leg ulcer and priapism. Viscosity-vaso-occlusive sub-phenotypes have predominant features of acute vaso-occlusive crisis, acute chest syndrome and avascular necrosis of the femur.

Sickle cell disease in children are not much different from adults, but some features are peculiar to children. Children with HbSS present with features of SCD from the age of 2 to 3 months, as HbF is gradually replaced by HbSS. Unless managed appropriately, these children have a lifelong course of severe intermittent illness, primarily due to the recurrent episodes of vaso-occlusive crises, upon an underlying persistently progressive vasculopathy and hemolytic anemia. The initial manifestation in the first months of life is anemia that, on an investigation, is hemolytic. A significant number of children also develop splenic dysfunction in early infancy with the concomitant risk of severe sepsis. By 5 years of age, an overwhelming majority of these children have functional asplenia.

B. Management of sickle cell disease

The goal for management should be patient centric focusing on life course approach with appropriate counseling of patient, educating regarding possible complications, pre-marital and pre-conception counseling.

General Principles of management

- To improve quality of life and life expectancy of the affected individuals.
- Prevent and reduce the number of crises and complications.
- Treat crises and complications promptly and effectively.
- Promote a healthy lifestyle.

i. Prophylactic management: Folic acid and penicillin treatment shall be considered for prophylactic management. Folic acid helps to prevent deficiency resulting from increased cell turnover. Recommended dose of folic acid is 5mg daily to all SCD patients above one year of age. For below one year of age recommended dose is 2.5 mg daily.

   SCD patients develop functional hyposplenia, therefore oral Penicillin is recommended for children upto 5 years of age or lifelong for those who had splenectomy.

The dosage of penicillin is as follows:

- Oral Penicillin V potassium 62.5mg/bd for 1 year
- 125mg/day after 1 year until the age of 2 years
- 250mg/day till 5 years

SCD patients are at risk of severe infections due to hyposplenia especially infections due to encapsulated bacteria therefore, early vaccination shall be instituted to prevent infection and complications in later stages of life. For newborn children, vaccination as per schedule in the National Immunization schedule. For adults vaccination, national guidelines needs to be followed.

ii. Preventive Management: To avoid sickle cell crisis, nutrition, health maintenance, and comprehensive care is important. Education of both patients and caregivers about sickle cell disease including 'Do's and Dont's in acute conditions before coming to the hospital shall be undertaken.

iii. Treatment for severe symptoms: Hydroxyurea should be considered for patients experiencing repeated episodes of acute chest syndrome or with more than three crises per year requiring hospitalization. Hydroxyurea has been proven to decrease complications in children, such as - pain crisis, acute chest syndrome and strokes. In case of children, hydroxyurea (suspension) is preferred.

C. Management of Sickle Cell Disease in Pregnancy

Pregnancy poses additional risks of complication for both pregnant mother suffering from SCD and the fetus. All pregnancies need to be managed as high-risk pregnancy. Complete health check-up, treatment of any anemia and medications prior to conception is ideal. Folic acid is recommended daily before and during pregnancy. Vitamin D should be prescribed as supplementation during pregnancy.

Risks during pregnancy

- Infection, thromboembolic events
- Increase in spontaneous miscarriage
- Increased risk of pre-eclampsia and pregnancy-induced hypertension
- Premature labor and acute painful crises during pregnancy
- Fetal growth restriction
- Antepartum hemorrhage
- Maternal mortality
- Increased incidence of perinatal mortality

Females who are planning a child should-

- Stop hydroxyurea (HU) 3 months prior to planned conception
- Avoid iron chelation medicines as these are potentially erotogenic. These drugs should be stopped before conception. If there is evidence of iron overload, that should be treated while the couple is planning for conception. Since sickle cell disease may also have iron deficiency, which should be treated as per guidelines for other pregnant women.

D. Complications of Sickle cell disease in Adults

i. Acute vaso-occulsive crisis

The acute vaso-occlusive crisis episode is the clinical manifestation of sickle cell disease. The pain episodes dominate the clinical picture throughout the lives of many patients with sickle cell disease. Acute painful episodes are the most common reason for hospital admission for sickle cell patients, and there is a correlation between the frequency of painful episodes and mortality.

Given the noted severity of sickle cell pain, treatment warrants the use of opioids. Failure to treat the acute painful episode aggressively can lead to a protracted episode, progression of an uncomplicated episode to one that is complicated, evolution of chronic pain syndromes, and aberrant pain behavior patterns.

While the inciting factor for most painful episodes cannot be determined, sickle cell painful episodes may be precipitated by temperature extremes, physical or mental stress, fatigue, infection, menses or dehydration. The pain associated with an acute painful episode may be located at any anatomic site. The most common sites for pain are the vertebral spine, abdomen, femoral shaft, distal limbs and ribs.

Assessment of Acute Painful Episode:

It is important to remember that all pain in patients with sickle cell disease is NOT sickle pain. Therefore, it is always necessary to consider other possible etiologies. At the time of the initial assessment of an acute painful episode, one should establish whether it is a "typical" or "atypical" episode. Most patients will be able to relate whether the characteristics of the present pain episode are similar to previous ones. If it is noted that the present episode is "atypical," this should alert one to the possibility of other etiologies for the pain.

During the initial assessment of the acute painful episode, it is also essential to determine whether the present episode is "Complicated" or "Uncomplicated." Findings such as tachycardia, tachypnea, hypoxia, fever >38.50C, hypotension, neurological deficits, priapism, recurrent emesis, or acute joint swelling indicate a complicated episode.

Initial work-up of an acute painful episode should include complete blood count (CBC) with reticulocyte count, serum chemistry including lactate dehydrogenase (LDH) and liver function test (LFT). If the patient has any respiratory symptoms, then a CXR should be obtained. Blood and urine cultures should be obtained if the patient is febrile or the total leucocyte count (TLC) is above baseline. During the admission, one should follow at least daily CBC with reticulocyte count (may follow every other day if an episode is without complications). Other daily required investigations as clinically indicated.

Management of Acute Painful Episode:

It should be noted that most of the pain episodes are managed at home. Therefore, aggressive regimens are recommended for the management of pain severe enough to require hospital admission.

- **Around the clock (ATC) dosing**

In around the clock (ATC) dosing, the opioid is administered IV or subcutaneously on a scheduled basis rather than on- demand basis. The frequency of the scheduled dose is based on the effective half-life of the opioid. For example, the plasma half- life of morphine is approximately 2½ hours; therefore, it is scheduled every three hours. On-demand dose may be given for breakthrough pain in addition to the scheduled dose. As noted earlier, if needed, a rescue dose of the IV opioids may be provided on demand basis for breakthrough pain at ¼ to ½ the scheduled dose. Limits should be set on the number of rescue doses that the patient uses before an adjustment is made in the scheduled dose. Once the painful episode has diminished, one can attempt to taper off the IV opioids and subsequently convert to oral analgesics in anticipation of discharge home.

- **Non-opioid Analgesics:**

Non-opioid analgesics such as acetaminophen or NSAIDs may be used to treat acute painful episodes.

These may be used alone, usually during a minor painful episode at home, or in conjunction with opioids for synergy and opioid sparing effect.

Patients with SCD are already at risk for renal complications because of their disease, therefore NSAIDs should be used cautiously.

Acetaminophen can also be used with opioids for synergy and opioid-sparing effect. This should also be used cautiously, given that many of the oral opioids already have acetaminophen and one need to be cautious about associated hepatotoxicity.

- **Fluid Management:**

Hydration is an integral part of the management of acute painful episodes. Oral fluids should be given if there is no IV access and the patient is not nauseated. IV hydration with ½ NS or normal saline is preferred. In- put/out- put charting should be done every 6 hours to avoid fluid overload.

- **Hydroxyurea in Sickle cell disease**

Hydroxyurea should be considered for patients experiencing repeated episodes of acute chest syndrome or with more than three crises per year requiring hospitalization. Hydroxyurea has been proven to decrease complications in children, such as - pain crisis, acute chest syndrome and strokes.

Indications for Initiation of Hydroxyurea therapy in Adults with SCD

- Three or more hospitalization for a vaso-occlusive crisis in the past 12 months.
- Severe or recurrent acute chest syndrome.
- Sickle cell-associated pain that affects daily activities and quality of life.

Evaluation of Patient before initiating Hydroxyurea

1. Explain indication, aims, benefits that can be expected, and possible side effects of hydroxyurea therapy to the patient and family members.
2. Order appropriate laboratory investigations: CBC including reticulocytes counts. Renal function test (RFT), LFT, pregnancy test for women, baseline quantitative HbF by Hb electrophoresis.
3. Prescribe contraceptive methods for a planned pregnancy.

Dose:

Starting dosage: 15 mg/kg/day, rounded up to the nearest 500 mg; 5-10mg/kg/day in the chronic kidney disease.

Monitoring: CBC, RFT, and LFT every four weeks until Maximum tolerated dose (MTD); also measure HbF every 3 months.

MTD: Highest dose at which neutrophil count is above 2000/µl (may reach 1250/ µl in young adults), with platelet and reticulocyte count above 80,000/ µl.

Dose escalation: Increase 5 mg/kg/day every 8 weeks until MTD is reached.

Toxicity: If neutrophil is < 2000/ µl (depending on age), platelet<80,000/ µl or reticulocytes < 80,000/ µl, stop hydroxyurea, monitor CBC weekly until recovery and restart hydroxyurea at a dose of 5mg/kg/day lower than previously.

Maximum dose: 35 mg/kg/day or MTD

Minimum duration of treatment: 6 months

Do NOT:

1 Double up doses if a patient misses a dose.
2. Stop HU during hospitalization or acute illness.
3. Stop HU due to lack of increase of Mean cell volume (MCV) or fetal hemoglobin.

Newer Drugs For Sickle Cell Disease

1. L glutamine oral powder
2. Voxelotor
3. Crizanlizumab

ii. Acute Chest Syndrome (ACS)

Acute Chest Syndrome is one of the leading causes of death in adult sickle cell patients. In addition, patients with ACS have the potential to progress to respiratory failure with an Acute Respiratory Distress Syndrome picture rapidly. Given the potential for rapid deterioration, patients with ACS need to be diagnosed promptly with appropriate and effective management.

Acute Chest Syndrome is diagnosed when there is a new pulmonary infiltrates consistent with consolidation involving at least one lung segment on CXR or CT scan with at least one or more of the following new symptoms: fever > 38.50C, chest pain, cough, sputum production, wheezing, or hypoxia. In addition, CBC may reveal an increase in TLC above baseline and worsening anemia as reflected by drop in hemoglobin concentration.

Management: Diagnosis:

Once the criteria for the diagnosis of acute chest syndrome are met, one should proceed with close monitoring and prompt appropriate treatment.

Monitor the following:

- Continuous monitoring of oxygen level by pulse oximeter.
- Serial/Daily Arterial blood gas (ABG).
- Serial/Daily CXR until improvement noted.
- Serial/Daily CBC and reticulocyte count.
- Pre/post-transfusion hemoglobin electrophoresis.

Institute the following treatment:

- IV hydration with ½ NS no more than 1½ of daily maintenance.
- Antibiotics consist of cephalosporin/macrolide or fluoroquinolone.
- Supplemental oxygen (O2) to maintain O2 >95%. If noted to have increasing O2 demands, FIO2 needs greater than 40%, then consider transfer to intensive care unit.
- Adequate pain control avoiding over sedation.
- Bronchodilator therapy in those with evidence of reactive airway disease.
- Incentive spirometer.
- Transfusions: Blood group typing and crossing matching should be sent immediately once ACS is suspected. RBC transfusions, whether simple or exchange, can be lifesaving in treating ACS as they may prevent its progression to acute respiratory failure. Simple transfusion of 1-2 units of packed red blood cell (PRBC) can be given to raise the hemoglobin level (NOT to exceed Hb level of 10gm/dl) as this will increase the oxygen-carrying capacity. Exchange transfusions, on the other hand can be used to remove sickle cells and replace them with normal red cells. The goal of exchange transfusion is to reduce the Hemoglobin S level to <30% with a total hemoglobin level of ~10gm/dl. The choice to proceed with simple versus exchange transfusion is based on the patient's clinical status, availability of units of PRBC, and hemoglobin level.

If a patient with ACS is noted to be unstable/deteriorating as indicated by low O2 saturation despite aggressive vent support, serial deterioration in O2 saturation with increasing O2 demands, and unstable vitals such as persistent tachypnea, then one should proceed with immediate rapid exchange transfusion.

iii. Acute Neurological Events

Central nervous system involvement in sickle cell disease is common in the hemolysis-endothelial dysfunction sub-phenotype. These features are less common in viscosity vaso-occlusion sub-phenotypes. Cerebrovascular accidents are a common complication seen in children and adults with SCD. The type of stroke varies with age, ischemic strokes are more common in the pediatric population, while hemorrhagic strokes are more commonly noted in adults.

Diagnosis/Management

Any of the mentioned signs or symptoms like headache, syncopal episode, change in the level of consciousness, motor or sensory deficit, changes in vision, or new-onset seizures noted in a patient with sickle cell disease one should raise suspicion of an acutely evolving neurological event. The concern for an acute neurological event is further raised if, in addition to the signs/symptoms, a prior history of a neurological event as repeat stroke, ischemic or hemorrhagic causes to be ruled out.

An adult patient with sickle cell disease with signs/symptoms suggestive of an acute cerebrovascular accident (CVA) should have an immediate evaluation of vital signs including general assessment. In addition, a CT of the brain without contrast should be obtained immediately, which will subsequently determine the course that must be followed.

▪ Hemorrhagic Stroke

As noted earlier, a hemorrhagic stroke is more common than ischemic stroke in the adult sickle cell population. These include subarachnoid, intraparenchymal, or interventricular hemorrhage. Patients with intracranial hemorrhage commonly present with severe headaches, nausea/vomiting and mental status changes. If the CT of the brain reveals hemorrhage, then neurosurgery needs to be consulted immediately. Neurosurgery is consulted as patients with intracranial hemorrhage will likely need angiography to identify the source of the bleed. An exchange transfusion should precede the angiography with a goal of HbS <30% and a total Hb of ~10gm/dl. Subsequent surgical intervention is to be determined by neurosurgery.

▪ Ischemic stroke:

Although less frequent than the hemorrhagic type, ischemic stroke may still occur in the adult sickle cell population. A non-contrast CT of the brain is less likely to reveal an acute/evolving ischemic stroke. If the non-enhanced CT does reveal an ischemic stroke (which is possible, particularly if the ischemia has been ongoing for several hours) , one may give Aspirin 325mg to the patient and subsequently proceed with an exchange transfusion with a goal of Hb S<30% with a total Hb level of ~10gm/dl. If the non-contrast CT is negative i.e no bleed or ischemia noted, one may still give Aspirin 325mg and proceed with obtaining the MRI/MRA. If the MRI is positive for an ischemic stroke, one would immediately proceed with the exchange transfusion to match with the mentioned goal of HbS % and Hb level.

▪ Transient Ischemic Attack (TIA)

If the imaging studies are negative and the clinical suspicion is high for a TIA, then one would still consider proceeding with an immediate exchange transfusion with the mentioned goal for HbS reduction and maintenance of total hemoglobin level. Conversely, if the clinical suspicion for TIA is low, one can keep the patient in observation.

▪ Aneurysm and Moya/Moya

Even though the imaging studies may not reveal an acute/evolving stroke, the MRA may still identify a vascular lesion such as an aneurysm (not uncommon for a patient to have multiple aneurysms). Injury to the endothelium by the sickle cells and the high viscosity associated with SCD likely promotes

the formation of aneurysms. If aneurysms are noted, then neurosurgery should be consulted for evaluation and possible intervention for clipping or coiling. The MRA may also reveal Moya Moya's collateral circulations formed due to significant vessel stenosis. Patients with moya/moya are at risk for recurrent hemorrhagic and ischemic strokes, so neurosurgery should again be consulted in this situation for evaluation and possible intervention.

iv. Fever/Infection

Both pediatric and adult SCD patients have increased susceptibility to infection mainly due to their afunctional spleen. Children with SCD have an increased risk for overwhelming sepsis with Streptococcus pneumonia, the most common cause of death in the sickle cell pediatric population. The mortality due to Streptococcus pneumonia sepsis has been significantly decreased in the sickle cell pediatric population because of pneumococcal vaccination and PCN prophylaxis.

Adults with SCD have matured immune system such that they are less susceptible to the overwhelming sepsis than those seen in the pediatric population. Despite this, they are still more prone to infection than normal adults. Therefore, any fever in adults with SCD requires an aggressive approach.

Management:

Acute painful episodes may cause low-grade fever with the temperature usually <38.50C, but fever over 38.50C are more likely due to an infectious etiology. Workup should include CBC, reticulocyte count, CXR, urine analysis and blood culture. A lumbar puncture may be considered if the clinical presentation suggests the possibility of meningitis. Given the increased susceptibility of SCD patients to infection, there should be a low threshold for initiating empirical antibiotics in those presenting with fever. The clinical presentation and findings on CBC, CXR and urine analysis determine the need and type of empirical antibiotics.

Common infections in adult SCD patients:

- **Pneumonia:** It is one of the most common etiologies of acute chest syndrome. If this is suspected by CXR findings, one should initiate ceftriaxone plus macrolide or fluoroquinolone. Ensure that there is atypical coverage since Mycoplasma and Chlamydia have been identified as the most common infectious agents resulting in acute chest syndrome.
- **Urinary Tract Infection (UTI):** Recurrent UTI are common, particularly with E. Coli as the most common pathogen. Inpatients with SCD, UTI should usually be treated as having a complicated one. Antibiotics are chosen based on their sensitivities.
- **Osteomyelitis:** It often occurs at the sites of necrotic bone where infection with Salmonella occurs more often in SCD patients but, overall Staph. Aureus remains the most common cause of infection. MRI is the preferred imaging study. Bone biopsy remains the gold standard for diagnosis. 2-6 weeks of parenteral antibiotics is the choice of treatment (choice of antibiotics based on isolated pathogen and susceptibilities).
- **Cholecystitis:** Cholelithiasis is common due to ongoing hemolysis with the formation of pigmented stones, and acute cholecystitis may be subsequently noted. Empiric broad-spectrum antibiotic with piperacillin and tazobactam or combined therapy with fluoroquinolone and metronidazole may be warranted before definitive surgical intervention.

v. Aplastic Crisis:

Given the significantly short life span of the RBC in sickle cell disease (~10 days in sickle cell anemia), any temporary suppression of the bone marrow may result in a rapid decline in hemoglobin levels. Any infection may result in bone marrow suppression, but the principal cause of transient red cell aplasia in sickle cell patients is parvovirus B19. CBC will reveal hemoglobin that is significantly below baseline, where the mean hemoglobin level at the presentation time is noted to be ~4gm/dl. Reticulocyte count <1% or an absolute count <10,000. Treatment consists of simple transfusion until the bone

marrow recovers. Recovery is noted when there is evidence of red cell production as determined by an increase in the reticulocyte count. One may consider treatment with IVIG, which may shorten the course of infection.

Other complications associated with infection with parvovirus B19 in SCD patients besides the aplastic crisis are nephrotic syndrome, stroke, acute chest syndrome and hepatic or splenic sequestration.

vi. Acute Sequestration Crisis

During a sequestration crisis, the sickled erythrocytes can become acutely entrapped in the spleen, liver, or lung with an acute drop in the hemoglobin/hematocrit (HCT).

▪ Acute Splenic Sequestration

Splenic sequestration is a life-threatening complication usually seen in the pediatric population of Hb SS patients between the ages of 3 months and 5 years. Acute splenic sequestration in Hb SS patients over the age of five is rarely seen, as the spleen usually has auto-infarcted by that age. In adult SCD patients, it is more common to see acute splenic sequestration in Hb SC and sickle/beta-thalassemia patients. These patients frequently have an enlarged spleen, therefore during vaso-occlusive episodes, they have the potential for sequestration. During splenic sequestration, Hb is usually noted to drop by more than 2gm/dl, accompanied by increased reticulocytes. Therefore, simple transfusion can be given to maintain hemoglobin levels. In this case, one must be careful with reverse sequestration as the entrapped RBCs may return to circulation and increase the viscosity. If more than one episode of sequestration is noted, then a splenectomy should be considered.

▪ Acute Hepatic Sequestration

This is an uncommon complication usually seen in Hb SS patients. During a vaso- occlusive episode, sickled erythrocytes become entrapped in the hepatic sinusoids. As a result, the Hb/HCT drops significantly below baseline accompanied by increased reticulocytes and hepatic size. Also, a dramatic rise in bilirubin may be noted, with the majority being conjugated.

The Alkaline phosphatase levels may rise, but the transaminases usually are not significantly elevated. Treatment consists of either simple or exchange transfusion. Again, one has to be cautious with the transfusions given the potential for reverse hepatic sequestration as entrapped RBCs return to the circulation increasing the potential for hyper-viscosity.

▪ Acute Pulmonary Sequestration

Acute pulmonary sequestration is noted to be one of the major causes of acute chest syndrome. The sequestration of sickled erythrocytes in the pulmonary capillaries results in pulmonary injury/infarction with subsequent rapid deterioration. In these cases, urgent exchange transfusion is warranted.

vii. Priapism

It is the least common clinical manifestation of sickle cell disease in Nepal. Priapism is a persistent, painful, and unwanted erection that occurs without sexual stimulation. It is a common complication of sickle cell disease which is encountered at least one episode in patients by the age of twenty. In sickle cell disease, priapism results from the failure of venous outflow (therefore, it is a low- flow state) involving the corpus cavernosum. As a result, the corpus spongiusum is spared.

The episode of priapism is either noted to be stuttering which spontaneously resolves within three hours (usually only last several minutes) or prolonged lasting more than three hours. Prolonged priapism is a medical emergency as it may result in loss of functionality. In addition, the risk of impotence is significantly increased if the priapism lasts longer than 24 hours. The goal of treatment is to relieve the pain, achieve subsidence of swelling and maintain functionality.

When a patient initially presents with priapism, they should be immediately started on IV fluids, preferably normal saline, and their pain controlled. The priapism may be associated with an acute sickle painful episode. Pain associated with the priapism, whether accompanied by a painful episode or not, will likely require treatment with IV opioids. If subsidence of swelling is not achieved with conservative treatment and has been prolonged and ongoing for 4-6 hours, then urology should be consulted for possible penile aspiration. If the priapism recurs despite urological evaluation/treatment, then exchange transfusion may be indicated at the time. The goal of exchange transfusion is to reduce the Hb S by less than 30%. If the priapism persists despite the above noted interventions, a shunting procedure may be considered. The process above is the last resort and, if possible, should be avoided.

E. Complications of Sickle cell disease in Children

Apart from adult features following features are more common in children with sickle cell disease.

▪ Dactylitis or Hand-Foot Syndrome

Dactylitis often referred to as a hand-foot syndrome, is often the first manifestation of pain in children with sickle cell anemia, occurring in 50% of children at the age of 2 years. Dactylitis often manifests with symmetric or unilateral swelling of the hands and/or feet. Unilateral dactylitis can be confused with osteomyelitis, and careful assessment to differentiate between the two is important because treatment differs significantly.

Dactylitis requires palliation with pain medications, such as acetaminophen with codeine, whereas osteomyelitis requires at least 4-6 weeks of IV antibiotics.

Figure 3: Dactylitis in Sickle cell disease

▪ Splenic Sequestration

Acute splenic sequestration is a life-threatening complication occurring primarily in infants and can occur as early as 5 weeks of age which is indicated by the rapid increase in the size of the spleen in a short period of time. Approximately 30% of children with sickle cell anemia have a severe splenic sequestration episode, and a significant percentage of these episodes are fatal.

Repeated episodes of splenic sequestration are common, occurring in 50% of patients. Most recurrent episodes develop within 6 months of the previous episode. Although blood transfusion has been used to prevent subsequent episodes, but evidence strongly suggests, this strategy does not reduce the risk of recurrent splenic sequestration when compared to no transfusion.

Splenic sequestration Prevention, Diagnosis, and Treatment

Anticipatory Guidance

Teach parents and primary caregivers how to palpate the spleen to determine if the spleen is enlarging.

Diagnostic Testing and Laboratory Monitoring

1. Increase of the spleen size
2. Evidence of hypovolemia
3. The decline in hemoglobin of ≥2 gm/dl from the patient's baseline hemoglobin
4. Reticulocytosis
5. A decrease in the platelet count may be present. These events can be accompanied by upper respiratory tract infections, bacteremia or viral infection.

Treatment

1. Early intervention and maintenance of hemodynamic stability using isotonic fluid or blood transfusions.
2. If blood is required, typically 5ml/kg of packed red blood cells is given.
3. Prophylactic splenectomy performed after an acute episode has resolved is the only effective strategy for preventing future life-threatening episodes.

Infectious Risk Management

1. Penicillin V orally from 2 months to at least 5 years of age.
2. Prompt administration of broad-spectrum or pneumococcal-specific antibiotics in case of possible bacterial infection
3. Malarial prophylaxis, when appropriate
4. Immunization against-
   - Streptococcus pneumonia
   - Haemophilus influenza
   - Meningococcus
   - Influenza
   - Salmonella typhi (for at risk individuals)
5. Elimination of recurrent focal infection (dental infection, sinusitis, acute recurrent tonsillitis, cholecystitis, urinary infections.

F. Transfusion

Red blood cell transfusion plays an integral part in managing sickle cell disease. PRBC transfusion are indicated in various clinical scenarios in SCD in acute and non-acute settings. In many cases, transfusions are lifesaving, however this does not mean that PRBC transfusion in SCD are a panacea. There are various serious complications related to transfusion therapy. Therefore, there must be a thoughtful approach before proceeding with any transfusions.

Special considerations for the blood that is to be transfused to SCD patients are the following:

- **Leukocyte depletion:** this can decrease the risk of alloimmunization. Also decreases the likelihood of febrile non-hemolytic transfusion reactions and transmission of Cytomegalovirus.
- **Phenotypically matched:** by matching for minor antigens, the risk of alloimmunization is reduced. Depending on the clinical scenario, patients may be either simple or exchanged transfused. Exchange transfusion offers the benefit of actually removing the sickle cells and replacing them with normal red blood cells without increasing the viscosity. Depending on the hemoglobin level, one may be limited in how aggressive the patient can be simply transfused, where we have to be cautious to avoid hyperviscosity by ensuring the hemoglobin level does not exceed12gm/dl. The other advantage of exchange transfusion besides the ones already noted is the fact that it limits the iron overload associated with the transfusions. So why not exchange everybody? As with any intervention, an exchange transfusion has its downside. The disadvantages associated with an exchange transfusion are the likely need for placing a large-bore central venous catheter, the need for several units of PRBC, which means an increased chance for alloimmunization and last but not the least, the cost.

Indications for transfusion in SCD

- **Episodic Simple Transfusion:**
  - Severe acute anemia as seen with hyperhemolysis (non-immune mediated), infection (any infection may result in suppression of the bone marrow as may be evident by a low reticulocyte count).
  - Acute splenic sequestration
  - Aplastic Crisis
  - Pre-operatively: the goal of hemoglobin level is ~10gm/dl prior surgery.
  - Acute chest syndrome: Simple transfusions are acceptable in patients who are stable with low hematocrit (should not exceed hematocrit of 30).

- **Episodic Exchange Transfusion:**
  - Acute chest syndrome.
  - Stroke.
  - Acute multi-organ failure.

- **Chronic Transfusion:**

Primary and secondary stroke prevention: The studies to justify chronic transfusion have been done mainly in the pediatric population and have been extrapolated to adults. Once the hemoglobin S has been reduced to 30% with an exchange transfusion, then this fraction may be maintained with a simple transfusion of 1-2 units every 3-4 weeks.

Complications of Transfusion

- **Alloimmunization:** This is one of the major complications of transfusion in SCD patients, where they form antibodies to the antigens in the transfused blood. Usually, many alloantibodies are present, which makes it rather difficult to find compatible blood. It has been noted that anywhere from ~5 to 50% of sickle cell patients will develop alloantibodies after several transfusions. The risk of alloimmunization has been estimated as ~3% per unit of PRBCs transfused. The high rates of alloimmunization in sickle cell patients have been attributed to the fact that the blood that they receive is likely from people of different ethnic/racial backgrounds who have a different antigenic frequency. The risk of alloimmunization can be reduced by transfusing only leuko-poor phenotype-matched RBC.
- **Delayed Hemolytic Transfusion Reaction (DHTR):** This usually occurs after 24 hours and more likely 5-14 days following the transfusion as a result of a primary or an anamnestic immune response in a previously all immunized patient. This may result in immune hyperhemolysis, where not only the transfused blood may be hemolyzed, but also autologous peripheral destruction may be noted (bystander hemolysis). During an episode of DHTR, the LDH and total bilirubin will rise significantly above baseline, and the direct antibody test (DAT) will be positive, if the cohort of cells has not been destroyed. In Sickle cell patients, the hemoglobin fractionation will likely reveal Hb A level of 0% as all of the transfused blood will have been hemolyzed. In this case, further transfusions should be avoided as they will exacerbate the situation. In addition to avoiding transfusions, the patient can be placed on corticosteroids (usually 1mg/kg of prednisone should suffice). The patient may also be given an erythropoietin injection to maintain/increase hemoglobin level. IVIG has also been used in this case.
- **Iron Overload:** Each unit of blood has approximately 250mg of elemental iron. The body does not readily excrete iron; hence it will accumulate with multiple transfusions of PRBCs. The accumulated iron may subsequently result in organ dysfunction, usually involving the heart, liver, and endocrine organs. MRI of the liver may be a more accurate estimation of iron stores/overload. The gold standard is a liver biopsy. Treatment of iron overload in sickle cell patients is problematic given that phlebotomy is not usually an option. The use of iron chelators such as deferoxamine or deferasirox should be encouraged.
- **Infections:** Viral infections such as hepatitis B and C, HIV1/2, HTLV type I and II and other transfusion transmitted virus and parasites may be transmitted with transfusion of PRBC.

CHAPTER
IV

GUIDELINE FOR MANAGEMENT OF THALASSEMIA

A. Genetic Basis and Pathophysiology

Hemoglobin Types

Oxygen is transported from the lungs to the tissues by a highly specialized protein molecule, hemoglobin, which is located in the red cells of the blood. Each red blood cell contains approximately 300 million molecules of this protein, in totality about 30 picograms in weight per cell. Each molecule of hemoglobin is formed by two pairs of identical sub-units, the globin chains, which are named with letters of the Greek alphabet and belong to two groups: the α-globin cluster, comprising the ζ- and α-globin chains, and the β-globin cluster, comprising the globin chains ε, γ, β and δ. The globin chains appear sequentially during ontogeny, and after pairing, form the following four major types of hemoglobin:

1. "embryonic" hemoglobins, which are detectable from the 3rd to the 10th week of gestation and represent ζ2ε2, α2ε2, and ζ2γ2 tetramers;
2. "fetal" hemoglobin (HbF α2γ2), which constitutes the predominant oxygen carrier during pregnancy;
3. "adult" hemoglobin (HbA α2β2), which replaces HbF shortly after birth, and;
4. a minor adult component, HbA2 (α2δ2).

Under normal conditions, the red cells of the adult human contain approximately 98% HbA, 2.0% HbA2 and traces of HbF.

Globin Genes and Globin Synthesis

The globin chains have an extremely precise structure, ensuring their prompt loading with oxygen in the lung alveoli and its controlled gradual delivery into the tissues. The precise structure of the globin chains is coded by genes contained in the DNA of chromosomes 16 (the α gene cluster) and 11 (the β gene cluster). Flanking the structural genes, i.e., in front (on the 5′ side of the DNA sequence, "upstream") and following them (on the 3′ side of the DNA sequence, "downstream"), lie several nucleotide sequences which have a "regulatory" role, i.e., they determine which gene is to be turned on and off, as well as how efficient its expression will be. In adult life, most of the globin synthesis occurs in the erythroblasts in the bone marrow. Hemoglobin must have the correct structure and be trimmed in such a way that the number of α-chains precisely matches that of the β-chains. When the above conditions are not met, the result is a complete or partial defect in one or both "allelic" globin genes.

Depending on which of the genes the defect occurs and the corresponding effect on the production of globin chains, α-thalassemia or β-thalassemia results.

β -thalassemia:

β-Thalassemia is due to a range of mutations associated with the β globin gene, resulting in reduced or absent production of β globin, one of the constituents of the adult hemoglobin molecule (HbA). The degree of globin chain imbalance is determined by the nature of the mutation of the β-gene. More than 200 thalassaemic mutations have been reported to date.

Figure 4. below outlines the pathophysiology of β-thalassemia and describes the chain of events following globin chain imbalance and the accumulation of excess α-chains i.e, ineffective erythropoiesis leading to anemia, bone marrow expansion, skeletal deformities, and increased gastro-intestinal iron absorption.

Figure 4: Pathophysiology of β–Thalassemia Major

β Thalassemia is categorized clinically into:

i. Transfusion-dependent thalassemia (TDT)
ii. Non- Transfusion-dependent thalassemia (NTDT)

i. Transfusion-dependent thalassemia (TDT)

Transfusion dependent is one essential factor in distinguishing the various thalassemia phenotypes and their severity. For example, the diagnosis of β-thalassemia major entails a lifelong regular transfusion requirement for survival, and the term transfusion- dependent thalassemia is conventionally used to describe such forms. The TDT require a regular blood transfusion to survive, and without adequate transfusion support, they would suffer several complications and a short life span. This category includes patients with β-thalassemia major, severe HbE/β- thalassemia, transfusion- dependent HbH disease or HbH hydrops, and surviving Hb Bart's hydrops.

β - thalassemia major patients develop clinical signs and symptoms from the age of 4 to 6 months. Clinically, the presentation is insidious, with poor feeding, faltering growth, pallor, and increased susceptibility to infection. Their hemoglobin level is reduced, usually below 7gm/dl. There is an enlargement of the liver and spleen. The ineffective expansion of the erythropoietic marrow results in bone thinning and deformity. If untreated with red cell transfusion, progressive anemia and metabolic stress eventually cause infection and heart failure, and death by 10 years of age.

ii. Non-transfusion-dependent thalassemia (NTDT)

It is used to label patients who do not require lifelong regular transfusions for survival, although they may require occasional or even frequent transfusions in certain clinical settings and for defined periods of time. NTDT encompasses three clinically distinct forms: β-NTDT, hemoglobin E/β-thalassemia (mild and moderate forms), and α-NTDT (hemoglobin H disease).

β- NTDT is usually diagnosed at the age of 3 to 15 years. There is a reduced amount of hemoglobin production, sufficient for growth and development without the absolute requirement for regular transfusions. Growth may fail, and other complications may develop in later childhood and adulthood requiring regular transfusions.

β-thalassemia trait/carrier is symptom-free with mild or no anemia, however the hemoglobin level may reduce under stress such as puberty, pregnancy or infection and may require treatment. The

primary defect is usually quantitative, consisting of the reduced or absent synthesis of normal globin chains, but there are also mutations resulting in structural variants produced at a reduced rate (e.g., HbE, HbLepore, HbE/β).

TDT and NTDT classification is based on clinical parameters, variations, and advances in clinical management, as well as other modifiers of disease which may shift a patient from one group to another during their lives. Thus, the TDT and NTDT designations should primarily represent patients' 'current' clinical status and entail the understanding that these two designations are interchangeable.

B. Transfusion-dependent thalassemia

Treatment of TDT

The supportive management of thalassemia is based on a program of regular blood transfusion and iron chelation. Regular blood transfusion from early childhood improves anemia and reduces the skeletal deformities associated with excessive erythropoiesis.

When to initiate transfusion and whom to transfuse:

For deciding when to initiate a regular transfusion regimen and whom to transfuse, the following should be considered;

- Confirmed laboratory diagnosis of thalassemia major
- Laboratory criteria: Hemoglobin <7gm/dl on two occasions, >2 weeks apart (excluding all other contributors causes such as infections) AND/OR
- Clinical criteria irrespective of hemoglobin level:
  - Haemoglobin >7 gm/dl with any of the following:
    - Significant symptoms of anemia
    - Poor growth/failure to thrive
    - Complications from excessive intramedullary hematopoiesis such as pathological fractures and facial changes
    - Clinically significant extramedullary hematopoiesis

Recommended blood products:

- Leuco-reduced packed red cells are recommended for eliminating the adverse reactions (see Table.1 below) attributed to contaminating white cells and for preventing platelets alloimmunization. The residual leucocytes count of filtered product should be 1 x 10^6/L or less per unit. There are several methods of leukoreduction (Pre-storage filtration, Pre- transfusion, and Bedside filtration), of which pre-storage filtration of whole blood is the preferred method.

Table 1: Adverse effects of leucocytes in blood products

REACTIONS	CAUSATIVE AGENTS
Febrile non-hemolytic transfusion reactions	HLA-antibodies in patients, cytokines produced by donor leucocytes
HLA- alloimmunization of recipients	HLA antigens on donor leucocytes
Transfusion-transmitted infections	Cell-associated infectious agents
Graft-versus-Host-Disease	Donor T-lymphocytes

Blood Products for special patient populations:

- Washed red cells may be beneficial for thalassemics who have repeated severe allergic transfusion reactions. Saline washing of the donor products removes plasma proteins that constitute the target antibodies in the recipient. Washing may be accomplished using manual or automated techniques. Washed red cells that are not suspended in storage solution must be transfused within 24 hours, and this shorter shelf-life creates the possibility of wastage if patients are not available for transfusion at the time the blood is prepared. Washing usually does not result in adequate leucocyte reduction and should not be a substitute for leucoreduction. Instead, washing should be used in conjunction with filtration. Washing of red cell units may remove some erythrocytes from the transfusion product, and it is therefore valuable to monitor post-transfusion hemoglobin levels to ensure attainment of the targeted hemoglobin level.
- Frozen (or cryopreserved) red cells is the component derived from whole blood in which red cells are frozen, preferably within 7 days of collection, using a cryopreservant and stored at -600C to -800C or below, based on the method used. These are used to maintain a supply of rare donor units for certain patients who have unusual red cell antibodies or who are missing common red cell antigens. Approximately 20% of the donor cells are lost in the washing after the freezing process.
- Red cells obtained by donor apheresis: This method whereby upto two units of red cells are collected from the same donor for transfusion of one patient is associated with a reduction of donor exposures and consequently, a decreased risk of transmission of infections and of developing alloimmunization and other transfusion-related complications.

Transfusion Program:

The recommended treatment for thalassemia-major involves lifelong regular blood transfusions, usually administered every two to five weeks, to maintain the pre- transfusion haemoglobin level 9-10 g/dl. This level of hemoglobin is considered adequate to suppress extra-medullary erythropoiesis and excessive absorption of iron from the gut.

A higher target pre-transfusion hemoglobin level of 11-12 gm/dl may be appropriate for patients with heart disease, clinically significant extramedullary hematopoiesis or other medical conditions, and for those patients who do not achieve adequate suppression of bone marrow activity at the lower hemoglobin level. The post-transfusion hemoglobin should not be greater than 14-15gm/dl as higher post- transfusion hemoglobin values risk hyper-viscosity and stroke. All patients with thalassemia should be transfused with ABO and Rh(D) compatible blood and a full cross-match and antibody screen should be performed prior to each transfusion. Regular blood transfusion from early childhood improves the anemia and reduces the skeletal deformities associated with excessive erythropoiesis. However, regular transfusion regimens are associated with some complications such as iron overload, platelet and RBC alloimmunization. The risk of alloimmunization appears to be greater in patients who begin transfusion therapy after the first few years of life.

Recommendations regarding the volume of transfused red cells are complicated by the use of different anticoagulant-preservatives and additive solutions. For CPDA-1 packed red cell units with a hematocrit of approximately 75%, the volume per transfusion is usually 10-15ml/kg, administered over 3-4 hours. Units with additive solutions may have lower hematocrits in the range of 60-70%, and consequently larger volumes with a higher hematocrit level are needed to administer the same red cell mass (see Table. 2).

Table 2: Guidelines for choosing how much blood to transfuse

Haematocrit of Donor Red Cells					
		50%	**60%**	**75%**	**80%**
Target Increase in Hemoglobin Level	1 gm/dl	4.2 ml/kg	3.5 ml/kg	2.8 ml/kg	2.6 ml/kg
	2 gm/dl	8.4 ml/kg	7.0 ml/kg	5.6 ml/kg	5.2 ml/kg
	3 gm/dl	12.6 ml/kg	10.5 ml/kg	8.4 ml/kg	7.8 ml/kg
	4 gm/dl	16.8 ml/kg	14.0 ml/kg	11.2 ml/kg	10.4 ml/kg

Assessing the effectiveness of blood transfusion regimen

The effectiveness of a blood transfusion programme is usually measured in terms of the rate of fall in levels of haemoglobin, which should not exceed 1gm/dl/week in splenectomised patients and 1.5gm/dl/week in non-splenectomised patients.

If Hb levels are found to fall at a greater rate, the following causes may be investigated:

- Antibodies (alloimmunization) to RBCs
- Enlarged spleen (hypersplenism) and/or liver (hepatomegaly), where a patient requires more than 200ml RBC/kg/year, for example, the possibility of an enlarged spleen should be investigated.
- Poor quality blood, meaning red blood cells have a shorter lifespan and function less effectively.
- Bleeding (e.g., from the gut)
- Increased red cell destruction from the use of medication (e.g., ribavirin)
- Increased red cell destruction from infection

Hemoglobin levels should ideally be measured before and after every transfusion in order to assess the effectiveness of the treatment regimen. If this is not possible, hemoglobin levels should be measured as often as possible – once a week, once every 15 days, or whenever the patient receives a transfusion.

Adverse Reactions:

Blood transfusion exposes the patient to a variety of risks. Thus, it is vital to continue to improve blood safety and to find ways of reducing transfusion requirements and the number of donor exposures. All the activities of transfusion chain are to be incorporated in National Hemovigilance system. (Refer to National Hemovigilance reporting Guideline in Nepal).

Following Adverse Reactions are noted;

Immune-mediated adverse transfusion reaction

Table 3: Broad categorization of immune-mediated transfusion related reactions

Acute	Frequency	Delayed	Frequency
Haemolytic (Intravascular)	1/25,000	Alloimmune	1/100
Anaphylactic	1/50,000	Hemolytic (Extravascular)	1/ 2,500
Febrile non-hemolytic	1/100	Graft Vs Host Disease	Rare
Allergic (Urticarial)	1/100		
Transfusion Related Acute Lung Injury	1/10,000		

Iron Overload and Iron Chelation

Iron overload occurs when iron intake is increased over a sustained period of time, either as a result of red blood cell transfusions or increased absorption of iron through the gastrointestinal tract (GIT). Both of these occur in thalassemia, with blood transfusion therapy being the major cause of iron overload in thalassemia major and increased GIT iron absorption being more important in NTDT.

Untreated transfusional iron overload in thalassemia major is fatal in the second decade of life, usually as a result of cardiac complications. Iron overload also causes pituitary damage, leading to hypogonadism and poor growth. Endocrine complications, namely diabetes, hypothyroidism and hypoparathyroidism are also seen. Liver disease with fibrosis and eventually cirrhosis, particularly if concomitant chronic hepatitis is present, is also a serious complication.

In the absence of any mechanism of the human body to excrete excess iron, chelation therapy is essential and constitutes the second important arm besides transfusion therapy in the clinical management of these patients.

Monitoring of Iron overload:

Monitoring closely and assessing as accurately the possible iron overload is essential in establishing effective iron chelation regimes.

▪ Serum ferritin

This is a relatively easy test to perform, well established, generally correlating with body iron stores, and prognostically relevant in thalassemia major. Observations with larger patient numbers have shown that maintenance of lower serum ferritin of 1,000 µg/L may be associated with a good outcome.

▪ Liver iron concentration (LIC)

Liver iron concentration is now regarded as the reference standard for estimating body iron loading and has been shown accurately to predict total body iron stores, using the formula:

Total body iron stores in mg/kg = 10.6 × the LIC (in mg/g dry wt)

Normal LIC values are up to 1.8 mg/g dry wt, with levels of up to 7 mg/g dry wt seen in some non-thalassemic populations without apparent adverse effects. Several studies link high LIC above 15–20 mg/g dry wt to worsening prognosis, liver fibrosis progression or liver function abnormalities.

▪ Myocardial iron estimation (T2*)

Estimation of myocardial iron using MRI is becoming increasingly available but requires expertise in its use and standardization. The T2* value in tissues shortens as the iron concentration increases. A shortening of myocardial T2* to <20 ms (implying increased myocardial iron) is associated with an increased chance of decreased left ventricular function. For example, patients with T2* values >20 ms have a very low chance of decreased left ventricular ejection fraction (LVEF). T2* values of 10–20 ms indicate up to a 10% chance of decreased LVEF; 8–10 ms indicate an 18% chance; 6 ms indicate a 38% chance, and T2* values of just 4 ms indicate a 70% chance of decreased LVEF.

Iron Chelation:

When to start iron chelation

As a general rule, patients should begin iron chelation

1. After the first 10-20 transfusions
2. Serum ferritin >1000mcg/L

▪ Desferrioxamine

- If before 3 years of age, monitoring of growth and bone development is recommended.
- Standard treatment: Slow subcutaneous infusion over 8–12 hours through infusion pump.

Standard dose:

- Children 20–40 mg/kg (not exceeding 40 mg/kg, until growth has ceased), and Adults 50–60 mg/kg. Infuse 8–12 hour intervals 5-7 days a week.
- Alternative route: subcutaneous bolus – two S.C. boluses/day to a total daily dose of 45 mg/kg.
- Vitamin C-dose limited to 2–3 mg/kg/day given orally at the time of infusion;
- Pregnancy – desferrioxamine can be used in pregnancy. It should be interrupted during the first trimester and can be used in the second and third trimesters in selected cases.
- Intensive chelation with desferrioxamine- continuous 24-hourly infusions , intravenous or subcutaneous.

Indications:

- Persistently high serum ferritin;
- LIC > 15 mg/g dry weight;
- Significant heart disease, and;
- Prior to pregnancy or bone marrow transplantation

▪ Deferasirox

Recommended dose:

Starting dose 20 mg/kg/day. After 10–20 transfusions (iron intake 0.3–0.5 mg/kg/ day)

If pre-existing iron overload (or iron intake > 0.5 mg/kg/day), the dose of 30 mg/kg/ day is recommended. For patients with a low rate of iron loading (<0.3 mg/kg/day), lower doses may be sufficient to control iron loading; some patients will still fail to achieve negative iron balance at a daily dose of 40 mg/kg/day of deferasirox, and studies are currently underway to assess the effectiveness and safety of higher doses.

- Administration: Tablet dissolved in water (or apple juice), using a non-metallic container and stirrer. Taken once a day before a meal.
- Continuous Monitoring
- Use in children > 2 (FDA) and >6 (EMEA) years of age
- Contraindicated in renal failure or significant renal dysfunction;
- It cannot be given during pregnancy

▪ Deferiprone

- Standard dose: 75-100 mg/kg/day in 3 divided
- Children above 10 years of age
- Vitamin C concomitant treatment not recommended
- Weekly blood counts (more frequently if signs of infection)
- Pregnancy – stop treatment. It is recommended that sexually active patients should use contraception.

▪ Combination Therapy

In patients for whom monotherapy with desferrioxamine or deferiprone is not controlling body levels of iron or myocardial iron or in the presence of significant heart disease, combined regimes offer an alternative that can reduce iron levels in both the liver and heart.

CAUTION: Agranulocytosis may be more frequent in combination therapy, especially in simultaneous use.

C. Medical problems and its treatment

Endocrine Complications in TDT

Endocrine abnormalities are among the common complications of thalassemia. Despite the early establishment of appropriate chelation therapy, problems such as delayed sexual maturation and impaired fertility may persist. Determining the prevalence of endocrine complications is difficult because of differences in the age of first exposure to chelation therapy and the continuing improvement in survival in well- chelated patients.

Growth:

Growth retardation is common in thalassemia. Patterns of growth are relatively normal until the age of 9–10 years, when growth velocity begins to slow. Key contributing factors to stunted growth in patients with thalassemia may include chronic anemia, transfusional iron overload, hypersplenism and chelation toxicity. Other contributing factors include hypothyroidism, hypogonadism, growth hormone deficiency/ insufficiency, zinc deficiency, chronic liver disease, under-nutrition and psychosocial stress.

Diagnosis and investigations

Diagnosis requires careful clinical evaluation to establish:

- Slow growth rates: growth velocity expressed in cm/year, below 1SD for age and sex (based on growth velocity charts)
- Short stature: height below the 3rd centile for sex and age (based on national growth charts)
- Signs of other pituitary hormone deficiencies (e.g., gonadotrophins)
- Other possible causes of retarded growth.

Evaluation of short stature/retarded growth

The first step in the investigation of short stature or retarded growth is the regular (six- monthly intervals) and accurate measurement of standing and sitting height, pubertal staging (Tanner 1962), and bone age, including examination of metaphyses. Interpretation of absolute height must take into account the height of the parents.

Additional endocrine studies that may be helpful include thyroid function tests (FT4, TSH), assessment of levels of sex hormones, growth hormone (GH) secretion, zinc, calcium, alkaline phosphatase, urine analysis, and investigation of glucose tolerance.

Possibly useful tests include Insulin Growth Factor-I (IGF 1) and Insulin Growth Factor Binding Protein-3 (IGFBP-3). The secretion of GH is normal in the majority of patients with thalassemia. However, an investigation of transglutaminase antibodies is also essential to exclude celiac disease.

It is important to bear in mind that desferrioxamine toxicity is an important cause of delayed growth.

Treatment

Anemia, folate deficiency, and hypersplenism are traditional causes of poor growth in patients with thalassemia receiving an irregular transfusion, as well as in those regularly using desferrioxamine. In addition, in peri-pubertal patients, hypogonadism should be carefully investigated before starting growth hormone treatment which may result in decreased insulin sensitivity and abnormal glucose tolerance.

Delayed puberty and hypogonadism:

Delayed puberty and hypogonadism are the most obvious clinical consequences of iron overload. Delayed puberty is defined as the complete lack of pubertal development in girls by the age of 13 and boys by the age of 14. Hypogonadism is defined in boys as the absence of testicular enlargement (less than 4 ml) and in girls as the absence of breast development by the age of 16.

Arrested puberty is a relatively common complication in moderately or grossly iron overloaded patients with thalassemia and is characterized by a lack of pubertal progression over a year or more. In such cases, the testicular size remains 6–8 ml, and breast size at B3. In such cases, annual growth velocity is either markedly reduced or completely absent.

Most women with thalassemia major present primary amenorrhoea, with secondary amenorrhoea developing over time, particularly in poorly chelated patients. Ovarian function in such cases is

generally normal, but gonadotrophin response to Gonadotrophin-Releasing-Hormone (Gn-RH) is low compared to patients with normal menstrual cycles.

Investigations

- Routine biochemical analysis
- Bone age (X-ray of wrist and hand)
- Thyroid function (TSH and FT4)
- Hypothalamic-pituitary-gonadal function: Gonadotrophin-Releasing-Hormone (Gn RH), stimulation test for Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH)
- Sex steroids (serum testosterone, serum 17-β, Estradiol)
- Pelvic ultrasound to assess ovarian and uterine size
- Transglutaminase antibodies
- Growth Hormone (GH) stimulation test, in selected cases
- Insulin Growth Factor-I (IGF-I), IGF Binding Protein-3 (IGFBP-3), plasma zinc, in selected cases.

Treatment

The treatment of delayed or arrested puberty and hypogonadotropic hypogonadism depends on factors such as age, the severity of iron overload, damage to the hypothalamic-pituitary-gonadal axis, chronic liver disease, and the presence of psychological problems resulting from hypogonadism. Therefore, a collaboration between endocrinologists and other doctors is critical.

For girls, therapy may begin with the oral administration of Ethinyl estradiol (2.5–5 µg daily) for six months, followed by hormonal reassessment. Suppose spontaneous puberty does not occur within six months after the end of treatment. In that case, oral estrogen is re-introduced in gradually increasing dosages (Ethinyl estradiol from 5–10 µg daily) for another 12 months. If breakthrough uterine bleeding does not occur, low estrogen-progesterone hormone replacement is the recommended treatment.

For delayed puberty in males, low dosages of intramuscular depot-testosterone esters (25mg) are given monthly for six months, followed by hormonal re-assessment. In patients with hypogonadotropic hypogonadism, treatment at a dose of 50 mg per month can be continued until growth rates wane. The fully virilising dose is 75–100 mg of depot-testosterone esters every 10 days, administered intramuscularly. The same effects can be achieved with topical testosterone gel.

For pubertal arrest, the treatment consists of testosterone esters or topical testosterone gel, administered for the treatment of delayed puberty and hypogonadotropic hypogonadism.

It is important that the treatment of pubertal disorders is treated on a patient-by- patient basis, taking into account the complexity of the issues involved and the many associated complications.

▪ Hypothyroidism:

This may occur in severely anemic and/or iron overloaded patients, usually appearing in the second decade of life. The condition is uncommon in optimally treated patients (de Sanctis, 1995; Sabato, 1983).

Signs and symptoms

Pre-clinical hypothyroidism is asymptomatic. In mild and overt hypothyroidism, symptoms such as growth retardation, decreased activity, above normal weight, constipation, reduced school performance, cardiac failure and pericardial effusion may be encountered. The incidence of hypothyroidism is slightly higher in females. Typically, the thyroid gland is not palpable, thyroid antibodies are negative and thyroid ultrasonography shows an irregular echo pattern with thickening of the thyroid capsule. Annual investigation of thyroid function is recommended, beginning at the age of 12 years. Free T4 and TSH are the key investigations. Bone age may help evaluate hypothyroidism. The majority of patients have primary thyroid dysfunction. Secondary hypothyroidism caused by iron-mediated damage to the pituitary gland occurs very rarely.

Treatment

Abnormal thyroid function may be reversible early through intensive chelation and good compliance.

Treatment depends upon the severity of organ failure. Sub-clinical hypothyroidism requires regular medical follow-up and intensive iron chelation therapy. In patients with mild or overt hypothyroidism, L-thyroxine is given.

▪ Impaired carbohydrate metabolism:

Impaired glucose tolerance and diabetes mellitus may be the consequence of β,-cell destruction secondary to iron overload, chronic liver disease, viral infection and/or genetic factors.

The pathogenesis resembles type-2 diabetes, with differences in the age of onset (it may start early in the second decade of life) and slow progression of disturbances in glucose metabolism and insulin secretion.

Diabetes in thalassemia is rarely complicated by ketoacidosis.

The type of glycaemia may be classified as diabetic, borderline or normal.

- Diabetic type: Fasting Plasma Glucose (FPG) ≥7.0 mmol/l (126 mg/dl) and/or plasma glucose 2 hours after 75 g glucose load (2hPG) is ≥11.1mmol/l (200 mg/dl). A casual Plasma Glucose (PG) ≥11.1 mmol/l (200 mg/dl) also indicates diabetic type. The persistence of "diabetic type" indicates that a subject has diabetes.
- Normal type: FPG <6.1 mmol/l (110 mgm/dl) and 2hPG <7.8 mmol/l (140 mg/dl)
- Borderline type: includes those who are neither diabetic nor normal types, according to cut-off values for venous PG measurements.

Investigations

The Oral Glucose Tolerance Test (OGTT) should be performed annually from the age of puberty. For children, a dose of 1.75 g/kg (to a maximum of 75 g) is used for OGTT. Treatment

- Impaired glucose tolerance may be improved by a strict diabetic diet, weight reduction, where applicable, and possibly intensive iron chelation therapy.
- In symptomatic patients, insulin treatment is normally required but metabolic control may be difficult to achieve.
- Where hyperinsulinism is insufficiently managed by diet alone, acarbose may be a useful first-line therapy for glycaemic control.
- The role of oral hypoglycaemic agents remains to be fully determined.

Monitoring diabetes and its complications

- Blood glucose (daily or on alternate days)
- Ketones – check if blood sugar is above 250 mg/dl
- Fructosamine estimation is more helpful than glycosylated haemoglobin level
- Urinary glucose is influenced by increased renal glucose threshold
- Renal function (serum creatinine)
- Serum lipids (cholesterol, HDL, LDL, triglycerides)
- Urinary protein
- Evaluation of retinopathy
- Hypoparathyroidism:

Hypocalcemia, due to hypoparathyroidism is a recognized late complication of iron overload and/or anemia and usually begins after the age of 16. Most patients show a mild form of the disease accompanied by paraesthesia. More severe cases may demonstrate tetany, seizures, or cardiac failure.

Investigations should begin from the age of 16 and should include serum calcium, serum phosphate, and phosphate balance. The parathyroid hormone (PTH) should also be evaluated in cases with low serum calcium and high phosphate levels. Parathhyroid hormone may be normal or low with low readings for 1,25dihydroxycholecalciferol (vitamin D). Bone radiology shows osteoporosis and malformations.

Treatment

- Oral administration of vitamin D or one of its analogs.
- Some patients require high doses of vitamin D to normalize their serum calcium levels.
- This should be carefully monitored, as hypercalcemia is a common complication of this treatment.
- Calcitriol, 0.25–1.0 µg, twice daily is usually sufficient to normalize plasma calcium and phosphate levels.
- Weekly blood tests are required at the start of treatment, followed by quarterly plasma and daily urinary calcium and phosphate measurements.
- In patients with persistently high serum phosphate levels, a phosphate binder (other than aluminum) may be considered.
- Tetany and cardiac failure due to severe hypocalcemia require intravenous administration of calcium under careful cardiac monitoring, followed by oral vitamin D.

Osteoporosis

Osteoporosis is a skeletal disease characterized by low bone mass and microarchitectural deterioration, resulting in bone fragility and susceptibility to fracture. With increased life expectancy, osteopenia-osteoporosis syndrome (OOS) is a major cause of bone pain in the hip and spine and fragility fractures, especially of the lumbar spine, which may be found in 70–80% of adult patients with β-thalassemia worldwide, accounting for significant bone morbidity.

Diagnosis and investigations

The commonest presentation is bone pain and backache with or without a past history of fractures. Patients may also be asymptomatic in 20% of cases.

i. DEXA Scan

The diagnosis is best confirmed by bone mineral density (DEXA) according to WHO criteria. Although bone mineral density remains the best available non- invasive assessment of bone strength in routine clinical practice, many other skeletal characteristics also contribute to bone strength. These include bone macro-architecture (shape and geometry), bone micro-architecture (both trabecular and cortical), matrix and mineral composition, as well as the degree of mineralization, micro damage accumulation, and the rate of bone turnover, which can affect the structural and material properties of bone which are complicated and difficult to assess in routine clinical practice.

ii. Biochemical

All patients must have endocrine and bone profiles including 25 (OH) vitamin D3, PTH, calcium, phosphate, liver function tests (alkaline phosphate, alanine aminotransferase, bilirubin, albumin), follicle stimulating hormone , luteinizing hormone, testosterone and oestradiol assays.

iii. Radiology

AP and lateral X-ray of the spine is important to rule out fractures even in asymptomatic patients who may have micro fractures.

iv. MRI

If available, spine MRI must be undertaken to determine extramedullary hematopoiesis, especially in TI patients, and check for degenerative changes, skeletal dysplasia and disc prolapse.

v. Assessment of iron load and chelation therapy

Management

Principles of management of OOS are the same as other patients with osteoporosis due to other conditions. The aim is to improve BMD score and prevent/reduce future risk or fracture with/without offering pain relief in thalassemia patients. General guidelines include assessment of other drugs, lifestyle issues, exercise, and diet.

Splenectomy in β-thalassemia

Many patients with thalassemia major require splenectomy. However, optimal clinical management from the time of diagnosis may delay or even prevent hypersplenism, thereby increasing the efficiency of transfusion therapy and reducing the need for splenectomy. Throughout the care of the patient with thalassemia, the size of the spleen should be carefully monitored on physical examination and, as needed by ultrasonography.

Splenectomy should be considered when:

- Annual blood requirements exceed 1.5 times those of splenectomized patients, provided that they are on the same transfusion scheme and have no other reasons for increased consumption. Such reasons include new alloantibodies, infection, and changes in the hematocrit of the transfused units. For patients maintaining a pre-transfusion Hb level of about 10 gm/dl, this increase in transfusion requirements represents consumption of more than 200–220 ml of red cells (assuming hematocrit of transfused cells is 75%)/kg/year. The rate of iron overload should also be taken into consideration. Splenectomy may be unnecessary for patients who maintain effective chelation therapy despite increased blood requirements. For patients with increasing iron stores despite good chelation therapy, reducing transfusional iron loading by splenectomy may be an important component of the overall management of iron overload.

- Splenic enlargement is accompanied by symptoms such as left upper quadrant pain or early satiety. Massive splenomegaly causes concern about possible splenic rupture.
- Leucopenia or thrombocytopenia due to hypersplenism causes clinical problems (e.g., recurrent bacterial infection or bleeding).

Splenomegaly due to periods of under-transfusion with the blood of inappropriately low hemoglobin may be reversible. Before considering splenectomy in this situation, the patient should be placed on an adequate transfusion program for several months and then re-evaluated.

It is generally advisable to delay splenectomy until patients are at least five years old because of the increased risk of overwhelming sepsis below this age.

D. Non-Transfusion Dependent Thalassemia

NTDT is a group of thalassemia which includes patients who usually do not require regular blood transfusions and demonstrate milder clinical symptoms than TDT patients. Such individuals manage to maintain hemoglobin levels between 6-9 gm/dl.

Considerable research into the condition has demonstrated that NTDT covers a wide range of clinical symptoms, some of which can be severe. In the most serious cases, patients may present clinical and laboratory evidence of the disease between the ages of 2-6. Although

growth and physical development are slower than normal, these patients may maintain a good quality of life without the regular blood transfusions required by TDT patients. In less serious cases, patients may not demonstrate symptoms until they are adults, suffering only mild anemia (8-10gm/dl) and rarely requiring blood transfusions if at all.

The spleen may become enlarged (splenomegaly) as in TDT because of the rapid breakdown and accumulation of red blood cells in the organ, and this may sometimes be the cause of more severe anemia in patients with NTDT. In such cases, patients may need to be transfused more regularly. Removing the spleen may also correct the complication. However, this is a very serious decision that should be taken with expert medical advice, taking into account the possible effect on other aspects of the patient's health besides relieving the anemia, such as the possibility of infection.

As described earlier, the main cause of symptoms in TDT is the excess amount of free α chains that accumulate inside the red blood cells, creating an imbalance between the α-beta chains and their usual partners, the alpha chains. The alpha chains alone interfere with almost every stage of red blood cells cycle of maturation, causing severe anemia and other conditions discussed earlier.

Given the above, it is reasonable to expect that the symptoms manifested by thalassemia patients will be less severe where conditions exist in which the number of excess alpha chains is reduced. Investigations at the molecular level have shown that a number of such conditions exist, including

(i) The presence of the β+ gene, which can produce some BETA chains although less than normal that in turn couple with alpha chains, thus reducing the number of free alpha chains. Mutations to the β+ gene that are associated with a very mild clinical outcome are sometimes designated β++.
(ii) A defect in the gene responsible for the synthesis of alpha chains, reducing the number of alpha chains produced and so improving the balance between alpha and beta chains.
(iii) A greater level of activity by the gamma genes responsible for producing gamma chains can bind ·alpha chains to produce fetal hemoglobin, thus reducing free, harmful alpha chains.

As the above points indicate, medical staff can greatly enhance their knowledge of a patient's condition by establishing the exact type of damage to that patient's DNA. It is then easier to set out the most appropriate treatment program for an individual patient, such molecular methods of investigation are proving invaluable aids to the treatment of thalassemia where available.

Diagnosis

In diagnosing NTDT, it is important to establish certain clinical and laboratory information to differentiate NTDT from thalassemia major. They generally present above 2 years with Hb around 8gm/dl with clinical features of the paleness of body and moderate fatigue, lethargy, poor appetite and hepato-splenomegaly.

Management of Non-Transfusion dependent thalassemia

In NTDT, hemoglobin level should not be an indicator for initiation of transfusion therapy, except in patients with Hb <5gm/dl. However, the following points may indicate occasional blood transfusions:

1. Delayed growth
2. Pathological bone fractures
3. Cardiac complications
4. Facial deformities
5. Decreased normal physical activity
6. Failure of secondary sexual development
7. Hypersplenism
8. Pregnancy

9. Infections

As in the case of transfusion-dependent thalassemia, it is important that patients are closely monitored through regular medical and laboratory check-ups aimed at promptly identifying the appearance of any complications. In addition, because patients with NTDT begin blood transfusions later in life than patients with TDT, it is important to pay particular attention to the possible development of reactions (alloimmunization) described earlier, as such reactions usually occur when transfusions begin at a later age. It is therefore essential that the patient and donor's blood are carefully typed and matched before every transfusion. It is also important to note that pregnant women with NTDT may require blood transfusions.

Iron chelation

As in TDT, iron overload in patients with NTDT may be due to:

i. Ineffective production of red cells.

ii. The breakdown of red cells.

iii. Increased quantities of iron absorbed by the gut.

There has been comparatively little research into iron accumulation in patients with NTDT. However, one study demonstrated that 2-5g of iron accumulate in the body of patients with NTDT each year – that is, 0.1mg/kg/day. This is a 20-70% higher rate of absorbing iron from the diet than normal. As they get older in most cases after a decade patients with NTDT, therefore, have almost the same risk of iron-associated complications as patients with thalassemia major receiving regular blood transfusions.

Difficulty in deciding when to start iron chelation in patients with NTDT is determining the patient's actual body iron overload, as serum ferritin levels may not provide an accurate measure as is the case with TDT. For this reason, it is advisable to assess iron concentration by means of a liver biopsy or MRI.

Once a decision has been taken to begin iron chelation therapy, it is recommended that Desferrioxamine be used, as in the case of TDT. However, patients with NTDT may require a subcutaneous infusion no more than 2 or 3 days a week. The same follow-up treatment recommended for patients with thalassemia major undergoing iron chelation should also be made available to patients with NTDT.

Patients with NTDT absorb significantly more iron from the gut than normal, so they should avoid foods rich in iron (e.g., spinach, liver, and some kinds of beans) and iron supplements. In addition, drinking black tea with meals may help reduce the amount of iron absorbed by the gut.

E. Medical problems in NTDT

i. **Bone changes.**

Hyperactive bone marrow– A result of the body's effort to produce more red blood cells to counteract anemia causes the bones to become distorted, fragile, and thinner, interrupting their growth and leaving patients vulnerable to fractures. However, severe bone problems can be overcome through regular blood transfusion therapy.

Osteoporosis- Patients are encouraged to exercise and increase the calcium in their diet to avoid severe bone disease (osteoporosis). Calcium and vitamin D capsules may provide additional benefits. Smoking should also be avoided. Some doctors have demonstrated beneficial results with bisphosphonates, administered orally or intravenously.

ii. **Hyperactivity or expansion of the bone marrow and folic acid:** Because the bone marrow of patients with NTDT works extra hard to combat the body's anemia by making more red blood cells, patients need extra amounts of specific vitamins, particularly folic acid. Insufficient folic acid can aggravate anemia in NTDT patients. Folic acid is found naturally in food such as meat and green vegetables. However, an additional amount usually a daily tablet should cover patient's extra needs.

iii. **Gall stones-** NTDT patients develop gallstones (Cholelithiasis) more frequently than normal. Gallstones are made from the by-products (bile pigments) released when red blood cells are broken down, accumulating in an organ next to the liver called the gall bladder, where they may cause an obstruction, prompting pain in the abdomen. The presence of gallstones can be confirmed by ultrasound examination. If pain in the abdomen persists, the gall bladder may be removed.

iv. **Leg ulcers-** Patients with NTDT frequently develop ulcers around the ankle, particularly older patients, due to poor circulation and oxygenation in some parts of the body. These ulcers tend to be persistent and very difficult to treat. However, regular blood transfusions raise hemoglobin levels and so improve the supply of oxygen to the tissues, as well as simple measures such as keeping the legs and feet raised above the level of the heart for 1-2 hours a day, sleeping with the end of the bed slightly raised and protecting the ankles by wearing socks, may offer some comfort. Drugs such as zinc sulphate tablets are also sometimes helpful, as well as hydroxyurea either alone or in combination with other agents that can increase foetal hemoglobin, such as erythropoietin and butyrates.

v. **Kidney complications-** Other medical problems reported among patients with NTDT include kidney damage, which may be the result of excess uric acid in the blood. Uric acid is the most important waste product formed due to over- active bone marrow. The drug Allopurinol may help reduce the amount of uric acid produced.

vi. **Thrombophilia-** Another complication is an increased risk of thrombosis, where thrombocytes or platelets accumulate in the blood vessels to form clots (aggregates) that prevent normal blood flow and so reduce the oxygenation of cells and tissues. Regularly counting the number of platelets allows the doctor to establish whether to prescribe anti-aggregates if these are raised or anti-coagulants if surgery is planned or if thrombosis occurs.

vii. **Extra-medullary erythropoiesis–** Unlike TDT patients, who receive regular blood transfusions from an early age which suppress the excessive activity of the bone marrow, patients with NTDT do not receive such regular blood transfusions and so continue to produce high levels of red blood cells, including in areas outside the bone marrow mainly in the chest area and near the spine. X-rays can reveal blood- forming tissue developing in masses in these areas. The production of red blood cells near the spine can cause neurological complications when extra pressure builds up around the spinal cord. Such activity can usually be identified through x-rays or with more sensitive methods such as MRI. Again, such conditions can usually be managed through blood transfusion therapy, which will suppress the extra formation of blood and, as a consequence, reduce the masses formed. Where serious neurological conditions occur, more active therapeutic measures may be needed, such as radiotherapy.

viii. **Heart and liver complications-** Chronic anemia may also cause heart problems, while both the heart and liver may be damaged by iron overload. Both conditions can be managed, as in the case of TDT.

F. Therapeutic regimes – established and future approaches

▪ Bone marrow transplantation (BMT):

Bone Marrow transplantation is at present the only proven treatment modality that can establish long-term normal hemopoiesis avoiding the need for transfusions and chelation treatment, and there is now long-term outcome data supporting its efficacy. Hence, the provision of related transplantation for patients and families with acceptable risks and benefits and who seek permanent cure has an adequate standard of care. However, the main constraint in offering this has been the availability of donors, and the risks are undertaken when considering alternative donors. Therefore, bone marrow transplantation in thalassemia should be considered for patients early or before complications due to iron overload have developed.

Three patient classes have been identified on the basis of the following risk factors, which have been found to have a significant influence on post-transplant outcome:

- inadequate iron chelation therapy,
- presence of liver fibrosis and
- hepatomegaly

Patients in Class I have none of the above characteristics, patients in Class II have one or two, and patients in Class III exhibit all three characteristics.

Other Approaches to Treatment

▪ Modulation of Foetal hemoglobin

Foetal hemoglobin is the predominant non-α globin produced in humans until around six months of age when it is typically suppressed, and the production of β-globin is increased. This pattern is the norm even when the genes are mutated, as in β-thalassemia.

Patients with β-thalassemia who continue to produce high levels of foetal globins, such as those with Hereditary Persistence of Foetal Hemoglobin, have less globin imbalance and less severe anemia. The therapeutic stimulation of foetal globin could benefit many patients, even rendering some transfusions independent. Several candidate therapies now offer the potential to correct or modulate the underlying pathology.

▪ Cytotoxic agents

Following observations that foetal hemoglobin synthesis is reactivated during recovery from bone marrow suppression after the use of cytotoxic drugs, attention has focused on the possible role of cytotoxic agents as therapies in treating severe hemoglobin disorders. In addition, several cytotoxic agents alter the pattern of erythropoiesis, favoring the expression of foetal (γ)-globin genes and so increasing the number of red cells containing HbF (F-cells) have been explored over the past 20–25 years.

▪ Demethylating agents

5-azacytidine and decitabine have been administered to a few β-thalassemia patients with good responses, raising total hemoglobin levels by a mean of 2.5 gm/dl above baseline and prolonging the lives of end-stage patients. The mutagenic potential and instability of formulations of 5-azacytidine have limited its investigation, but higher oral doses of decitabine have been effective in baboons, and studies are planned in selected patients.

Hydroxyurea

It has been studied in HbE/β-thalassemia patients, with lower responses but reduced hemolysis. Hydroxyurea has been less beneficial in NTDT than in sickle cell disease, in which the number of painful crises was reduced, and overall health indicators improved. The lesser benefits in thalassemia are perhaps because the cytostatic effects of hydroxyurea are limited in the disease.

Gene Therapy

The idea of using gene therapy to treat hemoglobinopathies (thalassemia and sickle cell disease) is in principle straightforward. Red blood cells are continuously replenished by bone marrow hematopoietic stem cells (HSC). Therefore, the stable transfer of a normal functioning copy of a β-globin therapy gene unit into the patient's own HSC would result in the generation of normal rather than diseased RBC for life.

A number of major discoveries and technical advances in gene therapy over the last 20 years, particularly since 2000, mean that, at long last, gene therapy for hemoglobinopathies looks like a serious possibility in the not too distant future.

G. Psychosocial problem

The common psychological problems encountered are:

1. Treatment burden
2. Influence of the disease on daily life, i.e., depression
3. A feeling of difference from peers
4. A feeling of dependence on other
5. A feeling of disfigurement and vulnerability to injury
6. Death anxiety

In terms of the psychological care of the patient, healthcare professionals should aim to:

- Provide information that promotes understanding of the illness.
- Help patient and parents to talk and to express feelings about the illness
- Help the patient to accept the illness and to take care of him/herself
- Maintain realistic hopes
- Facilitate a 'normal' lifestyle and encourage self-esteem
- Support the full development of an adult life.

Putting these goals into practice requires health professionals to be:

- Open-minded about psychological aspects of having and treating inherited disease
- Trained in normal psychosocial development from childhood to adulthood
- Sensitized to the special issues of this chronic hereditary disease
- Available to accompany and support the patient throughout his/her life path.

H. Diet in Thalassemia

Reducing the iron absorbed from food

In thalassemia, although most of the iron overload is due to blood transfusion, increased iron absorption from the diet is also important. This is because only a small amount of iron from the diet is absorbed into our bodies. The amount absorbed is higher when hemoglobin in the blood is low. People with low hemoglobin, such as those with NTDT or those with thalassemia major not regularly transfused could therefore adapt their diet so that not only the total amount of iron in their diet is low, but also the amount of iron in their body is low. There are two kinds of iron in the diet: iron which is present in red meat (meat iron), and iron which is widely distributed in the diet (non- meat iron).

Meat iron is present in red meat such as beef, lamb, and pork, and in seafood. The liver is a very rich source of meat iron. Choose the white part of the chicken rather than red meat as this contains less iron.

On average, after a meal with red meat, about 35% of iron will be absorbed into our bodies. However, this may vary between 10-40%, depending mainly on whether the meal contains milk or milk products. The calcium present in milk, cheese, yogurt, and cream decreases the absorption of meat iron. Milk intake should be at least one pint daily, particularly because it helps prevent osteoporosis.

Non-meat iron

Non-meat iron is widely distributed in the diet in eggs, chocolate, cereals, vegetables, fruits, roots (potatoes, parsnips), beans, and lentils. The absorption of non-meat iron from the diet into our body is much less than that of meat iron, but it may vary more than 20-fold, depending on the meal's composition. The foods that decrease their absorption are (i) cereals and (ii) dairy products. The foods which increase its absorption are (i) fruit and vegetables rich in vitamin C, (ii) meat, fish, shellfish, and poultry, and (iii) pickles, and soy sauce vinegar and alcohol.

Avoiding non-meat iron is very difficult because it is present in most foods. However, diet can be modified by taking more food than decreasing the foods, which increases the amount of iron absorbed into our body.

Food that decreases non-meat iron absorption

1. Cereals, Wheat bran, maize, oats, rice, and soy decrease the iron absorbed into our body and fight the effect of Vitamin C. Foods rich in vitamin C increase iron absorption. Eating a lot of cereals in your diet is good, but remember not to take vitamin C-rich food with them.

   Soy protein also decreases the amount of iron absorbed into your body.
2. Tea, Coffee, and Spices- Tea, coffee, and some spices (e.g., oregano) decrease iron absorption. Drink plenty of tea and coffee daily, particularly with your meals.
3. Dairy products- Milk, cheese, and yogurt decrease the iron absorbed into your body. Calcium is also important for osteoporosis, so it is good to include as many dairy products as you can in your diet. Lower fat varieties of milk (skimmed or semi-skimmed) and cheese are just as high in calcium and may be preferred if you are watching your weight.

Foods that increase non-meat iron absorption

1. **Vitamin C-** Vitamin C is present in fruit, juice, and vegetables. Beer increases iron absorption, so it is better to avoid drinking it with your meal too often, but you could always have it on its own with some nuts. Fruit and fruit juice are good sources of antioxidants and should be taken on their own as snacks. Boiled vegetables contain much less vitamin C because the vitamin leaks in the water.
2. **Meat, poultry, fish, and seafood-** Meat, poultry, fish, and seafood not only contain a lot of meat iron but also help absorb more of the non-meat iron from your food. It would be unwise to omit them from the diet altogether as they contain other vital nutrients essential for children and adolescents.
3. **Pickles,** soy vinegar, alcohol sauce, turnips, carrots, and fermented soy products (e.g., miso and soy sauce) enhance iron absorption. The iron absorbed is even higher when the pickled vegetables are added to bread and rye-containing meals.

Antioxidants in Food

Antioxidants are important in any diet because, as their name suggests, they prevent oxidative damage in the body. In doing so, they play an important role in preventing diseases such as coronary heart disease and cancer. In Thalassemia, there is a higher risk of oxidative damage because of the excess iron in the body.

1. **Vitamin E–** Vitamin E is the most important dietary antioxidant. Several studies have found that many patients with Thalassemia have lower levels of Vitamin E in their blood compared to non-thalassemics. Vitamin E is fat-soluble, which means it is present in foods with high fat. The best sources of Vitamin E are vegetable oils (olive, sunflower, palm, and soy oil). Other sources of Vitamin E are dairy products, cereals, nuts, eggs, and meat.
2. **Vitamin C–** Vitamin C increases the absorption of non-meat iron. Therefore, although Vitamin C is a very powerful antioxidant, the use of many foods containing Vitamin C in combination with foods that are high in non-meat iron should be limited. This is particularly important for those with Thalassemia intermedia who are not regularly transfused. Remember that non-meat iron is widely distributed in the diet in eggs, chocolate, cereals, vegetables, fruits, roots (potatoes, parsnips), beans, and lentils. Vitamin C is mainly found in fruit, fruit juices, and vegetables
3. **Carotenoids–** Common dietary sources of carotenoids are carrots, yellow squash, corn, tomatoes, papaya, oranges, and dark-green leafy vegetables. Again, most of these foods are high in Vitamin C; therefore, the same caution applies. It is worth pointing out that the absorption of carotenoids from the diet is much higher when the food contains fat or oil. Carotenoids can be destroyed at high temperatures, so keep the cooking temperature low and the time short if you can.
4. **Flavonoids** are found in tea, red wine, fruit, and vegetables.

CHAPTER

V

LABORATORY DIAGNOSIS OF HEMOGLOBINOPATHIES

A. Different Available Test

1. Baseline investigation in all cases:

A baseline blood investigation should be requested before a specific investigation of thalassemia. These investigations include:

- Complete blood count with red cell indices
- Reticulocyte count
- Peripheral smear examination

2. Antenatal Women or pre-pregnancy planning:

- For all women in first trimester
- If women is a carrier/ disease for hemoglobinopathy;
- Then testing of spouse is mandatory, preferably by High Performance liquid chromatography (HPLC)/Capillary Zone Electrophoresis (CE), if not available, r efer to higher center.
- If spouse, tests positive, counselling and pre-natal testing should be done.

3. Newborn Population screening:

- Validated Point of Care (POC) tests to initiate penicillin prophylaxis in baby for sickle cell disease.
- HPLC /electrophoresis, if available or at later date.

4. In endemic pockets/ high risk population:

- Validated POC tests for sickle cell
- OR HPLC/CE, if available.

5. Cascade screening in a disease/ carrier detected family:

- Screening of siblings and extended family members of patients and carriers of - thalassemia and variant hemoglobins.
- Enlisted in Essential In-Vitro Diagnostic test list.

6. Prenatal diagnosis:

- There are three methods available for fetal sampling.
- Chorionic villus sampling (CVS): Performed in the first trimester of pregnancy between 10-12 weeks of gestation.
- Amniocentesis: Collected after 15-17 weeks of gestation.
- Fetal blood sampling: Done in mid-trimester pregnancy at 18-20 weeks of gestation.
- In prenatal diagnosis, DNA based tests are essential for confirmation of mutation, as no tests are absolute (reported errors of 2%).
- These methods are beneficial in:
- Couples, where both partners are carriers.
- If the partner is not available for testing, where the women is a known carrier.

7. **Prenatal diagnosis follow-up:**
   A fetal DNA diagnosis should be confirmed at birth through a request for a cord blood sample.
8. **Pre-implantation Genetic diagnosis:**
   - To define the genotype of a single cell biopsied from cleaving embryos.
   - Useful in hemoglobinopathy carrier-couples.

B. Laboratory features of Thalassemias

α thalassemia:

- Heterozygotes for α thalassemia may have a completely normal blood count and peripheral blood film findings or trivial microcytic hypochromic anemia.
- Homozygotes have haematological abnormalities almost similar to those seen in β thalassemia heterozygotes.
- Definitive diagnosis requires DNA analysis.

β thalassemia:

Heterozygotes for β thalassemia:

- CBC shows normal or mild decrease in hemoglobin concentration, increased red cell count and decreased MCV and MCH , MCHC usually normal.
- Blood film shows almost normal to microcytic hypochromic red cells, may have basophilic stippling and target cells.
- Diagnosis requires detection of an increased hemoglobin A2 percentage.

Homozygous for β thalassemia:

- CBC shows hemoglobin concentration, red cell count, MCV, MCH and MCHC are reduced and RDW is increased.
- Blood film shows anisopoikilocytosis, microcytic hypochromic red cells, may have basophilic stippling, pappenheimer bodies, nucleated red cells and target cells.
- On HPLC/Capillary electrophoresis , only Hemoglobin A2 and Hemoglobin F percentage increased (β0β0) or along with variable amount of Hemoglobin A (β0 β+).

Sickle cell :

Heterozygotes for HbS:

- CBC shows normal hemoglobin concentration with normal MCV and MCH.
- Blood Film shows few plump cells that are pointed at both ends.
- On HPLC/ Capillary electrophoresis, demonstrate Hb S and Hb A, where Hb S comprising of 35-40% of total hemoglobin. The HbS identification must be supported by two independent tests.

Homozygous for HbS:

- CBC shows low hemoglobin concentration with normal MCV and MCH and slightly increased MCHC.
- Blood Film shows sickle cells, target cells and Howell-jolly bodies.
- On HPLC/ Capillary electrophoresis, demonstrate mainly HbS with decreased or absent Hb A , slight increase in HbF.

Compound Heterozygosity:

Various combination of α, γ, δ, β, or other globin chain shows changes in hematological parameters including separation and measurement of Hemoglobin fractions.

Hemoglobin E:

Heterozygotes for HbE:

- CBC shows normal or mild hemoglobin concentration, increased red cell count and decreased MCV and MCH and normal MCHC or occasionally increased.
- Blood film shows normal or microcytic hypochromic red cells with variable number of target cells, irregularly contracted cells, basophilic stippling.
- On Capillary electrophoresis, demonstrate HbE comprising of ≤ 25-30% of total hemoglobin.
- On HPLC, if Hb A2 > 9.0% , have to be confirmed by capillary electrophoresis to rule out Hb E.

Homozygotes for HbE:

- CBC shows normal hemoglobin concentration or with very mild anemia, increased red cell count, decreased MCV and MCH and normal MCHC.
- Blood film shows microcytic hypochromic red cells with variable number of target cells and irregular contracted cells.
- On Capillary electrophoresis, identification of Hb E as the sole variant hemoglobin.

C. Exclusion of Related Conditions:

1. Thalassaemic red cell indices with normal percentage of hemoglobin A2 and F; following conditions to be ruled out.
   - Coinheritance of β and δ thalassemia
   - γ δ β thalassemia
   - Polycythemia vera complicated by iron deficiency.
2. Normal/borderline reduced red cell indices with raised HbA2; following conditions to be ruled out.
   - Interaction of α- with β-thalassemia
   - HIV drug therapy
   - Hyperthyroidism
   - KLF1 gene mutations

D. Key points and recommendations

- Diagnosis of thalassemia should be considered in all those who have hypochromic microcytic anemia .
- An MCH of less than 27pg is an indication to quantify Hemoglobin A2 .
- In the diagnostic work-up for hypochromic microcytosis, iron deficiency anemia should always be excluded .
- In acquired causes of decreased percentage of Hemoglobin A2, iron deficiency and other causes of microcytosis and hypochromasia to be ruled out.
- In Hemoglobin H Disease, characteristic golf ball Hb H inclusion seen in red cells on Hb H preparation.
- Neonates with sickle cell anemia sometimes shows only HbF, so for diagnosis, repeat testing is required when the baby is few months of age.
- α thalassemia are mainly due to deletions of different length and they can be detected with methods such reverse dot blot (RDP), Gap-polymerase chain reaction (PCR), or multiplex ligation-dependent probe amplification (MLPA).

ANNEXES

Thalassemia Initial Assesment Sheet -I

Hospital Name: **Serial number:**

Name: **Age:**

Gender: **DOB:**

Blood Group: **Ethnicity:**

Father's Name: **Mother's Name:**

Permanent Address: **Ward No:**

Municipality/VDC:

Province:

District:

Local Address: **Ward No.:**

Municipality/VDC:

Province:

District:

Telephone Number:

Alternate Contact Number:

Date of Diagnosis:

Date of Registration:

Final Diagnosis:

Thalassemia Initial Assesment Sheet-II

Chief Complaints:

Family History:

Examination:

Height: Weight:

Liver: Spleen:

Anemia: Jaundice:

Facial Changes: Chest:

CVS: BP: Heart rate:

Pubertal Changes: Yes: No :

Age of onset: Vaccination:

Base Line Investigation:

Name of Investigations	Reports
Complete Blood Count (CBC)	
Peripheral Blood Smear (PBS)	
Liver function test (LFT)	
Renal Function Test (RFT)	
Iron Profile	

Thalassemia Initial Assesment Sheet-III

Family pedigree:

Name	Relation	Hemoglobin Electrophoresis			
		Hb A %	Hb A2 %	Hb F%	Others %

Record on Every Visit

Date	Pre-Transfusion Hb	Amount and Type of Blood Transfusion	Liver	Spleen	Iron Chelation*	Transfusion reaction	Next Visit

*Tests as per Iron Chelators	Reports		Date
• SGPT, SGOT	If on Defera-sirox		
• Urea, Creatinine			
• Urine R/E			
• Complete Blood Count	If on Deferi-prone		

Follow-up Parameters Every Six Months

TEST NAME	REPORT	DATE
Height		
Weight		
SGPT		
SGOT		
Urea		
Creatinine		
Ferritin		
Calcium		
Phosphorus		
Vitamin D		
Zinc level		

Follow-up Parameters in One Year

NAME OF INVESTIGATIONS	AGE OF IN-VESTIGATION	REPORTS	DATE
ECG			
Echocardiography			
DEXA Scan			
MRI CardiacT2*, MRI Liver Iron (where available)			
Anti-HIV ½			
Anti-HCV			
HBsAg			
Anti-HBs antibody			
Fasting Blood Sugar or Glucose Tolerance Test			
Thyroid Function Test	In > 5 years of age		
Parathormone			
FSH	In 12-14 years of age		
Estradiol/ Testosterone			
Dental Consultation			
Ophthalmology Consultation			

Note: Certain Specific tests may be required to be performed as per the Clinician Advice.

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