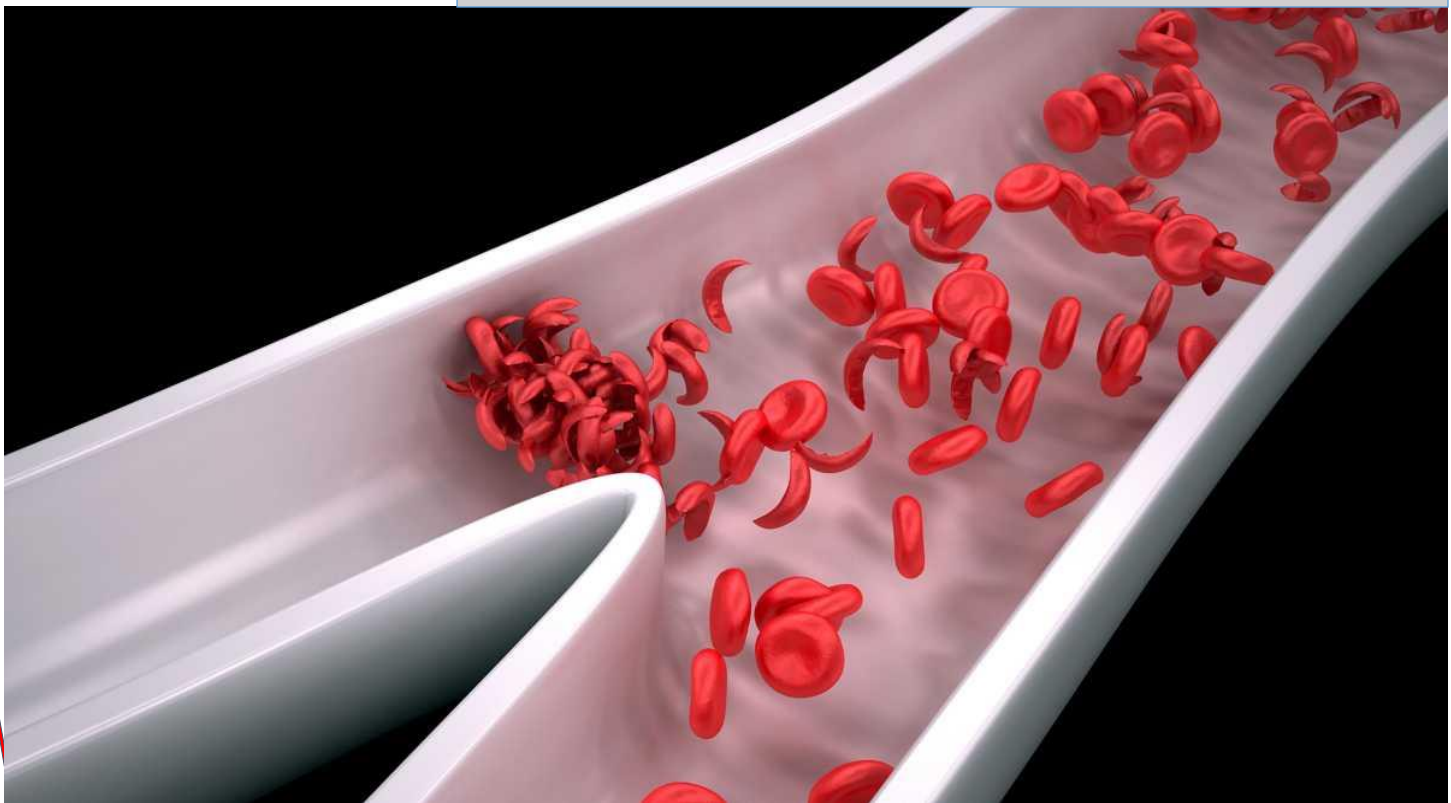


# National Guideline for Sickle Cell Disease and Thalassemia Management

(Clinical Guideline for doctors, nurses and paramedics)



MINISTRY OF HEALTH  
DEPARTMENT OF HEALTH SERVICES  
EPIDEMIOLOGY AND DISEASE CONTROL DIVISION  
TEKU, KATHMANDU  
2074

# National Guideline for Sickle Cell Disease and Thalassemia Management

(Clinical Guideline for doctors, nurses and paramedics)

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**Foreword**  
**By Health Secretary**

**Foreword**  
**By DG**

**Acknowledgement**  
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## Abbreviations

CBC	Complete Blood Count
CXR	Chest X-ray
DoHS	Department of Health Services
EDCD	Epidemiology and Disease Control Division
MoH	Ministry of Health
NPHL	National Public Health Laboratory
SCD	Sickle Cell Disease
SCT	Sickle Cell Trait
WBC	White Blood Cell
WHO	World Health Organization

## Glossary of Terms and Definitions

**HbSS:** People who have this form of SCD inherit two sickle cell genes (“S”), one from each parent. This is commonly called sickle cell anemia and is usually the most severe form of the disease.

**HbSC:** People who have this form of SCD inherit a sickle cell gene (“S”) from one parent and from the other parent a gene for an abnormal hemoglobin called “C”. Hemoglobin is a protein that allows red blood cells to carry oxygen to all parts of the body. This is usually a milder form of SCD.

**HbS beta thalassemia:** People who have this form of SCD inherit one sickle cell gene (“S”) from one parent and one gene for beta thalassemia, another type of anemia, from the other parent. There are two types of beta thalassemia: “0” and “+”. Those with HbS beta 0-thalassemia usually have a severe form of SCD. People with HbS beta +-thalassemia tend to have a milder form of SCD.

**HbSD, HbSE, and HbSO (raretypes):** People who have these forms of SCD inherit one sickle cell gene (“S”) and one gene from an abnormal type of hemoglobin (“D”, “E”, or “O”). Hemoglobin is a protein that allows red blood cells to carry oxygen to all parts of the body. The severity of these rarer types of SCD varies.

**Sickle Cell Trait (SCT),HbAS:** People who have SCT inherit one sickle cell gene (“S”) from one parent and one normal gene (“A”) from the other parent. This is called sickle cell trait (SCT). People with SCT usually do not have any of the signs of the disease and live a normal life, but they can pass the trait on to their children. Additionally, there are a few, uncommon health problems that may potentially be related to sickle cell trait.



# CHAPTER I: INTRODUCTION

## Background

Haemoglobinopathies are inherited blood diseases that affect how oxygen is carried in the body. Haemoglobin disorders fall into two main categories: sickle-cell disease and thalassaemias.

Sickle-cell disease is characterized by a modification in the shape of the red blood cell from a smooth, donut-shape into a crescent or half-moon shape. The misshapen cells lack plasticity and can block small blood vessels, impairing blood flow. This condition leads to shortened red blood cell survival, and subsequent anaemia, often called sickle-cell anaemia. Poor blood oxygen levels and blood vessel blockages in people with sickle-cell disease can lead to chronic acute pain syndromes, severe bacterial infections, and necrosis (tissue death).

Thalassaemias are also inherited blood disorders. People with thalassaemia are not able to make enough haemoglobin, which is found in red blood cells. When there is not enough haemoglobin in the red blood cells, oxygen cannot get to all parts of the body. Organs then become starved for oxygen and are unable to function properly. There are two major types of thalassaemia, alpha and beta, which are named for the two protein chains that make up normal haemoglobin. Alpha and beta thalassaemia have both mild and severe forms.

Approximately 5% of the world's population carries trait genes for haemoglobin disorders, mainly, sickle-cell disease and thalassaemia. Over 300,000 babies with severe haemoglobin disorders are born each year globally. These conditions are most prevalent in tropical regions. However population migration has spread these diseases to most countries. Thalassaemias are the most common in Asia, the Mediterranean basin, and the Middle East.

National burden of haemoglobin disorder has not been estimated in Nepal yet. Some of studies have been conducted based on hospital data. It seems that sickle cell disease is commonly detected in Banke, Bardiya, Dang, Kailali and Kanchanpur. Thalassaemia cases can be found any part of country.

Haemoglobin disorders are recognized as public health problems in Nepal. These disorder can be effectively reduced through a strategic balance of disease management and prevention programmes. This guideline is intended to serve as reference for disease management as well as for preventive interventions.

## **Rationale for guideline**

Haemoglobin disorders are one of the major public health problems in Nepal. Government of Nepal has been trying to reduce the burden of disease by two major interventions: a] effective case management and b] preventive measures. In order to effectively implement these interventions, we must enable our health system by strengthening technical and managerial capacity. For this, we do not have any guiding document. Therefore, a national guideline is required. This guideline is expected to full fil the gap.

## **Objectives of the Guidelines**

The general objectives of this guideline is to serve a reference for health managers, doctors, nurses and paramedics for case management and implementing preventive measures.

### **Specific Objectives**

- To provide basic information about haemoglobin disorders
- To provide guidelines for diagnosis, treatment and management of sickle cell diseases and thalassemia
- To make health care professionals be able to cope problems of haemoglobin disorders
  
- To improve health status and quality of life to increase life expectancy of patients with haemoglobin disorders
- To create awareness on haemoglobin disorders in the community

## **Sickle cell disease and thalassemia services in Nepal**

Government of Nepal (GoN), Ministry of Health has been providing financial aid for those patients who are impoverished. As of 2073, following disease and conditions are included for this provision.

1. Disease
2. Heart Disease
3. Kidney Disease
4. Cancer
5. Stroke
6. Head Injury & Spinal Injury
7. Alzheimer & Parkinson Disease
8. Sickle Cell Anemia
9. Aplastic Anemia
10. Thalassaemia

## **Designated for treatment centres for Sickle Cell Disease**

1. Civil Service Hospital, Min Bhawan, Kathmandu

2. Mid-Western Sub-regional Hospital, Ghorahi, Dang
3. Mahakali Zonal Hospital, Mahendranagar, Kanchanpur
4. Seti Zonal Hospital, Dhangadi, Kailai
5. Bheri Zonal Hospital, Nepalgunj, Banke
6. Lumbini Zonal Hospital, Butwal, Rupendehi
7. District Hospital, Kapilvastu
8. District Hospital Bardiya
9. District Hospital, Nawalparasi

**How can poor patient suffering from Sickle Cell Disease get financial aid?**

**Step1:**

**Step2:**

**Step3:**

**Step4:**

**Step5:**



## Public Messages on Sickle Cell Disease (SCD)

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

- ✓ Getting regular checkups with their doctor
- ✓ Following treatments prescribed by their doctor, such as taking 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 he or she receives 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, it is important for them to discuss this information with each other and with a doctor when making decisions about family planning.

\***Genes**, which are passed down from a parent to child, are instructions in each of our cells that determine a person's traits such as eye color, blood type, and risk of disease.

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

- ✓ In someone with SCD, red blood cells die early and not enough are left to carry oxygen throughout the body, causing anemia. Infection or enlargement of the spleen, an organ that stores red blood cells, may make anemia worse. 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 into a patient, can cure SCD. However, a bone marrow transplant may not be the best choice for all patients because it comes with serious risk. A bone marrow transplant expert can advise patients about whether or not it is a good choice for them.

## Public Messages on Thalassaemia

**Message 1:** Thalassaemia is a group of inherited blood disorders. There are two important things you should know:

- ✓ Thalassaemia is inherited. It is *not* something you “catch” by coming into contact with another person or from a virus. It is passed on to you through the genes of your parents
- ✓ If you have thalassaemia, your body is not able to produce enough of the protein needed to form hemoglobin. You need hemoglobin to carry oxygen throughout your body. When you don’t get enough oxygen in your bloodstream, you may notice certain signs and symptoms, like fatigue and other health problems

## Strategies for Control of haemoglobin disorders

The governing bodies of WHO have adopted two resolutions on haemoglobin disorders. The resolution on sickle-cell disease from the 59th World Health Assembly in May 2006 and the resolution on thalassaemia 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 thalassaemias, was adopted by the 63rd World Health Assembly in May 2010.

World Health Organization (WHO) has said that haemoglobin disorders can be effectively reduced through a strategic balance of disease management and prevention programmes.

Sickle-cell disease can be managed by simple procedures including: high fluid intake, healthy diet, folic acid supplementation, pain medication, vaccination and antibiotics for the prevention and treatment of infections, and a number of other therapeutic measures (*see in the sickle cell disease chapter for detail*). Similarly, thalassaemia major requires regular blood transfusions to maintain an adequate supply of haemoglobin and sustain life. As a result of multiple transfusions, organs become severely overloaded with iron and a specific treatment is needed to manage this condition. Thalassaemias can be cured by a successful bone-marrow transplant, however this procedure is expensive and not readily available in most settings. Recently, gene therapy has been successfully applied to a patient with thalassaemia (*see in the thalassaemia chapters for detail*).

The most cost-effective strategy for reducing the burden of haemoglobin disorders is to complement disease management with prevention programmes. Inexpensive and reliable blood tests can identify couples at risk for having affected children. This screening is especially opportune before marriage or pregnancy, allowing couples to discuss the health of their family. Subsequent genetic counselling informs trait carriers of risks that the condition may be passed along to their children, the treatment needed, if affected by a haemoglobin disorder, and the possible options for the couple. ***Prenatal screening of genetic diseases raises specific ethical, legal and social issues that require appropriate consideration.***

As per WHO's recommendation, following interventions should be implemented.

- Increase awareness on haemoglobin disorder in the community;
- Increase equitable access to health services including point of care testing, case management
- Implement preventive measures- prenatal screening and genetic counseling
- Promote and support research to improve quality of life for those affected

## CHAPTER II: BASIC GUIDELINES FOR CARE OF ADULTS WITH SICKLE CELL DISEASE

(This guideline includes some basic concepts in the approach and management of adult patients with sickle cell disease. The use of these guidelines is recommended in the treatment of adult patients with hemoglobin SS disease and sickle-beta thalassemia over the age of 18 years)

### I. Introduction

#### Sickle Cell Variants

The sickle cell mutation of the beta globin gene results in the production of abnormal hemoglobin S where valine is substituted for glutamic acid at the sixth position of the beta globin chain. Valine is hydrophobic resulting in a hemoglobin tetramer that is poorly soluble and polymerizes when deoxygenated. Polymerization of Hemoglobin S is the primary indispensable event in the pathogenesis of sickle cell disease.

The term sickle cell disease (SCD) refers to those hemoglobinopathies where two abnormal beta globin genes are inherited with at least one having the sickle mutation. Therefore, sickle cell disease refers to both homozygous hemoglobin SS disease (sickle cell anemia) and the compound heterozygote such as hemoglobin SC disease and sickle-beta thalassemia [sickle cell-beta (0) and sickle cell-beta (+)].

**Sickle cell anemia (Hemoglobin SS disease):** Both beta globin genes are the sickle mutation. The hemoglobin electrophoresis in a patient (non- transfused) with sickle cell anemia will reveal an Hgb A of 0%

**Sickle-Beta Thalassemia:** In beta thalassemia there is an absent or reduced production of the beta globin chain. When sickle hemoglobin is inherited along with beta thalassemia this results in a compound heterozygote where the disease severity depends on the amount of normal beta globin chain produced. In sickle cell-beta (0) thalassemia there is no production of normal beta globin therefore these patients are phenotypically similar to Hemoglobin SS disease. In sickle cell-beta (+) there is some production of normal hemoglobin A, but at a reduced level, resulting in a phenotype of intermediate severity. The hemoglobin electrophoresis in both S beta thal. (0) and Sbetathal. (+) will reveal an increase in hemoglobin A<sub>2</sub> (alpha<sub>2</sub>/delta<sub>2</sub>). **Sickle cell – beta thalassemia is frequently seen in our community. Most patient with sickle cell with beta thalassemia has splenomegaly.**

## II. Acute Painful Episode

The acute painful episode also known as sickle cell crisis is the hallmark 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 admissions for sickle cell patients and there is a correlation between the frequency of painful episodes and mortality.

**The acute painful episode in sickle cell disease has been described as more severe than post-operative pain and as intense as the pain associated with cancer, worse than the pain associated with child birth.** 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 painful 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. *During an acute painful episode, there are no physical changes. There are no objective findings than can be used to diagnose an acute painful episode.*

### Assessment of Acute Painful Episode:

*It is important to remember that all pain in patients with sickle cell disease is NOT sickle pain. It is necessary to always 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”, then 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 important 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 are indicative of a complicated episode.

Initial work-up of an acute painful episode should include CBC with reticulocyte count, serum chemistry, LDH, and LFTs. If the patient has any respiratory symptom, then a CXR should be obtained. Blood and urine cultures should be obtained if the patient is febrile or the WBC is above baseline. During the admission one would follow at least daily CBC with reticulocyte count (may follow every other day if an uncomplicated episode). Other daily labs as clinically indicated.

### **Management of Acute Painful Episode:**

It should be noted that the majority of 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 PRN. 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. A PRN dose may be given--in addition to the scheduled dose--for breakthrough pain. As noted earlier if needed, a rescue dose of the IV opioids may be provided PRN 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 abated, 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 in the treatment of acute painful episodes. These may be used alone which is 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 in conjunction with opioids for synergy and opioid sparing effect. This should also be used cautiously given that many of the PO opioids already have acetaminophen and one needs to be very cautious about associated hepatotoxicity.

#### **Fluid Management:**

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

### III. Acute Chest Syndrome

Acute Chest Syndrome (ACS) is one of the leading causes of death of adult sickle cell patients. Patients with ACS have the potential to rapidly progress to respiratory failure with an ARDS picture. Given the potential for rapid deterioration, patients with ACS need to be diagnosed in a timely manner with appropriate and effective management instituted immediately.

#### Diagnosis:

Acute Chest Syndrome (ACS) is diagnosed when there is a NEW pulmonary infiltrate 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  $> 38.5^{\circ}\text{C}$ , chest pain, cough, sputum production, wheezing, or hypoxia. CBC may reveal an increase in the WBC above baseline and worsening anemia as reflected by a drop-in hemoglobin.

#### Management:

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

Monitor the following:

- ❖ Continuous pulse oximetry.
- ❖ Serial/Daily ABGs.
- ❖ Serial/Daily CXR until improvement noted
- ❖ Serial/Daily CBC and reticulocyte count.
- ❖ Pre/post-transfusion hemoglobin electrophoresis.

Institute the following treatment:

- ❖ IV hydration with  $\frac{1}{2}$  NS no more than  $1\frac{1}{2}$  of daily maintenance.
- ❖ Antibiotic consisting of cephalosporin/macrolide or fluoroquinolone.
- ❖ Supplemental O<sub>2</sub> to maintain pulse ox  $>95\%$ . If noted to have increasing O<sub>2</sub> demands with FIO<sub>2</sub> needs greater than 40% then consider transfer to MICU.
- ❖ Adequate pain control while avoiding over sedation.
- ❖ Bronchodilator therapy in those with evidence of reactive airway disease.
- ❖ Incentive spirometer
- ❖ Transfusions: A type and cross should be sent immediately once ACS is suspected. RBC transfusions whether simple or exchange can be lifesaving in the treatment of ACS as they may prevent its progression to acute respiratory failure. Simple transfusion of 1-2 units or PRBCs can be given to raise the hemoglobin level (NOT to exceed Hgb level of 10g/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  $<30\%$  with total hemoglobin level of  $\sim 10\text{g/dl}$ . The choice to proceed with simple versus exchange transfusion is based on patient's clinical status, availability of units of PRBCs, and hemoglobin level.

If a patient with ACS is noted to be unstable/deteriorating as indicated by low O<sub>2</sub> saturation despite aggressive vent support, serial deterioration in O<sub>2</sub> saturation with increasing O<sub>2</sub> demands, and unstable vitals such as persistent tachypnea then one should proceed with immediate rapid exchange transfusion

#### **IV. Acute Neurological Events**

Central nervous system involvement in sickle cell disease is common. Cerebrovascular accidents are a common complication seen in both 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 following signs or symptoms noted in a patient with sickle cell disease should raise one's suspicion of an acutely evolving neurological event: headache, syncopal episode, change in level of consciousness, motor or sensory deficit, changes in vision, or new onset seizures. The concern for an acute neurological event is further raised if in addition to the aforementioned sign/symptoms, the patient has a prior history of a neurological event as repeat strokes whether ischemic or hemorrhagic are common.

An adult patient with sickle cell disease with signs/symptoms suggestive of an acute CVA should have an immediate general assessment including evaluation of ABCs and vital signs. A CT of the brain without contrast should be obtained STAT which will subsequently determine the course that will need to 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 headache, nausea/vomiting, and change in mental status. 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 source of bleed. The angiography should be preceded by an exchange transfusions with goal Hgb S <30% and total Hgb of ~10g/d. Subsequent surgical intervention is to be determined by neurosurgery.

##### **Ischemic stroke:**

Ischemic strokes although less frequent than the hemorrhagic type 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), then one may give the patient Aspirin 325mg and subsequently proceed with an exchange transfusion (goal as noted is Hgb S <30% with total Hgb level of ~10g/dl). If the non-contrast CT is negative (no bleed or ischemia noted), then one may still give ASA 325mg and subsequently proceed with obtaining the MRI/MRA. If the MRI is positive for an ischemic stroke, then one would proceed with the exchange transfusion immediately with the previous noted goals for Hgb S reduction and total hemoglobin.



### **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 noted goal for Hgb S reduction and total hemoglobin. If the clinic suspicion for TIA is low, then one can subsequently just observe the patient.

### **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 such as clipping or coiling. The MRA may also reveal Moya Moyas which are collateral circulations formed as a result of large vessel stenosis. Patients with moyamoyas are at risk for recurrent strokes--both hemorrhagic and ischemic. Neurosurgery should again be consulted in this situation for evaluation and possible intervention.

## V. Fever/Infection

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

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

### **Management:**

Acute painful episodes themselves may cause low grade fevers with the temperature usually <38.50C, but fevers over 38.50C are more likely to be from an infectious etiology. Workup should include CBC, reticulocyte count, CXR, UA, and blood culture. 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 empiric antibiotics in those presenting with fever. The clinical presentation and findings on CBC, chest x ray , urine analysis determine the need and type of empiric antibiotics.

### **Common infections in adult SCD patients:**

**Pneumonias:** One of the most common etiologies of acute chest syndrome. If this is suspected as by CXR finding, then one should initiate ceftriaxone plus macrolide or a floroquinolone. 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 Infections:** Recurrent UTIs are common particularly with E. Coli as the most common pathogen. Patients with SCD usually should be treated as having a complicated UTI. Antibiotics chosen based on the sensitivities.

**Osteomyelitis:** Often occurs at the sites of necrotic bone. Although Salmonella occurs more often in SCD patients, Staph. Aureus remains the most common cause of infection overall. MRI is the preferred imaging study. Bone biopsy remains the gold standard for diagnosis. Therapy is 2-6 weeks of parenteral antibiotics (choice of antibiotics based on isolated pathogen and susceptibilities).

**Cholecystitis:** Cholelithiasis is common as a result ongoing hemolysis with formation of pigmented stones and acute cholecystitis may be subsequently noted. Empiric broad spectrum antibiotic with zosyn or combined therapy with fluoroquinolone and metronidazole may be warranted prior to definitive surgical intervention.

## **VI. 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 a hemoglobin that is significantly below baseline. The mean hemoglobin at the time of presentation is noted to be ~4g/dl. Reticulocytes are <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 potentially shorten the course of infection.

Other complications associated with infection with parvovirus B19 in SCD patient beside the aplastic crisis are noted to be nephrotic syndrome, stroke, acute chest syndrome, and hepatic or splenic sequestration.

## **VII. 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 Hgb/HCT.

### **Acute Splenic Sequestration:**

Splenic sequestration is a life-threatening complication usually seen in the pediatric population of Hgb SS patients between the ages of 3 months and 5 years. Rarely does one see acute splenic sequestration in Hgb SS patients over the age of five as the spleen has usually auto infarcted by that age. In adults SCD patients it is more common to see acute splenic sequestration in Hgb SC and sickle/beta-thal (+) patients. These patients frequently actually have an enlarged spleen; therefore, during vaso-occlusive episodes they have the potential for sequestration. During splenic sequestration, the Hgb is usually noted to drop by more than two grams accompanied by increased reticulocytosis. Simple transfusions can be given in order to maintain hemoglobin levels. In this case, one has to 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 Hgb SS patients. During a vaso-occlusive episode, sickled erythrocytes become entrapped in the hepatic sinusoids. The Hgb/HCT is noted to drop significantly below baseline accompanied by increased reticulocytosis 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—increase potential for hyper-viscosity.

### **Acute Pulmonary Sequestration:**

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

## VIII. Priapism

This is the least common clinical manifestation seen in Nepal in sickle cell disease. Priapism is a persistent, painful, and unwanted erection that occurs without sexual stimulation. This a common complication of sickle cell disease with the majority of male sickle cell anemia patients noted to have had at least one episode 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. The corpus spongiosum is spared.

The episode of priapism are either noted to be stuttering which spontaneously resolve within three hours (usually only last several minutes) or prolonged lasting more than three hours. A prolonged priapism is a medical emergency as it may result in loss of functionality. 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 detumescence, and maintain functionality.

When a patient initially presents with a priapism, they should be immediately started on IV fluids preferably NS 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 detumescence is not achieved with conservative treatment and has been prolonged and ongoing for 4-6hours, 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 Hemoglobin S less than 30%. If the priapism persists despite the above noted interventions, then a shunting procedure may be considered. The aforementioned procedure is last resort and if possible should be avoided.

## IX. Transfusions

Red blood cell transfusions play an integral part in the management of sickle cell disease. PRBC transfusions are indicated in various clinical scenarios in SCD both in the acute and none acute settings. In many cases transfusions are lifesaving. This does not mean that PRBC transfusions in SCD are a panacea. There are various serious complications related with transfusion therapy, therefore there needs to be a thoughtful approach prior to proceeding with any transfusions.

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

**Sickle Negative:** this means that blood from sickle cell trait patients should not be transfused to SCD patients, since this will confound the hemoglobin electrophoresis results.

**Leukocyte depleted:** this can decrease the risk of alloimmunization. Also decreases the likelihood of febrile non-hemolytic transfusion reactions, and transmission of CMV.

**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 simple transfused given possibility of hyperviscosity at higher hematocrits (transfusing above a Hematocrit of 30 should be avoided). The other advantage of exchange transfusions 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, need for several units of PRBCs which means an increase chance for alloimmunization, and last but not least the cost.

## **Indications for transfusion whether simple or exchange in sickle cell patients**

### ***Episodic Simple Transfusions:***

- ✓ 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 is ~10g/dl prior to going to the OR.
- ✓ 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 Transfusions:***

- 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 simple transfusion of 1-2 units every 3-4 weeks.

## **Complications of Transfusion Therapy**

**Alloimmunization:** This is one of the major complications of transfusion therapy in SCD patients where they form antibodies to the antigens in the transfused blood. Usually many alloantibodies are present which makes it rather difficult in finding 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 background who have different antigenic frequency. The risk of alloimmunization can be reduced by transfusing only leukopoor, phenotype-matched RBCs.

**Delayed Hemolytic Transfusion Reaction (DHTR):** This usually occurs 5-14 days following the transfusion as a result of primary or an anamnestic immune response in a previously alloimmunized 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 episode of DHTR the LDH and TB 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 a Hgb 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). Patient may also be given 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 heart, liver, and endocrine organs. In Africans, and African-Americans, ferritin levels may not correlate well with the actual tissue stores. The 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 patient 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, HIV, and HTLV type I and II may be transmitted with transfusion of PRBCs. According to the American Red Cross, the risk of transmission of HIV is noted to be ~1 in 2.1 million transfusions. Hepatitis B transmission is ~1 in 200,000 transfusions. The risk of Hepatitis C on the other hand is ~1 in 2 million transfusions



## **CHAPTER III: BASIC GUIDELINES FOR CARE FOR SICKLE CELL DISEASE IN CHILDREN**

### **Introduction**

Features of Sickle cell disease in children are not much different from adults. But, some features are peculiar of 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 an anemia that on 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.

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 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 acetaminophen with codeine, whereas osteomyelitis requires at least 4-6 weeks of IV antibiotics.

**Figure 1: 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 week of age which is indicated by rapid increase in the size of 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 therapy has been used to prevent subsequent episodes, evidence strongly suggests this strategy does not reduce the risk of recurrent splenic sequestration when compared to no transfusion therapy.

## **Splenic sequestration Prevention, Diagnosis and Treatment**

### *Anticipatory Guidance*

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

### **Diagnostic Testing and Laboratory Monitoring**

1. Engorgement and increase of the spleen size
2. Evidence of hypovolemia
3. Decline in haemoglobin of  $\geq 2$ g/dL from the patient's baseline haemoglobin
4. Reticulocytosis
5. 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.

**Figure 2: Splenic sequestration in Sickle cell disease**



## **Infectious Risk Management**

1. Penicillin V orally from 2 months to at least 5 years of age (125 mg twice a day up to age 3yr, and then 250mg twice a day).
2. Prompt administration of broad spectrum or pneumococcal specific antibiotics in case of possible bacterial infection
3. Malarial prophylaxis when appropriate
4. Immunization
  - ✓ Streptococcus pneumonia
  - ✓ Haemophilus influenza
  - ✓ Meningococcus
  - ✓ Influenzae
  - ✓ Salmonella typhi (for at risk individuals)
  
5. Elimination of recurrent focal infection (dental infection, sinusitis, acute recurrent tonsillitis, cholecystitis, urinary infections)

## CHAPTER IV: CLINICAL GUIDELINE FOR MANAGEMENT OF THALASSAEMIA

(The main objective of this guideline is to train the physicians, nurses, lab technicians, clinicians, researchers and students and enable them to identify and manage thalassemia in Nepal in order to minimize disease burden in Society)

### Introduction

Haemoglobin (Hb) disorders are hereditary, genetic diseases consisting mainly of sickle cell disease and the thalassaemias, which account for a great proportion of births affected by a genetic disease.

The thalassaemias are a heterogeneous group of the haemoglobin disorders in which the production of normal haemoglobin is partly or completely suppressed as a result of the defective synthesis of one or more globin chains. Several types of thalassaemia have been described and named according to the affected globin-chain, the most common types of clinical importance being  $\alpha$ -,  $\beta\delta$ - and  $\beta$ -thalassaemia.

Thalassaemia, 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%. 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 haemoglobin 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).

In Nepal,  $\beta$  Thalassemia is more common. It is found mostly in low land Terai region and some in mid hill region and unlike sickle cell disease, it is seen prevalent in all ethnic community. At present only 186 thalassemia patients are registered at Nepal Thalassemia Society. The number of patients with thalassemia is estimated to be more, but because of various factors many patients are still under diagnosed. Significant number of children with  $\beta$ -thalassaemia die each year—undiagnosed or misdiagnosed, sub-optimally treated or not treated at all.

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 Hb disorders as a significant threat to public health.

### Genetic Basis and Pathophysiology

#### Haemoglobin Types

Oxygen is transported from the lungs to the tissues by a highly specialised protein molecule, haemoglobin, which is located in the red cells of the blood. Each red blood cell contains approximately 300 million molecules of this protein, totalling about 30 picograms in weight per cell. Each molecule of haemoglobin 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  $\alpha$ -globin cluster, comprising the  $\zeta$ - and  $\alpha$ -globin chains, and the  $\beta$ -globin cluster, comprising the globin chains  $\epsilon$ ,  $\gamma$ ,  $\beta$  and  $\delta$ . The globin chains appear sequentially during ontogeny and, after pairing, form the following four major types of haemoglobin:

1. “embryonic” haemoglobins, which are detectable from the 3rd to the 10th week of gestation and represent  $\zeta_2\varepsilon_2$ ,  $\alpha_2\varepsilon_2$  and  $\zeta_2\gamma_2$  tetramers;
2. “fetal” haemoglobin (HbF  $\alpha_2\gamma_2$ ), which constitutes the predominant oxygen carrier during pregnancy;
3. “adult” haemoglobin (HbA  $\alpha_2\beta_2$ ), which replaces HbF shortly after birth, and;
4. a minor adult component, HbA<sub>2</sub> ( $\alpha_2\delta_2$ ).

Under normal conditions, the red cells of the adult human contain approximately 98% HbA, 2.0% HbA<sub>2</sub> 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  $\alpha$  gene cluster) and 11 (the  $\beta$  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 which 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. Haemoglobin must have the correct structure and be trimmed in such a way that the number of  $\alpha$ -chains precisely matches that of the  $\beta$ -chains. When the above conditions are not met, the result is a complete or partial defect in one or both “allelic” globin genes.

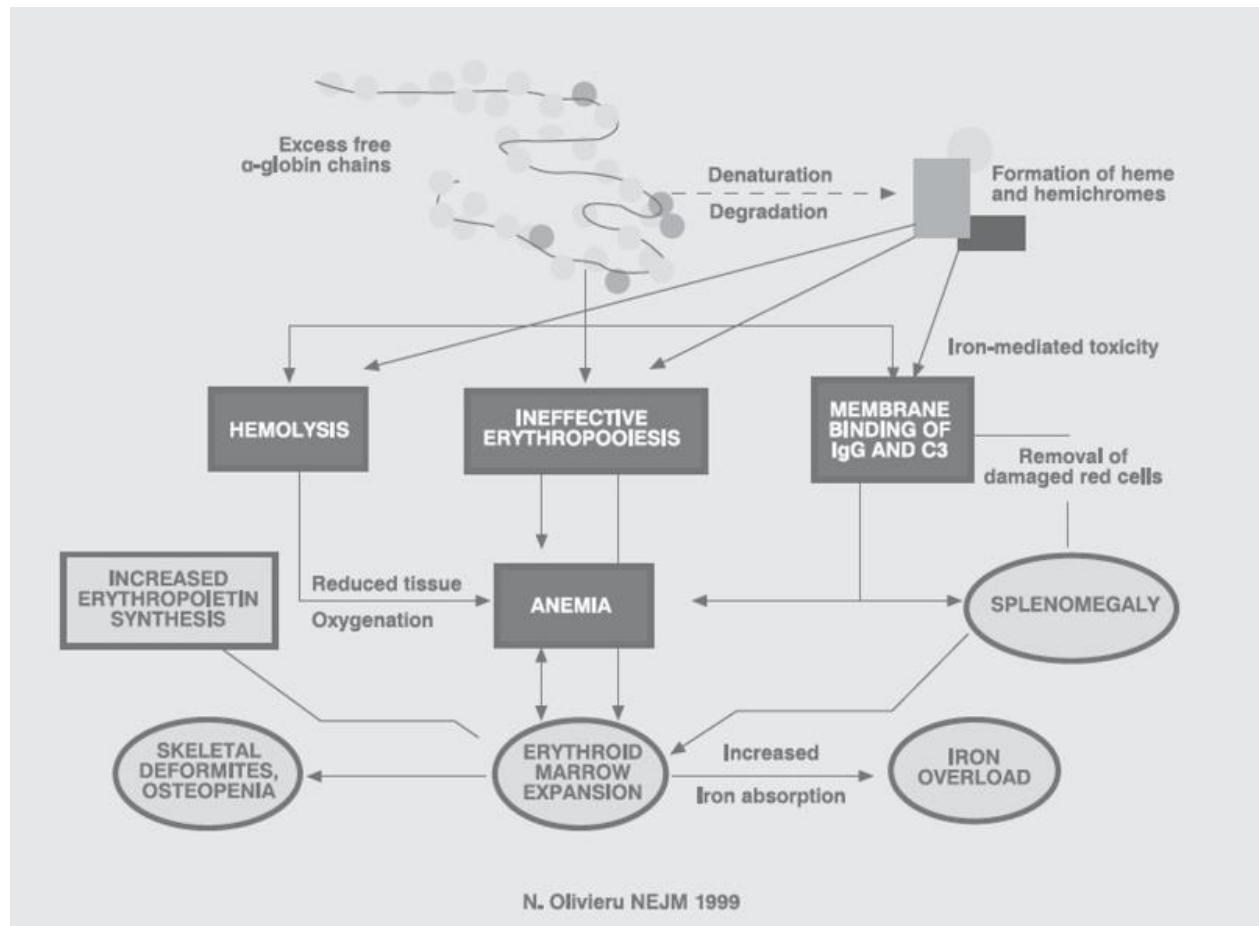
Depending on which of the genes the defect occurs and the corresponding effect on the production of globin chains,  $\alpha$ -thalassaemia or  $\beta$ -thalassaemia results. This book mainly addresses the latter group of thalassaemias.

#### **$\beta$ -thalassaemia:**

$\beta$  Thalassaemia is due to a range of mutations associated with the  $\beta$  globin gene, resulting in reduced or absent production of  $\beta$  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  $\beta$ -gene. More than 200 thalassaemic mutations have been reported to date.

Figure 3. below outlines the pathophysiology of  $\beta$ -thalassaemia and describes the chain of events following globin chain imbalance and the accumulation of excess  $\alpha$ -chains – that is, ineffective erythropoiesis leading to anaemia, bone marrow expansion; skeletal deformities and increased Gastro-Intestinal iron absorption.

**Figure 3: Pathophysiology of  $\beta$  -Thalassaemia Major**



$\beta$  Thalassaemia is categorized clinically into:  $\beta$  thalassaemia major,  $\beta$  thalassaemia intermedia and  $\beta$  thalassaemia trait/carrier.

$\beta$  thalassaemia 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 7g%. If untreated with red cell transfusion, progressive anemia and metabolic stress eventually cause heart failure and death. There is enlargement of the liver and spleen. The ineffective expansion of the erythropoietic marrow results in bone thinning and deformity. Untreated, children with Blood transfusion die from heart failure or infection before the age of five years.

$\beta$  thalassaemia intermedia is usually diagnosed at the age of 3 to 15 years. There is 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.

$\beta$  thalassaemia trait/carrier 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 reduced rate (e.g., HbE, HbLepore, HbE/ $\beta$ ).

### **Diagnosing thalassaemia**

A child born with thalassaemia will show no visible signs of the disease. Even laboratory tests may fail to diagnose thalassaemia, particularly if the parents have not been tested, no prenatal tests were carried out, and there is no other affected child in the family.

The diagnosis should be anticipated from antenatal screening, and established by prenatal diagnosis where requested or by neonatal testing. If not, affected infants may be identified through the newborn screening programme. The baby and parents should be seen for testing as soon as possible and preferably within two weeks. If the diagnosis has not been made at these stages, and the presentation is a clinical one, then assessment and treatment may be urgent, within one to two days.

Hematological and DNA diagnosis should be established as soon as possible by the following tests:

- Full blood count and blood film examination.
- Hemoglobin analysis by electrophoresis or high performance liquid chromatography (HPLC), and  $\beta$  and  $\alpha$  globin genotyping.

Family studies may be informative, and the parents should also be tested if results are not available from prior screening.

It should be emphasized that the clinical phenotype cannot be predicted accurately in the early stages, and that the child will be monitored carefully for clinical signs indicative of the need to commence transfusion, when that might be, and the implications of this.



## The treatment of -Thalassaemia major

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

### When to initiate transfusion therapy and whom to transfuse:

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

- Confirmed laboratory diagnosis of thalassemia major
- Laboratory criteria: Hemoglobin(Hb) <7g/dl on two occasions, >two weeks apart (excluding all other contributory causes such as infections or folic acid deficiency) or
- Clinical criteria irrespective of hemoglobin level:
  - Hemoglobin > 7g/dl with any of the following:
  - Facial changes
  - Poor growth
  - Fractures, and
  - 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 number of residual leucocytes should not be higher than  $1 \times 10^6$ . There are several methods of leucoreduction (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-haemolytic transfusion reactions (FNHTR)	HLA-antibodies in patients, cytokines produced by donor leucocytes
HLA- alloimmunisation of recipients	HLA antigens on donor leucocytes
Transfusion-transmitted infections	Cell-associated infectious agents
Graft-versus-Host-Disease (GVHD)	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 Hb 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 -60°C to -80°C 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. There is no good evidence about how long these can be stored though in many centers they are kept for 10 days.

**Red cells obtained by donor apheresis:** this method whereby two units of red cells are collected from the same donor for transfusion of one patient is associated with reduction of donor exposures and consequently to a decreased risk of transmission of infections, and of developing alloimmunisation and other transfusion related complications.

### **Transfusion Programme:**

The recommended treatment for thalassaemia-major involves lifelong regular blood transfusions, usually administered every two to five weeks, to maintain the pre-transfusion haemoglobin level above 9-10.5 g/dl. This transfusion regimen promotes normal growth, allows normal physical activities, adequately suppresses bone marrow activity in most patients, and minimizes transfusional iron accumulation.

A higher target pre transfusion hemoglobin level of 11-12 g/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 14-15g/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 alloimmunisation 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 CPD-A 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 Haemoglobin Level	1 g/dl	4.2 ml/kg	3.5 ml/kg	2.8 ml/kg	2.6 ml/kg
	2 g/dl	8.4 ml/kg	7.0 ml/kg	5.6 ml/kg	5.2 ml/kg
	3 g/dl	12.6 ml/kg	10.5 ml/kg	8.4 ml/kg	7.8 ml/kg
	4 g/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 1g/dl/week in splenectomised patients and 1.5g/dl/week in non-splenectomised patients.

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

- Antibodies (alloimmunisation) 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 use of medication (e.g. ribavirin)
- Increased red cell destruction from infection

Haemoglobin levels should ideally be measured before and after every transfusion, in order to assess the effectiveness of the treatment regime. If this is not possible, Hb 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.

Adverse events (see Table 3) associated with transfusion include:

**Table 3: Broad categorization of immune-mediated transfusion related (TR) reactions**

<b>Acute</b>	<b>Frequency</b>	<b>Delayed</b>	<b>Frequency</b>
Haemolytic (Intravascular)	1/25,000	Alloimmune	1/100
Anaphylactic	1/50,000	Haemolytic (Extravascular)	1/ 2,500
Febrile non-haemolytic	1/100	Graft Vs Host Disease	Rare
Allergic (Urticarial)	1/100		
TR 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 (GI). Both of these occur in thalassaemia, with blood transfusion therapy being the major cause of iron overload in thalassaemia major and increased GI iron absorption being more important in thalassaemia intermedia.

Untreated transfusional iron overload in thalassaemia 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, of the clinical management of these patients.

### **Monitoring of Iron overload:**

Monitoring closely and assessing as accurately as 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 thalassaemia major. Studies have identified a significantly lower risk of cardiac disease and death in at least two-thirds of cases where serum ferritin levels have been maintained below 2,500 µg/L (with desferrioxamine) over a period of a decade or more. Observations with larger patient numbers show that maintenance of an even lower serum ferritin of 1,000 µg/L may be associated with additional advantages.

### **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-thalassaemic populations without apparent adverse effects.

Several studies link high Liver Iron Content (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 LV function. For example, patients with T2\* values >20 ms have a very low chance of decreased LVEF. T2\* values of 10–20 ms indicate up to a 10% chance of decreased LVEF; 8–10 ms indicates an 18% chance; 6 ms indicates a 38% chance; and T2\* values of just 4 ms indicate a 70% chance of decreased LVEF .

### **Desferrioxamine**

- Initiate treatment after first 10–20 transfusions or ferritin level above 1,000 µg/l;
- If before 3 years of age monitoring of growth and bone development is recommended;
- Therapeutic index = mean daily dose (mg/kg) (Mean daily dose = actual dose of each infusion × doses/7 days) /ferritin (mg/l). Keep index < 0.025 at all times;

- Standard treatment: a) Slow subcutaneous infusion over 8–12 hours, b) 10% desferrioxamine solution (5 ml water for each 500 mg vial), and c) infusion pump (several types available);
- Standard dose: a) children 20–40 mg/kg (not exceeding 40 mg/kg, until growth has ceased), and b) adults 50–60 mg/kg. Infuse 8–12 hours 6 nights minimum per 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 IV or SC.

#### Indications:

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

Dose: 50 mg/kg/day (up to 60 mg/kg/day)

- In-dwelling catheters: danger of infection and thrombosis.

#### **Deferiprone**

- Standard dose: 75 mg/kg/day in 3 divided dose (up to 100 mg/kg/day, but as yet not enough information);
- 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. No recommendations as to which is the more effective combination can be made at present.

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

### 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 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 30mg/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 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;
- Cannot be given during pregnancy

## Medical problems associated with thalassaemia and its treatment

### Endocrine Complications in Thalassaemia Major

**Endocrine abnormalities** are among the common complications of thalassaemia. Despite 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 thalassaemia major. 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 thalassaemia may include chronic anaemia, 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.**

*Investigation of a child with thalassaemia who has stunted growth is generally similar to that of a child without thalassaemia*

### 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 thalassaemia. 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**

Anaemia, folate deficiency and hypersplenism are traditional causes of poor growth in patients with thalassaemia receiving irregular transfusion, as well as in those regularly using desferrioxamine. 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.

***Oral zinc sulphate supplementation should be given to patients with proven zinc deficiency.***

### **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 in 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 thalassaemia, 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 thalassaemia 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- $\beta$ , Estradiol)
- Pelvic ultrasound to assess ovarian and uterine size
- Transglutaminase antibodies

- In selected cases, Growth Hormone (GH) stimulation test
- In selected cases, Insulin Growth Factor-I (IGF-I), Insulin Growth Factor Binding Protein-3 (IGFBP-3), plasma zinc

### **Treatment**

The treatment of delayed or arrested puberty and of hypogonadotrophic hypogonadism depends on factors such as age, severity of iron overload, damage to the hypothalamo-pituitary-gonadal axis, chronic liver disease, and the presence of psychological problems resulting from hypogonadism. 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. If spontaneous puberty does not occur within six months after the end of treatment, oral oestrogen 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 oestrogen-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 hypogonadotrophic 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 as for the treatment of delayed puberty and hypogonadotrophic hypogonadism.

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

### **Hypothyroidism**

This may occur in severely anaemic 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 T<sub>4</sub> and TSH are the key investigations, and their interpretation, along with TRH test and TSH response, are shown in [Table 3](#). Bone age may be helpful in evaluating hypothyroidism. The majority of patients have primary thyroid dysfunction. Secondary hypothyroidism caused by iron-mediated damage of the pituitary gland occurs very rarely.

### **Treatment**

Abnormal thyroid function may be reversible at an early stage 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  $\beta$ -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.

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

- ✓ Diabetic type: Fasting Plasma Glucose (FPG)  $\geq 7.0$  mmol/l (126 mg/dl) and/or plasma glucose 2 hours after 75 g glucose load (2hPG) is  $\geq 11.1$  mmol/l (200 mg/dl). A casual Plasma Glucose (PG)  $\geq 11.1$  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 mg/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.

Diabetes in thalassaemia is rarely complicated by ketoacidosis.

### **Investigations**

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 levels
- ✓ 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**

Hypocalcaemia, due to hypoparathyroidism, is a recognized late complication of iron overload and/or anaemia and usually begins after the age of 16. The majority of 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. In cases with low serum calcium and high phosphate levels, parathyroid hormone should also be evaluated. Parathormone 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 analogues. Some patients require high doses of vitamin D to normalize their serum calcium levels. This should be carefully monitored, as hypercalcaemia 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 aluminium) may be considered.
- ✓ Tetany and cardiac failure due to severe hypocalcaemia require intravenous administration of calcium, under careful cardiac monitoring, followed by oral vitamin D.

## **Diagnosis and Management of Osteoporosis in $\beta$ -thalassaemia**

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

### **Diagnosis and investigations**

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

#### **(A) 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 .

#### **(B) Biochemical**

All patients must have endocrine and bone profile including 25 (OH) vitamin D3, PTH, calcium, phosphate, liver function tests, (alkaline phosphate, ALT, bilirubin, albumin) FSH, LH, testosterone and oestradiol assays.

#### **(C) Radiology**

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

## **(D) MRI**

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

## **(E) 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 thalassaemia patients. General guidelines include assessment of other drugs, lifestyle issues, exercise and diet.

### **Splenectomy in $\beta$ -thalassaemia**

Many patients with thalassaemia 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 thalassaemia, 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 splenectomised 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 haematocrit of the transfused units. For patients maintaining a pre-transfusion Hb level of about 10 g/dl, this increase in transfusion requirements represents consumption of more than 200–220 ml of red cells (assuming haematocrit of transfused cells is 75%)/kg/year. The rate of iron overload should also be taken into consideration. For patients who maintain effective chelation therapy despite increased blood requirements, splenectomy may be unnecessary. For patients with increasing iron stores despite good chelation therapy, reduction in the rate of 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 blood of inappropriately low haemoglobin may be reversible. Before considering splenectomy in this situation, the patient should be placed on an adequate transfusion programme 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.

## Thalassaemia intermedia and other thalassaemias

Thalassaemia intermedia is a medical condition in which individuals have inherited an affected  $\beta$ -gene from both the mother and father (i.e. they are homozygous for  $\beta$ -thalassaemia) but they demonstrate milder clinical symptoms than patients with thalassaemia major. Individuals with thalassaemia intermedia manage to maintain haemoglobin levels between 6-9g/dl and may not require regular blood transfusions.

However, considerable research into the condition has demonstrated that thalassaemia intermedia in fact 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 is slower than normal, these patients may maintain a good quality of life without the regular blood transfusions required by  $\beta$ -thalassaemia major patients. In less serious cases, patients may not demonstrate any symptoms until they are adults, suffering only mild anaemia (8-10g/dl) and only rarely requiring blood transfusions - if at all.

The spleen may become enlarged (splenomegaly) - as in thalassaemia major - because of the rapid breakdown and accumulation of red blood cells in the organ, and this may sometimes be the cause of more severe anaemia in patients with thalassaemia intermedia. 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 anaemia, such as the possibility of infection.

As described earlier, the main cause of symptoms in thalassaemia major is the excess amount of free  $\beta$ -chains that accumulate inside the red blood cells, creating an imbalance between the  $\beta$ -chains and their usual partners, the  $\alpha$ -chains. Alone, the  $\alpha$ -chains interfere with almost every stage of red blood cells' cycle of maturation, causing the severe anaemia and other conditions discussed earlier.

Given the above, it is reasonable to expect that the symptoms manifested by thalassaemia 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  $\beta$ - $\beta$  gene, which can produce some  $\beta$ -BETA chains - although less than normal - that in turn couple with alpha chains, thus reducing the number of free alpha chains. Mutations to the  $\beta$ - $\beta$  gene that are associated with a very mild clinical outcome are sometimes designated  $\beta$ - $\beta$ +

(II) A defect on 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, which can bind  $\alpha$ -chains to produce fetal haemoglobin ( $\alpha_2$ ,  $\gamma_2$ ), 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 programme for an individual patient.

Where available, such molecular methods of investigation are proving invaluable aids to the treatment of thalassaemia.

### **Diagnosis**

In diagnosing thalassaemia intermedia, it is important to establish certain clinical and laboratory information, in order to differentiate thalassaemia intermedia from thalassaemia major. However, this is not always easy or even possible, despite impressive improvements in molecular laboratory techniques. Nonetheless, some useful and simple criteria for differentiation are the following:

In conclusion, the term thalassaemia intermedia is used to describe a wide range of clinical and haematological findings in patients with less severe forms of the disease than homozygous  $\alpha$ -thalassaemia, but more severe than that of heterozygous carriers.

### **Management of thalassaemia intermedia**

In thalassaemia intermedia, the most important question is when to begin blood transfusion therapy. The following medical conditions may result from chronic anaemia, and certainly constitute reasons for initiating blood transfusion therapy: ñ delayed growth ñ pathological bone fractures ñ cardiac complications ñ facial deformities ñ decreased normal physical activity ñ hypersplenism

As in the case of thalassaemia major, 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 thalassaemia intermedia begin blood transfusions later in life than patients with thalassaemia major, it is important to pay particular attention to the possible development of reactions (alloimmunisation) 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 thalassaemia intermedia may require blood transfusions.

### **Iron chelation**

As in thalassaemia major, iron overload in patients with thalassaemia intermedia 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 thalassaemia intermedia. However, one study demonstrated that 2-5g of iron accumulate in the body of patients with thalassaemia intermedia 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 thalassaemia intermedia therefore have almost the same risk of iron-associated complications as patients with thalassaemia major receiving regular blood transfusions.

A difficulty in deciding when to start iron chelation in patients with thalassaemia intermedia is determining the patient's actual body iron overload, as serum ferritin levels



may not provide an accurate measure - again, as is the case with thalassaemia major. 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 thalassaemia major, although patients with thalassaemia intermedia may require a subcutaneous infusion no more than 2 or 3 days a week. The same follow-up treatment recommended for patients with thalassaemia major undergoing iron chelation should also be made available to patients with thalassaemia intermedia.

As patients with thalassaemia intermedia absorb significantly more iron from the gut than normal, they should avoid foods rich in iron (e.g. spinach, liver and some kinds of beans) as well as iron supplements. Drinking black tea with meals may help to reduce the amount of iron absorbed by the gut.

### **Medical problems in thalassaemia intermedia**

(1) **Bone changes. Hyperactive bone marrow** - a result of the body's effort to produce more red blood cells to counteract anaemia - 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 to increase the calcium in their diet in order to avoid serious bone disease (osteoporosis). Calcium and vitamin D capsules may provide additional benefit. Smoking should also be avoided. Some doctors have demonstrated beneficial results with the use of bisphosphonates, administered orally or intravenously, however their role in combating osteoporosis has yet to be confirmed.

(2) **Hyperactivity or expansion of the bone marrow and folic acid**- Because the bone marrow of patients with thalassaemia intermedia works extra hard in an effort to combat the body's anaemia by making more red blood cells, patients need extra amounts of certain vitamins, particularly folic acid. Insufficient folic acid can aggravate the anaemia in thalassaemia intermedia patients. Folic acid is found naturally in food such as meat and green vegetables. However, an additional amount - usually a tablet a day - should cover patients' extra needs.

(3) **Gall stones**-Thalassaemia intermedia 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.

(4) **Leg ulcers**-Patients with thalassaemia intermedia frequently develop ulcers around the ankle, particularly older patients, as a result of 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 to raise haemoglobin 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 haemoglobin, such as erythropoietin and butyrates.

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

(6) **Thrombophilia-** Another complication is an increased risk of thrombosis, where thrombocytes or platelets (see section in blood) 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.

(7) **Extra medullary erythropoiesis** - Unlike thalassaemia major patients, who receive regular blood transfusions from an early age which suppress excessive activity of the bone marrow, patients with thalassaemia intermedia 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.

(8) **Heart and liver complications.** Chronic anaemia 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 thalassaemia major.

## **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 those patients and families to whom the risks and benefits are acceptable and who seek permanent cure has an accepted standard of care. However, the main constraint in offering this has been the availability of donors and the risks undertaken when considering alternative donors. Bone marrow transplantation in thalassaemia should be considered for patients at an early age 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 haemoglobin is the predominant non- $\alpha$  globin produced in humans until around six months of age, when it is typically suppressed and the production of  $\beta$ -globin is increased. This pattern is the norm even when the genes are mutated, as in  $\beta$ -thalassaemia.

Patients with  $\beta$ -thalassaemia who continue to produce high levels of foetal globin, such as those with Hereditary Persistence of Foetal Haemoglobin, have less globin imbalance and less severe anaemia. The therapeutic stimulation of foetal globin could therefore benefit many patients, even rendering some transfusion independent.

Several candidate therapies now offer the potential to correct or modulate the underlying pathology.

### **Cytotoxic agents**

Following observations that foetal haemoglobin 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 the treatment of serious haemoglobin disorders. Several cytotoxic agents that alter the pattern of erythropoiesis, favouring the expression of foetal ( $\gamma$ )-globin genes and so increasing the number of red cells containing HbF (F-cells), have been explored over the past 20–25 years .

**The demethylating agents 5-azacytidine and decitabine** have been administered to a few  $\beta$ -thalassaemia patients with good responses, raising total haemoglobin levels by a mean of 2.5 g/dl above baseline and clearly 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** has been studied in HbE/ $\beta$ -thalassaemia patients, with lower responses but reduced haemolysis. Hydroxyurea has been less beneficial in thalassaemia intermedia than in sickle cell disease, in which the number of painful crises was reduced and overall health indicators improved. The lesser benefits in thalassaemia are perhaps due to the fact that the cytostatic effects of hydroxyurea are limited in the disease.

## **Gene Therapy**

The idea of using gene therapy to treat the haemoglobinopathies (thalassaemia and sickle cell disease) is, in principle, straightforward. Red blood cells (RBC) are continuously replenished by bone marrow hematopoietic stem cells (HSC). Therefore, the stable transfer of a normal functioning copy of a  $\beta$ -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 the haemoglobinopathies looks a serious possibility in the not too distant future.

## **Psychosocial support**

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

## **Life with Thalassaemia**

### **Diet and thalassaemia**

In general, patients with thalassaemia need not follow a special diet. Patients should, however, avoid food rich in iron. It is also wise to avoid alcoholic drinks, or to drink only moderately, because the liver is especially vulnerable in thalassaemia -- both because of the level of liver iron stores, and the possibility that patients have been exposed to hepatitis (read more about nutrition in the special section).

### **Sport and thalassaemia**

Thalassaemia patients can take part in most sports -- how often and what type of sport depends on the patient's clinical condition and the doctor's advice should be sought.

### **Holidays and thalassaemia**

TIF has compiled a list of medical experts and medical centres around the world (available from the TIF website) that can provide treatment to patients with thalassaemia major. Before travelling, patients should establish the location of the medical centre nearest to their destination and could also make contact with the local treating physicians of the centre before leaving home. Patients should also ensure they receive all necessary vaccinations (always in consultation with their doctor) before visiting a country and are aware of any specific infections prevalent in the area they plan to visit (see travel advice issued on the WHO website). All medicines required for the patient's treatment regime, including antibiotics and sterile equipment, should be carefully packed and carried with them in their hand luggage. Patients planning to visit high mountain areas should have normal haemoglobin levels and should give themselves time to acclimatize to higher altitudes. Patients may therefore consider restricting themselves to heights not greater than 11,000 feet, or make sure they have a transfusion immediately before travelling to higher altitudes. Otherwise, there are no restrictions on where a patient may travel.

### **Marriage and family**

Patients with thalassaemia major can certainly marry and have children. Whether their children will be healthy will depend on the thalassaemia status of their partner -- i.e. whether they are healthy, a carrier or a patient themselves. If a patient marries another patient with thalassaemia all children born will be affected. If a patient marries a carrier of  $\beta$ -thalassaemia 50% of the children will be affected and 50% will be carriers. On the other hand if a patient marries a non-carrier then all (100%) children born will be just carriers.

In short, patients who comply with recommended treatment regimens can live a near perfectly normal and happy life. In Cyprus, for example, 83% of thalassaemia patients have completed higher education, while 25% have graduated from university. Twenty-two per cent (22%) of patients are married, 73% of which have children -- some of them three or four. Seventy-nine per cent (79%) of patients in Cyprus work - in handicrafts and agriculture, as secretaries and teachers, and as nurses, medical or paramedical staff.

## **Epidemiology and prevention of thalassaemia**

Thalassaemia was originally thought to be a disease limited to the Mediterranean region, however it is now known that it occurs widely throughout many parts of the world including the Middle East, Southern and Eastern Asia, the South Pacific and South China,

Thalassaemia is particularly prevalent in areas in which malaria is or was once endemic. The malaria parasite is an infectious agent carried by the anopheles mosquito, enters the human body through a mosquito bite and causes disease in humans by attacking the red blood cells. It is thought that in areas where malaria was endemic, humans underwent a small genetic adjustment which gave them an advantage over those in whom this change did not occur. This is because important changes occurred in the environment of the red cells following this genetic change that did not allow the parasite to survive and multiply. This adjustment leads to  $\alpha$ , beta thalassaemia minor or  $\alpha$ , beta thalassaemia trait.

It is believed that as with beta thalassaemia and sickle cell disease carriers of the  $\alpha$ , beta thalassaemia trait were better able to survive malaria than healthy individuals, the number of carriers increased significantly over the years in malaria-endemic regions of the world as large numbers of healthy individuals died as a result of severe malaria infection. Although malaria eradication programmes in recent years have led to a steep fall in the incidence of malaria in many parts of the world, tackling thalassaemia and other severe haemoglobin disorders nonetheless remains a considerable challenge.

While reliable sources estimate that about 1.5% of the global population -- 80 million-90 million people -- are carriers of  $\alpha$ , beta thalassaemia, with about 60,000 affected children born annually, the great majority in the developing world, it is certain that these figures are gross underestimates: there is still little accurate data available on carrier rates (gene frequencies) in many population groups, particularly in areas of the world known or expected to be heavily affected. According to TIF records, however, only about 200,000 patients with thalassaemia major are alive and registered as receiving treatment around the world -- underlining the bitter reality that the majority of affected children, born in developing countries, die undiagnosed or misdiagnosed, receiving sub-optimal treatment or left untreated altogether.

The map indicates countries affected by  $\alpha$ , -thalassaemia. Together with other severe haemoglobin disorders such as sickle cell and HbE/ $\alpha$ , -thalassaemia, about 5% of the world's population is affected by such diseases.

### **Prevention**

Management of thalassaemia 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. It 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 thalassaemia major. Majority of patients have a poor quality of life with a high incidence of premature death. According to the Nepal Thalassaemia Society, the minimal annual cost for the treatment is estimated to be about 2,37,830 per patients. In this scenario, prevention strategies are important and an extremely cost- effectively public health measure.

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.

By contrast in the UK, where quality prevention programmes have been available for some time but where there was no national policy aimed at prevention, the rate of births of affected children has fallen by only 50%.

**Key aspects of the most successful prevention programmes includes**

- 1) Securing government will and commitment
- 2) Establishing powerful health education campaigns,
- 3) Raising public and health professional awareness
- 4) Establishing quality laboratories for screening and prenatal diagnosis and
- 5) Promoting genetic and obstetric services

**The importance of prevention**

According to the World Health Organization, the annual cost of a nationwide prevention programme in most countries is approximately equal to the cost of treating one annual birth cohort of patients for one year. Annual prevention costs are effectively constant while annual treatment costs rise year-on-year, so that the cost-effectiveness of a prevention programme increases with every year it is in place. World Health Organization projections of treatment costs have shown that without prevention programmes to limit the number of births of affected children, most countries will be unable to afford to provide optimal treatment to all patients with thalassaemia. An effective prevention programme is therefore essential in order to meet the cost of treating existing patients.

**Education and Counseling for Thalassaemia**

Patient as well as normal peoples should be educated and counselled properly to have sufficient knowledge about complications of Thalassemia and its management. They should be made able to recognize the earliest signs and symptoms of complications and seek necessary help and also identify environmental hazards. They should be guided properly to live their better life style, marriage, child bearing etc. All the Thalassemia patients should be counseled regularly by our trained counselors. Family counseling should be done at the door step of this community. All the Adolescents should be counseled for their marriage and future pregnancy. All the tribal persons should be given color coded card according to their Thalassemia Status, which will help them in marriage decision.

All parents and teachers should also counseled about disease and how to help the patients.

## **Diet and Thalassaemia**

### **Reducing the iron absorbed from food**

In thalassaemia, although most of the iron overload is due to blood transfusion, increased absorption of iron from the diet is also important. Only a small amount of iron from the diet is absorbed into our body. The amount absorbed is higher when haemoglobin in the blood is low. People with low haemoglobin such as those with thalassaemia intermedia or those with thalassaemia 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** Meat iron is present in red meat such as beef, lamb and pork and the dark meat of chicken as well as in seafood such as sardines, cockles and mussels. Liver is a very rich source of meat iron. Try to cut down on these and perhaps substitute meat with soy protein. It is not, however, a good idea to exclude meat, chicken and fish completely from your diet because they contain other important nutrients, particularly for children. 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 body. However, this may vary between 10-40%, depending mainly on whether the meal contains milk or milk products. The calcium present in milk, cheese, yoghurt and cream decreases the absorption of meat iron. Try to drink a glass of milk with a meat-containing meal and to use milk in cooking. Good examples are the white cheesy sauces in lasagne, pasticcio, mousaka and cannelloni, adding lots of cheese in spaghetti bolognese and using yoghurt and milk to cook your curries.

Milk intake should be at least one pint daily, particularly because it also helps to prevent osteoporosis, as will be discussed later. If you are worried about your weight, semi-skimmed milk or skimmed milk are just as rich sources of calcium as whole milk.

### **Non-meat iron**

Non-meat iron is widely distributed in the diet, present in eggs, chocolate, cereals, vegetables, fruits, roots (potatoes, parsnips), beans and lentils. In the UK several foods are fortified with iron, such as breakfast cereals, wheat flour and bread. However, this may not be the case in other countries.

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 composition of the meal. The foods that decrease its 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, sauerkraut, soy sauce, vinegar and alcohol.

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



### **Food that decrease 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. It is good to eat a lot of cereals in your diet, but remember not to take a vitamin C-rich food with them, like orange juice. Try to combine milk and cereals, (e.g., cheese sandwich, French toast, macaroni cheese, cereals and milk). In the UK all wheat flour other than wholemeal is required by law to be fortified with iron. The fortification of breakfast cereals is voluntary. It may, therefore, be better to choose unfortified wholemeal wheat flour and bread and to look carefully at the label of your favourite breakfast cereal. Unfortified breakfast cereals include porridge oats and some cereals in health shops but look at the label to make sure you choose an unfortified variety.

In other countries flour and breakfast cereals may not be fortified.

Soy protein also decreases the amount of iron absorbed into your body. Soy protein can work well in many recipes (e.g., spaghetti bolognese, stews and casseroles) and the taste can be improved by adding spices.

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. Better yet, if you take it with milk. Tea is also a very good source of antioxidants as will be discussed later.

3. **Dairy products-** Milk, cheese and yoghurt 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. At least one pint of milk should be taken every day.

### **Foods that increase non-meat iron absorption**

1. **Vitamin C-** Vitamin C is present in fruit, fruit juice and vegetables. It is better to avoid drinking fruit juice, such as orange juice, with your meal or your toast in the morning. Instead, a cup of tea or coffee is better options as they inhibit iron absorption. Alternatively, have a glass of milk! 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, however, 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 they also help to absorb more of the non-meat iron from your food! It would be unwise, however, to omit them from the diet altogether as they contain other vital nutrients, particularly important for children and adolescents.

3. Pickles, sauerkraut, soy vinegar, alcohol sauce Sauerkraut, pickled onions, turnips and carrots as well as fermented soy products (e.g., miso and soy sauce) enhance iron absorption. The amount of iron absorbed is even higher when the pickled vegetables are added to bread and rye- containing meals.

In general, a low iron diet would contain cereals (maize, whole-grain flour, beans) and root vegetables with little meat, fish or foods rich in Vitamin C. A moderate iron diet would consist of cereals and root vegetables but would also contain some vitamin C-rich foods and meat.

High iron diets contain generous quantities of meat, poultry and fish. They also contain foods with high levels of vitamin C such as citrus fruits and some vegetables. A high iron diet can be reduced to a moderate one by the regular consumption of foods which decrease the amount of iron absorbed by our body, such as dairy products, cereals, beans, coffee and tea.

### **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 the prevention of diseases such as coronary heart disease and cancer. In Thalassaemia, because of the excess iron in the body, there is a higher risk of oxidative damage. In this article, the author will concentrate on the four main antioxidants: Vitamin E, Vitamin C, Carotenoids and Flavonoids.

**1. Vitamin E** -Vitamin E is the most important dietary antioxidant. Several studies have found that many patients with Thalassaemia have lower levels of Vitamin E in their blood compared to non-Thalassaemics. This could be either because these patients do not take as much Vitamin E in their diet or because their needs are higher. In many studies, when Vitamin E was given as a supplement Vitamin E levels in the blood improved. However, even if your Doctor or Dietician recommends that you take a supplement, the best way for any vitamin to enter your body is through your food.

Vitamin E is fat-soluble which means that it is present in foods that have a high amount of fat. The best sources of Vitamin E are vegetable oils (olive, sunflower, and palm and soy oil). The best one to use is probably olive oil because the type of fat it contains can help to prevent heart disease. In Mediterranean countries where olive oil is used a lot (Greece, Portugal, Spain and Italy) heart disease is lower than in Northern Europe. Remember, however, that the vitamin is destroyed slowly with frying. Therefore, the best way to get the most out of your olive oil is to add it to food towards the end of cooking or even after it is cooked, as a dressing. Olive oil mixed with lemon, for example, can make a delicious dressing for fish, chicken, boiled vegetables and salads. Choose the extra virgin olive oil if you like the intense flavour and you tend to use it as a dressing, or experiment with more refined varieties if you want to use it for cooking, making cakes etc. Ghee also contains Vitamin E but since olive oil has additional health benefits, you may like to try using it in cooking.

Other sources of Vitamin E are dairy products, cereals, nuts, eggs and meat. Dairy products are particularly good to include in the diet not only because they contain Vitamin E, but also because they inhibit iron absorption from our food into our body and also because they contain a lot of calcium which can help to prevent Osteoporosis (weak bones). You can try to use milk in cooking or to have a glass of milk with your meal. Skimmed milk has lower levels of Vitamin E than full cream milk, although the amount of calcium is the same.

**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 Thalassaemia intermedia who are not regularly transfused.

Remember that non-meat iron is widely distributed in the diet, present in eggs, chocolate, cereals, vegetables, fruits, roots (potatoes, parsnips), beans and lentils. In the UK several foods are fortified with iron, such as breakfast cereals, wheat flour and bread, although this may not be the case in other countries.

Vitamin C is mainly found in fruit, fruit juices and vegetables. It might be better to have your piece of fruit or glass of fruit juice on their own, in between meals and not during or immediately after your meal. Health professionals recommend people 5 portions of fruit and vegetables to be consumed daily.

Examples of what is one portion are: a glass of fruit juice, a piece of fruit such as an apple, pear, banana, orange, half a grapefruit, one tomato, a helping of vegetables such as carrots, courgettes, French beans or a small salad. Vitamin C is water-soluble, so if vegetables are boiled it will leak out in the water. Light steaming preserves the vitamin better. Cooked vegetables with olive oil and lemon can make a very tasty snack or a light meal. Vitamin E and Vitamin C work well when they are together, so remember to fuel your vegetables with olive oil!

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 and therefore the same caution applies as above. It is worth pointing out that the absorption of carotenoids from the diet is much higher when the food contains fat or oil. So, keep adding that olive oil! Carotenoids can be destroyed at high temperatures so keep the cooking temperature low and the time short if you can.

4. **Flavonoids** -These are found in tea, red wine, fruit and vegetables. What better excuse to include a glass of red wine with your meal! If it is a more sober occasion, have your meal with a cup of tea! Tea will not only give you lots of antioxidants, but it will also inhibit the absorption of iron from your food, especially if you take it with milk. Try to have several cups of tea daily. Remember that we need about 8 glasses of fluid daily to be well hydrated.

## **CHAPTER V: LABORATORY DIAGNOSIS OF SICKLE CELL DISEASE**

### **What are different tests for screening sickle cell disease?**

Any patient suspected of suffering from sickle cell disease should get a basic laboratory test done. This includes complete blood count (CBC), red cell indices, reticulocyte count and peripheral blood smear examination

There are various specialized tests to screen or diagnose sickle cell diseases. The specialized analytical procedures employed must be capable of detecting and quantifying Hb A, Hb F and Hb S, along with other common clinically significant haemoglobin variants, i.e. C, D, E and O. In this way, the person will get screened not only for Sickle cell disease but for other hemoglobinopathies as well at the same time

Specialized tests for screening and diagnosis of sickle cell disease include cellulose acetate hemoglobin electrophoresis, isoelectric focusing, high-performance liquid chromatography, and capillary electrophoresis and DNA analysis. The testing method should be selected on the basis of local availability and cost.

First line test suggested in tertiary care centre is Capillary electrophoresis or High performance liquid chromatography (HPLC). As Hb S co-elutes with another hemoglobin variant in the first line test, abnormal results should be retested by a different technique

If Hb S seen by CE or HPLC, Perform Sickling test (preferable) or Rapid diagnostic test.

Solubility testing methods and sickle cell preparations are inappropriate diagnostic techniques as first line test if electrophoresis or HPLC is approachable. Although these tests identify sickle hemoglobin, they miss hemoglobin C and other genetic variants. Furthermore, solubility testing is inaccurate in the newborn, in whom fetal hemoglobin is overwhelmingly predominating

### **Who should be screened for sickle cell disease?**

- ✓ Anybody who requests to get screened
- ✓ History/family history suggesting sickle cell disease
- ✓ As part of antenatal tests in mother for high risk population
- ✓ Spouse and family members of known trait or diseased person

### **When can be screening test offered?**

Blood tests can be offered at any stage in life from newborn to elderly. For asymptomatic persons, the advantage of having tests before starting a family is that the person will know whether or not there is a possibility that their baby could inherit Sickle cell disease. This may be helpful when making decisions about pregnancy.

**Neonatal screening:** Neonatal screening will help in starting early management. If a baby is having sickle cell disease, Oral penicillin should be started before baby is 3 months old screening before the baby is 3 months old should be offered. For this newborn blood should be tested as soon as possible before 3 months age to identify the sickle cell disease status. Screening at this age however will not identify thalassemia and screening at later date will be required for that.

### **Antenatal screening of mother for sickle cell disease**

All patients coming for antenatal checkup in high risk districts should get screened for sickle cell disease if they are not tested before. This should be done within first 10 weeks or the very first visit. If tests show that the baby is at risk of inheriting a sickle cell disease then the parents need time to receive counselling and consider antenatal fetal screening or termination by 13 weeks. If both parents are trait or one is diseased and one is trait, The couple should promptly be offered counselling from a health care professional

### **Preoperative/pre-anesthesia screening**

This should be done in patients from ethnic groups where the prevalence of Hb S is high, as the presence of sickle Hb may influence preoperative techniques and clinical management. About 7% of all deaths among patients with sickle cell anemia are related to surgery. High risk population should be screened before anesthesia or operative procedures as they require proper planning and optimal perioperative preparation. Also high risk population should be screened for sickle cell disease if they present with osteonecrosis

### **Preconception testing:**

Preconception testing for hemoglobinopathies should always be performed on women being investigated for infertility and in those having assisted conception irrespective of risk prevalence

### **What will be the result of screening?**

The result will be one of the following

- Unaffected
- Sickle cell disease
- Sickle cell carrier
- Compound Heterozygotes for sickle cell and other haemoglobin variants
- Homozygotes or compound heterozygotes for non-sickling conditions

A homozygous adult patient will have hemoglobin S (HbS, 80-90%), hemoglobin F (HbF, 2-20%), and hemoglobin A<sub>2</sub> (HbA<sub>2</sub>, 2-4%). A carrier patient will have HbS (35-40%) and hemoglobin A (HbA, 60-65%).

In person with microcytic hemolytic anemia, if HbS is predominant, Hb F is less than 30% and Hb A<sub>2</sub> is elevated, a diagnosis of HbS–beta-0 thalassemia can be inferred but if the HbA<sub>2</sub> level is normal, consider the possibility of concomitant Sickle cell disease with iron deficiency. If HbS is greater than A and HbA<sub>2</sub> is elevated, a diagnosis of HbS–beta+ thalassemia can be inferred. If HbS and HbC are present in equal amounts, the diagnosis is HbSC disease.

### **Effect of recent blood transfusion on test result:**

It is best to avoid screening for hemoglobinopathies till 4 months after transfusion. If 4 months can't be awaited then at least wait till the next transfusion and get blood for test before transfusion. If person had transfusion and any of the transfused red cells are still present, misleading data and conclusions may result. A footnote should be present on all haemoglobinopathies results where transfusion history is not present, such as '**Result only valid if not transfused**'

### **Diagnosis by DNA analysis:**

Prenatal diagnosis can be offered to pregnant females when both partners have sickle cell disease or trait. DNA analysis can be performed using cells obtained by chorionic villus sampling or amniocentesis. CVS usually takes place at 10–12 weeks' gestation. Amniocentesis is usually done when a woman is between 14 and 16 weeks pregnant. DNA analysis provides the most accurate diagnosis in patients of any age, but it is still relatively expensive. Prenatal diagnosis is still not available in Nepal because of lack of experience gynaecologist who can perform Chorionic villus biopsy or amniocentesis and also there are at present no laboratory offers genetic diagnosis in prenatal samples.

### **Sickling test:**

It is method to detect sickle cells in blood microscopically

#### **Principle**

Sodium metabisulphite reduces the oxygen tension inducing the typical sickle-shape of red blood cells.

#### **Sample**

Fresh blood in any anticoagulant.

#### **Procedure**

- ❖ Mix 0.2 g of sodium metabisulphite in 10 ml of distilled water.
- ❖ Stir until dissolved. Prepare fresh each time.
- ❖ Mix 1 drop of blood with 1 drop of 2% sodium metabisulphite solution on a microscope slide.
- ❖ Cover with a cover slip and seal the edge with wax/vaseline mixture or with nail varnish.
- ❖ Allow to stand at room temperature for 1 to 4 hours.

- ❖ Occasionally the preparation may need to stand for up to 24°C. In this case put the slides in a moist Petri dish.
- ❖ Examine under a microscope with the dry objective.
- ❖ It is important to examine the preparation carefully and in particular near the edge of cover slip.

**Interpretation:**

- ❖ In positive samples the typical sickle-shaped red blood cells will appear
- ❖ False negative results may be obtained if the metabisulphite has deteriorated or if the cover slip is not sealed properly.
- ❖ A positive test does not distinguish the sickle cell trait from sickle cell disease.

**Advantages of Sickling Test:**

Can be carried out in a low resource setting  
 Easy to conduct and does not require intensive training.

**Limitations of Sickling Test:**

Will miss other hemoglobinopathies or compound heterozygotes

**Rapid diagnostic Test for sickle cell disease**

- ❖ 5 microlitre of blood required which can be obtained by finger prick or heel prick
- ❖ Instructions may vary from manufacturer to manufacturer. Follow manufacturers instruction
- ❖ One procedure is: mix 5 microlitre blood with solution provided. Mix well. Apply 2 drops of this blood mixed solution on application pad. Read the result after 5 minutes.



### **Advantages of rapid test**

- ❖ It is quick
- ❖ Does not require too much expertise for interpretation
- ❖ Can be performed in limited resource setting

### **Disadvantages of rapid test**

- ❖ It will cost more to the patient as kits are costly
- ❖ Will miss other hemoglobinopathies

If a person is tested by rapid diagnostic kit, then all positive and all negative with suspicious history should be referred to higher centre for HPLC or electrophoresis



## CHAPTER VI: LABORATORY DIAGNOSIS OF THALASSEMIA

### Whom to screen for thalassemia?

In high prevalence, thalassaemia screening should be offered to all areas of low prevalence will be required to offer screening for thalassaemia using the routine blood indices.

#### Test for thalassemia:

- ❖ when people request it
- ❖ History/family history suggesting thalassemia/hemolytic anemia or other hemoglobinopathies
- ❖ As part of antenatal screening for high risk population
- ❖ Spouse and family members of known trait or diseased person
- ❖ Persons with unexplained anemia
- ❖ When MCV less than 78 and MCH less than 27

#### Baseline investigation

- ❖ A baseline blood investigation should be requested in all cases before specific investigation of thalassemia. These investigations include
- ❖ Complete blood count with red cell indices and Red cell distribution width
- ❖ Reticulocyte count
- ❖ Peripheral smear examination : Microcytic hypochromic anemia, target cell and Heinz bodies are seen in thalassemia

#### *If MCV <78fL, MCH < 27pg, ask for quantification of Hb A2 and HbF*

- ❖ Hb A2 measurement
- ❖ For Hb A2 measurement, HPLC and Capillary Electrophoresis methods are acceptable for the quantitation of Hb A2. Scanning densitometry are not acceptable
- ❖ Hb F measurement
- ❖ HPLC, CE or two minute alkali denaturation is acceptable. The Kleihauer test is not appropriate for measurement

#### Action points of HbA2 and Hb F

- ❖ Hb A2 of equal to or greater than 3.5% should be taken as action point in the diagnosis of carriers of  $\beta$  thalassaemia.
- ❖ There are two action points for Hb F.
- ❖ If the MCH is equal to or greater than 27pg, the action point for Hb F is greater than 10%.

- ❖ If the MCH is less than 27 pg, the action point is greater than 5.0%.
- ❖ Hb A2 values less than 3.5 % with normal red cell indices and an Hb F level of less than or equal to 10% can usually be regarded as not significant for screening purposes.

### **What will be the result of screening?**

- ❖ No hemoglobinopathy detected
- ❖ Thalassemia trait
- ❖ Thalassemia major/Intermedia
- ❖ Other hemoglobinopathy

### **How to interpret screening the report of and advise further**

- ❖ No confirmatory test is necessary if the Hb A2 more than 3.5 % (but not greater than 8% if HPLC is method used for quantification.) and the red cell indices are typical of a carrier of  $\beta$  thalassaemia. HPLC cannot differentiate between Hb A2 and HbE so if Hb A2 is more than 8 %, it's likely Hb E. However capillary electrophoresis can differentiate between Hb A2 and Hb e
- ❖ Hb A2 may be overestimated in the presence of Hb S. However, this is not a problem as long as the percentage of Hb A is greater than Hb S.
- ❖ MCV 78fL or above, MCH 27pg or above, Hb A 2 less than 3.2 -----No beta thalassemia
- ❖ MCV 78fL or above, MCH 27pg or above, Hb A 2 more than 3.5---screening of family and spouse
- ❖ MCV less than 78pg, MCH less than 27fL, Hb A2 more than or equal to 3.5 ---beta thalassemia trait
- ❖ MCV less than 78fL, MCH less than 27pg, HbA2 less than 3.2, iron study..(if iron decreased, IDA; if iron normal alpha thalassemia carrier likely)
- ❖ MCV less than 78fL, MCH less than 27pg, HbA2 between 3.2 & 3.5: reanalyze CBC - repeat Hb A2 to confirm the value - consider B12/folate deficiency or liver disease/alcohol or HIV infection. If the results remain the same offer screening of spouse and family
- ❖ In a pregnant female or married person if the Hb A2 is equal to or greater than 3.5% or if the person's haemoglobin is less than 8g/dL and the Hb A2 is between 3.0% and 3.5%, testing of spouse should be done.
- ❖ Hb A2 values less than 3.5 % with normal red cell indices and an Hb F level of less than or equal to 10% can usually be regarded as not significant for screening purposes.

Diagnosing Thalassemia Major;

The presentation will be early as soon as Hb F will starts to get replaced by HbA . In CE/HPLC Hb F will be elevated (may be more than 90%) and there will no Hb A and variable HbA2. Peripheral blood smear will show marked anisopoikilocytosis, fragmented RBCs and polychromasia. Since level of HbF in neonate is high, thalassemia major should not be tested for in neonatal period. If thalassemia major is diagnosed, screening of siblings should be advised

#### Effect of transfusion on test result

It is best to avoid screening for hemoglobinopathy till 4 months after transfusion. If 4 months can't be waited then at least wait till the next transfusion and get blood for test before transfusion. If person had transfusion and any of the transfused red cells are still present, misleading data and conclusions may result. A footnote should be present on all haemoglobinopathy results where transfusion history is not present , such as 'Result only valid if not transfused'

#### Effect of Iron deficiency on HbA2 value

- The Hb A2 level may be lowered by up to 0.5% in cases of severe iron deficiency anaemia, however screening for haemoglobin variants and thalassaemia should proceed without regard to iron deficiency, suspected or proven. Any decrease in MCH should be regarded as potentially due to a haemoglobinopathy and the Hb A2 should be measured.
- In pregnant women there is no justification for delaying the investigation of haemoglobinopathies whilst treating iron deficiency, as this will delay the process of identifying at-risk carrier couples who should be offered prenatal diagnosis
- Screening test results do not enable definitive differentiation of iron deficiency and  $\alpha$  thalassaemia. Within the context of the screening programme,  $\alpha$ + thalassaemia is not regarded as a significant risk.

#### **Effect of alpha thalassemia on HbA2 value**

In the presence of an alpha chain variant reliable Hb A2 value may not be obtained .For this reason, where the person has suggestive red cell indices and HbA2 is normal or reduced , testing of spouse should be done

## Who will be missed by suggested methods?

- ❖ Possibly some  $\beta$  thalassaemia carriers obscured by severe iron deficiency anaemia.
- ❖  $\beta$  Thalassaemia carriers with a co-existing  $\delta$  chain mutation which is silent with the first line screening technique, or who have co-existing  $\delta$  thalassaemia.
- ❖  $\beta$  Thalassaemia carriers with co-existing Hb H Disease, as some cases have normal Hb A2 values.
- ❖  $\alpha 0$  Thalassaemia occurring outside the defined at risk family origins or in those women with an  $MCH \geq 25$ pg.
- ❖  $\delta\beta$  Thalassaemia carriers with Hb F  $\leq 5\%$ .
- ❖  $\gamma\delta\beta$  Thalassaemia carriers.
- ❖ Any significant haemoglobin masked by an unreported bone marrow transplant and/or adoption

## Screening for Alpha thalassaemia

Within the context of the screening programme,  $\alpha^+$  thalassaemia is not regarded as a significant risk. However it's important to screen for Alpha zero thalassaemia

There are three forms of alpha zero thalassaemia:

- ❖ Carriers of Alpha zero thalassaemia
- ❖ Alpha zero thalassaemia major (hydrops fetalis)
- ❖ Haemoglobin H disease.

1. Carriers of Alpha zero thalassaemia. People who carry alpha zero thalassaemia are perfectly healthy in themselves, but if both members of a couple carry alpha zero thalassaemia, they may pass alpha zero thalassaemia major on to their children.

2. Alpha zero thalassaemia major (Hb Barts hydropsfetalis). This can happen if a baby inherits alpha zero thalassaemia from both parents. It is a very severe anaemia that affects the unborn baby in the womb. The baby cannot make enough blood, and dies either before birth, or within a few hours of birth.

3 Haemoglobin H disease. This can happen when one parent has alpha zero thalassaemia trait and the other carries a milder form of alpha thalassaemia called alpha plus thalassaemia. People with Haemoglobin H disease are anaemic, but can usually lead a normal life without the need for any treatment.

Screening for carriers of alpha zero thalassaemia ( $\alpha 0$  thalassaemia)

The lack of a specific biomarker for the diagnosis of  $\alpha$  thalassaemia carriers creates particular problems in the context of a screening programme. 99% of  $\alpha 0$  thalassaemia cases have an  $MCH < 25$  pg. Many have occasional red cells containing Hb H inclusions but these are not always detectable by routine screening. Effort should be made to detect couples at risk of hydrops fetalis. Hydrops fetalis can result in a stillbirth. A mother carrying a fetus with

hydrops fetalis is at risk of obstetric complications such as toxemia and hypertension, particularly in the third trimester of pregnancy. Usually a baby only has Hb Bart's hydrops fetalis if both parents are carriers of  $\alpha^0$  thalassaemia. In cases where the fetus is at risk of Hb Bart's hydrops fetalis, ultrasound can identify fetal hydrops and measure the fetal middle cerebral artery peak systolic velocities, which are increased in fetal anaemia. If the middle cerebral artery peak systolic velocities are normal, the fetus is not significantly anaemic and the couple can be reassured, avoiding invasive treatment and the associated risks

### **When to perform genetic counseling?**

Genetic counselling with DNA studies should be performed when one spouse has suggestive red cell indices but normal or low Hb A2 and other is known to have beta thalassaemia, or has Hb S or E or Lepore and/or is at risk of alpha zero thalassaemia.

## Bibliography

## **Annex 1:**