HAEMOGLOBIN DISORDERS HAEMOGLOBINOPATHIES

BOOKLET THREE (3)

ABOUT Sickle Cell Disorders

about thalassaemia

BY Androulla Eleftheriou (B.Sc., M.Sc., Ph.D, MBA)
Michael Angastiniotis (MD, DCH)

THALASSAEMIA INTERNATIONAL FEDERATION1986
"In official relation with the W.H.O. - 1996"

THALASSAEMIA INTERNATIONAL FEDERATION - PUBLICATION No 13

CONTENTS

FOREWORD BY THE PRESIDENT

This booklet (number three) contains basic information about sicle cell disease (SCD). Whether you are a carrier or a patient, or simply interested in finding out more about SCD, we encourage you to read this booklet. Every effort has been made by the authors to include valuable information regarding the disease, its inheritance, prevention and treatment.

If you need to know more details on any aspect described in this booklet, we advise you to consult your physician or national health authority. The authors of this booklet, will also be very happy to answer your questions as far as possible.

> *I hope that this booklet, which constitutes part of our educational material, will contribute significantly to TIF's efforts in spreading awareness across the world about Haemoglobin disorders, their prevention and treatment.*

TIF is greatly indebted to Dr. Androulla Eleftheriou and Dr. Michael Angastiniotis, members of TIF's Scientific Advisory Panel, for their invaluable contribution to the preparation of three booklets including this one, which aim to provide important information in a simple manner to everyone interested in learning about β-thalassaemia (booklet one), α-thalassaemia (booklet two) and sickle cell disease (booklet three).

PANOS ENGLEZOS
PRESIDENT, TIF

ABOUT THE THALASSAEMIA INTERNATIONAL FEDERATION

The Thalassaemia International Federation (TIF) was established in **1987** with the mission to promote the establishment of national control programmes for the effective prevention and appropriate clinical management of thalassaemia, in every affected country of the world. TIF is today, a Federation "umbrella", comprised of *98 national thalassaemia associations from 60 countries*, embracing hundreds of thousands of patients worldwide.

TIF has been in official relations with the World Health Organisation (WHO), since 1996, and works closely with scientific and medical professionals in this field from more than 60 countries, as well as with international and European health bodies, pharmaceutical companies and agencies and other disease orientated patients' organisations.

TIF's educational programme is one of its most important and successful activities. It includes the organisation of local, national, regional and international workshops, conferences and seminars, as well as the preparation, publication and translation of leaflets, magazines and books for health professionals, patients/ parents and the community at large, *distributed free in more than 60 countries of the world.*

"UNITY IS OUR STRENGTH"
our Motto

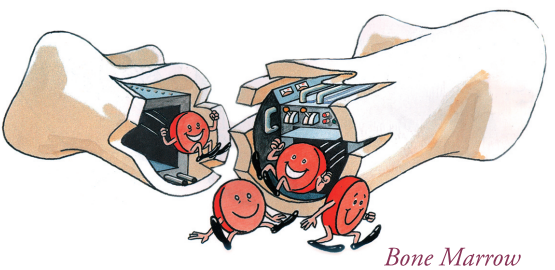
"*Equal Access to quality health care for every patient with Thalassaemia across the world*"
our mission

HAEMOGLOBIN DISORDERS HAEMOGLOBINOPATHIES

ABOUT SICKLE CELL DISORDERS

Introduction:

Haemoglobin disorders are a group of conditions affecting the red blood cells - an important part of the human blood - the vital fluid that brings nourishment, such as oxygen (O_2), hormones, proteins, fats and carbohydrates, to the body's organs and tissues and carries away waste substances such as Carbon Dioxide (CO_2), urea and uric acid.

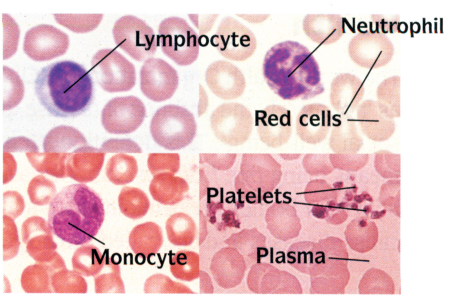


Bone Marrow

Blood (Whole Blood):

In adults, blood is exclusively produced in a special tissue called marrow, which is found in the central cavity of the bones (bone marrow). Blood consists of two major components:

I. the plasma, the yellow liquid, that constitutes about 55% of the volume of blood and contains water, salts and important proteins, and;



Composition of blood

II. the part that contains three types of cells, microscopic building blocks, trillions of which make up the human body, and which are:

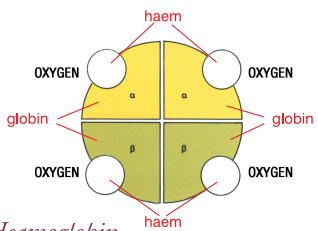
- The white cells or leucocytes
- The platelets or thrombocytes, and
- The red cells or erythrocytes

Each type of blood cell has specific functions and each contributes, in its own special way, to the well-being of the human organism, including protection against infection (white cells); limiting blood loss when a vessel is damaged (platelets) and provision of oxygen to tissues and vital organs (red cells).

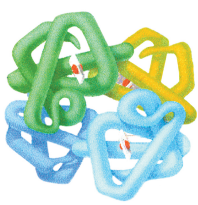
Many diseases in humans are caused by abnormalities in the blood and these are categorized according to the component of the blood affected (white cell diseases, platelet diseases and red cell diseases) Red cell diseases include amongst others the hereditary Haemoglobinopathies or Haemoglobin disorders, the most severe of which are the thalassaemias [alpha (α-) and beta (β-) and sickle cell disease, and are so called because they result from abnormalities of a special protein inside the red cells of blood called haemoglobin.

Haemoglobin:

4,500,000 - 5,000,000 red cells circulate in human blood and each one of them is packed with, 300 million molecules of Haemoglobin. Haemoglobin gives the red blood cells their oxygen carrying capacity, which is their most important function in blood. (Oxygen is essential for the growth and performance of the cells and organs of the human organism). The haemoglobin molecule itself consists of two major parts (i) the **globin** and (ii) the **haem**:



Heamoglobin



(i) The **globin** is a protein made up of smaller units, referred to as chains –the alpha (α) and the non-alpha such as Beta (β), Gamma (γ), Delta (δ), chains. The alpha (α) chains couple with beta (β) chains to make up the haemoglobin (HbA) which is the dominant one in adults, and up to 10% of the haemoglobin of the fetus. Alpha (α) chains also couple with other chains making up the haemoglobins found at various stages of human life, from conception, through fetal life to birth.

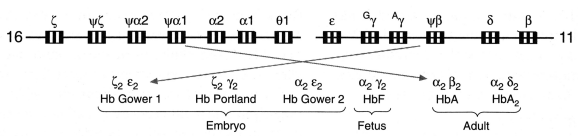


Fig. 2.11 The α- and β-globin gene clusters on chromosomes 16 and 11, respectively. In the extended α- and β-globin genes the introns are shaded dark, the 5′ and 3′ non-coding regions are hatched, and the exons are unshaded.

NOTE: The words alpha, beta, gamma, delta where met in this text will be referred to with their respective abbreviations α, β, γ, δ.

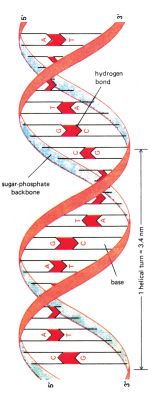
(ii) The **haem** part contains iron - a metal that is essential for the growth and normal functioning of the cells. Iron has the capacity to easily bind and lose oxygen, providing the haemoglobin molecule the capacity to carry and distribute easily oxygen to tissues and organs of the body. Adults have about 4g of iron in their body, 75% of which is used to synthesize the haemoglobin molecules of the red cells.

The level of haemoglobin found in a routine laboratory blood examination will, therefore, reflect the level of the individuals' iron.

Inheritance:

Haemoglobinopathies are genetic disorders, that are passed on from parents to children according to what is referred to in biology as *"Mendelian recessive autosomal pattern of inheritance"*. i.e. all characteristics are passed on from parents to children through genes -- the biological units of inheritance that provide all the information needed for controlling growth and development throughout human life. The contribution of genes from both of the parents (recessive) is essential for the inheritance of these disorders, which can affect both male and female children alike (autosomal).

Deoxyribonucleic Acid, a chemical substance often referred to by its abbreviation, DNA, constitutes the key part of genes, of which a great number are needed to carry out the many and complicated biological functions of the human organism. Genes linked together in the cell on long piles of DNA are called chromosomes, of which there are 23 pairs, half inherited from one, and half from the other, parent.



DNA DOUBLE HELIX

In the case of adult Haemoglobin, for example, the production and synthesis of its α and β chains, which constitute its major component, is controlled by genes on specific chromosomes. Four (4) α-globin genes on chromosome 16 and two (2) non-α-globin such as (β, γ and δ) genes on chromosome 11, are responsible for the production, in exactly equal numbers, of α and β chains, respectively.

Chromosomes

Any defect in a gene responsible for the production of α-chains (or as referred to in scientific terms "coding" for α-chains), may cause reduced production of these chains, resulting in α-thalassaemia carrier status. If the defect involves more genes then less α-chain is produced and the individual may be affected more significantly. Similarly, a defect in the gene coding for β-chains (the β-globin gene) may cause a reduction or total loss of β-chains. The degree of β-chain reduction will determine whether an individual is a β-thalassaemia carrier, or a patient with β-thalassaemia intermedia or major.

In contrast to the thalassaemias in which the production of a globin is affected, there are conditions in which the defect in the gene results in the production of wrong kinds of proteins - called abnormal or structural haemoglobin variants - whose structure as well as their function, are different from that of the common adult haemoglobin (HbA). Reference is made to their inheritance and clinical outcome in booklets 1 and 3.

The major Haemoglobin disorders are:

α- chain disorders	β- chain disorders
α-thalassaemias	**Sickle cell disorders**
HbH disease	Sickle cell anaemia (HbSS)
α-thalassaemia Hydrops Fetalis	HbS/β-thalassaemia
(=Hb Bart's Hydrops Fetalis)	HbSC disease
α-chain variants	HbSD disease
	Other rare sickling disorders
	HbS/E
	HbS/O Arab
	β-thalassaemias
	β-thalassaemia major
	β-thalassaemia intermedia
	HbE/β-thalassaemia
	Other rare thalassaemias

This booklet describes how the variant haemoglobin S (HbS) is passed on to children, according to their parents' genetic characteristics. Reference is also made to variants HbE, HbC, HbD and HbO Arab. In booklets 1 and 2 the inheritance of β- and α-thalassaemia, as well as other variants in combination with these, are described respectively.

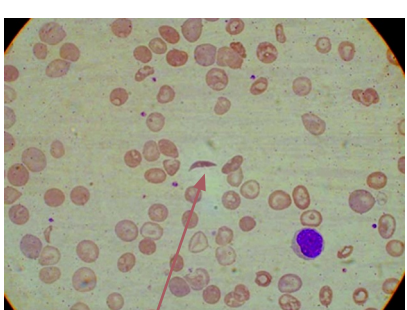
1) **HbA/HbA**
   If both parents have the common adult haemoglobin (HbA), each and every time they are expecting a child there is a 4 in 4 chance (100%) that their child will inherit this common haemoglobin. There is no possibility of their children inheriting any unusual, abnormal haemoglobin.

2) **HbS/HbA**
   When one of the parents is a carrier of the variant sickle haemoglobin (HbS for short) and the other parent carries the common haemoglobin (HbA) each child born to these parents has a one in two (or a 50%) chance of inheriting the HbS variant i.e. he/she will be a carrier of HbS, just like one of his/ her parents. Other names describing the individual who carries the HbS variant include:
   I. Carriers of the Sickle cell trait
   II. Individuals heterozygous for haemoglobin S (HbS)
   III. Sickle Cell carriers

About carriers of the Sickle Cell trait

Carriers of haemoglobin S do not have a disease. They have no physical or mental symptoms and do not require a special diet, medical advice or treatment.

Their red blood cells are usually similar in size to those of non-carrier individuals, since the quantity of haemoglobin is not reduced. Smaller red cells are sometimes seen in those carriers of HbS who have co-inherited α-thalassaemia, a combination common in many populations. Otherwise, the red cells examined under the microscope may be indistinguishable from normal red cells.



Sickle Cell

Sometimes altered shapes (poikilocytosis) and cells with pointed ends are seen but the typical sickle shaped cells, characteristic of this disorder in the homozygote state, are not often seen in carriers.

The carrier status, cannot, (as in the case of β and α-thalassaemia) become a disease over time. Indeed, most will be unaware that they are carriers unless specifically tested.

Carrying haemoglobin S has no effect on health, length or the quality of life. The rare exception to this occurs when a carrier is in a situation of severe lack of oxygen in which case pain and blood vessel blockage may be experienced.

What about pregnant women who are carriers?
Like other pregnant women, women who carry HbS can become iron deficient and may need extra iron. The anaemia will improve after the baby is born. Carrier pregnant women are also more prone to urine infections compared to non-carriers.

Is there any treatment to stop being a carrier?
No, a person who is born carrying HbS will always carry it throughout his/ her life.

Can the Sickle Cell trait be transmitted or acquired at a later stage in life?
The Sickle cell trait cannot be acquired or transmitted through the environment, transfusion or other means by which people become infected.

Can carriers donate blood?
Carriers may be suitable blood donors if their haemoglobin level is according to national donors' inclusion criteria.

How does one know whether he/ she is a carrier?

In most cases, simple but specific laboratory tests can identify whether a person carries the sickle cell trait or trait of any other Haemoglobin disorder. Genetic counselling before and after the tests i.e. provision of reliable information, advice and guidance, by specialists in the field, will cover important aspects of prevention, including:

- Where to be tested
- What the test results mean

- What it means to be a carrier
- What options are available to couples where both are carriers
- The nature and treatment of sickle cell disorder

Laboratory Testing to establish whether one is a carrier.

Laboratory testing for sickle cell and other haemoglobinopathies, includes a routine blood test known as a Complete Blood Count (CBC), which involves measuring blood parameters related to the size and volume of red blood cells, Mean corpuscular Volume (MCV), and the content of haemoglobin in the red cells, Mean Corpuscular Haemoglobin (MCH) The MCV and MCH may be normal in individuals carrying the sickle cell trait, in contrast to those carrying α and β-thalassaemia trait.

Other tests to determine the presence of the carrier state, include a laboratory process known as haemoglobin electrophoresis, which enables measurement of the quantity of HbA and HbA2, the main and minor components of common adult haemoglobin respectively and other haemoglobins including fetal haemoglobin (HbF) and HbS. In the case of sickle cell carriers the HbA2 level is normal, but HbS fraction will constitute up to 40% of the total haemoglobin.

Other ways to diagnose the disease include another method of electrophoresis known as isoelectric focusing (IEF), and another methodology known as High Pressure Liquid Chromatography (HPLC)*. This latter is an expensive laboratory technology but which due to its sensitivity and specificity and its rapid daily output, is considered today as a method of reference for confirming the diagnosis of haemoglobinopathies. Samples from laboratories which are not equipped with specialised technologies such as IEF or HPLC may be referred to a National Reference Laboratory which can deal with difficult to identify cases and use such specialised tests.

If the above tests are inconclusive, especially if combinations with other variants or thalassaemias are involved, and do not allow the laboratory scientists to provide a confirmed diagnosis, other more specialised tests are available. These are genetic tests i.e. tests which examine the genetic material, the DNA of blood, from the individual but also often from other members of the family so that a definite diagnosis can be made.

*Bio-Rad HPLC for example is considered by many as the Gold Standard for Hb determination.

3. HbS/HbS

If both parents have sickle cell trait (HbS), each and every time they are expecting a child there is a one in two or (50%) chance that the child will also be a carrier of the sickle cell trait, and a one in four (25%) chance that the child will be completely unaffected. i.e. inherit the common haemoglobin (HbA) and a one in four (25%) chance that their child could inherit Sickle Cell Disease (HbSS), otherwise referred to as sickle cell disease or homozygous for Sickle Cell disease.

Other possibilities include the following combinations:

(FIG A)

4. HbS/HbSS

If one parent has sickle cell trait (HbS) and the other parent has sickle cell anaemia (HbSS), each and every time they are expecting a child:

There is a two in four chance (50%) that their child could inherit sickle cell trait (HbS) and a two in four chance (50%) that their child could inherit sickle cell anaemia (HbSS).

5. HbS/β-thalassaemia

If one parent has sickle cell trait (HbS) and the other parent has β-thalassaemia trait (HbA,Thal), each and every time they are expecting a child:

There is a one in four chance (25%) that their child could inherit the usual haemoglobin (HbA), a one in four chance (25%) that their child could inherit sickle cell trait (HbS), a one in four chance (25%) that their child could inherit

β-thalassaemia trait and a one in four chance (25%) that their child could inherit sickle cell β-thalassaemia anaemia (HbS/βThal).

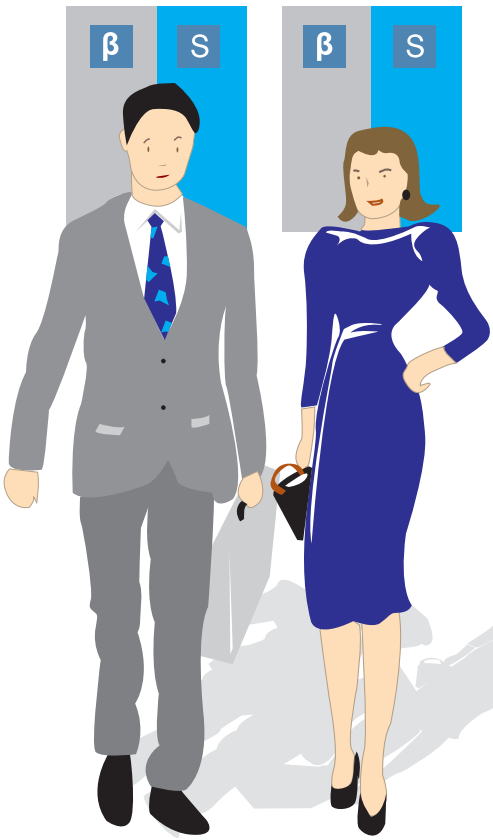
FIG 1

6. HbS/HbC disease

If one parent has sickle cell trait (HbS) and the other parent is a carrier of another haemoglobin variant, haemoglobin C (HbC), each and every time they are expecting a child:

There is a one in four chance (25%) that their child could inherit the common haemoglobin (HbA), or a one in four chance (25%) that their child could inherit haemoglobin C trait (HbC), or a one in four chance (25%) that the child could inherit sickle cell trait and a one in four chance (25%) that their child could inherit sickle haemoglobin C disease (HbS/C).

BOTH PARENTS HAVE SICKLE CELL TRAIT



UNAFFECTED

β-THALASSAEMIA

HbS

CHANCES ARE:

25% PATIENTS WITH SCD DISEASE (HbSS)

50% CARRIERS OF SICKLE CELL TRAIT

25% UNAFFECTED

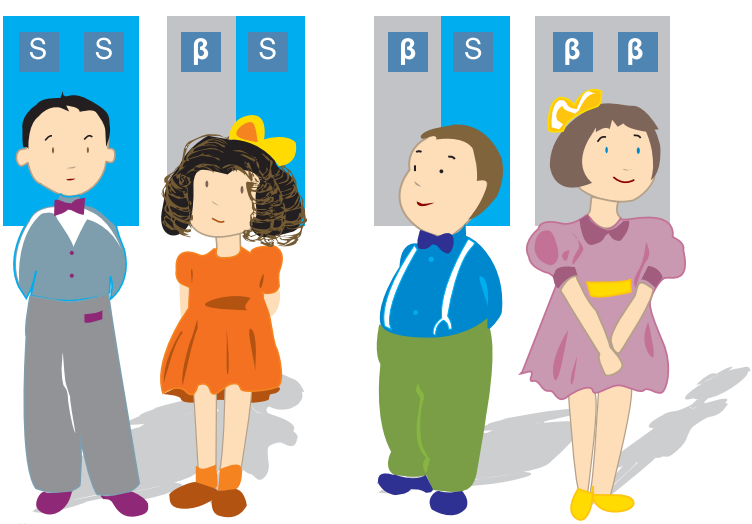
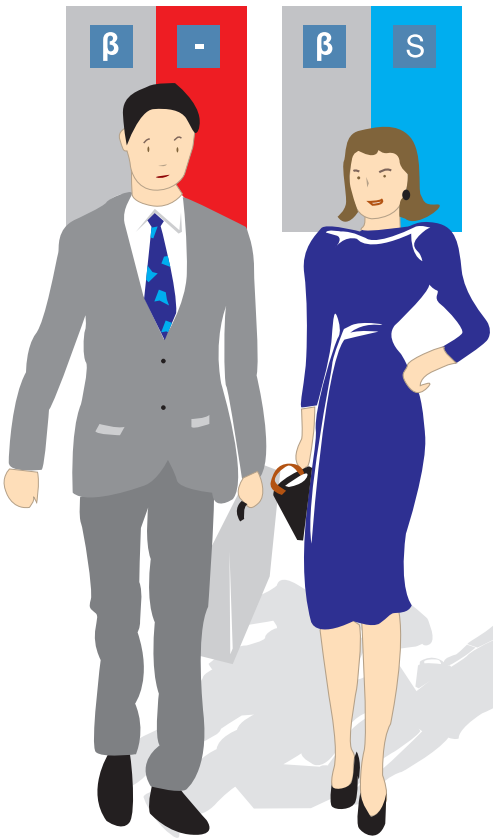


FIG1

HbS AND β-THALASSAEMIA

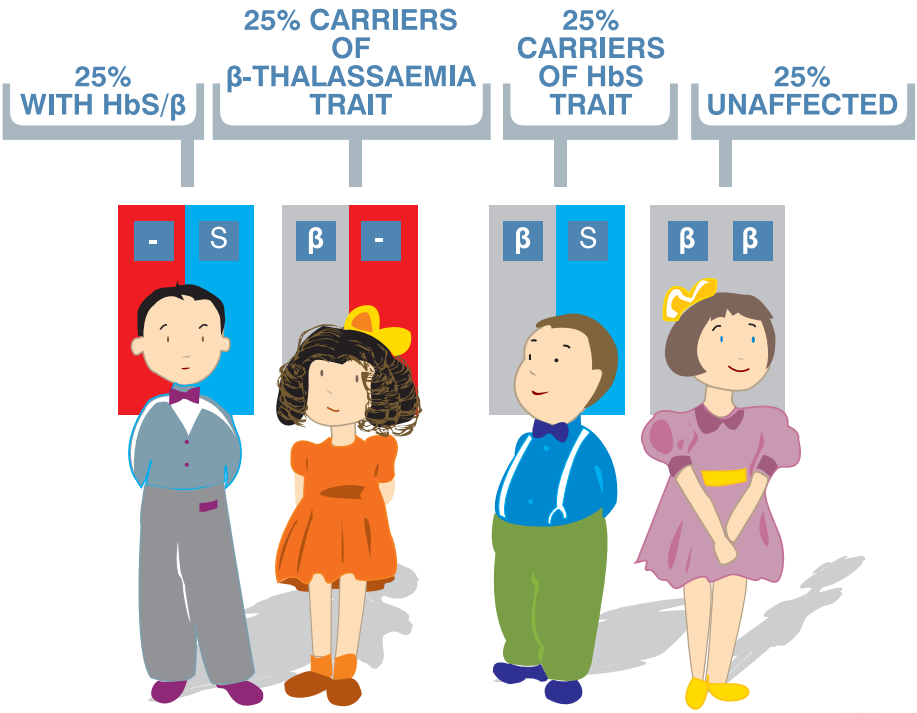


UNAFFECTED

β-THALASSAEMIA

HbS

CHANCES ARE:



7. HbS/HbD Punjab

If one parent has sickle cell trait (HbS) and the other parent has haemoglobin D (Punjab), each and every time they are expecting a child:

There is a one in four chance (25%) that their child could inherit the common haemoglobin (HbA), a one in four chance (25%) that the child may inherit haemoglobin D Punjab trait (this is harmless), a one in four chance (25%) that the child may inherit haemoglobin S trait (this is harmless) and a one in four chance (25%) that the child may inherit haemoglobin D Punjab from one parent and haemoglobin S from the other. This child would have haemoglobin S/D disorder.

8. HbS/HbE

When one parent carries Haemoglobin S and the other carries Haemoglobin E, in each pregnancy there are four possibilities: There is a one in four chance (25%) that the child may not be a carrier at all, a one in four chance (25%) that the child may carry haemoglobin E (this is harmless), a one in four chance (25%) that the child may carry haemoglobin S (this is harmless) and a one in four chance (25%) that the child may inherit haemoglobin E from one parent and haemoglobin S from the other. This child would have haemoglobin S/E disorder.

9. HbS/HbO Arab

Where one parent carries haemoglobin S and the other parent carries haemoglobin O Arab, in each pregnancy there are four possibilities:

There is a one in four (25%) chance that the child will inherit the common haemoglobin, a one in four (25%) chance that the child may carry haemoglobin O Arab (this is harmless), a one in four chance (25%) that the child may carry sickle cell (this is harmless) and a one in four (25%) chance that the child may inherit haemoglobin O Arab from one parent and haemoglobin S from the other. This child would have haemoglobin S/O Arab disorder.

Clinical Outcome of inheriting HbS with other variants

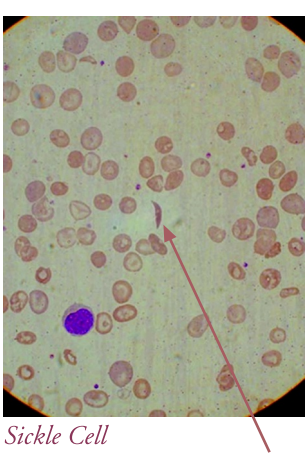
HbS/HbC, HbS/HbD Punjab, HbS/HbE and HbS/HbO Arab disorders are sickle cell disorders. They are rare and are generally a mild type of

sickle cell disorder. Some, however, may develop various degrees of anaemia and have attacks of severe pain in joints or other parts of the body from time to time. A few may even experience severe health problems and need frequent admissions to hospital. People with these disorders should attend a sickle cell clinic regularly for a check-up and advice.

At present it is not possible to predict whether a particular couple could have children with mild, moderate or severe HbS/HbC, HbS/HbD Punjab, HbS/HbE or HbS/O disorder. It is possible, however, to test a baby for these disorders early in pregnancy. "At risk" couples should see an expert counsellor in haemoglobin disorders to discuss their options. They should do this before starting a pregnancy, or as early in pregnancy as possible.

Inheritance of β-thalassaemia trait from one parent and HbS from the other, will result in a haemoglobin disorder (HbS/β), the clinical outcome of which is similar to sickle cell disease, which is very different from β-thalassaemia major/intermedia and, consequently is managed in the same way as sickle cell disease. The clinical severity of HbS/ β-thalassaemia varies just as it does in sickle cell disease.

What is Sickle Cell Disease or Anaemia



Sickle Cell

In this condition almost all of the haemoglobin in the patient's blood is HbS. The red cells which contain this variant will change in shape, from a biconcave disc to a crescent or sickle shape, but also in flexibility, becoming considerably more rigid. This means that they cannot change their shape, as red cells with common Hb (HbA) do, so that passage through small blood vessels is difficult. Loss of flexibility can become serious in conditions of lack of oxygen or in the case of an infection with fever, or when dehydrated, and in these situations the sickle red cells greatly increase in number and can block the passage of blood (sickling crisis); this results in the patient feeling pain in the area of the body which is not receiving blood. Such events can also be severe enough to damage tissues such as joints, spleen, kidneys and even the brain. In addition, as these altered red cells (sickle cells) do not survive in the circulation for as long as normal cells, and are thus continuously destroyed, the patients

experience a degree of anaemia, which may become severe under certain circumstances, leading to a need for blood transfusion.
As a chronic disorder, Sickle Cell Anaemia requires treatment in specialized centres aimed at both preventing and managing complications. This includes preventing infections by means of immunizations and regularly taking penicillin. It also means managing pain which may be severe enough to require hospital treatment. In order to prevent effectively some of these complications, it is necessary to have the patient under observation from early childhood. To provide the affected child with appropriate medical care aimed at preventing and minimizing the development of complications, a policy of newborn screening is recommended so that such a child may be identified from birth.

Developments in the Management of Sickle Cell Disease

Sickle cell disease affects people in different ways. Some people are mildly affected, whilst others are severely affected, even within the same family. The reasons for this are not always clear but several things have been linked with the severity of sickle cell disease. For example, the level of baby haemoglobin F (HbF), which some people continue to make into adulthood is important. Normally, the level of haemoglobin F falls to about 1% by the end of the first year of life and stays at this level right through adulthood, but children with sickle cell anaemia may go on producing higher that usual levels of haemoglobin F for longer and levels above 7% appear to be related to less sickling crisis and fewer complications of sickle cell disease. The level of HbF may be boosted by the use of certain drugs, which is currently the most promising method for reducing sickling in the blood cells. The advantage of fetal haemoglobin is that it does not sickle and its presence prevents red blood cells from sickling.

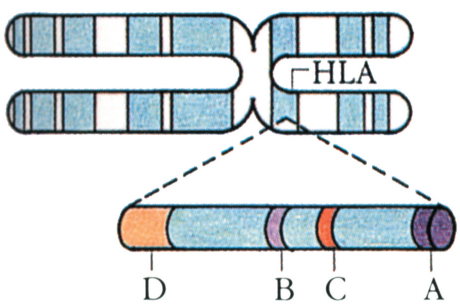
Hydroxyurea

Several drugs have been shown to increase production of fetal haemoglobin; of these Hydroxyurea is the most promising, and the one that is currently being prescribed. There is good evidence now that Hydroxyurea reduces sickling crises and the need for blood transfusions. Hydroxyurea may not be suitable for all patients with SCD. It has the potential to reduce bone marrow activity, which increases the risk of infection. It should not be used for patients who are likely to become

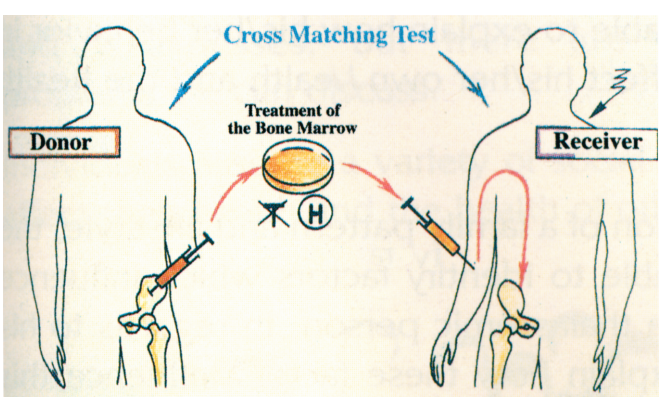
pregnant or those who have difficulty following the instructions given for the treatment.
Careful follow-up is needed, with regular visits to the clinic. It is important to understand that Hydroxyurea is not a cure for sickle cell disease. It is an effective treatment for preventing or reducing sickling crisis and its effect will only last as long as the person is taking the drug.

Bone Marrow Transplantation (BMT)

This is the only cure for sickle cell disease at the moment. BMT involves taking normal bone marrow from a 'matched' relative or donor and giving it to the person with sickle cell disease. This is after they have had treatment with strong medicines to wipe out their own bone marrow which is producing the sickle red blood cells. Whilst this treatment has been performed successfully on many patients with sickle cell disease, there are several problems. It is best done using a matched brother or sister, possibly before any complications have arisen, but are expected to develop in the future. As the severity of sickle cell disease can be so variable it is difficult to justify a high risk treatment like BMT where some will die, and some will survive with long term problems, without some way of selecting those likely to benefit or those more in danger of serious complications. One test which measures the blood flow in the brain (Transcranial Doppler) can identify patients at risk for developing a stroke and so these may be good candidates. The risks and benefits of transplantation must be assessed in each individual case.



HLA - typing



Procedure of BMT

As in the case of β-thalassaemia, it is often difficult to find a relative with a matching bone marrow, making this curative method applicable only to a proportion of patients. Rates of success and complication free survival vary and depend not only on the patients health status prior to BMT, but on the quality and expertise of the BMT centre.

Gene Therapy

In future it is likely that gene therapy will be offered, by replacing the defective gene with a normal gene. Research in this area has made significant advancements in recent years, but application of these to humans for a final cure still has a long way to go. To find out more about these developments talk to your counsellor, or doctor.

How is Sickle Cell Disease diagnosed?

A child born with Sickle cell disease will show no visible signs of the disease. The baby may be diagnosed if a Neonatal screening programme is available in the country where the family live. This is particularly important if the parents have not been tested, no prenatal tests were carried out, and there is no other affected child in the family. It is possible to diagnose Sickle cell Disease at this very early age by means of simple, but specific laboratory tests, including electrophoresis, IEF and HPLC, as described earlier for the diagnosis of sickle cell trait.

Haematological methods commonly used to diagnose Sickle Cell Disease

(i) Haematological indices. These haematological parameters are measured by electronic equipment - a red cell counter - which assesses the size and volume of red blood cells and the amount of haemoglobin contained in them.
Some haematological indices most commonly found in patients with SCD are shown below:

	Range, (Mean)
Hb g/dl	4.1 - 13 (8)
MCV fL	70 - 90 (87)
MCHC g/dl	27 - 41 (33)

(ii) Blood film and RBC morphology. Observed under a microscope, the red blood cells in the majority have abnormal shapes: mainly the sickle- shaped red cells (around 10% of all the cells) and the

marked poikilocytosis (varied shapes) and of different sizes (anisocytosis).

(iii) Haemoglobin electrophoresis. This is a process that separates the different proteins that make up a haemoglobin molecule - i.e. HbA, HbA2, and HbF. A diagnosis of Sickle Cell Disease is indicated where levels of HbS are higher than normal and may vary between 20-90%. HbA2, which usually accounts for up to 3% of normal adult haemoglobin, ranges from 1.5 to 3.6% and HbF ranges from 5 to 10% (occasionally up to 40%). The variability of these measurements depends on whether there is HbS/S or if there are other combinations such as with β- and α-thalassaemia.

(iv) Genetic tests. These are specialised ways of confirming diagnosis and/ or obtaining more specific information using DNA investigation. For example, with such tests the mutations (the genetic changes) that cause the condition are identified, information that in addition to confirming a diagnosis, may also provide an indication of the clinical severity of the disease.

It is very important to confirm an early and accurate diagnosis of sickle cell disease to prevent development of, and treat promptly any serious complications.

Can an unborn baby be tested in the womb?

There are a number of ways of testing to find out if the unborn baby has a genetic condition. These tests are called pre-natal diagnosis (PND). They will tell you which haemoglobin type your baby has inherited from you and your partner. It is worth thinking about these options before you become pregnant so that you will have had a chance to find out as much as possible about the tests beforehand.

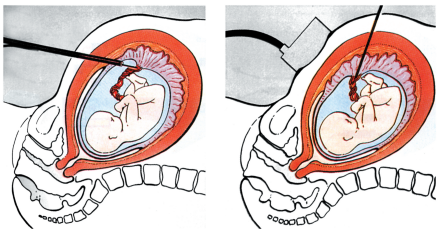
(i) Amniocentesis:

Amniocentesis is performed in the second trimester of pregnancy, after about 15 weeks' gestation. Using ultrasound as a guide, a trained obstetrician inserts a very thin needle through the mother's abdomen. A small amount of amniotic fluid containing cells from the fetus is withdrawn. This is then analysed in the laboratory to determine whether the fetus has sickle cell disease.

The risks that this test pose to the mother and the fetus are not

significant. There is a small risk of miscarriage, which occurs in 1:200 - 1:400 cases (less than 0.5%). The specialist Obstetrician, however, will be able to explain and discuss in detail all aspects of this test.

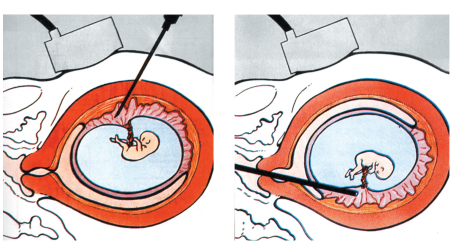
Amniocentesis *Cordocentesis*



(ii) Cordocentesis (sampling of fetal blood)

Under ultrasound guidance, a fine needle is inserted through the abdomen into the fetal umbilical cord, through which a small volume of blood is aspirated. Fetal blood is separated out and analysed in the laboratory. In skilled hands as much as 100% of pure fetal cells are obtained from the first attempt in the majority of cases. The Obstetrician specialising in prenatal examination will be best able to explain and discuss with you causes of failure in obtaining pure fetal blood as well as any other potential risks, when undergoing the procedure. Cordocentesis is performed after 18 weeks into pregnancy. The risks include miscarriage (1 – 2 %), blood loss, infection and leaking of amniotic fluid. Early and specific diagnosis by molecular methods has almost completely replaced cordocentesis which is now mainly indicated only in pregnant women who report late.

(iii) Chorionic Villus Sampling (CVS)



CVS

CVS is a method of diagnosing haemoglobin disorders in the fetus and can be performed earlier than amniocentesis, at about 10-11 weeks' gestation. Using ultrasound as a guide, the specialist obstetrician removes a small sample of the chorionic villi i.e. cells that contain the same genetic information as the fetus and which will eventually form the placenta. The cells are removed either by a thin needle inserted through the mother's abdomen (transabdominal) or a thin catheter inserted through the vagina (transcervical). The cells are then analysed and a diagnosis made. There is a small risk of miscarriage (up to 2%, but much less if the Obstetrician is experienced) and an even smaller risk of infection or bleeding. There is, in addition, a very small risk of limb abnormalities, which is virtually excluded if CVS is performed after 10 weeks.

As with other prenatal diagnosis methods, information on potential risks and benefits of using this procedure are provided to the couple by the specialist Obstetrician.

How is the diagnosis of the fetus made after obtaining samples using the above methods?

Amniocentesis and CVS are both based on DNA, otherwise known as genetic testing and involve identifying the genetic abnormality (mutation) present in parents. This kind of testing constitutes the most accurate means of diagnosing inherited diseases, and, as with all tests, there is a possibility of error, albeit a very small one.

In the case of CVS, for example, laboratory scientists study the haemoglobin genes contained in the DNA of cells from the chorionic villi to see if the baby will be healthy, with unaffected genes, whether it will be a sickle cell carrier or whether it will have affected Hb genes and have sickle cell disease.

If the test shows that the baby is affected, the couple may decide to either proceed with the pregnancy, accepting that lifelong treatment will be necessary, or to end the pregnancy. If pregnancy termination is the choice, however, this is done in one of two ways, depending on the stage of the pregnancy.

Termination of Pregnancy

Early termination

Early termination can be carried out when a woman is less than 14 weeks pregnant. The couple should be given all information and their concerns and worries should be addressed by appropriate counselling. They should, for example, be informed by the Obstetrician and/ or Counsellor that termination will not reduce the woman's chance of having another baby and that each pregnancy conceived by an at-risk couple carries the same risk of producing an affected child.

In addition, it should be clearly explained that If the couple wishes to know whether any subsequent babies conceived carry sickle cell disease prenatal diagnosis will have to be carried out again, involving exactly the same procedures and with the same benefits and risks.

Late termination

The procedure for terminating a pregnancy at over 14 weeks involves inducing labour of the pregnant woman by introducing hormones (prostaglandin). The labour may last for several hours and the procedure

is much more psychologically disturbing for the woman than an early termination. Again, as with early pregnancy, this type of termination does not affect the woman's ability to become pregnant again, and the Obstetrician will provide all information and answer all questions regarding this choice of termination.

Other approaches

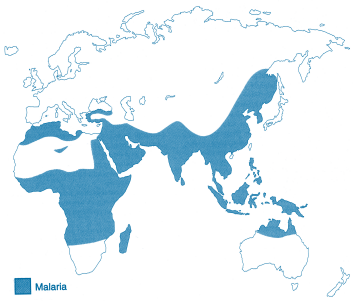
Prenatal diagnosis and the termination of pregnancy are methods that may not be acceptable to every couple at risk or to certain populations due to religious and cultural beliefs.
Unfortunately, however, prevention cannot rely on the identification of carriers alone, and screening cannot be effective and successful in the absence of prenatal diagnosis and pregnancy termination.

Other methods of prevention have been developed, while others are still in the research stage, both to minimise intervention and psychological stress, as well as to be more culturally and religiously acceptable by certain populations and individual couples. For example, analysis of **fetal cells circulating in the mother's blood** is a test where significant research has focused in the last decade. This however, has limitations and cannot offer to date a reliable alternative to classical prenatal testing. **Pre-implantation genetic diagnosis (PGD)**, is another aproach which involves the use of in-vitro fertilisation techniques and DNA technology. A few cells are taken from the very early embryo or an egg free of Hb disorders is selected from a woman carrier, which is then fertilized in the laboratory and introduced into the womb.

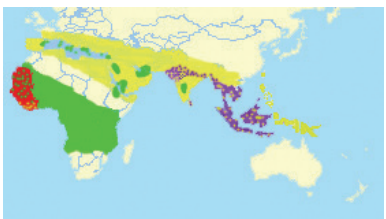
PGD may prove more acceptable to those individuals opposed to the termination of pregnancy, and may thus become more widely used. The technique is still costly and technologically demanding, but it has begun to be more and more widely accepted and used. Replacing the tested embryo in the womb does not always result in a pregnancy (only in about 20 – 30%) and many choose to place 2 or 3 embryos sometimes resulting in multiple pregnancies.

Where do we find Sickle Cell and other Hb disorders

The Sickle cell haemoglobin variant is believed to have originated in Africa where it is most commonly encountered, while India is considered as an additional origin. From these areas migrations have taken the gene to almost all regions of the world.



Countries affected by malaria before establishment of control programmes



Map of haemoglobin disorders worldwide "Guidlines to the clinical Management of Thalassaemia " 2000

It is believed, and scientifically demonstrated for some of the major Hb disorders, that in these areas of the world, where the haemoglobin genes originally occurred which were endemic for malaria, the human organisms underwent a change in their genes - a genetic adjustment, or a mutation, as called in biology. This led to important changes in the environment of the red cells that prevented malaria parasites from growing and multiplying in them, giving these people an advantage over those in whom this genetic change did not occur. Carriers of the thalassaemia trait (α and β) as well as carriers of other Hb disorders, such as sickle cell anaemia, were thus 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.

Population migration and intermarriage between different ethnic groups has introduced thalassaemia and sickle cell, as mentioned earlier, into almost every country of the world, malaria endemic or not, including northern Europe and other countries where thalassaemia did not previously exist.

According to recent epidemiological information about 7% of the global population carries an affected haemoglobin gene, with between 300,000-500,000 affected children born annually. About 80% of them have sickle disorder and the majority live in the developing world, mainly Africa. Even to date a significant number of affected children, born in the developing regions of the world, die undiagnosed or misdiagnosed, receiving sub-optimal treatment or left untreated altogether. ("World Bank 2006, report of a joint WHO - March of Dimes meeting 2006)

The World Health Organisation and patient's organisations like TIF work closely to support national health authorities to promote the establishment of effective national control programmes, to reduce the number of annual affected births and to improve the medical care and the quality of life of patients with severe Hb disorders across the world.

THALASSAEMIA INTERNATIONAL FEDERATION'S PUBLICATIONS

1. "Blood Safety Kit" (1999)
   [In English]

2. "Guidelines to the clinical Management of Thalassaemia" 2000
   [Translated into 6 languages]

3. "Compliance to Iron Chelation therapy with Desferrioxamine" 2000 - Reprint 2005
   [Translated into 4 languages]

4. "About Thalassaemia" - 2003
   [Translated into 11 languages]

5. "Prevention of Thalassaemias and other Haemoglobinopathies " Volume I (2003)
   [Translated into 2 languages]

6. "Prevention of Thalassaemias and other Haemoglobinopathies" Volume II (2005)
   [Translated into English]

7. "Patients' Rights" 2007
   [In English]

8. "A guide to the establishment and promotion of non-government patients/parents' organization" 2007
   [In English]

9. Updated version of the book "Guidelines to the Clinical Management of Thalassaemia" May 2007
   [In English]

10. Children's dialogue: "Thalassaemia and Me" 2007
    [In English]

11. Booklet One: About β-thalassaemia 2007

12. Booklet Two: About α-thalassaemia 2007

13. Booklet Three: "About Sickle Cell Disease" 2007

14. TIF's Educational Folder 2007

HOW TO CONTACT TIF

Thalassaemia International Federation
P.O Box 28807, 2083 Nicosia, Cyprus
Tel: 00357 22319129/ 22319134
Fax: 00357 22314552
E-mail: thalassaemia@cytanet.com.cy
Web-site: www.thalassaemia.org.cy

Published by the:
Thalassaemia International Federation

No. 13

ISBN: 978-9963-623-47-1