












































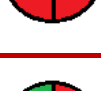

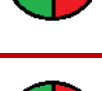










A Hand Book on Sickle Cell Disease



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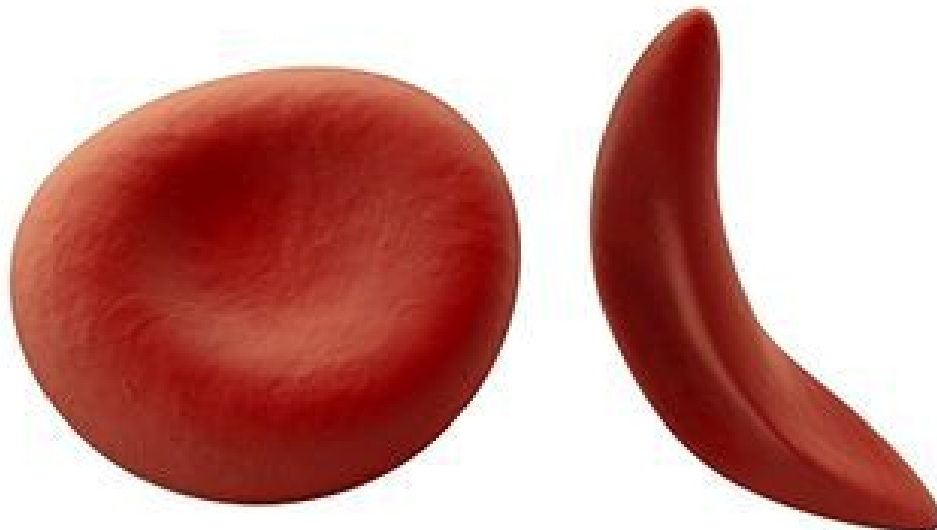
Please match following 'Sickle Kundli' before marriage

S. No.	Father	Mother	Possible Haemoglobin pattern of children (This does not show the order of child births)					Advice related to marriage
1							All Normal	Get married
2							50% Normal, 50% Carriers	Get married
3							50% Normal, 50% Carriers	Get married
4							All Carriers	Get married
5							All Carriers	Get married
6							25% Normal, 25% Patients, 50% Carriers	Think
7							50% Patients, 50% Carriers	Should not get married
8							50% Patients, 50% Carriers	Should not get married
9							All Patients	Should not get married

Note: Men and women belonging to serial numbers 6, 7, 8 and 9, should not get married.



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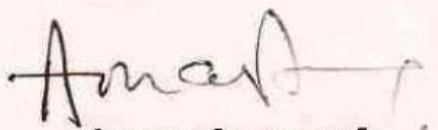
Date **11 2 NOV 2020**

Approximately, 10% of Chhattisgarh population carries the Sickle Cell Disease (SCD) gene and constitutes a large socio-economic burden on the economy of the state. However, planned measures were not taken to manage and treat the SCD patients since long. As a state, we are obligated to take action in the best interest of public health and have established a dedicated institute to cater the needs of SCD in Raipur, the capital city of the state.

The Institute is committed to improve the health status and quality of life of the sickle cell disease patients through state-of-the-art patient care system and innovative research along with training and counseling programs to improve the awareness regarding this devastating disease. The Institute will maximize the accessibility to healthcare by providing diagnosis, treatment and research. In addition, intensive training will be given to the health care staff as well as to the family members to support the sufferers.

The Institute will also provide an interface for networking among the experts in different fields to pursue interdisciplinary research to reduce the menace of SCD in the state and country.

Further, this initiative will reduce the psycho-social and economical burden on patients and their family. I am confident that Sickle Cell Institute Chhattisgarh would turn out to be a milestone in our quest for sickle cell disease patients and also pledge my wholehearted support to this key initiative.


Amar Agrawal

Prologue



In India, the sickle cell gene is distributed across the country, predominantly in Chhattisgarh, Madhya Pradesh, Orissa, Jharkhand, Maharashtra, Gujarat, Andhra Pradesh, Kerala, Karnataka, Tamil Nadu and some Northeastern states. Initial screening for sickle cell allele across the state of Chhattisgarh has revealed that its prevalence is 10% in Chhattisgarh population. In some communities, the prevalence of the sickle cell allele is as high as 30%.

Although its prevalence is high, most of the individuals are unaware of basic scientific facts of this inherited genetic disorder. This condition poses a serious medical as well as socio-economic burden to the patients and their families. The social stigma associated with this disease further aggravates their suffering.

In pursuance of the suffering associated with this disease, the government of Chhattisgarh has established a dedicated Sickle Cell Institute in 2013 at Raipur to cater the needs of patients. The institute provides specialized treatment and counseling facilities to patients and their family members. Further, to maintain the availability of trained manpower to deal with patients of sickle cell disease, the institute provides education and training to healthcare professionals of the state.

Globally, India contributes significantly to the overall disease load, however a very little research work has been carried-out on highly diverse Indian populations. Being a disease prevalent in developing countries, much attention has not been paid by developed countries and requires special attention. Therefore, the 'Sickle Cell Institute Chhattisgarh' is dedicated to pursue cutting edge research to develop markers, drugs as well as specialized treatment modalities.

This Handbook is a sincere attempt to meet one of our goals of generating trained workforce of health professionals to fight with sickle cell disease. I hope this book will be helpful not only to health professionals and researchers but also to the general public to get acquainted with the current knowledge regarding SCD. Comments and suggestions are invited for further improvement of this handbook.

A handwritten signature in blue ink, appearing to be 'P. K. Patra'.

Prof. (Dr.) P. K. Patra

Director General

Sickle Cell Institute Chhattisgarh

Raipur, C.G.

A Handbook on Sickle Cell Disease

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Unit-1

Introduction to Sickle Cell Disease and Pathophysiology

1.1 Sickle Cell Disease/Anaemia

Sickle cell disease (SCD) is a life threatening autosomal recessive genetic disorder resulting from inheritance of abnormal genes from both parents. Normal red blood cells (RBCs) are biconcave disc shaped and move smoothly through the blood capillaries. The RBCs are produced in bone marrow and average life of normal RBCs is about 120 days. Biconcave disc shape of RBCs changes to sickle shape under low oxygen tension due to polymerization of faulty haemoglobin called HbS arising out of a point mutation in beta globin gene. The life span of RBCs in SCD patients is only about 10 to 20 days and the bone marrow can't replace them fast enough. As a result there is decrease in number of RBCs in the body and the RBCs don't contain sufficient amount of haemoglobin (hypochromia). In SCD the RBCs become sickle or crescent shaped which are stiff & sticky and tend to block the blood flow in small capillaries. Blocked blood flow causes ischemia leading to severe pain and gradual damage to organs.

Sickle cell gene is commonly believed to be associated with African ancestry and malaria endemic areas. Besides Africa, it is found around Mediterranean, Middle-East and India. The disease gene has spread also to Europe, America & Caribbean through migration of human populations. In India, it is prevalent in Chhattisgarh, Odisha, Maharashtra, Gujarat, Madhya Pradesh, Telangana, Andhra Pradesh and some parts of Tamil Nadu & Kerala.

1.2 Inheritance

As discussed before, being an autosomal recessive disorder, abnormal beta globin gene from both mother and father are required to be inherited (homozygous) in offspring to

cause SCD. If a person has only one abnormal beta globin gene inherited (heterozygous) either from mother or father, it is referred as sickle cell trait and are usually asymptomatic, but can pass the abnormal beta globin gene to their progeny.

1.3 Situation

The Sickle cell anaemia has no available cure. However, treatments to improve the anaemia and lower complications can help with the symptoms and complications of the disease in both children and adults. Blood and marrow stem cell transplants may offer a cure for a small number of people.

The Sickle cell anaemia varies from person to person. Some people who have the disease have chronic (long-term) pain or fatigue (tiredness). However, with proper care and treatment, many people who have the disease can have improved quality of life and reasonable health.

Because of improved treatments and care, people who have sickle cell anaemia are now living into their forties or fifties, or longer.

1.4 Pathophysiology

A single nucleotide substitution in the sixth codon of the β globin gene results in the substitution of valine for glutamic acid on the surface of the variant - β globin chain. This change causes HbS to polymerise when deoxygenated, the primary event in all sickle cell pathology.

Polymerisation is dependent on intra-erythrocytic HbS concentration, the degree of haemoglobin deoxygenation, pH and the intracellular concentration of HbF. The polymer is a rope-like fiber that aligns with others to form a bundle, distorting the red cell into characteristic sickled forms (Figure 1).

These deformed sickle red cells can occlude the micro vascular circulation producing vascular damage, organ infarcts, painful episodes and other symptoms associated with SCD.

There are two essential pathological processes: *haemolysis* and *vaso-occlusion*.

- *Haemolysis* results in anaemia and a functional deficiency of nitric oxide which results in vascular endothelial damage and may be responsible for complications such as pulmonary hypertension, priapism and stroke.
- *Vaso-occlusion* causes acute and chronic ischaemia and is responsible for acute pain and organ damage.

Sickle cell disease refers to not only patients with sickle cell anaemia but also to compound heterozygotes where one β globin gene mutation includes the sickle cell mutation and the second β globin allele includes a gene mutation other than the sickle cell mutation, such as mutations associated with HbC, HbS β -thalassaemia, HbD, and HbO Arab. In sickle cell disease, HbS is >50% of total haemoglobin.

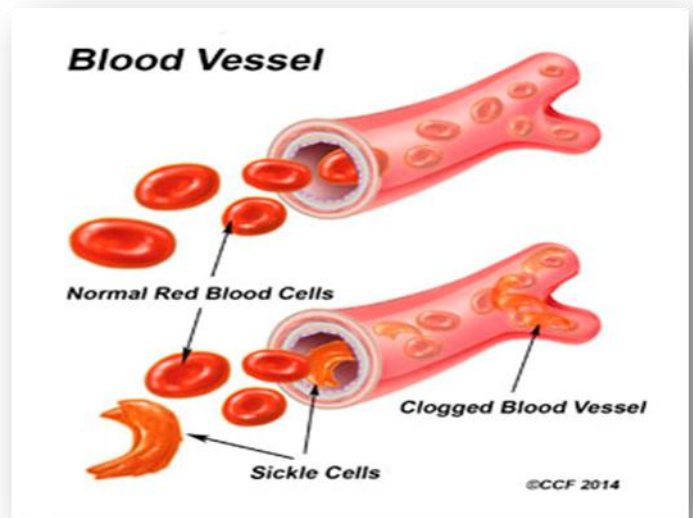


Figure 1 : The normal Red Blood Cells and Sickle Cells

1.5 Sickle Cell Trait

People who inherit a sickle haemoglobin gene from one parent and a normal gene from the other parent have sickle cell trait. In sickle cell trait the HbS is <50% of total haemoglobin. People who have sickle cell trait usually have few, if any, symptoms and lead normal lives.

People who have sickle cell trait can pass the sickle haemoglobin gene to their children. The following image shows an example of an inheritance pattern for sickle cell trait.

Example of an Inheritance Pattern for sickle cell trait and disease are given in figure-2

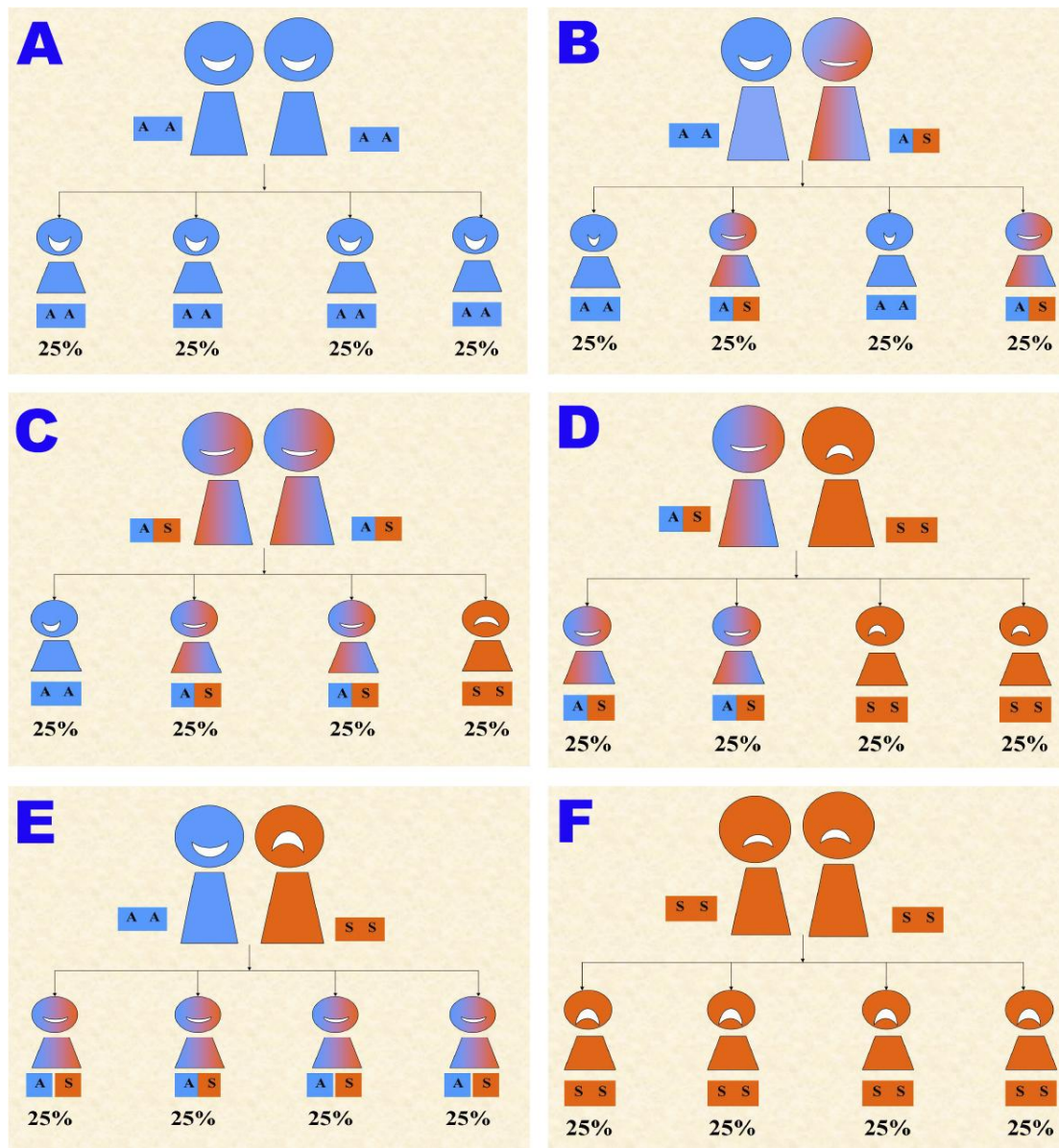


Figure 2 : Shows Different Patterns of How Sickle Haemoglobin Genes are Inherited: A person inherits two haemoglobin genes one from each parent. A normal gene will make normal haemoglobin (A). A sickle haemoglobin gene will make abnormal haemoglobin (S).

When both parents have a normal gene and an abnormal gene, each child has a 25 percent chance of inheriting two normal genes; a 50 percent chance of inheriting one normal gene and one abnormal gene; and a 25 percent chance of inheriting two abnormal genes (Figure 2).

Unit 2

Laboratory Diagnosis of Sickle Cell Disease

2.1 Haemoglobin Structure and Forms

Haemoglobin (Hb) is the iron-containing protein made up of four globin chains. Depending on their structure these globin chains are known as alpha, beta, gamma, and delta. The function of haemoglobin and its ability to transport oxygen is mainly determined by the globin chains that are present in it. The normal haemoglobin types include Haemoglobin A, Haemoglobin A₂ and Haemoglobin F (fetal haemoglobin/HbF). Haemoglobin A contains two alpha and two beta protein chains ($\alpha_2\beta_2$) and is comprised 95%-98% of Hb found in adults. Haemoglobin A₂ has two alpha and two delta protein chains ($\alpha_2\delta_2$) and makes up to 2%-3% of Hb found in adults. Haemoglobin F has two alpha and two gamma protein chains ($\alpha_2\gamma_2$) and, is mainly produced by the fetus during pregnancy its production usually falls shortly after birth and reaches 1%-2% of Hb found in adults by 1-2 years. Newborn infants have two types of γ chains, G γ (HbG₂) and A γ (HbG₁) which has glycine and alanine residue respectively at position 136. Furthermore, the ϵ chain has been observed in early human embryos (Table 1). An investigation of a haemoglobin disorder typically involves tests that determine the types and amounts of haemoglobin present in a person's sample of blood.

<i>Greek Designation</i>	<i>Greek Name</i>	<i>No. of Amino Acids</i>	<i>Observed in</i>	<i>Chromosome</i>
α	Alpha	141	Fetal and adult life	16
β	Beta	146	Adult life	11
Δ	Delta	146	Adult life	11
γ	Gamma	146	Fetal liver, spleen and bone marrow	11
ϵ	Epsilon	146	Embryonic yolk sac	11
Z	Zeta	146	Early embryonic Yolk sac	16

Table 1: Globin Chains in Hmoglobin

2.2 Haemoglobin S

Sickle haemoglobin (HbS) is a haemoglobin variant in which two normal α -globin chains and two abnormal β -globin chains that contain a single amino acid substitution, from glutamic acid to valine, on the 6th position of the beta globin chain, hence the nomenclature of $\beta 6 \text{ glu} \rightarrow \text{val}$. The mutation in the β -globin chain results in decreased solubility of de-oxygenated haemoglobin S that then forms rigid polymers that distort the red cells into the characteristic sickle shape. Classically, these red cells appear in the form of a thin crescent with two pointed ends and lack central pallor. The polymerization of deoxygenated haemoglobin S may cause the red cells to appear in one or more of the following forms: envelope cells filament-shaped and crescent shaped.

2.3 Rapid Screening of Sickle Haemoglobin

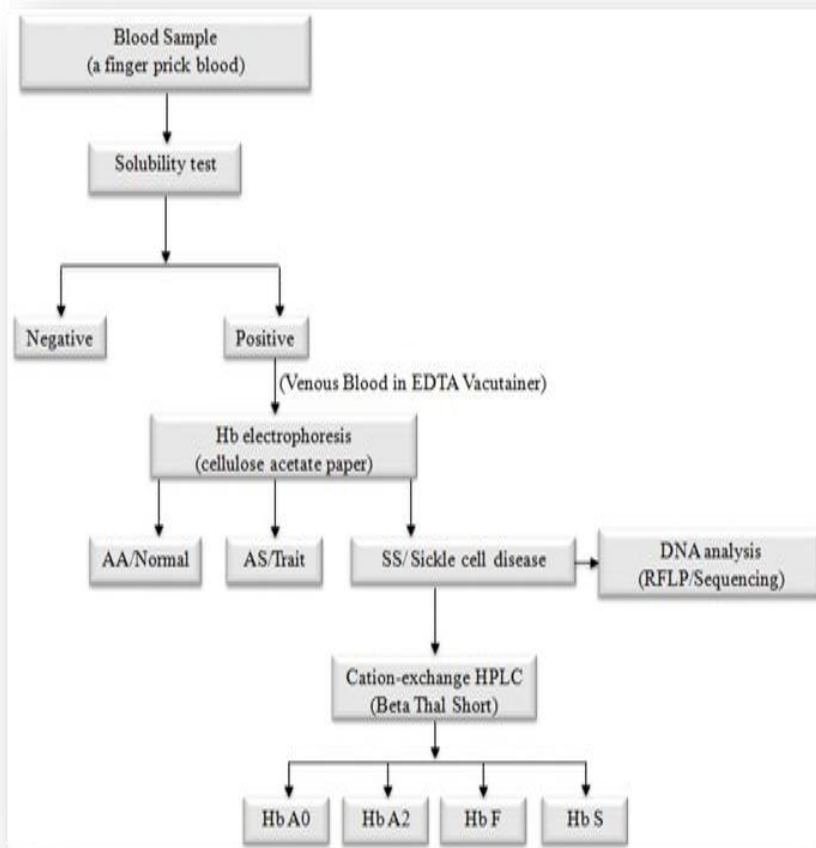


Figure 3: Flow Chart for Sickle Cell Disease

To identify the presence of sickle haemoglobins, sickling and solubility tests are used. Further diagnosis is done by using electrophoresis, HPLC and molecular markers. Sequence of the screening and diagnostic tests to be used for sickle cell disease is depicted in figure 3.

2.3.1 Solubility Test

In the solubility test is based on the relative insolubility of haemoglobin S in the reduced state in high phosphate buffer solution (metabisulfite is a reducing agent). When whole blood is mixed with the reducing agent, the haemoglobin S forms liquid crystals and give a cloudy appearance to the phosphate buffer solution. A transparent solution is seen with other haemoglobins that are more soluble in the reducing agent. A positive result is indicated by a turbid suspension through which the ruled lines are not visible. A negative result is indicated by a transparent suspension through which the ruled lines are visible (Figure 4).



Figure 4: The Sick Cell Solubility Test
Widely Used in Screening Projects

Whole blood anticoagulated with EDTA, heparin, or sodium citrate is acceptable. Specimens may be stored at 4°C up to three weeks before testing. A positive control (AS) containing 30-45% HbS and a negative control (AA) should be analyzed with each patient specimen. Solubility test is a qualitative test and does not distinguish the difference between haemoglobin S disease (SS) and haemoglobin S trait (AS). Preparation of reagents and Methodology for solubility test in brief is given in Box 1.

Other abnormal haemoglobin variants (HbC) are known to cause sickling and will give a positive solubility test. There are some physiologic sources of error that may lead to false positive or false negative results. Erythrocytosis, hyperglobulinemia, extreme leukocytosis, or hyperlipidemia may cause false positive results. Further an anemic individual with Hb of < 7.0 g/dL may give a false negative result. Use of packed erythrocytes (0.01 mL) for solubility test will correct for this error. In infants younger than 6 months and individuals with history of recent transfusion with normal erythrocytes, false negatives may occur due to low concentration of HbS. Hence to confirm the presence of HbS and differentiate between the two states (AS and SS), a haemoglobin electrophoresis at an alkaline pH should be performed.

Box 1: Preparation of Reagents and Methodology for Solubility Test

Reagents

- **Stock 2.58 M phosphate buffer:** Dissolve 215 g K_2HPO_4 and 169 g KH_2PO_4 , 5g sodium dithionate and 1g saponin in distilled water. Then make up to 1Litre with distilled water. Adjust pH to 6.5-6.8.
- Buffer may be kept at 4°C and used as long as they are clear and uncontaminated.

Method

- Pipette 1 ml of phosphate buffer into a properly labelled tube.
- Add two drops (~20 μ l whole blood) blood into the tube with a disposable Pasteur pipette and mix well.
- Add a pinch (about 10-20 mg) of sodium dithionite or sodium metabisulphite powder to the tubes.
- Mix and read immediately.
- Run positive and negative control bloods by following the same steps given above.

2.3.2 Sickling Test

In the Sickling test we create the conditions at which oxygen tension decline to induce the Sickling process of HbS in RBCs. When a drop of blood is sealed between a cover slip and a slide, the decline in oxygen tension due to oxidative processes in the blood cells leads to sickling. In this method blood drop is added with sodium metabisulfite, a chemical reducing agents which rapidly reduces oxyhaemoglobin to reduced haemoglobin to accelerate sickling. In positive samples the typical sickle-shaped red blood cells will appear (Figure 5). Occasionally the preparation may need to stand for up to 24°C. In this case put the slides in a moist Petri dish to maintain temperature. 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. It is important to examine the preparation carefully and in particular near the edge

of cover slip. Preparation of reagents and methodology to perform sickling test is described in Box 2.

Box 2: Preparation of Reagents and Methodology for Sickling Test

Reagents

- 2% Metabisulphite: 0.2 g of sodium metabisulphite in 10 ml of distilled water and stir until dissolved.
- Prepare fresh each time.

Steps of Sickling Test

- Fresh blood in any anticoagulant.
- 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.
- Incubate at room temperature for 1 to 4 hours.
- Examine under a microscope with the dry objective.

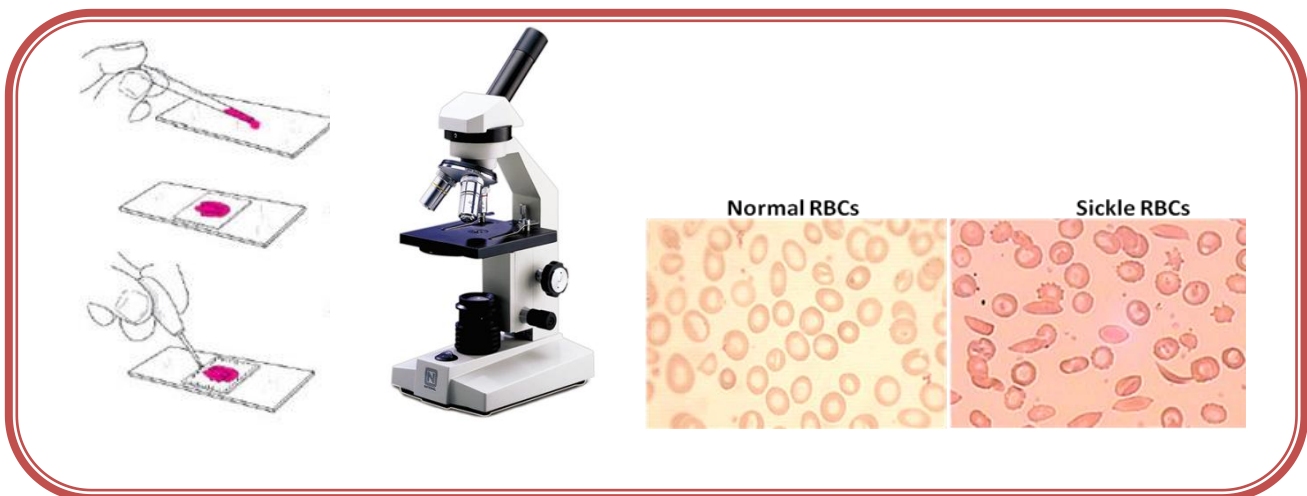


Figure 5: Schematic presentation of sickling test

2.4 Identification of Normal and Abnormal Haemoglobin Types

There are several methods of evaluating the type and relative amounts of various normal and abnormal haemoglobin types. Haemoglobin electrophoresis is traditionally used as the method to identify the presence of various haemoglobins. Haemoglobin fractionation by HPLC is the most frequently used method to screen for haemoglobin variants, including HbS. Isoelectric focusing is a highly sensitive method that is often used at large reference laboratories. These methods evaluate the different types of haemoglobin based on the physical and chemical properties of the different haemoglobin molecules.

2.4.1 Electrophoresis

The electrophoresis process takes advantage of the fact that different haemoglobin types have different electrical charges and move at different speeds in an electric field. In an electrical field proteins that have a net negative charge will migrate from the cathode ("black," "negative") to the anode ("red", "positive") of the field. The positions of the protein products are detected either directly by staining, or by coupled enzymatic reactions. Substitution mutations that result in the replacement of one amino acid by another with a different electrical charge can lead to slight changes in the overall charges of the protein. In the case of HbS, replacement of a negatively-charged glutamate in the standard HbA beta-globin by a neutral valine in HbS results in a protein with a slightly reduced negative charge. Hence homozygous AA and SS individuals yield respectively "fast" and "slow" single bands and heterozygous individual yield two bands (both alleles) (Figure 6). Preparation of reagents and methodology to perform electrophoresis test is described in Box 3.

Box 3: Preparation of Reagents and Methodology for Electrophoresis

Reagents and Equipment

- Electrophoresis buffer. Tris/EDTA/borate (TEB) pH 8.5. Tris-(hydroxymethyl) aminomethane (Tris), 10.2 g, EDTA (disodium salt), 0.6 g, boric acid, 3.2 g, water to 1 litre. The buffer should be stored at 4°C and can be used up to 10 times without deterioration.
- Fixative/stain solution, PonceauS 5g, trichloroacetic acid 7.5g, water to 1Litre.
- Destaining solution 3% (v/v) acetic acid 30ml, water to 1Litre.
- Haemolysing reagent. 0.5% (v/v) Triton X-100 in 100mg/l potassium cyanide.
- Power supply capable of delivering a constant current, 0-80mA and up to 400volts.
- A horizontal electrophoresis tank with adjustable bridge gaps and a polarity indicator.

Method

- Centrifuge samples at 1200g for 5min. Dilute 20µl of the packed red cells with 150µl of the haemolysing reagent. Mix gently and leave for at least 5 min. If purified Hemolysate are used, dilute 40µl of 10g/dl Hemolysate with 150µl of lysing reagent.
- With the power supply disconnected, prepare the electrophoresis tank by placing equal amounts of TEB buffer in each of the outer buffer compartments.
- Wet two chamber wicks in the buffer, and place one along each divider/bridge support ensuring that they make good contact with the buffer.
- Soak the cellulose acetate by lowering it slowly into a reservoir of buffer. Leave the cellulose acetate to soak for at least 5 min before use.
- Remove the cellulose acetate strip from the buffer and blot twice between two layers of clean blotting paper. Do not allow the cellulose acetate to dry.
- Load the applicator by depressing the tips into the sample wells twice, and apply this first loading onto some clean blotting paper. Reload the applicator and apply the samples to the cellulose acetate.
- Place the cellulose acetate strip across the bridges to maintain good contact.
- Electrophorese at 350 V for 25 min.
- After 25 min electrophoresis, immediately transfer the cellulose acetate to Ponceau S and fix and stain for 5 min.
- Remove excess stain by washing for 5 min in the first acetic acid reservoir and for 10 min in each of the remaining two. Blot once, using clean blotting paper, and leave to dry.
- Label the membranes and store in a protective plastic envelope.

Electrophoresis of haemoglobin variants with similar motilities has inherent limitations. The identification of variants is dependent on the technical performance of electrophoresis, which has many variables, e.g., haemoglobin concentration, amperage, running temperature, and length of electrophoresis run. These variables can affect the quality of separation and relative positioning of the bands. Variants that migrate similarly would be very difficult to evaluate without haemoglobin mixture or known stored specimen being electrophoresed adjacent to the unknown specimen.

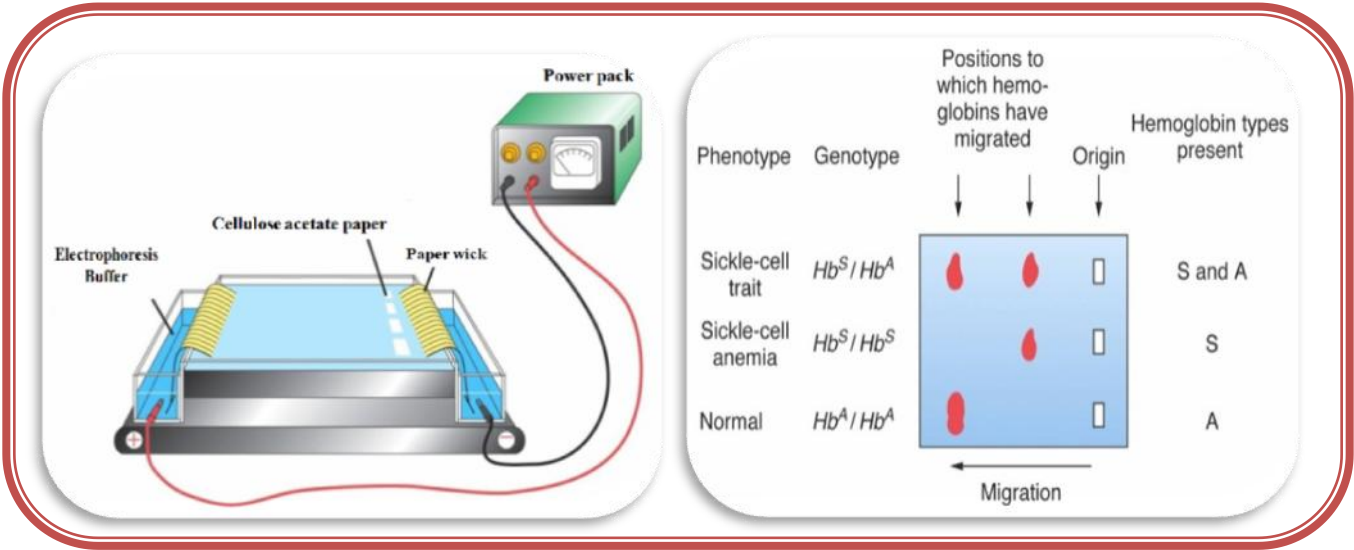


Figure 6: Electrophoresis Unit and Hypothetical Banding Pattern for SS, AS and AA Individuals.

Alkaline and acid haemoglobin electrophoresis on cellulose acetate are the two most widely used methods for investigating common haemoglobin variants and *haemoglobinopathies* (Figure 7). At alkaline pH, some haemoglobin variants co-migrate with HbA2 (e.g. HbC, HbE, HbO Arab) while some co-migrate in the HbS region (e.g. HbD Punjab, HbD Iran, HbG). At acidic pH, it is possible to separate HbC from HbE, HbO Arab and HbS from HbD and HbG. HbE cannot be separated from HbO Arab and HbD from HbG.

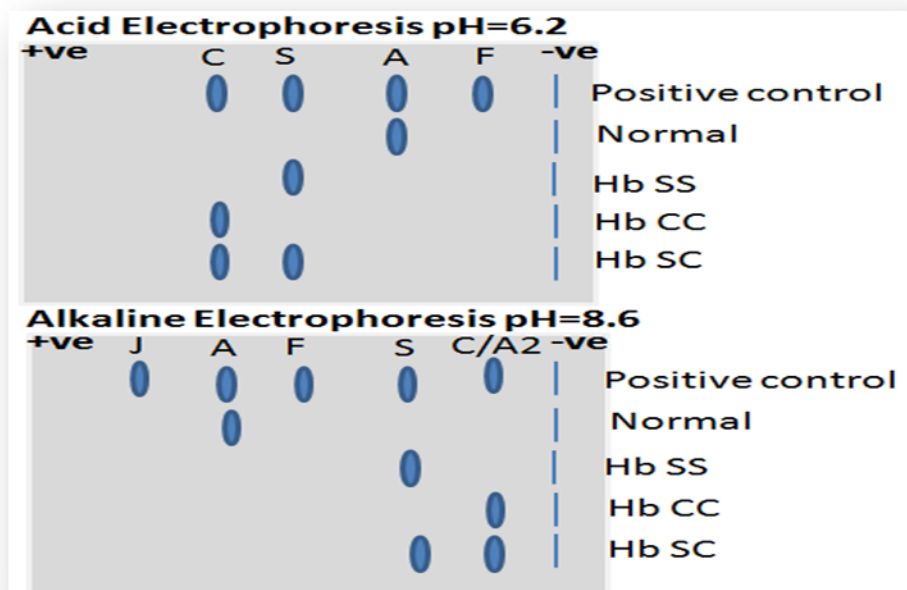


Figure 7: Pattern of Haemoglobin Separation in Acid and Alkaline Electrophoresis

At acidic pH most common abnormal haemoglobins, HbS and HbC, are effectively separated from HbA, as well as most others that migrate in similar locations by alkaline electrophoresis. At alkaline pH mobility within the gel differs based on the overall charge of the haemoglobin. This method allows separation of many types of haemoglobin from normal HbA; although multiple abnormal haemoglobins may migrate in the same position.

2.4.2 Isoelectric Focusing

To overcome these limitations and increase quality of separation, it is possible to use isoelectric focusing (IEF), an electric current is passed through a supporting medium such as a precast agarose or polyacrylamide gel containing carrier ampholytes. These ampholytes migrate through the medium to form a stable pH gradient ranging from pH 6.0 at the anode to pH 8.0 at the cathode. Hemolysate is applied to the gel at the cathode end, and haemoglobin fractions migrate through the pH gradient until they are “focused” into a sharp, distinct band at the pH equal to the isoelectric point (pI) at which the haemoglobin is neutrally charged. It has better resolving power than many other electrophoretic techniques as it can distinguish haemoglobin variants on the basis of their minute differences in pI. However, HbOArab is not well separated from HbE, nor HbDPunjab from

HbG Philadelphia. Isoelectric focusing uses more specialized reagents and lengthy procedure, hence it has largely been replaced by high performance liquid chromatography (HPLC).

2.4.3 Capillary Electrophoresis

Most recently, automated capillary electrophoresis (CE) instruments have been making their way in to the clinical laboratory for haemoglobin analysis. By this method, electrophoresis is performed by adding patient sample to a thin capillary tube containing a buffer, most often an alkaline buffer. Voltage is applied to allow separation of haemoglobins based on their charge, similar to the traditional gel electrophoresis methods mentioned above. Multiple samples undergo an eight-minute high-resolution separation, concurrently.

A high-resolution haemoglobin separation is obtained, similar to IEF separation. The ideal wavelength of 415 nm is utilized for haemoglobin detection with CE. The electropherogram is made up of 300 consecutive readings (dots) and is divided into 15 zones. To facilitate interpretation, results are automatically positioned with regard to the HbA and HbA2 fraction in the sample. Haemoglobins (normal and variant) are displayed as peaks, and the zone to which a variant belongs is identified automatically by the system. An on-board haemoglobin library is present in the form of a drop-down list and lists all of the normal and variant haemoglobins that may be present within a particular zone. This method has the advantage over HPLC of allowing accurate quantification of HbA2 in the presence of Hb E. Packed red blood cell samples can be utilized for capillary electrophoresis analysis. After removing plasma from samples, the bar-coded primary sample tube can be loaded onto the instrument. Rest of the steps in sample processing and separation are performed automatically by the system.

2.4.4 High Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is an excellent, powerful diagnostic tool for the direct identification of haemoglobin variants with a high degree of precision in the quantification of normal and abnormal haemoglobin fractions. Cation-exchange HPLC has the advantage of quantifying HbF and HbA2 along with haemoglobin variant screening in a single, highly reproducible system, making it an excellent technology to screen for haemoglobin variants. In cation-exchange HPLC, hemolysate is injected into a chromatography column containing a negatively charged resin onto which the positively charged haemoglobins are adsorbed. As the ionic strength of the eluting liquid phase increases, haemoglobin variants will come off the column at a

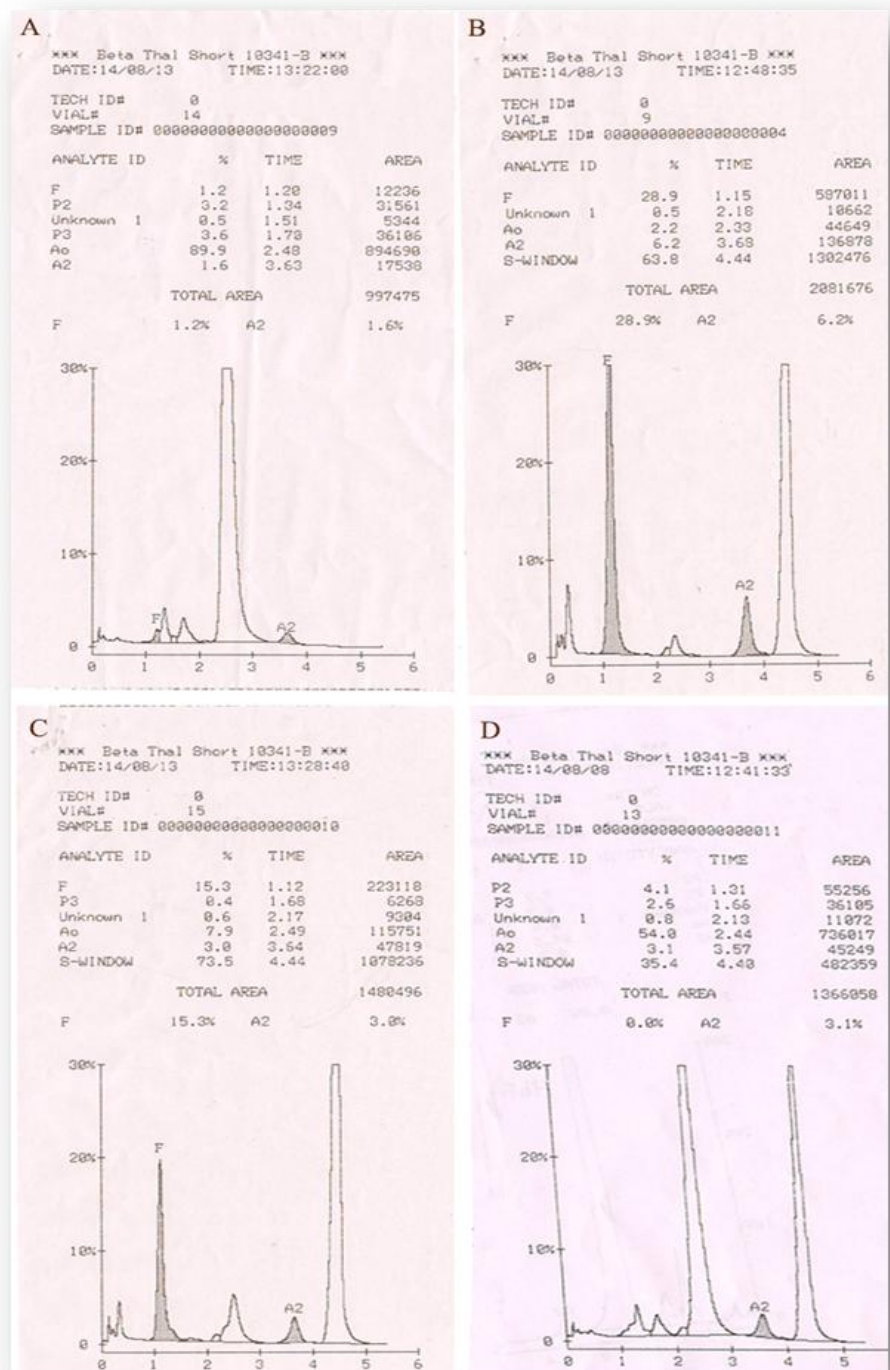


Figure 8: Chromatogram Examples for Normal, AS, SS and SS β thal Conditions.

A. normal individual, B. SS β thal disease, C. HbSS disease, and D. HbAS trait. Elution times on these plots shows Hb F at 1.12-1.20 minutes, HbA at 2.33-2.49 minutes, HbA2 at 3.57-3.69 minutes and HbS at 4.40-4.44 minutes. It is obvious that in HbSS disease patient a dominant peak in the S window is present, and no detectable HbA was observed.

specific retention time, thus allowing identification of the haemoglobin variant based on the overall charge characteristics of the protein and is continuously monitored by an optical detector. The chromatogram is stored in and analyzed by a microcomputer. The pattern seen by alkaline electrophoresis demonstrates some correlation with retention time by HPLC since both methods are dependent on the charge of the haemoglobin molecule; although the specific retention time by HPLC is dependent on the column and eluting solution used in the instrument. In general terms, amino acid substitutions leading to more overall negative charge will result in faster migration by alkaline electrophoresis and a shorter retention time on the column by HPLC.

One advantage of this method is that HbC does not migrate with HbA₂ as it does on alkaline electrophoresis, thus allowing measurement of HbA₂ in a patient with heterozygous or homozygous HbC. Unfortunately, Hb E does elute with HbA₂ by this method, precluding accurate measurement of HbA₂ when HbE is present. Examples of a normal adult haemoglobin pattern by HPLC, as well as AS, SS and SS/*Bthal*, are shown in Figure 8.

2.5 Newborn Screening

Early diagnosis may reduce morbidity, premature death, intellectual disability, and other developmental disabilities because treatment can begin before the condition can cause health problems. Newborn screening for sickle cell can be performed via the more sensitive haemoglobin HPLC fractionation or automated capillary electrophoresis and identifies the specific types of haemoglobin present. This test uses a few drops of blood from pricking the baby's heel. These drops are absorbed on a screening card (Guthrie Cards) that is taken to a laboratory for testing. Newborn dried blood spot samples are screened for the presence of normal haemoglobins (F and A) and common haemoglobin variants to include S, C, D, E, and Bart's. A fully automated instrument is fast and can analyze 96 samples in few hours. The fast throughput is accomplished due to simultaneous analyses of samples. Result interpretation is aided by automatically color-coded curves (normal or abnormal results) and on-board haemoglobin library arranged by zone.

2.6 Molecular Diagnostics

Molecular diagnosis combines laboratory medicine with the knowledge and technology of molecular genetics for the detection of the various pathogenic mutations in DNA samples in order to facilitate detection, diagnosis, sub-classification, prognosis, and monitoring response to therapy. It has been revolutionized over the last decades, benefiting from the discoveries in the field of molecular biology. The rate of disease gene discovery is increasing exponentially, which facilitates the understanding diseases at molecular level. Molecular understanding of disease is translated into diagnostic testing, therapeutics, and eventually preventive therapies. To face the new century, the medical practitioners not only understand molecular biology, but must also embrace the use of this rapidly expanding body of information in their medical practice.

2.6.1 Restriction Fragment Length Polymorphism (RFLP)

The change in the sequence of the gene can be detected by cutting DNA with the restriction endonuclease Bsu36I, which recognizes the sequence CC/TNAGG. The restriction site is present in the gene for normal haemoglobin, but is lost in the sickle cell haemoglobin gene. A portion of DNA (268 bp), surrounding the mutation site of the haemoglobin gene is amplified by using the following PCR primers. Forward: 5'-CAACTTCATCCACGTTTACC-3' and reverse

5'-GAAGAGCCAAGGACAGGTAC-3'. Cutting normal haemoglobin DNA with Bsu36I will result in two DNA fragments (215 bp and 53 bp). Sickle cell haemoglobin DNA is resistant to Bsu36I, resulting in a single DNA fragment because it lacks the Bsu36I recognition site.

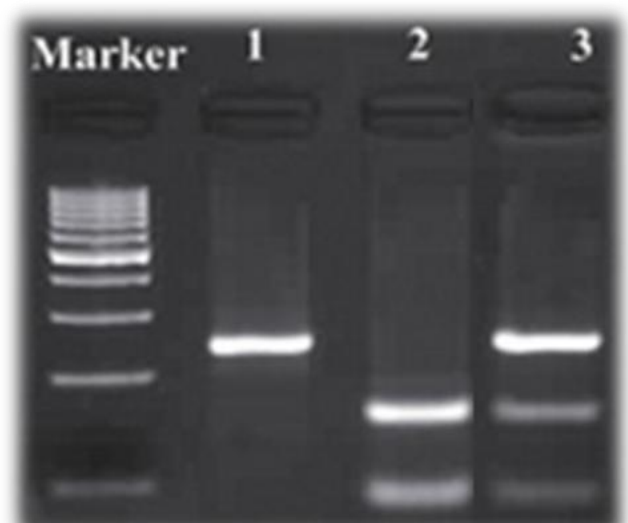


Figure 9: Characterization of Sickle Cell Anaemia Using Bsu36I RFLP. Marker is 100bp DNA Ladder, 1 is AA, 2 is SS and 3 is AS.

DNA from a heterozygous sample, with both sickle and normal haemoglobin DNA, will give all three DNA fragments upon digestion with Bsu36I (Figure 9).

2.6.2 DNA Sequencing

Although majority of the common haemoglobin variants can be identified by other methods such as IEF, HPLC and capillary electrophoresis, it is very difficult to characterise unstable haemoglobins and low or high oxygen affinity haemoglobins due to their neutral charges. This type of uncommon haemoglobin variants

requires DNA sequencing for further identification. In general, polymerase chain reactions (PCRs) are performed to amplify the coding regions of the β and/or α -globin genes. Then these PCR products are directly sequenced to determine the nucleotide sequence of these genes. The nucleotide substitution that leads to change the amino acid sequence (non-synonymous mutation) is usually easily identified to indirectly characterise the uncommon haemoglobin variants (Figure 10). In addition to its use in sickle cell anaemia, DNA sequencing can also be used to identify several other nucleotide substitutions associated with β -thalassemia.

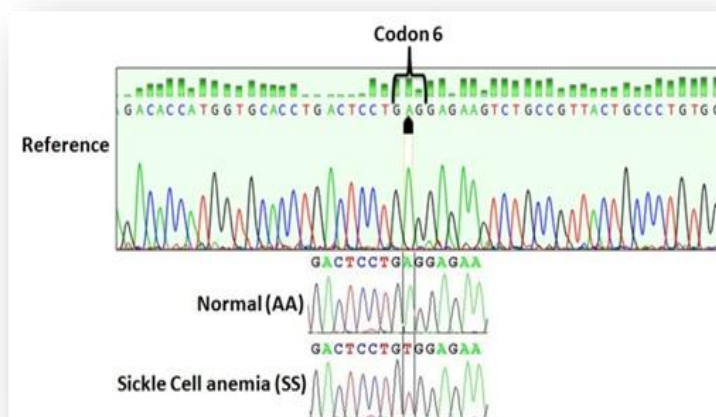


Figure 10: DNA Sequence of HBB Gene glu6val Region.

2.7 The Future of SCD Diagnostics

The year 2010 was celebrated as the centenary year of the diagnosis of sickle cell disease. Only just before this event UNESCO and World Health Organization recognized the SCD as a public health priority. Although Hb electrophoresis, IEF and cation-exchange HPLC can reliably distinguish between the different forms of Hb and provide the information necessary for objective, definitive SCD diagnosis, currently each of these

assays must be performed in a specialized laboratory. The development of a low-cost, portable, easy-to-use diagnostic test for SCD could make it possible for more local health clinics to offer SCD screening, decreasing the burden on overcrowded hospitals and centralized laboratories. Further development of novel highly-selective and efficient approaches for isolation of nucleated fetal cells from mother's blood could enable prenatal genetic screening for early detection of SCD.

Unit-3

Clinical Manifestations and Treatment of Sickle Cell Anaemia

Children with sickle cell disease should be followed by experts in the management of this disease, most often by pediatricians. Comprehensive medical care with evidence-based strategies delivered by experts in sickle cell disease and anticipatory guidance of the parents about the most common complications can decrease sickle cell disease–related mortality and morbidity. Medical care provided by a pediatrician can also decrease frequency of emergency department visits and length of hospitalization when compared to patients who were not seen by a pediatrician within the last year.

Infants with sickle cell anaemia have abnormal immune function and may have functional asplenia at as early as 6 months of age. Bacterial sepsis is one of the greatest causes for morbidity and mortality in this patient population. By 5 yr of age, most children with sickle cell anaemia have functional asplenia.

Children with sickle cell anaemia have an additional risk factor, the deficiency of alternative complement pathway serum opsonins against pneumococci. Regardless of age, all patients with sickle cell anaemia are at increased risk of infection and death from bacterial infection, particularly encapsulated organisms such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b.

Children with sickle cell anaemia should receive prophylactic oral penicillin VK until at least 5 yr of age (125 mg twice a day up to age 3 yr, and then 250 mg twice a day). No established guidelines exist for penicillin prophylaxis beyond 5 yr of age, and some clinicians continue penicillin prophylaxis, whereas others recommend discontinuation. Continuation of penicillin prophylaxis should be considered for children beyond 5 yr of age with previous diagnosis of pneumococcal infection, due to the increased risk of a recurrent infection. An alternative for children who are allergic to penicillin is erythromycin ethyl succinate 10 mg/kg twice a day. In addition to penicillin prophylaxis, routine childhood immunizations as well as the annual administration of influenza vaccine are highly recommended.

Human parvovirus B19 poses a unique threat for patients with sickle cell anaemia because such infections limit the production of reticulocytes. Any child with reticulocytopenia should be considered to have parvovirus B19 until proved otherwise. Acute infection with parvovirus B19 is associated with red cell aplasia (aplastic crisis), fever, pain, splenic sequestration, acute chest syndrome (ACS), glomerulonephritis, and strokes.

3.1 General Health Management

Box 4: General Health Maintenance

1. Environmental

- Altitude: less than 1500 meters
- Avoid cold exposure
- Avoid hot exposure

2. Way of Life

- Regular hydration
- Avoidance of alcoholic beverages
- Suppression of active (or passive) tobacco use
- No cannabis or other illegal drugs
- Avoidance of strenuous exercise
- Adoption of a quiet way life

3. Nutrition

- Folic acid supplementation 5 mg/day,
- Zinc supplementation until puberty

4. Education

- Health education for the patient and relatives
- Information on symptoms requiring medical advice
- Genetic counseling
- Appropriate use of analgesia at home

5. Psycho-Social Management

- Implementation of care pathways
- Easy access to social workers
- Open access to psychologist
- Avoidance of stress

6. Occupational Orientation

- Avoid physically tiring jobs
- Avoid occupations with cold exposure

General health management must begin with neonatal (or prenatal) diagnosis so that parents can be informed and the disease explained to them and the necessary collaborative network set up, including the parents and other care givers. Disease

management must take into account the familial and genetic dimensions of the disease. Some of the tips for general health maintenance are given in Box 4.

Females with sickle cell anaemia maintain a lower average height and weight than those females with normal haemoglobin. This lower than average height and weight continues until late adolescence. Puberty is usually delayed by several years. Menarche (beginning of the menstrual period) is also delayed. It is important to reassure the adolescent that she will eventually catch up with her peers.



Figure 11: *Patients with Typical Hemolytic Face and Stunted Growth*

Males with sickle cell anaemia maintain a lower average height and weight than those males with normal haemoglobin (Figure 11). This lower than average height and weight continues until late adolescence. Puberty is usually delayed by several years. It is important to reassure the adolescent that he will eventually catch up with his peers.

3.2 Fever and Bacteremia

Fever in a child with sickle cell anaemia is a medical emergency, requiring prompt medical evaluation and delivery of antibiotics due to the increased risk of bacterial infection and concomitant high fatality rate with infection. Several clinical management strategies

have been developed for children with fever, ranging from admitting all patients with a fever for IV antimicrobial therapy to administering a 3rd-generation cephalosporin in an outpatient setting to patients without any of the previously established risk factors for occult bacteremia (Box 5).

Given the observation that the average time for a positive blood culture with a bacterial pathogen is <20 hr in children with sickle cell anaemia, admission for 24 hr is probably the most prudent strategy for children and families without a telephone or transportation, or with a history of inadequate follow-up. Outpatient management should be considered only for those with the lowest risk for bacteremia, and treatment choice should be considered carefully.

Children who have sickle cell disease and who are treated with ceftriaxone can develop severe, rapid, and life-threatening immune hemolysis; the established risks of outpatient management must be reasonable against

the apparent benefits. Regardless of the clinical management strategy, all patients with any type of sickle cell disease and fever should be evaluated and treated immediately for occult bacteremia with either IV or IM antibiotics. Those with poor adherence, limited financial resources, or established risk factors for bacteremia should be admitted for at least 24 hr. The patients with positive blood cultures, pathogen-specific therapy should be well thought-out. In the incident that *Salmonella* spp. or *Staphylococcus aureus* bacteremia occurs, strong consideration should be given to evaluation of osteomyelitis with a bone scan, given the increased risk of osteomyelitis in children with

Box 5: Clinical Factors Associated with Increased Risk of Bacteremia Requiring Admission in Febrile Children with Sickle Cell Disease

Seriously ill appearance
Hypotension: systolic BP <70 mm Hg at 1 year of age or <70 mm Hg + (2 × tage in years) for older children
Poor perfusion: capillary-refill time >4 seconds
Temperature >40.0°C
A corrected white-cell count >30,000/cubic mm or <500/cubic mm
Platelet count <100,000/cubic mm
History of pneumococcal sepsis
Severe pain
Dehydration: poor skin turgor, dry mucous membranes, history of poor fluid intake, or decreased output of urine
Infiltration of a segment or a larger portion of the lung
Haemoglobin level <5.0 g/dl

sickle cell anaemia when compared to the general population. Details of Infectious risk management in sickle cell patients were provided in Box 6.

Box 6: 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 3 yr, and then 250 mg 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 pneumoniae
 - Haemophilus influenzae
 - Meningococcus
 - Influenzae
 - Salmonella typhi (for at risk individuals)
5. Elimination of recurrent focal infection(dental infection, sinusitis, acute recurrent tonsillitis, Cholecystitis, urinary infections)

3.3 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 anaemia, occurring in 50% of children by the age of 2 years. Dactylitis often manifests with symmetric or unilateral swelling of the hands and/or feet (Figure 12 and Figure 13). Unilateral dactylitis can be confused with osteomyelitis, and careful assessment to



Figure12: Dactylitis affecting the proximal phalanx of right index finger of a 5 month old child (Courtesy: Dr. Graham Serjeant, Sickle Cell Trust, Jamaica.

differentiate between the two is important, because treatment differs significantly. Dactylitis requires palliation with pain medications, such as acetaminophen with codeine, whereas osteomyelitis requires at least 4-6 wk of IV antibiotics.

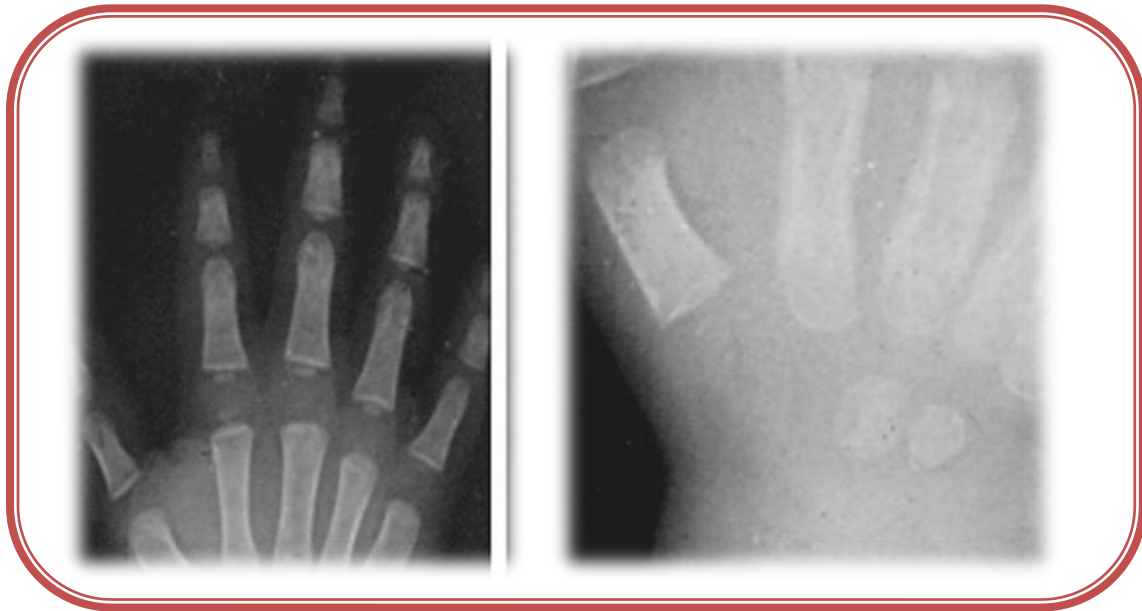


Figure 13 : Roentgenograms of an Infant with Sickle Cell Anaemia and acute dactylitis

3.4 Aplastic Crisis

Aplastic crisis is a temporary cessation of bone marrow activity affecting predominantly the red cell precursors (Figure 14). The destruction of red cell precursors by human parvovirus B19 is the main etiology. Bone marrow aplasia, predominantly affects red cell series but also lowers platelets and white

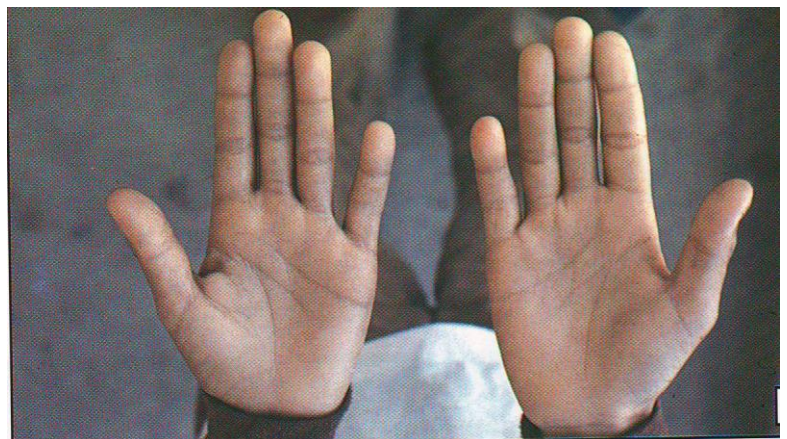


Figure 14: Hands of a 12 Year Old Boy in Aplastic Crisis with Pallor (Curtesy: Dr. Graham Serjeant, Sickle Cell Trust, Jamaica)

cells, lasting 7-10days. The bone marrow always recovers with the development of viral immunity but because of shortened red cell survival in SS disease may cause death. The prevention, diagnosis and treatment strategy for aplastic crisis was given Box 7.

Box 7: Aplastic Crisis Prevention, Diagnosis and Treatment

Prevention

Human parvovirus vaccine is under development but not yet available. SS siblings of affected case have over a 50% chance of aplasia simultaneously or within 3 weeks.

Diagnosis

Marked lowering of Hb – usually to 2-4 g/dl over a few days.

Reticulocytes 0% or if present, a daily marked increase consistent with the recovery phase.

Treatment

Blood Transfusion (BT) as emergency if reticulocytes 0% and Hb > 2g/dl below steady state level; transfusion may be performed in day care centre if uncomplicated aplasia (no features other than pallor).

Review after 3-4 days to ensure reticulocytosis of recovery phase has occurred.

Patients may be closely monitored without transfusion if they are already in recovery phase - daily marked increase in reticulocytes count and rising Hb.

Monitor urine for proteinuria; watch for signs of stroke.

3.5 Splenic Sequestration

Acute splenic sequestration is a life-threatening complication occurring primarily in infants and can occur as early as 5 wk of age which is indicated by rapid increase in the size of spleen in a short period of time (Figure 15). Approximately 30% of children with sickle cell anaemia have a severe splenic sequestration episode, and a significant percentage of these episodes are fatal.

Box 8: Splenic sequestration Prevention, Diagnosis and Treatment

Anticipatory Guidance

1. Teaching parents and primary caregivers how to palpate the spleen to determine if the spleen is enlarging.
2. The etiology of splenic sequestration is unknown.

Diagnostic Testing and Laboratory Monitoring

1. Engorgement and increase of the spleen size
2. Evidence of hypovolemia
3. Decline in haemoglobin of ≥ 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 5 ml/kg of packed red blood cells (RBCs) is given.
3. Prophylactic splenectomy performed after an acute episode has resolved is the only effective strategy for preventing future life-threatening episodes.

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 (Box 12) 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. The prevention, diagnosis and treatment strategy for splenic sequestration was given Box 8.

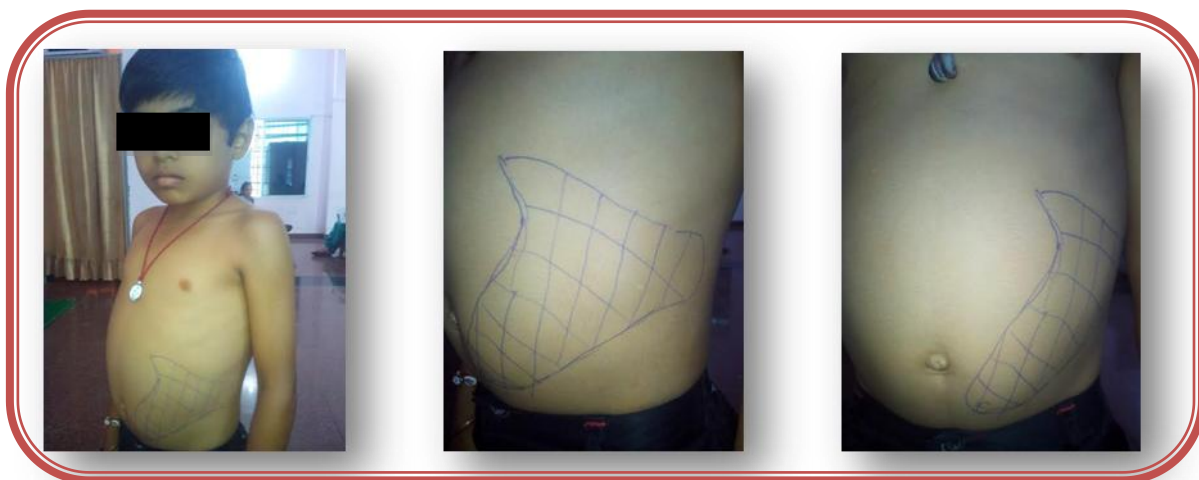


Figure 15: Patient of Sickle Cell Anaemia with Chronic Massive Splenomegaly

3.6 Pain

The chief clinical feature of sickle cell anaemia is pain. The pain is characterized as constant discomfort that can occur in any part of the body but most often occurs in the chest, abdomen, or extremities. The only gauge for pain is the patient. Child's pain can be assessed in different ways. The Wong Baker FACES Pain Rating Scale is one among them (Figure 16). Patients with these conditions often present with debilitating primary effects under conditions of unclear etiology and powerful secondary effects that can alter emotional states and facilitate misdiagnosis (Figure 17).

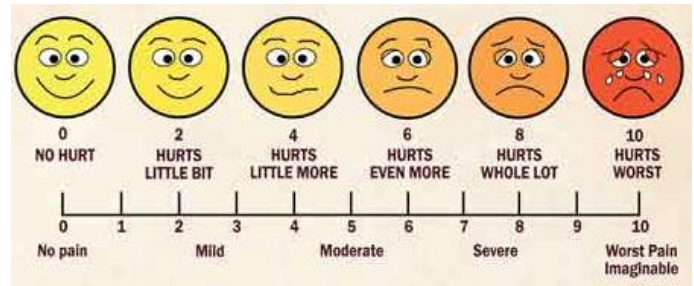


Figure 16: Wong Baker Faces Pain Rating

Box 9: Diagnostic Testing of Pain, Laboratory Monitoring and Treatment

Guidance

1. Precipitating causes of painful episodes can include physical stress, infection, dehydration, hypoxia, local or systemic acidosis, exposure to cold, and swimming for prolonged periods.
2. Should develop a consistent, validated pain scale, such as the *Wong-Baker FACES Scale* for determining the magnitude of the pain.
3. Sleeping through the night might be an indication for decreasing pain medication by 20% the following morning.
4. Successful treatment of painful episodes requires education of both the parents and the patients regarding the recognition of symptoms and the optimal management strategy.
5. At home with comfort measures, such as heating blanket, relaxation techniques, massage, and pain medication.

Diagnostic Testing and Laboratory Monitoring

1. Given the absence of any reliable objective laboratory or clinical parameter associated with pain, trust between the patient and the treating physician is paramount to a successful clinical management strategy.

Treatment

1. Patient who has ~1 painful episode per year that requires medical attention.
2. Specific therapy for pain - the use of acetaminophen or a non-steroidal agent early in the course of pain, followed by escalation to acetaminophen with codeine or a short- or long-acting oral opioid.
3. Hospitalization for administration of IV morphine or derivatives of morphine.
4. BT - reserve for patients with a decrease in Hb resulting in hemodynamic compromise, respiratory distress, or a falling Hb concentration, with no expectation that a safe nadir will be reached, such as when the child has both a falling Hb level and reticulocytes count with a parvovirus B19 infection.
5. IV hydration does not relieve or prevent pain and is appropriate when the patient is unable to drink as a result of the severe pain or is dehydrated.
6. Opioid dependency in children with SCD is rare and should never be used as a reason to withhold pain medication.
7. Hydroxyurea, a myelosuppressive agent, is the only effective drug proved to reduce the frequency of painful episodes.

The incremental increase and decrease in the use of the medication to relieve pain roughly parallels the 8 phases associated with a chronology of pain and comfort. The average hospital length of stay for children admitted in pain is 4.4 days. The American Pain Society has published clinical guidelines for treating acute and chronic pain in patients with sickle cell disease of any type. Summary of the Chronology of Pain in Children with Sickle Cell

Disease, diagnostic testing, laboratory monitoring and treatment is provided in Box 9. Characteristics of pain in sickle cell disease and suggested measures to be used are also documented in Table 2.

Phase	Pain Characteristics	Suggested Measures
(Baseline) 1	No vaso-occlusive pain; pain of complications may be present, such as that connected with avascular necrosis of the hip	No comfort measures used
(Pre-pain) 2	No vaso-occlusive pain; pain of complications may be present; prodromal signs of impending vaso-occlusive episode may appear, e.g., “yellow eyes” and/or fatigue	No comfort measures used; caregivers may encourage child to increase fluids to prevent pain event from occurring
(Pain start point) 3	First signs of vaso-occlusive pain appear, usually in mild form	Mild oral analgesic often given; fluids increased; child usually maintains normal activities
(Pain acceleration) 4	Intensity of pain increases from mild to moderate. Some children skip this level or move quickly from phase 3 to phase 5	Stronger oral analgesic are given; rubbing, heat, or other activities are often used; child usually stays in school until the pain becomes more severe, then stays home and limits activities; is usually in bed; family searches for ways to control the pain
(Peak pain experience) 5	Pain accelerates to high moderate or severe levels and plateaus; pain can remain elevated for extended period.	Oral analgesics are given around the clock at home; combination of comfort measures is used; family might avoid going to the hospital; if pain is very distressing to the child, parent takes the child to the emergency department

Phase	Pain Characteristics	Suggested Measures
	Child's appearance, behavior, and mood are significantly different from normal	After child enters the hospital, families often turn over comforting activities to health care providers and wait to see if the analgesics work Family caregivers are often exhausted from caring for the child for several days with little or no rest
(Pain decrease start point) 6	Pain finally begins to decrease in intensity from the peak pain level	Family caregivers again become active in comforting the child but not as intensely as during phases 4 and 5
(Steady pain decline) 7	Pain decreases more rapidly, becomes more tolerable for the child Child and family are more relaxed	Health care providers begin to wean the child from the IV analgesic; oral opioids given; discharge planning is started Children may be discharged before they are pain free
(Pain resolution) 8	Pain intensity is at a tolerable level, and discharge is imminent. Child looks and acts like "normal". Self mood improves	May receive oral analgesics

Table 2 : Characteristics of Pain in Sickle Cell Disease

Many myths have been propagated regarding the treatment of pain in sickle cell anaemia. The idea that painful episodes in children should be managed without opioids is without basis and results in unnecessary suffering on the part of the patient. There is no evidence that blood transfusion therapy during an existing painful episode, decreases the intensity or period of the painful episode. However, patients with multiple painful episodes requiring hospitalization within a year or with pain episodes that require hospital stay for more than 7 days, should be evaluated for comorbidities and psychosocial stressors that might contribute to the frequency or duration of pain.

Hydroxyurea can decrease the rate of painful episodes by 50% and the rate of ACS episodes and blood transfusions by ~50%. Hydroxyurea is safe and well tolerated in children >5 yr of age. The primary toxicities are limited to myelosuppression that reversed upon cessation of the drug. The long-term toxicity associated with hydroxyurea in children has not been established; but all evidence to date suggests that the benefits far outweigh the risks.

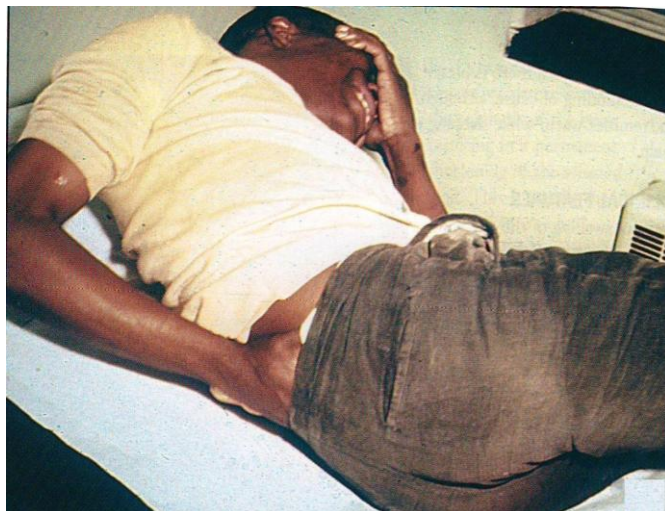


Figure 17: A Young Patient in Painful Crisis : Patient clutches his lower back where the pain is worse (Curtsey: Late Professor Lemuel Diggs, Memphis, USA)

The dose of hydroxyurea being prescribed at our institute is 10 mg/kg body weight daily. If required, dose can be increased upto 20-30 mg/kg per day gradually, if tolerated by patient. Monitoring children on hydroxyurea is rigorous, with initial visits every 2 weeks to monitor for hematologic toxicity with dose escalations and then monthly after a therapeutic dose has been identified.

3.7 Priapism

Priapism is defined as an involuntary penile erection lasting for longer than 30 minutes and is a common problem in sickle cell anaemia (Figure 18). The persistence of a painful erection beyond several hours suggests priapism. On examination, the penis is erect. The ventral portion and the glans of the penis are typically not involved, and their involvement necessitates urologic consultation based on the poor prognosis for spontaneous resolution. Priapism occurs in 2 patterns, stuttering and refractory; with both types occurring in patients from early childhood to adulthood. No formal definitions have been established for these terms, but generally stuttering priapism is defined as self-limited,



Figure 18: Twenty Seven Year Old Male under State of Priapism since Two Days (Curtsey: Dr. Graham Serjeant, Sickle Cell Trust, Jamaica)

intermittent bouts of priapism with several episodes over a defined period. Refractory priapism is defined as prolonged priapism beyond several hours.

Approximately 20% of patients between 5 and 20 yr of age report having at least 1 episode of priapism. Most episodes occur between 3 AM and 9 AM. The mean age at first episode is 12 yr, and the mean number of episodes per patient is ~16, with a mean duration of ~2 hr. The actuarial probability of a patient's experiencing priapism is ~90% by 20 yr of age. Acute and preventive therapy for priapism is given in Box 10.

Box 10: Acute and Preventive Therapy for Priapism	
Acute Treatment	Preventive Therapy
<ol style="list-style-type: none">1. Sitz bath or pain medication.2. Priapism lasting >4 hr should be treated by aspiration of blood from the corpora cavernosa followed by irrigation with dilute epinephrine to produce immediate and sustained detumescence.3. Urologic consultation is required to initiate this procedure, with appropriate input from a hematologist.4. Either simple blood transfusion therapy or exchange transfusion is proposed for the acute treatment of priapism.	<ol style="list-style-type: none">1. For the prevention of recurrent priapism, hydroxyurea appears to have promise;2. The use of etilefrine, a sympathomimetic amine with both $\alpha 1$ and $\beta 1$ adrenergic effects, appears safe and promising in the secondary prevention of priapism.3. The long-term effects of recurrent or prolonged priapism episodes in prepubertal children are not known. In adults, infertility and impotence are potential consequences.

3.8 Neurologic Complications

Neurologic complications associated with sickle cell anaemia are varied and complex. Children with other types of sickle cell disease such as HbSC or HbS β -thalassemia plus might have overt or silent cerebral infarcts as well. Details of neurological complications, diagnosis, treatment and prevention measures in sickle cell anaemia patients are given in Box 11.

Box 11: Management of Neurological Complications

Features & Diagnosis:

1. Focal neurologic deficit that lasts for >24 hr and/or increased signal intensity with a T2-weighted MRI of the brain indicating a cerebral infarct.
2. Absence of a focal neurologic deficit lasting >24 hr in the presence of a lesion on T2-weighted MRI indicating a cerebral infarct indicates silent cerebral infarct.
3. Evidence of a stroke can be found as early as 1 yr of age.
4. Other complications include headaches, anaemia, seizures, cerebral venous thrombosis, and Reversible Posterior Leukoencephalopathy Syndrome (RPLS).
5. CT brain to exclude cerebral hemorrhage.
6. MRI of brain with diffusion-weighted imaging to distinguish between ischemic infarcts & RPLS.
7. MR venography to evaluate the possibility of Cerebral Venous Thrombosis.

Treatment:

Focal Neurologic Deficit

1. A prompt pediatric neurologic opinion.
2. Oxygen administration to keep SPO₂ >96%.
3. Simple BT within 1 hr of presentation with goal of Hb maximum 10 g/dl. Excess Hb might limit O₂ delivery to Brain due to hyper-viscosity of blood can decrease O₂ delivery.
4. Prompt exchange transfusion either manually or erythrocytapheresis to reduce the HbS % to at least <50% and ideally <30%.
5. IV hydration does not relieve or prevent pain. Give iv. hydration only when the patient is unable to drink due to the severe pain or dehydration.
6. Opioid dependency in children with SCD is rare and should never be used as a reason to withhold pain medication.
7. Hydroxyurea, a myelosuppressive agent, to reduce the frequency of painful episodes.

Prevention:

Primary

Transcranial Doppler (TCD) assessment of the blood velocity in the terminal portion of the internal carotid artery (ICA) and the proximal portion of the middle cerebral artery (MCA). Two methods are used *viz.*, non-imaging and imaging techniques.

The scan results can be divided into five categories depending on the time averaged maximal mean (TAMM) velocity recorded, whether in the ICA or MCA or the bifurcation of the two arteries:-

- Inadequate image
- Unusual low velocity
- Normal velocity - 'low risk'
- Borderline velocity - 'conditional'
- High velocity - 'high risk'

The TAMM blood velocities used as cut-offs to define the risk limits are as follows:

Normal velocity - 'standard risk'	<170 cm/s
Borderline velocity - 'conditional'	170 to 199 cm/s
High velocity - 'high risk'	>200 cm/s

TCD scanning decision

Inadequate scan/ low velocities	Rescanning or alternative technique is suggested.
Normal scan <170 cm/sec	Repeat TCD in 1 year.
Conditional 170 to 199 cm/sec	Rescan between 1 & 4 months. Children <10 years & those with higher velocities are considered to be at higher risk & should be scanned earlier
Abnormal >200 cm/sec	Discuss risk of stroke & consider chronic transfusion. A rescan might be considered appropriate depending on the blood velocity & individual clinical circumstances.

Secondary

The approach is BT therapy aimed at keeping the maximum HbS conc. <30% in first 2 years following any new stroke and <50% thereafter. The primary toxic effect of BT is excessive iron store.

Deferoxamine, chelating agent, is administered subcutaneously 5 of 7 nights per week for 10 hr a night , or Deferasirox can be used as a tablet taken by mouth daily.

3.9 Excessive Iron Stores

The assessment of excessive iron stores in children receiving regular blood transfusions is difficult.

- Biopsy of the liver is the gold standard for diagnosis of excessive iron stores but it is an invasive procedure exposing children to the risk of general anesthesia, bleeding, and pain.
- The most commonly used and least-invasive method of estimating total body iron involves serum ferritin levels; however, ferritin measurements have significant limitations, because ferritin levels rise during acute inflammation and correlate poorly with excessive iron in specific organs after 2 yr of regular blood transfusion therapy.
- MRI of the liver is a reasonable alternative to biopsy and more accurate than serum ferritin in measuring iron content in heart and liver, the two most commonly affected organs associated with increased total body iron stores. MRI T2 and MRI R2 and R2 sequences are used to estimate iron levels in the heart and liver.

Erythrocytapheresis is the preferred method because there is a minimum net iron balance after the procedure. Simple transfusion therapy is the least preferable method because this strategy results in the highest net positive iron balance after the procedure. Despite being the preferred method, erythrocytapheresis is less commonly performed

because of the requirement for technical expertise, access to a large vein, and an available apheresis machine. For patients who either will not or cannot continue blood transfusion therapy to prevent subsequent strokes, hydroxyurea

therapy may be a reasonable alternative. The efficacy and toxicity of hydroxyurea as an option for preventing secondary stroke is being addressed in a clinical trial setting.

Box 12: Methods of Blood Transfusion Therapy

1. Erythrocytapheresis
2. Manual exchange transfusions (phlebotomy of a set amount of the patient's blood followed by rapid administration of donated packed RBCs)
3. Simple transfusion



Figure 19: Hemolytic Facies

Alternatively, human leukocyte antigen (HLA) matched hematopoietic stem cell transplantation from a sibling donor is a reasonable approach for patients with strokes, although only a few children have suitable donors. Hematopoietic stem cell transplantation using unrelated donors is the subject of an open clinical trial that is too premature to comment on.

3.10 Lung Disease

The second most common reason for admission to the hospital and a common cause of death in children with sickle cell anaemia is acute chest syndrome (ACS). ACS refers to a constellation of findings that include a new radiodensity on chest radiograph, fever, respiratory distress, and pain that occurs often in the chest, but it can also include the back and/or abdomen only. Even in the absence of respiratory symptoms, all patients with fever should receive a chest radiograph (Figure 20) to identify ACS because clinical examination alone is insufficient to identify patients with a new

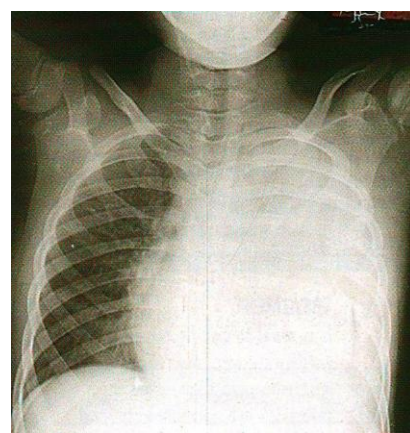


Figure 20: An X-ray Image Showing Complete White Out of Left lung in a Patient with ACS

radiographic density, and early detection of acute syndrome will alter clinical management. The radiographic findings in ACS are variable but can include involvement of a single lobe (predominantly the left lower lobe) or multiple lobes (most often both lower lobes) and pleural effusions (either unilateral or bilateral).

Given the clinical overlap between ACS and common pulmonary complications such as bronchiolitis, asthma, and pneumonia, a wide range of therapeutic strategies have been used (Box 13). Oxygen administration and blood transfusion therapy, either simple or exchange (manual or automated), are the most common interventions used to treat ACS. Supplemental oxygen should be administered when the room air oxygen saturation is >90%. The decision about when to give blood and whether the transfusion should be a simple or exchange transfusion is less clearly defined. Commonly, blood transfusions are given when at least one of the following clinical features is present: decreasing oxygen saturation, increase work of breathing, rapid change in respiratory effort either with or without a worsening chest radiograph, or previous history of severe ACS requiring admission to the intensive care unit.

Box 13: Acute Chest Syndrome: Diagnosis, Laboratory Monitoring and Treatment

PREVENTION

1. Incentive spirometry and periodic ambulation in patients admitted for vaso-occlusive crises, surgery, or febrile episodes
2. Watchful waiting in any hospitalized child or adult with sickle cell disease (pulse oximetry monitoring and frequent respiratory assessments)
3. Avoidance of overhydration
4. Intense education and optimum care of patients who have sickle cell anaemia and asthma

Diagnostic Testing & Laboratory Monitoring

1. Blood culture
2. Nasopharyngeal samples for viral culture (respiratory syncytial virus, influenza)
3. Blood counts every day and appropriate chemistry
4. Continuous pulse oximetry
5. Chest radiograph

Treatment

1. Blood transfusion (simple or exchange)
2. Supplemental O₂ for drop in pulse oximetry by 4% over baseline, or values <90%
3. Empirical antibiotics (cephalosporin and macrolide)
4. Continued respiratory therapy (incentive spirometry and chest physiotherapy as necessary)
5. Bronchodilators and steroids for patients with asthma
6. Optimum pain control and fluid management

3.11 Leg Ulcer

Leg ulcers are one of the complications of sickle cell disease. They start in adolescence and eventually appear in 75% of adults (Figure 21). Low steady state haemoglobin values are associated with a higher incidence of ulcer formation. A high fetal haemoglobin production correlates with a lower incidence of leg ulcers. The ulcers usually present over the medial surface of the lower tibia or just posterior to the



Figure 21: Non Healing Leg Ulcer in 32 Years Male with Sickle Cell Disease.

medial malleolus. Treatment is in many instances temporary and recovery may take a long time. The current recommendations for management of leg ulcers are given in box 14. To prevent ulceration pay close attention to improved venous circulation by using above the knee elastic stocking.

Box 14: Management of Leg Ulcers	
Acute Ulcer	Chronic Ulcer
1. Surgical debridement if there is unhealthy tissue, especially if the ulcer is chronic and there is slow or minimal healing.	1. Chronic transfusion program (every 4 weeks for 6 – 12 months)
2. Scrupulous hygiene	2. Consider oral zinc sulfate to promote healing
3. Topical antibiotics	3. Split thickness skin grafts
4. Moist-wound dressing – one to four times a day (with wet to dry gauze – saline dressings) it helps in gentle debridement and healing.	4. Treatment with hydroxyurea, with or without erythropoietin, depending on response to hydroxyurea.
5. Rest	
6. Elevation of leg	

3.12 Deep Jaundice

The obstruction of excretory pathway for bilirubin is main cause, which is because of increased bilirubin production due to hemolysis. It may cause stone in gall bladder and bile duct. The differential diagnosis of deep jaundice (Figure 22) includes acute cholestasis, viral hepatitis, and a stone obstructing the common bile duct. Investigation of choice must be Ultrasound of the common bile duct.



Figure 22: Patient with Deep Jaundice

3.13 Loss of Vision

The loss of vision may occur when blood vessels in the eye become blocked with sickle cells and the retina (the thin layer of tissue inside the back of the eye) gets damaged. Some patients grow extra blood vessels in the eye from the lack of oxygen. Management of loss of vision in sickle cell patients is given in Box 15.

Box 15: Management of Loss of Vision

Prevention

The eyes should be checked every year to look for damage to the retina. Primarily it should be done by an ophthalmologist who specializes in diseases of the retina.

Treatment

If there is retinal damage by undue blood vessel growth, laser treatment often can prevent further vision loss.

3.14 Pregnancy & Contraception

SCD is related with both maternal and fetal complications and is associated with an increased incidence of perinatal death, premature labour, fetal growth restriction and acute painful crises during pregnancy. Some studies also portray an increase in spontaneous miscarriage, antenatal hospitalisation, maternal mortality, delivery by caesarean section, and antepartum haemorrhage (Box 16). An increased risk of pre-eclampsia and pregnancy-induced hypertension has been described in some studies but not in others (Box 17). In HbSC there are fewer reported adverse outcomes, but there is evidence of an increased incidence of painful crises during pregnancy, fetal growth restriction, antepartum hospital admission and postpartum infection.

Box 16: Effect of Sickle Cell Disease on

Sexual Development	Fertility	Contraception
Frequently delayed in SS disease. The median age of menarche usually increased compared with AA controls.	No good data on fertility but similar time between initial sexual exposure and initial pregnancy suggests that fertility for first child closed to normal.	Patients should be offered the most reliable methods; none are contraindicated in SS disease.

Box 17: Effect of Sickle Cell Disease on Pregnancy

On 'SS' Mother	On Child of 'SS' Mother
Greater risk of ACS & painful crisis especially in 3 rd trimester and early postpartum. Also preeclampsia and maternal death	Greater risk of fetal loss at every stage of pregnancy. IUGR, LBW baby.

Women and men with SCD should be encouraged to have the haemoglobinopathy status of their partner determined before they plan for pregnancy. If identified as an 'at risk couple' they should receive counseling and advice about reproductive options. Effective contraception should be offered if requested.

- **Penicillin prophylaxis** or the equivalent should be prescribed.
- **Vaccination** status should be determined and updated before pregnancy. Patients with SCD are hyposplenic and are at risk of infection, in particular from encapsulated bacteria such as *Neisseria meningitides*, *Streptococcus pneumonia* and *Haemophilus influenzae*.

Once pregnant, patients should register early for antenatal care and be monitored closely during pregnancy. Give iron and folic acid supplementation for duration of pregnancy. Monitor fetal growth and consider operative delivery if evidence of fetal distress, otherwise delivery by vaginal route.

3.15 Newborn Screening

Most of the SS patients die before completing one year of their life, because detection of SCD is not possible using routine screening and diagnostic protocols. Rationale of newborn screening is to increase the chances of detection of SCD in the first year of life. Several of the causes of early death may be prevented and patients may be more effectively treated. Prophylaxis and education may only be put in place if the underlying diagnosis is known (Box 18).

Box 18: Management of Newborn Screening

- Confirm Diagnosis on capillary sample at age 1 month; once confirmed, children should be registered for regular follow-up, education, and counseling of family.
- Offer screening tests for parents to affected children.
- Ensure regular immunization schedules (Sickle cell patients often miss immunization because of transient fevers, illness), including Haemophilus influenzae type b vaccine.
- Teach parents how to feel for the spleen which should be done daily; or more often if child is sick.
- Advise purchase of thermometer and teach its use.
- Start regular pneumococcal prophylaxis from 4 months to 4 years.
Oral penicillin-V 125 mg twice a day up to age of 3 years, and then 250 mg twice a day; or erythromycin 125 mg bd up to age of 3 years and then 250 mg bd if allergic to penicillin.
- Pneumococcal vaccine given at age 4 years in Jamaica.
- Explain about delayed physical and sexual development and slim build: advise plenty of fresh fruit and vegetables and advice against expensive tonics to fatten child.
- Arrange regular review of child every 3 months or immediately if sick. Stress importance of attendance when well in order to establish steady state blood level and clinical feature.
- Provide walk-in facilities or immediate contact if sick.

3.16 Models of Management

Early Diagnosis and Follow-up

Newborn diagnosis essential to prevent many early deaths. Regular follow-up in dedicated clinic with staff educated in sickle cell disease and its complications patients must have immediate access to care and treatment if sick. Establish baseline for patients' clinical state and hematology by review when well. Provide programme of management including pneumococcal prophylaxis and education on splenic palpation.

Diet

Give regular dietary advice. Regular folate supplementation is necessary in when there is evidence of megaloblastic change or poor diet.

Haematology

Monitor hematology with reticulocytes and red cell indices every year while well until 5 years then every 2 year. Monitor renal function every 3-5 year in patients over age 30 years to detect renal failure. Always identify and treat cause for failing haemoglobin levels.

Gallstones

Common in SS disease (50% by 25 year) but rarely cause trouble no indication for removal of asymptomatic stones. Remove if evidence of acute or chronic Cholecystitis or blockage of the cystic or common bile duct.

Fever

Investigate febrile patients urgently and assume septicemic until proved otherwise, acute chest, syndrome is common cause of fever,

Stroke

It is a catastrophic complication in childhood. Chronic transfusion programs may prevent recurrence but many difficulties with such programs in the Caribbean.

Bone Pain

Find out precipitating factors for painful crisis and explore methods of avoidance. Provide day care facilities for management of uncomplicated painful crises, remember pain

the ribs and sternum may precede acute chest syndrome. Investigate pain in hip with limp as possible avascular necrosis of femoral head.

Leg Ulcers

Avoid trauma around the ankles if possible, Treat ulcer actively with bed rest, we dressing, supportive elastic bandages.

Acute Chest Syndrome

It is a major cause of morbidity and mortality in sickle cell disease. Monitor cases closely with frequent clinical and radiological appraisal and pulse oximetry. Chronic transfusion programmes may delay onset of respiratory failure.

Priapism

Ask about priapism because it is common treatable, may have serious sequelae and is under-reported because of embarrassment or not realized as sickle cell problem.

Renal Failure

Progressive glomerular damage associated with failing haemoglobin level increasingly common in patients over 30 years. Treat with top-up transfusions.

3.17 Outlook

Current Treatment modalities available for sickle cell disease are as follows:

- Hydroxyurea may reduce painful crises and ACS in selected seriously affected patients but must be monitored carefully with frequent blood tests.
- Bone marrow transplantation may cure symptoms but is expensive requiring high expertise, only applicable to approximately 15% of Patients with HLA matched siblings, and has a 10% short-term mortality longer term effects on reproduction and other aspects are unknown.
- Chronic transfusion programmes reduce recurrent strokes and may prevent some other pathology but there are potentially serious complications.

3.18 Conclusions

- In malarious areas, malaria continues to be a major cause of early death and complications in sickle cell disease patients. Control of malaria in the Caribbean has been associated with better survival.
 - Serious infections may be prevented by prophylaxis or more effectively treatment by early presentations to clinic and appropriate antibiotics.
 - Education and supportive services should empower the patient to cope with their disease. More effectively. A greater sense of control by the patient is vital to disease management.
- .Painful Crises, an important cause of recurrent and severity in most patients after the age of 30 years.
- Cumulative end-organ damage especially of the kidneys. And the lungs become more important with advancing age.
 - With good care many patients with SS disease are living productive lives and surviving to 70 years and beyond. Median survival in US and Jamaica currently estimated at 45-55 years.

Unit-4

Counseling

For counseling to be effective, you have to first identify the patient.

The patient should know:

- What Sickle Cell is?
- The difference between sickle cell Trait and sickle cell disease
- That the Severity of pain & frequency of crisis are unpredictable.
- That this is incurable.
- That Only amelioration of conditions can be done.
- That this is a genetic disorder.

He/She should try:

- Try to reduce pain and crisis episodes.
- Try to live life normally/ try to increase life span

4.1 Spread

- Approximately 12% of total population is affected in (*Chhattisgarh*)
- Found mostly in semi-tribal areas of Chhattisgarh
- High incidence of SCA/SCD in certain castes

4.2 Identification of Patient

- Delayed growth, weight & height sub-normal
- General complains of weakness, weak body
- Highly anemic appearance, severe anaemia
- Pale skin, colorless nails
- Yellow skin & eyes, long time jaundice
- Flat bones (*forehead structure*)
- Continuous mild fever, long term sufferance
- Short breaths/breathlessness, laborious breathing

- Loss of appetite
- More tired than usual
- Frequent urination, dark urine
- Pain in bones & ribs
- Irritability & prominent mood swings
- Swelling in hands & legs
- Priapism
- Infertility
- Frequent viral & bacterial infections
- Swollen spleen

4.3 Age-wise Counseling

4.3.1 Parent's Role in Adolescents & Young Adults

- Role of parents very important
- Discuss nature of the disease/trait & its affect on them
- Learn to identify onset of pain/crisis by self
- Discuss & review ways to manage pain/crisis
- Counsel them about adverse effects of tobacco, smoking, alcohol & drugs
- Review school & work-place performance, watch out for drops in performance
- Parents to give praise frequently, hug often
- Create a safe positive & loving home environment
- Watch what words you say to the children
- Encourage your adolescent to try different activities to see what he/she enjoys (*divert attention*)
- Friends also play an important role in this

Counseling focuses on 3 things:

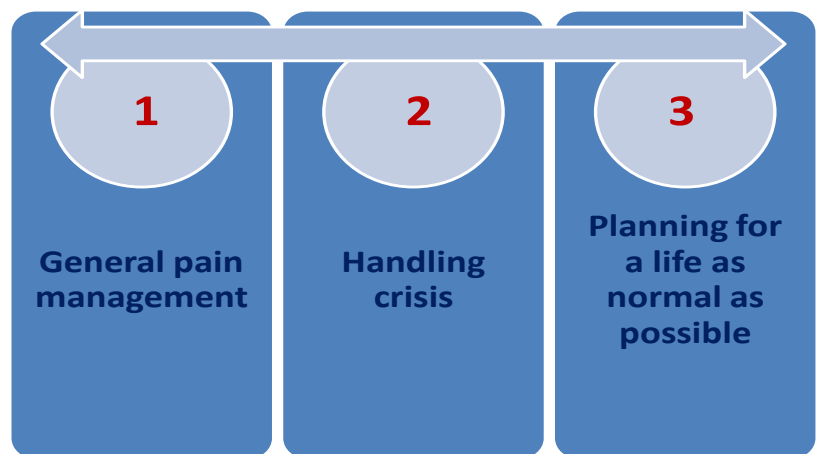


Figure 23: Component of Counseling Patients with Sickle Cell Disease

4.3.2 Adolescents & Young Adults

- Patient should know who to contact, when to contact-doctor's name, hospital, etc.
- Should know how to make appointments
- Should have a personal health summary at all times
- Should carry own health insurance, cards, etc.
- Should understand what complications can arise, & under what conditions
- Should discuss plans for independent living, vocational & economic self-reliance
- Should undergo Pre-marriage screening & genetic counseling
- Parents wanting patient's marriage & unmarried patient himself/herself should consider the inheritance patterns

4.3.3 Young Couples/Just Married

- Genetic counselor will not tell the patient what to do. Partners are free to choose the path which is most comfortable to them.
- Option for having a child should be based on inheritance pattern
- Give the parents knowledge of how SCD affects the newborn baby

4.3.4 Pregnancy Phase/Pre-natal

- Planning & early pre-natal care is the key
- Pre-natal care should be done by an Obstetrician, who is an expert in **high-risk** pregnancy
- Avoid infections of all types- viral, bacterial
- Healthy, high protein diet, fibrous diet is required
- Pre-natal vitamins, folic acid supplements may be required
- Preventing dehydration is a must
- There is high possibility of premature/pre-term baby
- During labour, IV fluids should be given to prevent dehydration
- Oxygen (*through mask*) may be given during labour (*depends on patient condition*)
- Give the parents knowledge of how SCD affects the newborn baby

4.3.5 Neonatal/Early Infancy

- High possibility of premature/pre-term baby– so prepare accordingly in advance
- Newborn screening for SCA/SCD: HPLC test and other tests
- Test for sepsis
- High risk of infections
- Vaccinations– penicillin, anti-tetanus, pneumococcal vaccination till age 5

4.3.6 Childhood and School Going Children

- Should have affection, safe environment at home and in school
- Two sets of books/copies advisable , one at home, one at school, so that they do not have to carry load daily
- Should be allowed to drink water frequently in school
- Should be allowed to go to the bathroom as many times as required.
- No strenuous exercise, long distance movement or running desirable
- Should be exempt from outdoor activities when temperatures are very high or low, instruct for alternate activity
- Should be given Moderate work load in schools desirable
- Should be given extra time to complete class work, tests, etc.
- Children should be **allowed** to carry extra water bottles, medications, umbrella, etc.
- At the hint of fever/ pain/ change in complexion or pallor/ breathing difficulty/ dizziness/ differential palpitation/ swelling/ muscular weakness, they should be immediately allowed to go home or referred to a doctor / Should know who to contact in emergency.

4.4 Counseling for Diet Management

- Take a high fibre diet, plenty of roughage
- Take large amount of proteins in diet
- Choose diet rich in anti-oxidants
- Avoid oily and fatty foods (masala)
- Take plenty of water

4.5 Do's & Dont's

- Drink plenty of water
- Modify dietary habits
- Take rest and relax
- Do not overwork
- Avoid extreme temperature
- Avoid infections
- Maintain regular schedules of work and play

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Who We Are

The Sickle Cell Institute Chhattisgarh (SCIC), an autonomous institute established by Government of Chhattisgarh in July 2013, is committed to increase knowledge and awareness among the people about Sickle Cell Disease (SCD), enhance methods of identification, diagnosis, and treatment to improve the quality of life of affected individuals and their families.

To fulfill this mission, the SCIC will support the development and growth of member organizations/institutions/centers in the state of Chhattisgarh and across India.

The SCIC is establishing a dedicated platform for clinical services and research initiatives that will support treatment of SCD patients. We believe that attracting experts in clinical and research fields to undertake and further the activities in the field of SCD is essential call of the time. SCIC is determined to pursue clinical and research activities in this field.

As an institution with a dedicated team clearly paying attention on the provision of improved and enhanced treatment options for patients with SCD, the Sickle Cell Institute Chhattisgarh is working for its mission and vision diligently.



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