



Prologue

Sickle cell disease is the major hemoglobinopathy seen in central india, inherited in an autosomal recessive fashion, and is responsible for significant burden of illness and morbidity especially among tribals and forest dwellers where malaria is common. The prevalence of disease in these areas is due to survival advantage relative to normal individuals with HbA especially in early childhood.

Patients with this illness suffer acute painful complications such as acute chest syndrome, dactylitis, bone pain and bone infection, stroke and splenic sequestration crisis, since early childhood. Splenic dysfunction early on, leads to increased chances of premature death due to overwhelming infections. Those who are fortunate to survive into adulthood often have crippling avascular necrosis of femoral head and some times head of humerus. Chronic pain, anemia, renal complications, stroke sequelae, retinopathy and pulmonary hypertension are the hallmarks of untreated patients who often suffer and die in silence and ignominy.

If only we as health care providers learn to suspect and screen for sickle cell disease and manage illness from an early age using simple principles and evidence based practices, the lives of most of these patients can become pain free, with fewer hospitalizations and hence health care expenses and longer fruitful lives.

We hope this resource book entuses many clinicians to take on this challenge to manage these patients effectively.

Jan Swasthya Sahyog team

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What is Sickle cell disease ?

Sickle cell disease is caused by the inheritance of abnormal beta-globin alleles carrying the sickle mutation . The mutation is a structural variant of haemoglobin in which glutamic acid , an amino acid at position 6 of b-globin chain of Hb is replaced by valine. The most common and severe form is homozygous HbSS (sickle cell anaemia) with inheritance of Beta S from both parents which permits formation of the pathological sickle hemoglobin tetramer ($\alpha_2\beta_2(s)$, HbS . Sickle hemoglobin gets polymerised at low oxygen tension and deforms the red blood cell from discoid shape to sickle like form and hence the name

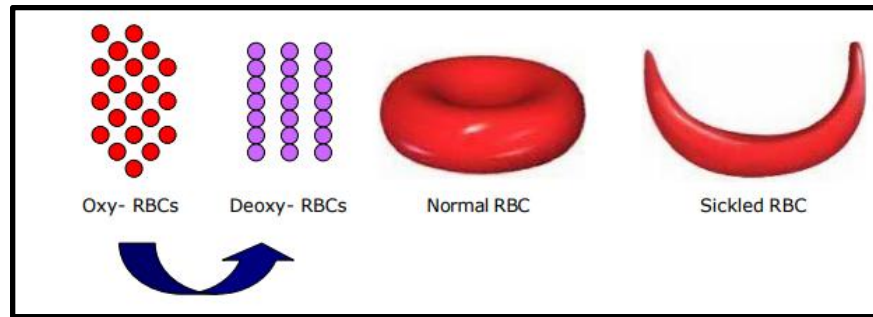
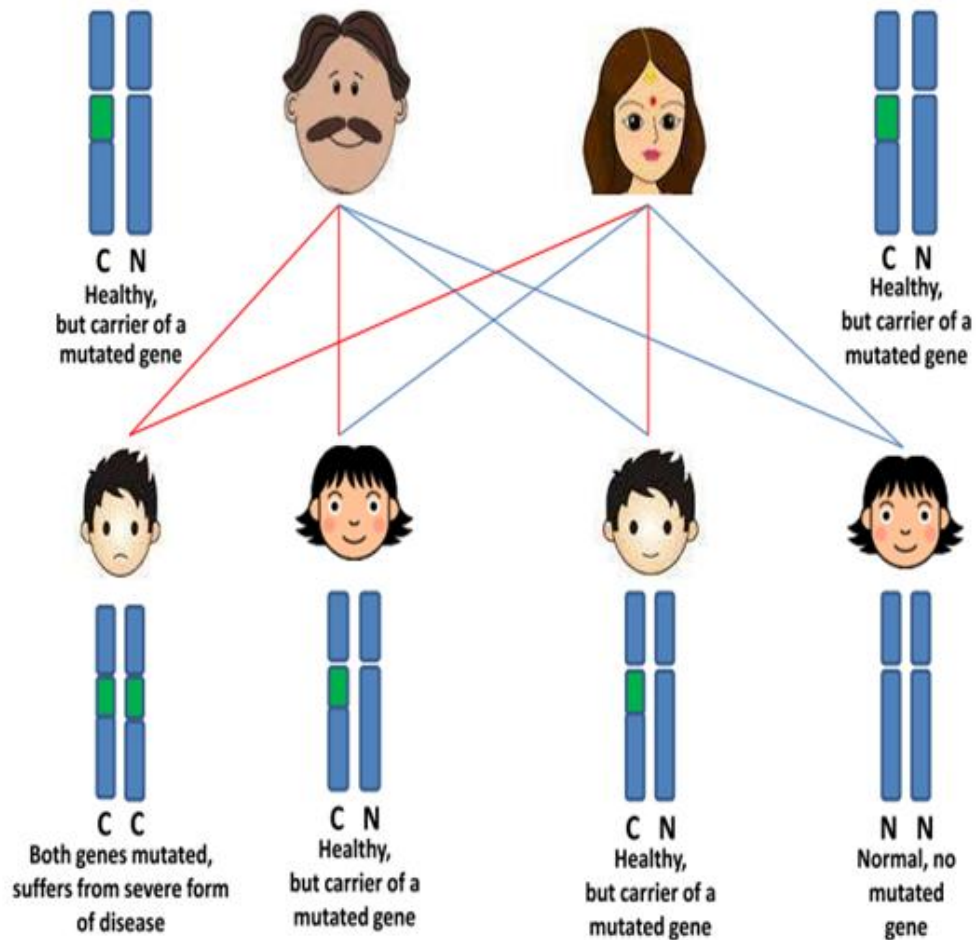


Fig - Normal and sickle RBC

Source - Gupta R.B(2006)

It is transmitted as autosomal recessive character . If a person receives only one gene responsible for sickle hemoglobin from either of parent , the condition is called carrier or trait . If one inherits two defective genes , one from each parent , the condition is called sickle cell disease . There are also compound heterozygotes who may have one beta globin gene carrying S while the others is B thal + , B thal 0 or Hb C . HbSBthal0 behaves almost like HbSS in severity while HbSBthal+ and HbSC are milder but more variable course .

The inheritance and risk of having a child born with Sickle cell disease when both parents carry the mutant allele for sickle cell HbS. (both parents are sickle cell trait or carriers). The blue colour depicts normal allele, mutant allele is green in colour.



Burden of sickle cell disease in India

With a population of close to 1.30 billion it is estimated that India is home to over 50% of the world's sickle cell disease patients (Shrikhand et al 2014) . Prevalence of sickle gene as per the hospital data is 22.5%-44.4% in central India (Gorakshakar 2006) . In some ethnic groups , it is specifically prevalent due to endogamy . In a study done in district hospital of Kalahandi Odisha , Hb of cord blood of 761 newborns was analysed in which 14.7% were heterozygous (sickle cell trait) and 1.7% were homozygous for sickle cell disease (Sujata et al 2015). It is widely believed that the disease in India differs from that in Africa , a milder clinical course resulting from high HbF levels and frequent association of alpha thalassemia characteristics of Indian patients .

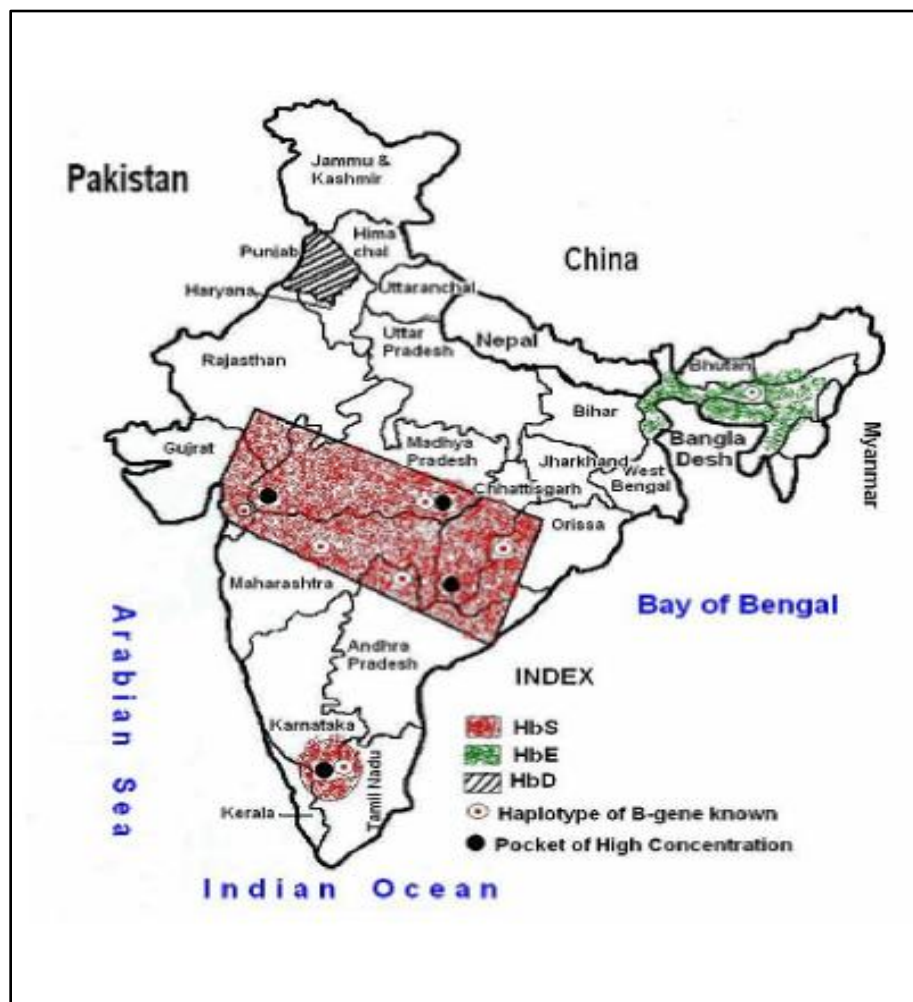


Fig - Distribution of hemoglobinopathies in India

In Madhya Pradesh , 27 districts fall under the sickle cell gene belt which include Dindori , Mandla , Dhar , Anuppur , Shahdol , Umaria . Madhya Pradesh has the highest load with an estimated number of 9,61,492 sickle heterozygotes and 67,861 homozygotes .It has been estimated that 13,432 pregnancies would be at risk of having a child with sickle cell disease in this State and the expected annual births of sickle homozygotes would be 3358.

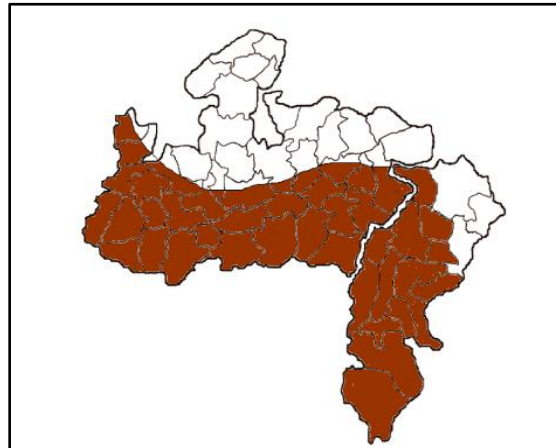


Fig - Sickle cell belt in Madhya Pradesh and Chhattisgarh

Gupta R.B (2006)

Pathophysiology

Vaso-occlusive crisis

In sickle cell disease erythrocytes undergo rapid but reversible shape change on deoxygenation and the shape changes from biconcave to an elongated sickle shape . Sickled erythrocytes cause vaso occlusion together with many other cellular and plasma factors leading to a broad range of acute and chronic clinical complications caused by repeated ischaemia and inflammation .

Reoxygenation of erythrocytes breaks down the HbS polymer and restores the normal shape . This process of sickling and unsickling continues until the erythrocyte membrane is no longer flexible and irreversibly sickled cells undergo intravascular hemolysis or extravascular removal by the spleen .

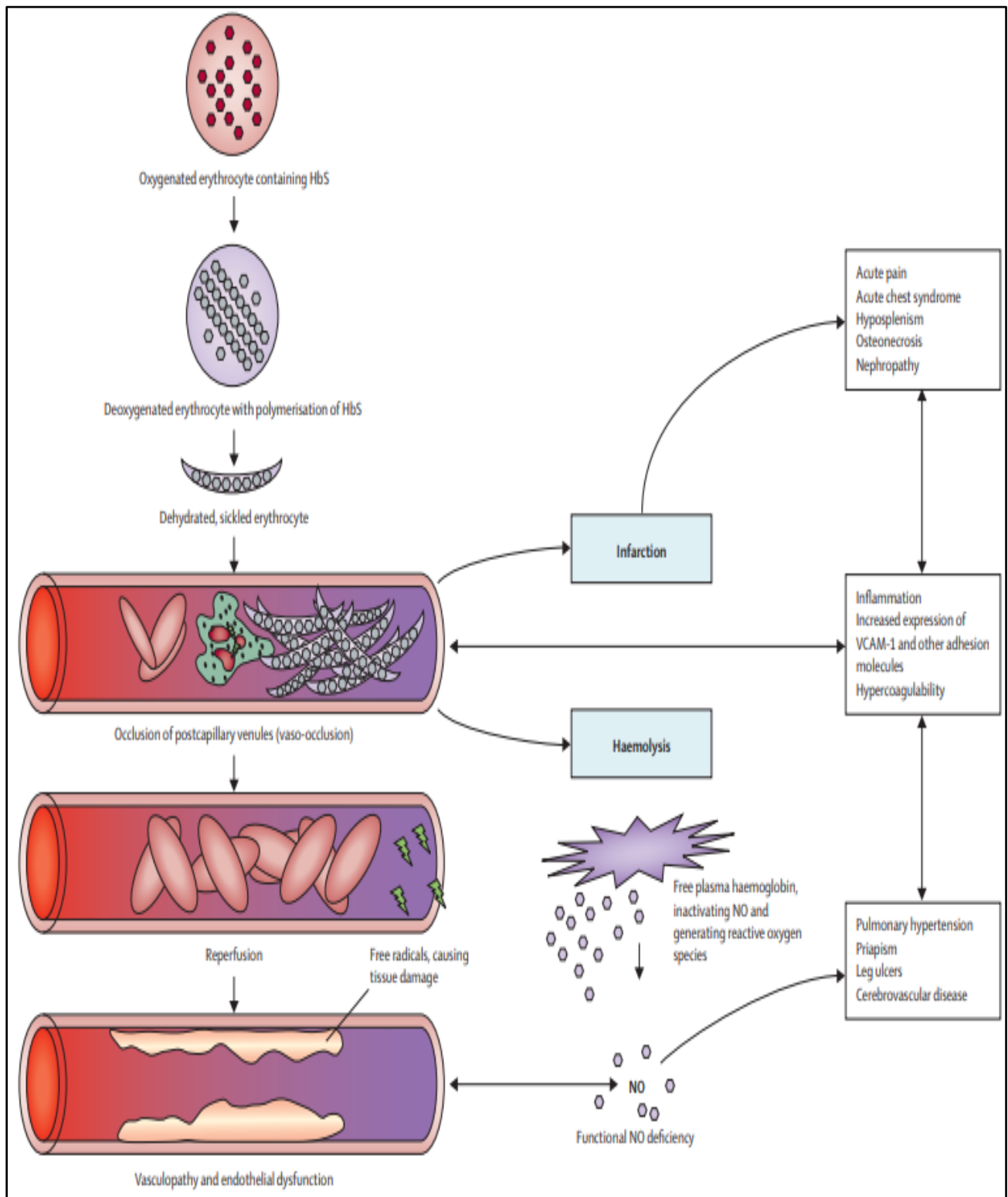


Fig - Vasoocclusion - The Lancet , 2010

Hemolysis

The second pathophysiological process in sickle cell disease is hemolytic anaemia which is also driven by HbS polymerisation . Hemolysis can cause anaemia , fatigue and cholelithiasis but now there is evidence that it contributes to the development of progressive vasculopathy . Cholelithiasis , cutaneous leg ulceration , priapism and pulmonary hypertension are associated with low steady state hemoglobin concentrations and an increased rate of intravascular hemolysis – an average RBC life span is of 17 days in HbSS(SC Disease) patients .

Common complaints

1. Pain

Pain is the most commonly reported symptom and is due to vaso-occlusion . Patients frequently present with acute bony pains manifesting in newborns and infancy as dactylitis (involving small bones of hands and feet) . Abdominal and chest pain can also occur which can represent dreadful complications like abdominal crisis and acute chest syndrome respectively .The initial process of sterile ischaemia /necrosis in tissues may get complicated with infections , which may be life threatening. There may be associated fever , dehydration and hypoxia .

2. Frequent Fever

3. Frequent hospitalization

4. Frequent jaundice

5. History of sibling deaths due to jaundice or severe pains

6. Blood transfusions

7. Poor growth and development , delayed puberty

8. Chronic pain , general fatigue and reduced ability to do manual work

Complications and their management

Cardiothoracic System	Nervous System	Reticuloendothelial System
<p>Chronic restrictive lung disease</p> <p>Left ventricular diastolic disease</p> <p>Pulmonary hypertension</p> <p>Acute chest syndrome</p> <p>Dysrhythmias</p> <p>Sudden death</p>	<p>Hemorrhagic stroke (brain)</p> <p>Venous sinus thrombosis</p> <p>Silent cerebral infarction (brain)</p> <p>Chronic pain</p> <p>Acute ischemic stroke (brain)</p> <p>Proliferative retinopathy (eye)</p> <p>Orbital infarction (eye)</p> <p>Cognitive impairment</p>	<p>Splenic sequestration</p> <p>Functional hyposplenism</p> <p>Anemia</p> <p>Hemolysis</p>
Musculoskeletal System	Urogenital System	Gastrointestinal System
<p>Avascular necrosis</p> <p>Leg ulceration (skin)</p>	<p>Papillary necrosis</p> <p>Proteinuria</p> <p>Renal failure</p> <p>Hematuria</p> <p>Nocturnal enuresis</p> <p>Priapism</p>	<p>Cholelithiasis</p> <p>Cholangiopathy</p> <p>Hepatopathy</p> <p>Mesenteric vaso-occlusion</p>

1. Acute chest syndrome

- Second most common cause of hospitalisation and commonest cause of death
- In 50%, it develops during hospitalisation for another cause like voc, post operatively, post-partum, etc.
- Peak incidence in children 2-4 yrs.
- Risk factors- asthma, smoke exposure, chronic hypoxemia

- **Diagnosis**
- New infiltrate on chest x ray with one or more of the following:
- Chest pain.
- Temp. > 38.5 degC
- Hypoxemia
- Tachypnea, wheezing, cough
- Increased work of breathing

- **Management**
- Treat infection- Ceftriaxone + Azithromycin
- Analgesia, maintenance fluids
- Respiratory support to maintain SpO₂ > 92%
O₂, CPAP, NIV, Mech. Ventilation
- Bronchodilators and steroids for asthma
- Early transfusion to bring Hb to 11g/dl
- Exchange transfusion in severe disease

- **Prevent recurrence of Acute Chest Syndrome**
- Prevent infection
- Treat asthma
- HYDROXYUREA

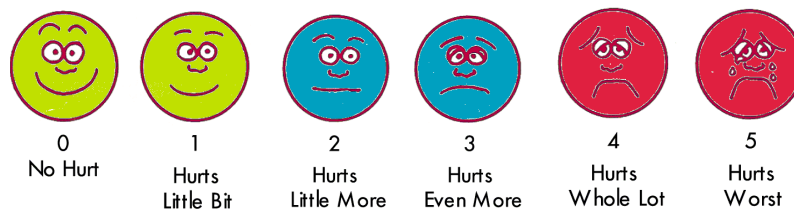


Case presentation - Chest X-ray of a 7 year old tribal girl who presented with history of fever , cough and pain in the legs for 4 days . She had history of recurrent episodes of pain in limbs , chest and abdomen for one year

- **Vaso-occlusive pain**

- Commonest reason for seeking care
- Acute pain – vaso-occlusion, ischemia, inflammation
- Chronic pain-due to complications such as compression fractures, AVN, arthropathies, leg ulcers, etc.
- Location, duration, severity
- Associated conditions- Acute chest syndrome, Bone infarction, splenic sequestration
- Rapid assessment, rely on patient's report of severity

- **Management**
- Home management
- Mild pain-PCM, NSAIDS
- Moderate pain- opioids (Codeine, Tramadol)
- Severe pain- Admit
- Rapid initiation of analgesia within 30 minutes
- Treat with morphine, pentazocine, fentanyl
- Look for assoc. fever, cough, tachypnea, symptomatic anemia, sequestration, stroke
- Fluids - Will prevent further pain crisis , maintain hydration . Can be given oral or IV (if required) . Maintain urine output more than 0.5ml/kg/hour
- Blood Transfusion, Oxygen - only if indicated
- Venous Thrombo-embolism prophylaxis in adults - Heparin
- Psychosocial support, distraction, relaxation exercises
- **HYDROXYUREA**- reduces frequency and intensity of pain episodes
- Treat chronic pain with hydroxyurea, opioids , NSAIDS. It is useful to use a visual pain scale such as the one given below
- Prevention – Adequate Hydration and rest, avoid strenuous activity



- Mild pain (0,1) - NSAIDs
- Moderate pain (2,3) - NSAIDs and/or Opiates
- Severe pain (4,5) - Morphine (Opiates) , Round the clock treatment with complete pain relief

Fever in sickle cell disease

- First indication of life threatening infection
- Temp.> 101 deg F –evaluation – early, quick (qSOFA) and within 4 hrs.
- History and exam to localize site of infection, hemodynamic instability, assoc. complications
- Lab tests-CBC, retics, Blood culture if available
- Chest X ray in presence of respiratory signs
- Urine exam, CSF if indicated

- **Treatment**
- Empiric antibiotic therapy within an hr.
- I\V Ceftriaxone 50-75 mg/kg/day, 100 mg/kg in meningitis
- Admit if age less than 2yrs., Temp.> 104 F, TLC> 30,000 or <5000, Hb fall >2gm from baseline, previous history of invasive infection, associated complications
- If above absent, patient . looks well, can follow up on out-patient Management

- **Prevention of infection**
- Immunisation - routine childhood immunisation through at least 5 years including HIB, pneumococcal, meningococcal
- Prophylactic penicillin
 - Twice-daily prophylactic penicillin beginning in early infancy and continuing through at least age 5
 - Tab. oral penicillin prophylaxis (125 mg BD for age <3 years and 250 mg BD for age ≥3 years)
- Empiric management of fever

Pulmonary hypertension

- occurs in 6-11 % of patients
- risk factor for mortality
- Dyspnoea on exertion , fatigue, palpitations, chest pain,
- edema, syncope

Diagnosis

- SpO₂ < 94% or drop of > 4% on activity
- Loud P₂
- Chest X Ray , ECG
- Echocardiography- Tricuspid valve regurgitation jet velocity > 2.5 m/sec
- Pulmonary Artery and RV systolic pressure are increased
- Serum NT pro BNP level
- 6 min walk test
- Confirm with Right Heart Catheterization

Treatment

- O₂, SpO₂ > 90%
- Treat obstructive sleep apnoea , asthma, nocturnal hypoxemia
- HYDROXYUREA
- Chronic transfusion
- Pulmonary Vasodilators- ERA, prostenoids
- Sildenafil and calcium channel blockers are not recommended
- Anticoagulation if VTE and no bleeding risk

- **Stroke**

- Ischaemic and haemorrhagic stroke
- 11% by 20 yrs., 25% by 45 yrs, if untreated
- Primary prevention- measurement of transcranial doppler velocities between 2-16 yrs. followed by chronic transfusion in at risk children
- Treat hypertension
- Suspect if delayed development, neurological deficit, altered consciousness , seizures
- **Diagnosis and Management**
- Neuroimaging
- Treatment- transfusion simple/exchange
- Aspirin in ischemic stroke
- Thrombolytic therapy not used generally
- Chronic transfusion and HYDROXYUREA to prevent recurrence
- Other neurological complications- Transient ischaemic attacks, seizures, Posterior reversible encephalopathy syndrome (PRES)

Anaemia

- Chronic compensated hemolytic anemia
- Hb 8-10 g/dl, mild leucocytosis and reticulocytosis, normocytic, normochromic, or mild macrocytosis, microcytic if assoc. thalassemia or Iron deficiency
- Acute exacerbations caused by
 - Aplastic crisis
 - Splenic sequestration

Aplastic crisis

- Due to infections

Parvo virus B19, Pneumococcus, Salmonella,

EBV

- Fall in Hb by >2 g/dL from the steady state
- Low Hb, low Retics < 1%

- Transient 2-14 days
- Treat with transfusion

Splenic sequestration

- Due to pooling of a large quantity of blood in an organ (spleen, liver)
- Infants and children
- 10-15% mortality

Clinical features-

- Tender splenomegaly
- Hypovolemic shock
- Anemia, thrombocytopenia, reticulocytosis
- Treat with fluids and blood transfusion
- 50% chance of recurrence- prevent recurrence by teaching spleen self palpation, splenectomy



Fig - Splenomegaly in a child with Splenic sequestration

- **Skeletal complications**

- Vaso-occlusive pain
- Dactylitis – 6 months to 4 yrs.
- Painful symmetrical swelling of hands and feet associated with erythema and mild fever
- X-ray shows soft tissue swelling
- Differential - osteomyelitis
- Rx- hydration, analgesics, anti inflammatory drugs, warm packs, HYDROXYUREA



Fig - Dactylitis

- **Osteomyelitis and Septic arthritis**

- Infection in areas of infarcted bone and due to splenic hypofunction
- Prolonged fever and pain, swelling at a single site
- Diagnosis- X-ray changes appear at 7-10 days
 - Biopsy and aspiration of suspected focus
- Salmonella, E.coli, Staph. aureus
- Treat with antibiotics (ceftriaxone and ampiclox) and drainage

Avascular Necrosis

- Occurs due to infarction of bone trabeculae
- 10% of SCD pts. affected, prevalence increases with age
- Humeral and femoral heads affected, femoral head undergoes progressive destruction due to chronic weight bearing
- Reduced mobility, gait abnormalities, limb length discrepancies, B/L involvement

- **Management**
- *Early* - Conservative
- Analgesia, rest, reduced wt. bearing, physiotherapy
- Core decompression if no relief with conservative Rx
- Arthroplasty- high failure rate with serious peri-op. and post op. complications
- Early detection is important to delay progression
- High HbF, protective- HYDROXYUREA



Fig - Avascular necrosis involving right femoral head

Hepatobiliary complications

- Acute ischemia
- Cholestasis
- Hepatic sequestration
- Cholelithiasis
- Iron overload
- Viral hepatitis

Renal complications

- Defect in urinary concentrating ability leading to enuresis
- Hematuria due to papillary infarcts
- Proteinuria leading to progressive renal disease
- Hypertension
- FSGS leading to ESRD
- Nephrogenic Diabetes Insipidus

Leg ulcers

- Painful ulcers due to vaso-occlusion in the skin
- Present after 10 yrs. of age, males more commonly affected
- Spontaneous or after trauma,
- Medial and lateral malleolus, B/L
- Prevention- well fitting shoes, aggressive Rx of skin injury
- Antibiotics, local wound care, debridement

Retinopathy

- Due to retinal artery occlusion and ischemia followed by neovascularisation
- Reduced visual acuity
- Annual ophthalmologic examination starting at 10 yrs. of age
- Rx- laser photocoagulation

Pregnancy and Sickle Cell disease

Due to sickle cell disease , risks of the following complications increases in pregnancy

- Infections - UTI
- Vaso-occlusive crisis
- Thromboembolism
- Placental insufficiency which can lead to the following complications
 - Maternal - Pregnancy induced hypertension (PIH) , HELLP syndrome , PPH , premature placental separation (APH)
 - Fetal - Prematurity , IUGR
- Puerperal sepsis

Reasons for admission

- Pain
- Fever more than 100 F
- Severe anaemia
- Shortness of breath
- Preeclampsia , PIH
- Infection
- Labour
- Symptomatic in the post partum period

Management

- Keep warm and hydrated
- Maintain strict fluid balance chart
- If SpO2 below 94% _ humidified Oxygen
- Appropriate pain relief
- VITALS RECORDING AND MONITORING
- Infection screen - Malaria , Total counts , UTI

Transfusions

- Low tolerance to Anaemia - Hb <9g/dL
- Hypoxemia (saturation <94%)
- Clinically indicated
- Multiple pregnancy
- Complicated pregnancy
- Recurrent pain crisis

Hematinics

- Folic acid - 5mg/day
- FeSo4 - Can give oral iron and see for response to oral iron

Analgesia

- PCM and Fluids - For mild to moderate pain
- NSAIDs like Ibuprofen , Diclofenac - Between 12 to 28 weeks only
- Opiates
 - Low potency - Codeine
 - Tramadol 50mg BD or TID PO or IM or IV
 - Morphine if severe pain
 - Pentazocine (Fortwin) : 0.3-0.5 mg/kg/dose for max 60mg / day
 - Avoid pethidine - associated with high risk for seizures
 - Thromboprophylaxis with heparin

Fluids

- Oral intake encouraged
- Hydration

Delivery and early post partum (48 H)

- Prefer vaginal delivery - Verify CPD
- Avoid prolonged labour - Use partograph
- Induction if more than 38 weeks of gestation
- Oxygen - 2L/min
- IV Antibiotics - Must
- Hydration : Oral and if needed IV

- Heparin - Prophylactic dose 5000 U SC BD for min 2 days
- Arrange for 2 units of blood - Be ready
- High risk of acute chest syndrome
 - Measure respiratory rate in post partum period
 - SpO2 measurement
 - If any doubt do a chest x ray
 - Avoid hydroxyurea while breast feeding
 - Contraception : Must counsel

Lab investigation

The cornerstone of investigating any patient suspected of having a sickle cell disorder or one of its complications are

- Reliable Hb estimation – This could be done using a coulter counter (complete CBC) or a digital Hemoglobinometer (such as Handyspan or other)
- Complete blood count
- Screening for sickle disorder using
 - Solubility test
 - Sickle prep
- Reticulocyte count
- Confirmation of diagnosis using
 - Hb Electrophoresis
 - HPLC
 - RFLP
 - Isoelectric focussing
 - Capillary electrophoresis
 - DNA Sequencing

In addition , availability of safe blood and testing by grouping and cross match must be available . We discuss the ones that must be available at secondary care facilities (FRUs and District Hospitals) where most such patients will be seen . While Hb electrophoresis and HPLC are

frequently available for clinical use , the other techniques of IEF , Capillary electrophoresis , RFLP and DNA sequencing are more demanding and expensive , mostly confined to research laboratories .

Solubility test

The solubility test is based on the relative insolubility of HbS in the reduced state in high phosphate buffer solution (metabisulfite is a reducing agent). When whole blood is mixed with the reducing agent , the HbS forms liquid crystals and gives a cloudy appearance to the phosphate buffer solution.

Reagents:

Phosphate buffer (pH 7)

Sodium dithionate powder

Normal Saline

Preparation of Phosphate Buffer:

K₂HPO₄ : 250gm

KH₂PO₄ : 143.5gm

Saponin : 2 gm

Benzoic acid: 2.5 gm

Distilled water: 1 lt

This reagent buffer is stable for 6-7 days at room temperature and for 1 month at 4 degree celsius.

Sample required: 2 ml EDTA sample

Preparation of packed cell:

- Fill a small test tube with approximately 2-3 ml Normal Saline.
- Add 2 to 4 drops of well mixed whole blood.
- Mix thoroughly and centrifuge at 3000 rpm for 2 minutes.
- Discard the supernatant and repeat cell washing process three times.
- Discard supernatant after 3rd washing.

Method:

- Take a pinch of sodium dithionate powder in test tube.
- Add 1 ml phosphate buffer and mix well till sodium dithionate powder dissolves completely.
- Add approximately 20 micro l washed RBC's and shake gently.
- Allow it to stand for 5 minutes.

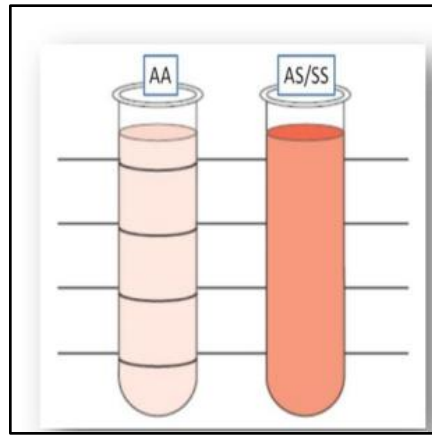


Fig - The Sickle cell solubility test

Interpretation:

- Once RBC's dissolve colour will be pinkish violet.
- Look for turbidity against two dark black lines on white paper against a bright source of light.
- Keep tubes approximately 1 cm away from paper.
- If turbidity seen- solubility test is positive - dark lines do not appear clearly through tubes.
- If turbidity not seen- solubility test is negative - dark lines appear clearly through tubes.
- If rings are allowed to stand for more than half n hour, a ring formed by insoluble HbS is seen on the surface of the test reagent.
- Known Positive control and Negative Control should be kept to compare the result.

Sickle prep test

Sickle prep (slide method):

Principle:

RBCs become sickle shape in Sickle cell disease. Due to its Oxygen carrying nature, sickle shape RBCs also appear in normal RBC shape on routine microscopy. The sickle shape appears only in deoxygenated sickle RBCs. In Sickle prep method, sodium dithionate helps in deoxygenate the RBCs. Wax sealing prevents further air/oxygen contact of RBCs, thus sickle RBCs remain in sickle shape. These can be easily identified on microscopy. De-oxygenation with sodium dithionate takes about an hour. In sickle prep investigation, blood sample is subjected to de-oxygenation after sealing the sample with wax followed by microscopy to identify sickle RBCs.

Requirement:

- Slide
- Sickle buffer
- Cover slip
- Wax
- Match box
- Dropper
- Lancet
- Needle
- Spirit
- Bunsen Burner
- Electronic balance

Chemicals required:

- Disodium hydrogen phosphate (Na_2HPO_4)
- Sodium dithionate ($\text{Na}_2\text{S}_2\text{O}_4$)

Process of making buffer and working solution:

Buffer: Mix well - Disodium hydrogen phosphate (Na_2HPO_4) – 8.1 grams and distilled water 833 ml. This mixed solution can be kept in dark brown bottle for 4-5 month.

Working Solution: Mix well a solution of 12.5 ml of buffer and 110 mg sodium dithionate ($\text{Na}_2\text{S}_2\text{O}_4$) in a screw cap test tube. This working solution can be stored in refrigerator upto a week.

Process of sickle prep testing:

1. Take a drop of working solution on the slide and mix it well with small drop (5 micro lit) EDTA or finger prick blood.
2. Cover this mixture with cover slip and seal its borders with liquefied wax.
3. Observer the slide under 40X zoom of microscope after 1 hour.
4. If sickle test is positive - we would find sickle shaped red blood cell (as marked in the slide here). In case of negative sickle test, there would be no change in RBC shape.

Precautions:

- Heat the wax only until it liquefies. Don't heat the wax at high temperature.
- Quantity of sodium dithionate to be taken is 110 mg strictly. Higher quantities can crenate RBCs resulting in mistake in result.
- More than 5 micro liters of blood quantity may cause overlapping of RBCs.

Reticulocyte countStaining of reticulocytes

Principle - Brilliant cresyl blue in an isotonic medium selectively stains nucleic materials of erythrocytes called reticulocytes which can be seen under a microscope.

- Important Equipment - Microscope, Slide, Test tube, Retic Reagent, etc.
- Sample - EDTA Sample / whole blood .
- Procedure - 5ml Glass test tube in 200ul Blood Sample in 100ul Retic Stain(2:1) mix and incubate at 37°C for 20 minutes after mix and prepare few Smears on mycroscopy glass slide reported by 100x (oil immersion less) .

migration fronts that appear as bands on the electrophoresis medium (agarose gel or cellulose acetate paper). For example, in a mildly alkaline medium (pH 8.2 or 8.4), normal adult haemoglobin (HbA) and sickle haemoglobin (HbS) both move towards the anode but HbA moves faster leaving HbS behind. Ultimately all the HbA molecules accumulate in a distinct band ahead of the HbS molecules, which also move together in a trailing band. The different bands can be identified by comparing their positions with those obtained from control samples of known varieties of haemoglobin. These can also be clearly visualized and identified on an agarose gel stained with Amido black 10B Stain.

Materials needed are:

- Electrophoresis unit
- Electrophoresis power supply
- Spatula (for detaching the gel from the casting tray for staining purpose)
- Petri dish for staining and de-staining
- Centrifuge machine
- Pipettes(1000 and 10 micro lt)
- 50ml beaker
- Bunsen Burner, Tripod Stand, Wire Mesh
- Conical Flask

Storage

Alkaline Hemoglobin Electrophoresis Buffer can be stored in fridge for 1 week and used.

Specimen Handling and Collection

Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future). Ensure that the blood sample is at room temperature before beginning the protocol.

Procedure

I. Sample preparation

The quantity of sample required for the electrophoresis testing is 250 μ l of blood, if patient's hemoglobin is less than 7 gm% then take 500 μ l sample. For testing purposes red blood cells are

to be separated from the fresh blood sample to isolate hemoglobin from them. For doing that, Centrifuge the fresh whole blood sample at approximately 3000 rpm for 10 minutes at room temperature. Discard the supernatant plasma carefully such that the red blood cell pellet obtained, is not disturbed. Wash the red blood cell pellet with 2 ml of Normal Saline. Centrifuge the resuspended pellet at approximately 3000 rpm for 10 minutes. Again discard the supernatant carefully and wash the pellet with another 2ml of Normal Saline. Perform this wash step 5 times with Normal Saline to obtain a red blood cell pellet. Discard the supernatant carefully at the end of the washing steps such that the red blood cell pellet remains undisturbed. Make sure that there is no normal saline remaining in the tube.

II. Lyse

1. Add 200-500 μ l of Distil Water, mix the suspension well and let it stand for 10 minutes.
2. Add 200 μ l of CCL₄ (Carbon Tetra Chloride), Mix well and centrifuge at approximately 3000rpm for 10 minutes.
3. Take the tubes carefully out of the centrifuge without disturbing the supernatant and pellet.

III. Preparation of Working Buffer

1. Mix 8 gm Tris buffer pre-weighed and 3.6 gm Glycine to 500ml of distilled water in a conical flask.
2. Check pH of the buffer, it should be 7.4.
3. This working buffer can be stored in fridge and reused 3-4 times, provided its pH is maintained at 7.4.

IV. Agarose gel preparation

1. Prepare agarose gel by adding pre-weighed 320mg of Agarose in 40ml working buffer. Dissolve it completely by boiling in the working buffer.

NOTE: Prepare fresh diluted Gel Running Buffer as indicated in general preparation instructions

NOTE: The agarose powder should be dissolved in diluted working buffer by boiling and swirling intermittently such that the agarose dissolves completely. Do not over boil the agarose so as to minimize water loss due to evaporation.

2. Cool the melted agarose for about 10 minutes, cover the open sides of the boat with cello tape. Pour the melted agarose while hot in the casting unit of electrophoresis unit with the two combs placed in their respective notches. Ensure that the gel poured spreads evenly on the surface of the casting tray to form a thin gel. Allow the gel to set. The gel will solidify completely in 15 minutes.

NOTE: Do not pour the gel when it is boiling hot as it leads to water loss due to evaporation which will alter the concentration of agarose in the gel. The formation of a thin uniform gel is essential to minimize resistance produced, which leads to generation of heat due to high voltage and high current required for the electrophoretic run.

3. Position the casting tray after the gel has set such that the wells are oriented towards the cathode.

4. Pour 450 ml of working buffer into the electrophoretic tank. Ensure that the agarose gel is submerged completely in the working buffer.

5. Load 5 μ l of supernatant from each sample into each well.

6. Connect the electrodes of the Electrophoresis unit to Electrophoresis power supply unit and run the gel at 150 to 200 V and 90 mA for 1 hour. To ensure that the run has started the user can observe bubbles in the buffer from the sides of the electrophoresis unit.

Usually migration of protein band can be read after 1 hour without staining but if the bands are lightly coloured and for clear reading we can go ahead with staining.

V. Staining of gel for visualization of hemoglobin protein bands

1. Slice the gel carefully along the edges of the casting tray using a spatula, such that gel can slide down easily into the staining tray. Avoid breakage of gel during handling.
2. Pour Amido Stain onto the gel such that the gel is completely submerged in the staining liquid. Allow staining by shaking the gel in the staining solution for 10 minutes.
3. Decant the stain used in a container. This stain can be reused 8-10 times. It should be stored in brown glass bottle.

4. Rinse the gel with 100 ml distilled water by pouring it in the tray and shaking the gel intermittently.
5. Decant tap water and pour 5% acetic acid into the tray for 30 minutes. Shake the gel intermittently.
6. Discard the acetic acid after 30 minutes.
7. Discard the acetic acid and take reading of the bands.

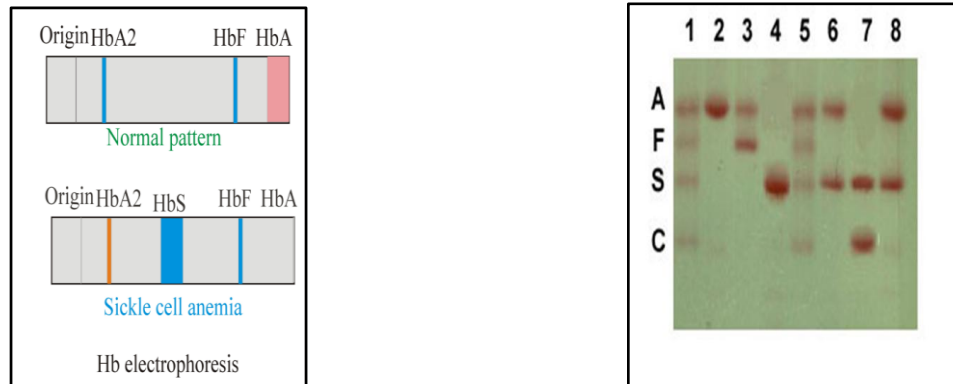


Fig - Electrophoresis

HPLC

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 a useful technology to screen for haemoglobin variants. The pattern seen by alkaline electrophoresis demonstrates some correlation with retention time by HPLC since both methods are dependent on the charge of the hemoglobin molecule; although the specific retention time by HPLC is dependent on the column and eluting solution used in the instrument. In general term, amino acids substitutions leading to more over all negative charge will result in faster migration by alkaline electrophoresis and a shorter retention time on the column by HPLC.

Guidelines for Prescribing Hydroxyurea at Primary & Secondary care Centre.

Hydroxyurea also known as Hydroxycarbamide, is a Ribonucleotidase Reductase Inhibitor mainly acts by increasing the HbF levels, thus increasing the Oxygen - O₂ carrying capacity and reducing the sickling episodes.

Other minor mechanisms of action are as follows:-

1. Decreases the number of Circulating WBCs and Reticulocytes as well as Adhesion molecules à reduction in Vaso-occlusion
2. Metabolism of Hydroxyurea à Releases NO (Nitric Oxide) à vasodilatation of small and large blood vessels
3. Raises MCV
4. Reduces the cellular deformability and Rheology.

Trade names of Capsule Hydroxyurea:-

Cap. Hydrea ; Cap. Droxia; Cap. Durea

Strength of Capsule:-

Hydroxyurea is available only in the form of capsule of 500 mg strength. For administering the lower strength of capsules, the powder in capsule could be divided equally in 2 or 3 portions according to the requirement.

Evidence for Prescribing Hydroxyurea:-

The drug is listed in the World Health Organization (WHO) Model List of Essential Medicines for Children in section 10.3 (Hydroxycarbamide) (April 2015) [3]. An expert review of the WHO supported the use of hydroxyurea as a disease modifier in sickle cell anemia [4]. Hydroxyurea is also listed in Essential drug list of Chhattisgarh & Madhya Pradesh, but not dispensed in Government Hospitals. It is prescribed & provided in the state of Gujarat & Maharashtra under state health programme

Four major trials were conducted in India for evaluating safety & efficacy of the Hydroxyurea, of which the Trial by Singh et. al., prescribed Hydroxyurea at 22 mg/kg/day with

escalating doses based on pain frequency & intensity. This was the only trial in India where Adults (more than 18 years of age) were included in the trial.

Safety:-

Hydroxyurea is relatively nontoxic, with myelosuppression as the major dose-limiting toxicity. In various studies in children and ones including infants, the occurrence of severe cytopenias has been uncommon. Short term toxicities of hydroxyurea are mainly those associated with myelosuppression (e.g., neutropenia, thrombocytopenia). These are generally rapidly reversible by holding the dose temporarily, for about two weeks and restarting at a lower dose. There is certainly a need for regular monitoring i.e. minimum 2-3 monthly monitoring of the hematological parameters to detect any abnormality in white cell counts anytime during the continuation of treatment [1, 2].

Indications for use of hydroxyurea in children with sickle cell disease:-

All infants 9 months of age or older, children, adolescents as well as adults with sickle cell anemia.

Dose:-

The starting dose: 15-20 mg/kg per day.

The dose can be increased in increments of 5 mg/kg/d increments every 8 weeks till maximally tolerated dose is reached NOT exceeding 25-35mg/kg/day.

Following Table could be used for prescribing Hydroxyurea:

Weight Range (Kg)	Dose (mg)	Quantity of Drug to be taken
5 – 7.5	125	¼ Cap OD
7.6 - 12.5	180	⅓ Cap OD
12.6 – 17.5	250	½ Cap OD
17.6 – 22.5	300	½ Cap OD
22.6 – 27.5	375	1 Cap & ½ Cap Alternate Day
27.6 – 32.5	450	1 Cap OD
32.6 – 37.5	525	1 Cap OD
37.6 – 42.5	600	1 ⅓ Cap OD
42.6 – 47.5	675	1 ½ Cap OD
47.6 and onwards	750	2 Cap OD

Who can prescribe?:-

The care of individuals with sickle cell anemia/ disease needs a comprehensive protocol, involving Analgesia, Hydration, Hematinic (Folic Acid) administration & Hydroxyurea prescription; of which treatment with hydroxyurea is an important component.

Given the burden of the disease, and the need to reach out to every affected individual, it is suggested that the treatment with Hydroxyurea can be started by any physician or medical officer working in Primary or Secondary care setting, for a patient with Sickle Cell Disease (SS).

Within the State health services, the treatment could be started at the CHC as well as PHC level. All the blood parameters could be checked at District Hospital or CHC at minimum 3 monthly intervals.

The Physician or Medical Officer should carefully assess the patient for the evidence infection, pain frequency & intensity & signs of chronic complication of Sickle Cell Disease. On occurring of Myelosuppression i.e. Low TLC (less than 4500/cu.mm) & Low platelets (less than 80,000/cu.mm), the Hydroxyurea can be stopped for 2 weeks & then restarted again within 2 weeks after rechecking the Blood Parameters (CBC).

Monitoring parameters:-

Following blood parameters must be monitored at least every 3 month visit

1. CBC_ Hemoglobin; Total Leukocyte Counts(TLC); Differential Leukocyte count(DLC); Platelets; Mean Corpuscular Volume (MCV)
2. Serum Creatinine
3. SGPT or Serum Alanine Phosphate (ALT)

When to stop the Medicine?

If the Total Blood Count is less than 4500/cu.mm then, one must STOP Hydroxyurea for 2 weeks minimum & Restart in weeks after checking the Total Leukocyte counts again.

If Platelet count is less than 80,000/ cu.mm then also one must STOP for 2 weeks & Restart in 2weeks after checking the Platelet counts.

If Serum Creatinine is increased i.e. more than 1.2 mg/dl; then Creatinine Clearance (CrCl) must be calculated. If CrCl < 60 mL/min, Hydroxyurea should be started in half dose & then should be referred to Tertiary care centre for Paediatric consultation.

Hydroxyurea is CONTRAINDICATED in pregnancy & lactation period

SICKLE CELL DISEASE PATIENT PROFORMA

An aid to better working

PATIENT PROFILE :

Name
Age
Village
Religion
Father's name
Mother's name
Married Yes / No

Sex - Male / Female
DOB
Post Office
District

OP #
IP #
Date

HISTORY

1. Age of onset
2. Symptoms - jaundice
Pallor
Painful episodes- joints/ chest/ abdomen
Recurrent infections
Neurological
Others
3. Frequency of symptoms - (in last one year)
4. Admissions - if any number of admissions
5. Treatment so far - Folic acid
Fe
Antibiotics
Blood transfusions if any- total number of times transfused
- 6 Family history

EXAMINATION

Weight
Height
Pallor
Icterus
Chest
Spleen
Size
CNS
Others

INVESTIGATION

Hb
TLC/ ANC
Platelets
Serum Creatinine
SGOT/SGPT
Blood Group

TREATMENT PLAN

Folic Acid
Deworm
Fersolate for 2 months
Oral Penicillin till 5 years of age
Pneumovac

Hydroxyurea
Indication

Hydroxyurea	10mg/Kg		15mg/Kg		20mg/Kg		25mg/Kg	
Date / dose								
* SS								
* Hb >6								

Further Reading

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