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Sickle Cell Disease: A Single Gene Mutation with Varied Presentations

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Abstract

Sickle cell disease is one of the commonest severe monogenic disorders seen worldwide. Hemoglobin S (Hb S) is the result of a single base-pair change, thymine for adenine, at the sixth codon of the β globin gene. This change encodes valine instead of glutamine in the sixth position in the β globin molecule. In the United States, sickle cell disease occurs in African Americans at a rate of 1: 396 births and in Hispanics at a rate of 1: 36,000 births. In the UK, the prevalence is 1:2000 live births. In India SCD gene frequency varies from 2 to 14 % of at risk population and is as common as thalassemia, but less highlighted, due to the predominantly underprivileged, tribal population of Central India (Vidharbha, Marathwada, M.P., AP, West-Odisha., Chhattisgarh and Gujrat).

Key Words: Homozygous HbSS Beta globin gene.

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SICKLE CELL DISEASE:

A Single Gene Mutation with Varied Presentation

Sickle cell disease is one of the commonest severe monogenic disorders seen worldwide. Hemoglobin S (Hb S) is the result of a single base-pair change, thymine for adenine, at the sixth codon of the β globin gene. This change encodes valine instead of glutamine in the sixth position in the β globin molecule. Sickle cell anemia, homozygous Hb SS, occurs when both β globin genes have the sickle cell mutation. Sickle cell disease refers to not only patients with sickle cell anemia 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 Hb C, Hb S β-thalassemia, Hb D, etc. In sickle cell anemia, Hb S is commonly as high as 90% of the total hemoglobin. In sickle cell disease, Hb S is >50% of all hemoglobin, while the heterozygous state of AS is associated with less than 50% sickle hemoglobin and has originated from families with ancestral roots in malaria endemic regions. In the United States, sickle cell disease occurs in African Americans at a rate of 1:396 births and in Hispanics at a rate of 1:36,000 births. In the UK, the prevalence is 1:2000 live births. In India SCD gene frequency varies from 2 to 14 % of at risk population and is as common as thalassemia, but less highlighted due to the predominantly underprivileged, tribal population of Central India (Vidharbha, Marathwada, M.P., AP, West-Odisha., Chhattisgarh and Gujrat).

In the homozygous HbSS state, the formation of hydrophobic motifs in the mutant β-chain leads to bonding between β1 and β2 chains of the Hb tetramer. The resultant crystallization of the Hb molecule then produces a polymer nucleus, which fills up the red blood cells and causes a disruption of its architecture. At the cellular level, this results in a loss of flexibility, leading to cellular dehydration and physical & oxidative stress. HbS polymerization is 1) directly proportional to the duration and degree of Hb deoxygenation, 2) the concentration of intracellular HbS, 3) viscosity of blood, and 4) inversely proportional to the concentration of fetal Hb in the RBCs. The Asian/Indian haplotype is associated with more than 50% coinheritance of "Deletional Alpha Thalassemia" and persistence of high Fetal hemoglobin levels; and hence milder course than the African haplotype. Initially, the formation of these polymers is reversible, at least partially, with reversal of the deoxygenated state. However, recurrent cycle of deoxygenation or prolonged RBCs hypoxia leads to an irreversible polymerization, thus effectively rendering the RBC effete. The polymers form bundles that lead to RBC distortion into the classic sickle or crescent shape. Also the RBC dehydration leads to membrane damage; these in turn increase the HbS concentration and lead to further sickling. Sickle cells are stiff, less deformable and have abnormal Cation permeability leading to Intracellular dehydration. These cells also generate potent oxidants such as super oxides, peroxides, OH radicals. Loss of Free Heme depletes nitric oxide and damages RBC membrane by oxidizing its proteins and lipids. Hemolysis leads to multiple changes including altered nitric oxide metabolism and oxidant stress, leading to endothelial dysfunction and vascular occlusion. Sickle cell disease is also an inflammatory disease due to the complex interaction between RBC receptors (VLA4, CD36), WBC count, and cytokines such as VCAM-1(Vascular cell adhesion molecule), Platelets, and plasma coagulation factors, culminating in vaso-occlusion in post-capillary venules.

It is clinically categorized as being due to 1) Chronic Anemia, 2) Various crisis, 3) Infections, and 4) Chronic organ damage. As early as 2 to 3 months of age, HBSS patients manifest with pallor, growth failure, irritability, due to progressive decrement of Hb F.

i) Intermittent **Vaso-occlusive** painful crises are the commonest clinical features at 6 months onwards till adolescence. Hand foot painful swelling due to ischemic necrosis of small bones of extremities; recurrent abdominal and chest pain, and cerebrovascular occlusion leading to stroke are common during infancy and early childhood.

ii) Acute Chest Syndrome (ACS) characterized by fever, cough, wheezing, chest pain or tachypnea, hypoxia and sometimes progressing to white out lung on chest radiograph, leading to respiratory insufficiency and death in as high as 25 % of HbSS children. Factors responsible for ACS are Infections [viral, mycoplasma, chlamydiae], pulmonary fat embolism, acute pulmonary sequestration pulmonary infarction, fluid overload, bronchospasm, and hypoventilation due to sedation.

iii) Oste-oarticular complications are seen in early childhood and adolescents, in the form of recurrent necrosis of bone, particularly avascular necrosis of Femoral and Humerus head. Osteomyelitis due to Salmonella species is seen in older children

iv) Acute splenic sequestration crisis occurs in up to 30% of preschool children, and a leading cause of reported death in 20% of hospitalized HbSS children. The majority of children manifest with rapid enlargement of spleen, severe pallor, circulatory collapse, and mandating cautious blood transfusion. Recurrent episodes of splenic sequestration crisis lead to infarcts, auto splenectomy and functional asplenia. This predisposes to risk of life threatening sepsis due to encapsulated organisms such as Pneumococcus and meningococcus.

v) Cerebro-vascular strokes occur in 10 % of children with SCD and amount to a 200-fold increased risk as compared to normal children before 20 yrs of age; peak incidence at 6 to 9 yrs of age. Cerebral stroke and infarct could be overt (11%) or silent (peak age less than 4 years) in as many as 35 to 40 % of total strokes. However, intracranial hemorrhage predominates in Adults in the 3rd decade of life. Risk of stroke is high in children with low steady state Hb level, history of Transient ischemic attacks, recent attack of acute chest syndrome, systolic hypertension, nocturnal hypoxemia, past h/o strokes, family history of sibling with SCD-SS and history of stroke. Transcranial Doppler Ultrasound time averaged the mean velocity of more than 200 cms/s. Other neurological features include, TIA, headaches, seizures, cerebral venous thrombosis, Posterior Reversible Encephalopathy Syndrome (PRES), and Fat embolism syndrome

vi) Renal manifestations in SCD children include hyposthenuria and enuresis in as high as 40 % of children between 6–8 yrs of age, Sickle cell nephropathies include Gross hematuria, papillary necrosis, renal infarction, Hypertension, Nephrotic syndrome, Pyelonephritis, renal medullary carcinoma, Chronic renal failure, and ESRD in as high as 4.5% adults having SCD.

v) Priapism, a painful prolonged involuntary erection due to acute vaso-occlusion in the penile corpora cavernosa is a distressing and emergency condition seen more often in sexually active SCD adolescent boys.

vi) Anemia may be chronic with an average Hb of 6 to 9 gms due to reduced (12 days) survival of SS RBCs and is referred to as “Steady state level”. But acute exacerbation may occur due to

sequestration crisis, recurrent malarial infection, and due to oxidant drug use in co-morbid G6PD deficiency condition (with reticulocytosis). It may get precipitated also by Folate deficiency, and Parvo virus infection (with reticulocytopenia). Human parvovirus B19 has profound effects of destroying RBC precursors in bone marrow and causes transient aplasia leading to life threatening severe anemia, referred to as **"Aplastic crisis"**, which lasts for a period of about one week.

vii) Pulmonary hypertension is seen in 20% (mild PH) and 9% (severe PH) in adolescents and young adults. Risk factors are hypoxemia, sleep apnea, pulmonary thrombo-embolic disease, LV dysfunction, severe anemia, and Iron overload.

viii) Growth impairment is almost always seen in the majority of children. The responsible factors are reduced energy intake, recurrent infection and hyper-catabolic status, increased cardiac output, leading to excess demand for calories. Weight is more severely retarded than height, puberty and sexual development is delayed by 1-2 yrs, bone age is retarded by as much as 4 yrs.

ix) Ocular morbidity could be due to 1) Vision sparing Non-proliferative changes such as occlusion of conjunctival vessels, iris atrophy, and retinal pigmentary changes; 2) Proliferative changes seen in 5–10% of patients are due to stimulation of angiogenesis following retinal ischemia; and 3) Abnormal vasculature which may rupture leading to large vitreous hemorrhage and loss of vision.

x) Other problems include Sickle hepatopathy due to impaction of hepatic sinusoids with sickled RBCs, patchy areas of hepatic necrosis, engorgement of kupffer cells and bile stasis, and frequent blood transfusion which can add to hemosiderosis. Cholelithiasis is seen in as high as 33%, and chronic leg ulcer in up to 20% of older children and adults. Psycho social, scholastic, and vocational impairments leading to progressive decrement in quality of life are usually underestimated and prompt recognition and corrective measures are needed for improving self-esteem and peer relationship.

Laboratory diagnosis

1) Prenatal:

a) PCR for detection of sickle cell mutation genes in fetal fibroblasts obtained by amniocentesis or on fetal cells sample obtained by chorionic villous biopsy

b) Preimplantation genetic tests after isolating fetal cells from maternal peripheral blood for DNA analysis

2) Newborn screening: Universally accepted for early diagnosis, treatment and prevention of complications

a) Thin Layer/Isoelectric Focusing (IEF),

b) High Performance Liquid Chromatography (HPLC), and

c) PCR amplification of DNA extracted from dried blood on filter paper. (Perinatal transfusion can give false results)

3) Lab investigations beyond perinatal period:

a) CBC, Peripheral blood film,

b) Sickling test by 2% Sodium metabisulfite as reducing agent.

c) Sickle solubility test using deoxygenating substance such as buffered solution of Sodium dithionite where in mixing of HbSS blood will give rise to turbidity. False –in the Newborn period and hence not useful. False +ve in leucocytosis, hyperlipidemia, and hyperproteinemia due to plasma turbidity, useful for community screening of population.

d) Diagnostic tests: i) Hb Electrophoresis,

ii) HPLC**,** iii) Mass spectrometry iv) Iso-electric focusing, v) DNA testing for identification of mutation.

Table-1: Diagnostic investigations on the disease

Disease	Alleles	Main Hb	Hb (Gms/dl)	MCV	Reticcount	Usual phenotype
Sickle cell Anemia HbSS	$\beta^s\beta^s$	Hb S	6 – 9	N	10-25	Severe
Sickle-β^0 thalassemia Hb Sβ^0	$\beta^s\beta^0$	Hb S	6 – 9	□□	10-25	Severe
Sickle- Hb C disease	$\beta^s\beta^c$	Hb S, Hb C	9 – 12	N - □	5-10	Mild-Mod.
Sickle Sickle-β^+ thalassemia Hb Sβ^+	$\beta^s\beta^+$	Hb S >Hb A	10 – 12	□□	2-10	Mild
Sickle Cell trait Hb ASβ	$\beta\beta^s$	Hb A >Hb S	11 – 13	N	N (< 2.5)	Asymptomatic

As some investigations are described in Table 1, Investigation for suspected complications and prevention: "Focused Tailor made" investigation guided by clinical scenario are as follows:

1) Periodic CBC, Hb%, Retic count, ESR, S. Ferritin, Red cell antigen typing

2) Urine analysis

3) Periodic transcranial Doppler study to evaluate middle and anterior cerebral artery blood flow velocity. [N <170 – 200 cms/s]

4) Imaging: X ray chest, long bones, hip and shoulder joints, bone age assessment, bone scan, MRI scan of brain, bone, MR angiography, Imaging for liver and heart iron content MRI R2, R2* and MRI T2*

5) USG abdomen, lungs . +++

6) CRP, Blood culture, and bone marrow culture

7) Liver and kidney function test, pulmonary function tests, Echo study

8) Screening for HIV, Hepatitis B, and C. Parvovirus B 19

Management of Sickle cell disease:

1) Early establishment of diagnosis, comorbidities, complications, immune status

2) Monitoring for CBC, Growth and Development, Quality of life and Psychodynamics of family members

3) Vaccination against common VPD, HB, HIB, Pneumococcus, Meningococcus, Typhoid fever, and Influenza

4) Prevention of pneumococcal infection by daily chemoprophylaxis with Penicillin 125 mg BD [for 2 mths to 3 yrs; and thereafter 250 mg BD at least up to 5 yrs of age or throughout life

5) Supplements with Folic acid [5 mg OD], Zinc 20-50 mg OD;

6) Promotion of Fetal hemoglobin by daily Cap Hydroxyurea 15 to 35 mg /kg OD starting from as early as 9 months of life; and to monitor CBC every 3 to 6 monthly

7) Counseling of parents about nature, cause, course, complications, importance of splenic palpation, liberal fluid intake, and treatment options. Advice about Pre marital, pre implantation, prenatal testing, and genetic counseling

8) Regular Blood screening of prospective mothers during ANC, cord blood, adolescents, and blood donors

9) Psycho social counseling of parents, siblings, and patients, to improve the morale, future opportunities, prospects for

education, vocations, job opportunities, and quality of life.

Specific management:

1) Recurrent infections : Due to impaired immune status (Autosplenectomy, decreased opsonin activity, zinc deficiency associated impaired cellular immunity, interleukin-2 production and T-helper cell dysfunction) patients with recurrent febrile illnesses present with 1) Sepsis, 2) Pneumonia, 3) Osteomyelitis, caused by HIB, Pneumococcus, more often in preschool age children and by E. Coli, Klebsiella, staphylococcus, Mycoplasma, and chlamydiae in teen age group children. Early institution of empiric corresponding antibiotics after collection of Blood sample for Blood culture and PCR studies is warranted.

2) Acute sickle cell crisis:

a. **Vaso-occlusive crisis:** The most common, distressing painful crisis involving limbs, abdomen, chest , spleen, brain is managed by i) Adequate Hydration, ii) Prevention & treatment of Hypoxia iii) Correction of excess H^+ ions i.e. Acidosis, iv) Non narcotic / Narcotic analgesics. V) Treatment of underlying infections.

Use of Corticosteroids and inhaled Nitric oxide may be offered in selected severe painful conditions. Regular use of Hydroxyurea and other disease modifying agents have a salvaging effect on VOC.

Periodic transcranial Doppler study and chronic blood transfusion therapy can abort the risk of strokes in at risk patients from 10 % to < 1%. Early use of facility based blood transfusion within two hrs of incipient stroke is likely to abort the stroke in these SS patients. Aim should be to reduce Hb S percentage to less than 30% and total Hb of 9 gms %. (ASH 2020 guidelines).

b. Acute Splenic Sequestration: Acute life threatening major episode, presents in shock with severe pallor. Prompt cautious Packed RBC or whole blood transfusion to control shock and prevent hyperviscosity as well as convalescent volume overload and CCF; (Hb not more than 10 gms/dl.) is life saving. Training of parents about splenic palpation and prompt treatment in nearby health facilities can abort death in these children by as much as 90%. Splenectomy to avoid future life threatening episodes is recommended after the 1st episode of the sequestration crisis. Chronic transfusion therapy is recommended to reduce HbS less than 30%, till splenectomy is done.

c. Anemia of varied nature, severity and etiology has to be managed by the following information:

Table-2: Clinical scenario based Blood transfusion

Scenario	Hb gms %	Complications	Nature of Blood transfusion
Acute Febrile illness	6 – 9 Gms%	Nil	Only optimum hydration; oral fluids
Vaso –occlusive crisis	6 – 9 Gms%	Nil	Only optimum hydration: oral / IV fluids
	< 5 gms	Severe pain > 5 - 7 days, ACS, Stroke, TIA, Sequestration	Whole blood Transfusion
	8 to 9 gms	ACS, Stroke, TIA	Exchange transfusion (ET)
Multi organ failure	Less than 6 - 9 gms/dl.		Whole blood Transfusion or ET
Preoperative			

Clinical scenario based Blood transfusion could be either Whole Blood, Packed RBC, "Top Off", or Phlebotomy transfusion or Automated Exchange

transfusion (Erythrocytapheresis) (Table 2).

Benefits of blood transfusion: Preferably fresh blood with red cell phenotype analysis and screened for transfusion induced infections should be preferred. Advantages are as follows: It decreases the percentage of Hb S (target being Hb S concentration less than 30%), suppresses Hb S synthesis, and reduces hemolysis. **Chronic transfusions** given every 3 to 4 weeks are beneficial in 1) Primary and 2ndary stroke prevention, 2) Severe debilitating pain, 3) Splenic sequestration while awaiting splenectomy, 4) Recurrent / stuttering persistent Priapism, 5) Anemia associated with Chr. Renal failure and Pulmonary hypertension.

Risk of Acute and chronic blood transfusions including Alloimmunization, Iron overload, Hyperviscosity, Congestive failure, and Transfusion transmitted infection should always be prevented. Delayed transfusion reactions and transfusional hemochromatosis should be prevented by iron chelator therapy.

d) Pulmonary hypertension: Hydroxyurea, regular chronic blood transfusion, are usually tried. Other therapies include i] endothelin receptor antagonist (Bosentan), ii) Sildenafil, iii) epoprostenol, iloprost.

e) Surgery in Sickle cell children: i) Perioperative hydration, ii) Oxygenation and iii) early mobilization and ambulation give rewarding results with or without Pre surgical Transfusion therapy. Common surgical issues are 1) Splenectomy, 2) Osteoarticular diseases, 3) Clolelithiasis, 4) Photocoagulation or Cryotherapy to induce regression of neovascular tissue. For major surgeries such as splenectomy planned after the age of 3 yrs, a patient's immune status has to be improved with vaccination for HIB, HB, Pneumococcus, Meningococcus, Influenza almost 2 to 6 months before planned surgery.

f) Newer therapeutic modalities:

a. Hydroxyurea: i) Increases the HbF concentration and hence reduces relative concentration of Hb S. ii) Increases total Hb concentration, iii) Decreases white cell and platelet count, iv) Decreases number of total reticulocytes, stress reticulocytes, and young low density SS RBCs, which are more likely to adhere to vascular endothelium. v) It also increases the concentration of nitric oxide generation and changes the expression of adhesion molecules.

Hydroxyurea is expected to decrease the frequency of painful episodes, acute chest syndrome, and the need for blood transfusion; it can have protective effects against cerebrovascular disease, and reduce hypoxemia and proteinuria; thus, reduce admission to hospital in-patients with sickle cell anemia and increase life expectancy. Hydroxyurea has to be started as early as 9 months of age at a dose 15 – 20 mg/kg/day and escalated by 5 mg/kg/day increments over a period of 6 - 12 weeks, until the dose of 35 mg/kg/day is reached. Myelosuppression a common side effect, should be checked by discontinuing the drug for a period of 2 weeks, if absolute neutrophil count is < 1500/cu mm, and/or platelet count < 80,000, and/or absolute reticulocyte count < 100,000/cu mm are observed.

b. L-glutamine is also used as an add-on drug for reducing sickle cell crisis and hospitalization.

c. Other experimental therapeutic agents for Sickle cell disease are

i. Agents to increase HbF: Decitabine, Butyrates

ii. Nitric oxide promoters: Nitric oxide, Arginine, Citrulline

iii. Red Cell membrane channel blockers: Magnesium, Arginine, Clotrimazole derivatives

iv. Endothelial interactions / decreased activation: Anti adhesion molecules, sulfasalazine

b. Hematopoietic Stem Cell Transplantation (HSCT): Planned to replace sickle cell gene with cells containing normal gene, by autologous HLA matched sibling bone marrow. The indications for HSCT are i) history of previous CNS stroke, or abnormalities on MRI or neuropsychological functions, ii) Recurrent ACS or VOC, iii) Sickle Nephropathy, iv) AVN of multiple joints, and v) Recipient's age less than 16 yrs. Overall post transplant survival is more than 90%; and event free survival of more than 80 %. However, less than 20% of patients have a HLA matched sibling donor; and hence significant interest in matched unrelated donor transplants is underway.

c. Gene based therapies: Attractive but still experimental. A lentiviral mediated gene transfer , and use of genetically engineered restriction enzymes "Zinc finger nucleases" are being tested in human trials.

Summary

1. Sickle cell disease is a common severe monogenic lifelong devastating disorder all over the world and in Central India as well as in other Asian and African countries.

2. Sickle hemoglobin polymerizes and produces reactive oxidative species, causing pleiotropic effects on Red Blood Cells (RBCs): dehydration, membrane damage, increased RBC microparticles and hemolysis, all resulting in enhanced erythropoiesis with increased reticulocytes and young RBC that avidly adhere to leukocytes and endothelium, and enhance inflammation.

3. Novel agents are being developed to target sickling, oxidative stress, and cell-cell adhesion to ameliorate these processes and reduce vascular occlusion and ischemia reperfusion injury.

4. Curative therapies such as Hematopoietic stem cell transplant are available for a miniscule percent of sickle cell patients due to non availability of HLA matched sibling donor and socio-economic factors.

5. Iry Prevention in the form of Educating masses about genetic disorder and risk of consanguineous marriages, premarital, pre-implantation, prenatal & neonatal screening is of utmost importance.

6. For the unfortunate sickle cell disease patients and their families, 2ndary prevention in the form of education about the need of chemoprophylaxis, targeted Immunization, periodic clinical, Laboratory and Imaging (TCD) check up is of great importance to reduce morbidity and mortality, and improve quality of life.

7. Collaborative efforts by parents, teachers, social workers, pediatricians, physicians, Obstetricians, Hematologists, Geneticist, Government agencies are warranted to boost up the multipronged quality research in this field of Sickle cell disease, so as to offer affordable, beneficial therapy to these underprivileged population.

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