Sickle cell anemia: Review and remedial hope

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ABSTRACT

Genetic diseases and disabilities have been described as the most ubiquitous and burdensome to the populations. The hereditary anemia especially due to sickle cell is common globally with variably high prevalence in indigenous populations. In India, several endogamous tribal pockets that have been malaria endemic harbor sickle cell gene in strikingly high frequency. In this background management of sickle cell patients in context of contemporary health care system remains limited and requires integration of appropriate strategies into the existing health care delivery system. This may involve research, diagnosis, training, genetic counseling and possible remedial interventions for urban as well as rural populations. Remedial interventions that are possible in existing health care delivery system and potential therapies under investigation have been discussed.

Key Words:
Sickle cell anemia, infant mortality, pre-reproductive mortality, Indian tribes, malaria, sickle cell crisis management, remedial hope for sickle cell patients.

Human adult hemoglobin consists of two components, Hb A and Hb A2. All the human hemoglobins have a similar overall structure with two distinct pairs of peptide chains, each related to a single haem unit. In Hb A, α-chains are paired with β-chains (α2β2) and in Hb A2 with δ-chains (α2δ2). All seven types of polypeptide chains (α, β, γGγγ, δ, ε and ζ) combine to form the various hemoglobins found during human development1. Intrinsic defects of the erythrocytes or hemoglobinopathies are inherited changes of hemoglobin molecule leading to metabolic disorders. They fall into two major groups, viz.

i. Diseases resulting from an inherited structural alteration in one of the globin peptide chains and

ii. Those due to inherited defects in the rate of synthesis of the globin chains.

Sickle cell hemoglobin (Hb S) is the most common erythrocytic abnormality which belongs to the first group, having structural alteration at molecular level in β globin chain. A single base pair in the β globin gene is mutated from adenine to thymine, producing a corresponding change in the sixth amino acid of protein from glutamic acid to valine resulting in its altered structure2,3. Since early 20th century very many reports appeared on prevalence of this disorder in various
pockets of the world populations thus, studies on sickle cell haemoglobin have been important for its high frequency in many indigenous populations of the world.

Reports on genetics, physiological and molecular basis, clinical consequences as well as possible explanation of the existence of this molecular abnormality in high frequencies have also been advanced. It seems reasonable to recall these important investigation reports in light of the fact that previously reported population groups which harbored sickle cell gene may still possess this abnormal gene in more or less same frequencies besides slow but continuous alterations in population structure due to many dynamic factors, hence imposing serious health burden in contemporary world.

Apart from this, in view of the vastness of the country and the multiracial nature of the peoples of India, the information is meager therefore; systematic in-depth investigations are still required taking into account other important variables which may involve the ethnic origin, the social, environmental and nutritional aspects as well as prevalent diseases in various pockets of human populations. Moreover, simple diagnosis method and possible remedial interventions in case of sickle cell crisis must be communicated to the medical professionals; especially those who are working in rural tribal areas where high prevalence of sickle cell anaemia has been found.

Physiological Basis and Clinical Consequences:

Clinically the sickle cell disease resulting from homozygous state of Hb S gene has shown a wide spectrum of morbidity in different regions of the world. The disease appeared in severe form with mortality in childhood in West and North Africa. Various factors have been suggested to influence the morbidity and the disease crisis. V.E. Emmele (1927) suggested that disease crisis is primarily due to precipitation of Hb S resulting in sickle shape under deoxygenated condition. J.G. Huck, noted that sickling was a reversible process. V.P. Sydenstricker (1924) reported additional findings, including the variability in the course of illness.

E.V. Hahn and E.B. Gillespie confirmed the intimate relationship between the sickling of red cell and a reduced supply of oxygen and also demonstrated that sicked red cells could revert to their normal shape when exposed to adequate amount of oxygen. Significantly these two workers, as early as in 1927, also rightly attributed the defect in the sickling phenomenon to the hemoglobin contained within the red cells and not to the red cell itself.

Early major contributions by L.W. Diggs and R.E. Ching included the first detailed post-mortem reports on patients with sickle cell disease in the USA. Five years later, L.W. Diggs and associate J. Bibb also reported on certain hematological parameters (hemoglobin content, red cell fragility and detailed morphology of sickled cells) in patients suffering from sickle cell anemia. They noted for the first time the presence of red cells which were irreversibly sickled even after adequate re-oxygenation.

The period between 1940 and 1960 witnessed rapid advances in the understanding of various facets of this
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disease. T.H. Ham and N.B. Castle\textsuperscript{10}, elaborated on the pathogenesis of the vicious cycle of sickling in vivo, that is sickling results in increased viscosity of blood, which leads to reduced circulation and increased deoxygenation, which in turn causes further sickling\textsuperscript{10}. I. J. Sherman\textsuperscript{11} observed that deoxygenated sickled cells exhibited optical bi-refringence, a phenomenon which was not seen in normal red cells\textsuperscript{11}. This was probably the first evidence that the deoxygenated form of sickle hemoglobin has an orderly structure.

The cells sickle at the oxygen tension normally found in the venous blood. When the level of healthy red cells becomes too low, this can lead directly or indirectly to a variety of complications which include hemolytic crisis and anaemia, jaundice, coelithiasis, aplastic crisis, autosplenectomy, sequestration crisis, dactylitis, priapism and renal papillary necrosis etc. Pulmonary hypertension, conjunctival and retinal vessel alterations have also been reported in sickle cell disease\textsuperscript{12-13}. The cells with increased rigidity move slowly or become jammed in capillaries, with resultant damming up of blood which eventually leads to infarction\textsuperscript{14}. Overt and incomplete (silent) cerebral infarction has also been reported in selective cases of sickle cell anemia\textsuperscript{15}. For sickle cell homozygotes, the mortality rate has been reported very high and nearly half die in the first year of life whereas, the majority of children who are carriers of sickle cell gene die during their first 10 years of life from complications\textsuperscript{1}. The crises may mimic acute rheumatism or an acute abdominal lesion. Incidences of osteomyelitis and bilateral leg ulcers have been noted in nearly 40% patients\textsuperscript{16}.

Sickling phenomenon greatly depends on the amount of Hb S hence, The heterozygotes with 50% or below remain free from the clinical problem whereas, those with more than 50% as in SS, SC, S-Thalassaemia, SO, SE and SD (Arab) have normally greater risk of sickle cell crisis. Moreover, person with Hb S as well as HPFH enjoys an advantage due to persistent Hb F as an efficient oxygen carrier\textsuperscript{17}. HPFH is a condition characterized by the fetal hemoglobin in adult life in the absence of any marked hematological abnormality or apparent ill effect even in persons who are homozygous for this mutation and have only Hb F.\textsuperscript{2}

It has been interesting that a “switch” gene seems to be involved in the change from synthesis of \(\gamma\) chains (Hb F) in fetal life to the synthesis of \(\beta\) chains (Hb A) in postnatal life. The regulation by the “switch” gene must be at the level of the chromosome as evident by the fact that person heterozygous for the high-F gene has nearly half Hb F and half Hb A. Furthermore, the person heterozygous for both high-F gene and the sickle gene has no Hb A but shows no interference with Hb S synthesis. The homozygote for high-F not only exhibits absence of Hb A but also lack the minor component normally found in the adult, Hb A\(_2\) (\(\alpha_2\delta_2\)). The \(\delta\) locus is closely linked with the \(\beta\) locus representing a plausible model envisions an “operator” gene that controls the function of at least two structural genes, the \(\beta\) locus and the \(\delta\) locus. In addition, other gene possibly like \(\gamma\) as well constitutes a unit called operon. It is suggested that there may be one or more regulator loci in other chromosomes that influence the function of individual structural genes.\textsuperscript{2}
Diagnosis:

The diagnosis is made by observing characteristic sickle cells in the peripheral blood. Normally, sickle cells are observed in the stained blood film used for routine microscopy only in homozygotes. Heterozygotes do not show sickling on the film unless reducing agent like sodium meta-bi-sulphite (2% solution) is added to the blood. This simple and rapid method of demonstrating sickling of red cells without stain, using reducing agent on microscopic slide has been earlier suggested\(^{18}\) and can be conveniently adopted for diagnosis by Primary Health Centers of rural areas. Sickle cell hetero- and homozygotes can be recognized by electrophoresis on cellulose acetate plates or other medium involving starch or paper conventionally used for electrophoretic separation. Homozygotes demonstrate a single main band of Hb S whereas, heterozygotes show a mixture of Hb A and S\(^2\). Isoelectric focusing, cation exchange and high performance liquid chromatography (HPLC) are also being used for laboratory diagnosis of sickle cell anemia.\(^{19,20}\)

Transmission and Molecular Basis:

The transmission of the trait was shown by J.V. Neel\(^{22}\), to follow the simple Mendalian principle in which those severely affected were homozygous for the mutant gene\(^{22}\). In this instance, the heterozygotes could also be recognized clinically by a mild form of the disease referred to as sickle cell trait. L. Pauling and associates\(^{23}\), reported a chemical difference between the hemoglobin of sickle cell patients (Hb S) and that of normal individual (Hb A). The two types of molecules could be separated by electrophoresis which indicated a difference in the net charge on the two types of molecules\(^{23}\). The persons who were heterozygous were found to possess both molecular species in a ratio approaching one to one (60:40), resulted due to presence of one gene for Hb A and one gene for Hb S. They are usually normal hematologically and clinically, although hematuria and splenic infarction can occur under anoxic conditions. Normal erythrocytes become sickled in the trait when oxygen saturation falls below 40%, a level which is rarely reached in venous blood.\(^{16}\)

Molecular techniques are generally employed for prenatal diagnosis of sickle cell anemia which involve simple strategies based on amplifying the β globin gene using polymerase chain reaction (PCR), followed by restriction analysis\(^3\). Beside this, microarray based human globin gene mutation detection methods are also available and may gradually substitute conventional methods to the extent that their widespread use reduces their cost, which at present is high\(^{21}\). Moreover, a family study is always of fundamental importance to establishing diagnosis.\(^{20}\)

The work on hemoglobin was significant because this was the first demonstration of an inherited change in the molecular structure of a protein. Since the change was inherited in simple Mendalian fashion, the alteration was thought to be due to a single mutation. Although, one could not define the alteration with certainty, the work of Sanger on sequences of amino acids in insulin polypeptide chains indicated that the difference might be due to amino acid substitution.

In spite of magnitude of the problem,
the alteration in hemoglobin S was demonstrated within a remarkably short time on the basis of experiments on digestion of haem-protein with trypsin, which cleaved polypeptide chains on paper by a combination of chromatography and electrophoresis. V.A. Ingram\textsuperscript{24} demonstrated that the difference between hemoglobin A and S were due to the substitution of single amino acid. In haemoglobin A, glutamic acid, which has a free carboxyl group, had been replaced by the neutral residue of valine to form hemoglobin S.\textsuperscript{24}

A few years later when V.M. Ingram and his colleague, J. A. hunt, examined another mutant hemoglobin C, they found that it had a different substitution at the same site in the polypeptide chain. In this instance, the basic amino acid, lysine was substituted for glutamic acid\textsuperscript{25}. By this time hemoglobin had been shown to be composed of four peptide chains, two identical $\alpha$-chains and two identical $\beta$-chains. Both substitutions mentioned were in the $\beta$-chains.

**Infant or Pre-reproductive Mortality:**

A.C. Allison\textsuperscript{26} had mentioned about the virtual lethality of homozygous sickle cell conditions in Africa\textsuperscript{26}, whereas, H. Lehmann and R.G. Huntsman\textsuperscript{27} stated that in West Africa a number of sickle cell homozygotes survived to become adults and their number was greater amongst sophisticated regions of West Africa, particularly amongst the upper class\textsuperscript{27}. In India, a few studies on haemoglobinopathies have also demonstrated the survival of sickle cell homozygotes to adulthood.\textsuperscript{28-31}

Apart from this, a clear declining trend of sickle cell trait in ascending age groups was noted in Bhilala, Barela and Korku tribes. This trend and some family studies in these tribal groups strongly suggested infant or pre-reproductive mortality. This has also shown the possible adverse effects of both homozygotes and heterozygotes on the health of the tribals living in poor environmental conditions\textsuperscript{32}. Recently, susceptibility of severe cases of sickle cell patients to chronic pain, strokes and premature death has been reported by CBS News, U.S.A. in background of the fact that nearly 7 million African Americans suffer from sickle cell anemia.\textsuperscript{33}

**Origin of Sickle Cell Gene in India:**

There has been much debate about the origin of sickle cell gene in India. Whether it is indigenous in origin or it had migrated from Africa to India in prehistoric times is not clear\textsuperscript{34-37}. With the help of endonuclease restriction enzymes, Y.W. Kan and A. M. Dozi\textsuperscript{38}, showed that the association of sickle cell gene with 7.6 kb recognition site found among the Valmiki, Konda Reddy and Koya Dora tribes in southern India (probably Andhra Pradesh) is different from its association with 13.0 kb recognition site among the people of western Africa\textsuperscript{38}. They were in favor of view that the Indian and West African sickle gene mutations arose by separate events. Afterwards no attempts were made to further studies on origin of sickle cell gene by advanced molecular biology techniques.

**Malaria-Sickle Cell Hypothesis: Possible Explanation of the Existence of Sickle Cell Gene:**

Malaria is an acute infectious disease caused by malarial parasites, Plasmodium vivax or Plasmodium falciparum,
transmitted by mosquitoes of the genus Anopheles. This illness is characterized by a cycle course with periods of acute febrile attacks and paroxysm free intervals as well as by splenohepatomegaly, anemia and occasional severe lesions of the nervous system, kidneys and other organs. Relapses are also common. Even today this is one of the most wide-spread transmissible diseases in the world, with the African continent being the largest malaria stronghold on the globe. Furthermore, the disease is also rather wide spread in a number of countries of South-East Asia and South America.

Transmission of malaria is possible when a combination of several factors is available, including sources of infection, vectors, susceptible population and favorable natural and climatic conditions. The source of infection is a malaria patient or parasite carrier whose peripheral blood contains mature sexual forms of malarial plasmodia (gametocytes). In vivax, quartan and oval malaria, gametocytes appear in the blood in the first days of the disease, their number growing after a few attacks. In falciparum malaria, gametocytes are discovered in the peripheral blood on the 8th-10th day from the onset of disease reaching the peak values by the 14th-15th day.39,40

In highly endemic malarial areas where the adult population possesses the acquired immunity, children present the major source of malaria. Children do not have acquired immunity, hence their parasitaemia is usually heavy, the disease course is rather frequently atypical and the disease diagnosis is late41. Parasite carriers in endemic areas may serve as sources of infection for a long time because malaria in such individuals is rarely identified and they receive no treatment. Yet, the infectivity of gametocytes in immune individuals appears to be less marked than in non-immune children.

A mosquito female of the genus Anopheles serves as a host for the sexual cycle of parasite development, sporogony which may be complete only at a temperature higher than 16ºC. At higher temperatures, the process of sporogony accelerates. In conditions of hot climate, blood digestion by mosquito females occurs faster, they more often feed on humans and produce more oocytes which accounts for the higher intensity of infestation of the vector by sporozoites.

The sickle cell homozygotes apparently suffer from severe anemia and die before attaining reproductive age without contributing to the gene pool of the population. Therefore, observation of very high frequencies of sickle cell trait in various pockets of world populations called for an explanation. A number of considerations were advanced to explain the high frequencies of a deleterious gene like sickle cell27. The most acceptable possibility was the mechanism of balanced polymorphism as was suggested by J.B.S. Haldane (1949) in connection with thalassaemia42 and later developed by A.C. Allison26 in populations with high incidence of sickling in Africa.43,44

A.C. Allison26 put forward the malaria-sickle cell hypothesis which states that in the presence of Plasmodium falciparum malaria, the heterozygote sickle cell trait carriers enjoy a selective advantage over the normal homozygotes as the former are resistant to this particular type of malaria43. It has been
suggested that Hb S offers protection against P. falciparum probably because of deoxy-Hb S aggregates with RBC interfering with intra-erythrocytic schizogony and their subsequent removal by macrophages. This selective advantage has given rise to balanced polymorphism in relation to the Hb S and is mainly responsible for maintenance of high frequencies of sickle cell gene in malaria endemic regions of Africa.

Though the malaria-sickle cell hypothesis appeared to be the best plausible explanation of the high frequencies of sickle cell trait yet, some objections to the validity of the hypothesis became evident when hypothesis was examined in relation to the distribution of sickle cell trait in some population groups of India. In India, the first report on the role of selective advantage of sickle cell gene was published in 1974 among Mahars of Aurangabad where a good correlation between the endemicity of malaria and the incidence of Hb S trait was observed. In another study on Bhilala and Barela tribes of West Nimar and Korku tribe of Pachmarhi hills of Central India, significant correlation in the distribution of sickle cell trait and incidence of malaria has been noted.

**Distribution of Sickle Cell Gene with Special Reference to India:**

J.B. Herrick (1910), first recognized the presence of red cell which was shaped like a sickle in an anemic, West Indian medical student and it was considered to be a Negroid character. However, it is now well established that this particular gene is widely distributed in other parts of the world including India with its highest incidence among black Africans and descendants of those who emigrated from equatorial Africa. It was earlier reported that nearly 8% of black population in the United States, Latin America and Caribbean carry sickle cell gene. Incidences were first reported in India from Nilgiri hills, South India and from Assam simultaneously. Since then large data on the incidence of sickle cell gene have been collected in different tribal populations from Indian subcontinent.

The frequency of Hb S ranging from 0.5% among Tadas of Nilgiri hills in Tamil Nadu to 34.43% among Paniyans has been reported from southern India. The frequency of Hb S in western India ranges from 0.02% to 30.6%. The highest frequency at 30.6% has been reported among Sorathis and the lowest at 0.02% among Parsis of Gujarat. Some hospital cases of Hb S associated with thalassemia had been reported from Maharashtra.

In eastern India, frequency of Hb S has been reported from 1.7% among Santhals of West Bengal to 29% among Griza Oriahans of Assam. Non-tribals of Assam however, rarely show a frequency of Hb S more than 10% particularly among upper castes. Frequency of sickling ranged from 1.4% among the unknown community of Mirzapur, in Uttar Pradesh to 23.64% among Garasias of Sirohi in Rajasthan in northern India.

Many tribal groups of central India have been reported to possess Hb S in considerably high frequencies. In Bastar and Bilaspur districts, frequencies of Hb S ranging from 3.45% among Dhurva to as high as 48.58% among Panka (mixed) have been reported. A few surveys have however, shown complete absence of sickle cell trait in a
number of tribal groups of India which include few tribes of central region involving Dhanwar, Manjhwar, Oraon, Kol and Pando and Kanwar.64,70-74,37

Bhils in central region have shown variations in frequency of Hb S ranging from 5.90% to 23.91%.75,76 A comparatively high frequency has been reported among Bhilala ranging from 9.51% to 28.50% in different pockets of their population77,78,32. Among Patelia of the same region, the frequency of Hb S ranged from 20.48% to 31.10%.77 Frequency of Hb S ranging from 14.68% to 24.64% was observed among Barela in Khargone of this region32,78. A tribe Pardhan of Mandla district was reported to harbor 9.0% Hb S79. Korku of Pachmarrhi hills in Hoshangabad district were found to have considerably high frequency of Hb S being 16.86%.32 Whereas, in neighboring Chhindwara district in central India, Korku and Bharia tribes exhibited comparatively lower frequencies of Hb S, 7.20% and 2.60%, respectively80. Gond, Halba and Kawar tribes of Raipur have also been investigated for Hb S. These tribes have shown the frequencies of sickle cell trait to be 15.92%, 13.90% and 5.50%, respectively77. Hence, persistence of sickle cell gene in considerably high frequencies in various indigenous population groups of India remains a serious health burden in background of their poor environmental and economic status.

Remedial Hope for Sickle Cell Patients:

Management of sickle cell patients in context of existing contemporary health care system is limited and requires integration of appropriate strategies into the existing health care delivery system. Management strategies may involve development of research, diagnosis, training, genetic counseling and possible remedial interventions in such a way that both urban as well as rural populations are benefited. Strategy for prevention and possible remedial interventions as suggested by H.M. Bhatia62 is considerable in case of sickle cell crisis17. This involves following points:

1. Rapid diagnosis and avoidance of unnecessary surgical intervention.
2. Prevention of infarcts and formation of thrombi in circulatory system.
3. Avoid vascular stasis.
4. Avoid cold and cold bath or drinking cold water.
5. Avoid excessive heat to prevent dehydration.
6. Give magnesium sulphate injection during crisis. It helps in clotting process, delays thrombi formation and acts as vasodilatory agent in dislodging nests of sickle cells from small venules.
7. Avoid intravascular sickling, pH is kept alkaline with oral administration of sodium bicarbonate, 2-3 gm per day in divided doses as a precaution to prevent crisis especially during infections.
8. Avoid infections as well as malaria.
9. Provide adequate fluid intake to avoid dehydration.
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10. Prophylactic antibiotics and antimalarial drugs are useful in preventing crisis.

11. Selection of these drugs should take note of the G6PD deficiency.

12. Provide folic acid supplements without iron supplements. Iron supplement should follow the serum iron estimation.

13. Maintain haemoglobin level between 6-9 gm per cent.

14. Give transfusion only if haemoglobin level falls to or below 5.0 gm per cent.

15. Avoid protracted labor or caesarean operation.

16. Leg ulcers may be resistant to treatment. Keep under warm stockings in cold weather and give conservative treatment to avoid infections.

Moreover, these days some promising therapies for sickle cell anemia are being investigated through research which involve the following methods:

1. One method for treating sickle cell anemia is to reduce the concentration of defective hemoglobin by stimulating the body to make other kinds of hemoglobin. Hydroxyurea (Droxa), a cancer medication, has been shown to reduce pain and complications in sickle cell anemia in adults. It is still being investigated for use in children, with special concern for how hydroxyurea may affect child growth and development. Two other drugs being investigated to stimulate hemoglobin production are butyrate and arginine. Studies have shown both drugs not only increase healthy hemoglobin but also reduce the symptoms of sickle cell anemia as well.

2. Since the pain of sickle cell anemia comes from blood vessels being blocked off, another method of treatment would be to get more oxygen to the painful areas. Poloxamer 188 (Flocor), made by CytRx Corp., has been shown in studies to decrease the length of painful episodes in sickle cell anemia by improving blood flow in the tiny blood vessels around the painful area. In June 2001 the U.S. Food and Drug Administration (FDA) granted CytRx permission to put Flocor on the fast track for development, for treatment of acute chest syndrome.

3. Sickled red blood cells could also be made less “sticky” and thus less likely to block off blood vessels. A study published in 2001 showed that the drug sulphasalazine could reduce the number of “sticky” molecules on red blood cells in sickle cell anemia.

Apart from this, cord blood transplant as a permanent remedy for sickle cell patient has recently made a medical history. Cord blood contains stem cells, the building blocks of the body’s blood-making system. Cord blood is extracted from the umbilical cord and placenta at child birth. Cord blood transplants are especially promising for treating blood diseases like severe anemia or leukemia because it is easier to match the immature cord stem cells of donors to unrelated patients whereas, finding two unrelated people with matching bone marrow cells remains extremely diff-
Difficult. A half, full or partially matched unit can in many cases take hold and form a new normal blood cell forming tissue after transplants.

Keone Penn, a 12 year old sickle cell patient in US in 2006 became the first child to receive a cord blood transplant as reported by CBS News Correspondent R. Pinkston. New transplanted blood-making system needs time to take root but by the time report appeared he had shown no sigh of sickle cell anaemia since the transplant was performed. It is encouraging that his cure may become viable dream for other people with sickle cell anaemia in near future.

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