A SYSTEMATIC REVIEW ON ABNORMAL HEMOGLOBIN OF SICKLE CELL DISORDER PATIENTS

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With a populace of over 1.2 billion people, it is anticipated that our country is domestic to over fifty percent of the arena's SCD (Sickle Cell Disease) sufferers (Kate et. al., 2002). Likewise, it is seen that sickle cell is widespread in lots of tribal groups, the very best prevalence seems to be in 3 specific socioeconomically deprived communities, recognized as the Scheduled Castes (SC), Scheduled Tribes (ST), and other Backward classes (OBC) (Kate et. al., 2002). Each of those categories includes numerous distinct big ethnic groups, which have practiced marrying within a selected group, for millennia, and for this reason constitutes genetic isolates. Therefore, it is likely to be beneficial for the functions of making plans for genetic counselling services, manage packages, medical provider provision or genetic epidemiological studies, screening ought to generate prevalence estimates of the βs gene in every ethnic group. Therefore, the superiority of the βs gene has been well described within the ST populace (Italia et. al., 2009).

Central India, that's one of the high occurrence areas for the βs gene, has occurrence rate of 9.4-22.2% for this mutation in diverse groups. (Kamble et. al., 2000, Shukla et. al., 1985). The state of Maharashtra in central India has a populace of 112.3 million of which the SC and ST incorporate 10.2% and 8.9% of the population respectively. Moreover, the OBC population incorporates 52% of the population.

The lack of red blood cell elasticity is significant to the pathophysiology of sickle disorder. Regular red blood cells are elastic, which permits the cells to deform to bypass via capillaries. In sickle cell disorder, low oxygen tension promotes red blood cell sickling and repeated episodes of sickling harm the cell membrane and reduces the cell's elasticity. Those cells fail to go back to regular shape whilst regular oxygen tension is restored. For this reason, these inflexible blood cells are not able to deform as they goes through slim capillaries, leading to vaso occlusion and ischemia. Sickle Cell Disease (SCD) is one of the first molecular disease become understood at the genetic degree wherein the bone marrow produce odd sickle hemoglobin (HbS) that polymerizes in low oxygen concentrations. Sickle genes are unusual genes, inherited from each of the parents. Being a recessive genetic ailment, the sickle cell is seen, when both the parents have the disease or either of the mothers or father has the ailment and the opposite is a carrier (called the sickle cell trait) or both dad and mom are providers for the disease. Humans with sickle trait do have no longer the signs and symptoms of sickle cell anemia however have the capacity to bypass the disease to their youngsters. The medical signs and symptoms of SCD are severe and differ from patient to patient, but chronic vasoocclusive procedures can cause massive organ damage, ensuing in elevated morbidity and mortality in these people. Polymerized HbS contributes to the property of sickle shape to the RBC, in affiliation with different cell changes; moreover, those RBCs are equiprobable to rupture, releasing broken cellular-loose hemoglobin (Hb) into the flow (known as haemolysis), which leads to vascular Nitric oxide (NO) intake and oxidative stress (Kato et. al., 2009).

Ordinary RBC is biconcave disc formed and has elasticity ability, which incorporates Hb. Bone marrow produces ordinary RBCs with common lifespan of a hundred and twenty days (Pandey et.al 2012), furthermore, it shows extra affinity in the direction of oxygen. In sickle cell disease below, low oxygen level referred to as hypoxia the biconcave RBC turns into crescent or sickle shape (Lakhkar et.al 2015), that is triggered via a factor mutation in the sixth codon of the B-globin gene in which 'T' replaces 'A', changing a glutamic acid residue right into a valine residue and causing an altered B- globin chain, denoted as sickle globin (Abdul Rahman et.al., 2016). The hemoglobin molecule contains an iron atomat its center located within a porphyrin ring. LIkewise, in the normal hemoglobin A, this iron-porphyrin complex is surrounded by two alpha and two beta globin chains. Moreover, this beta globin chain is a linear protein composed of 146 amino acids, coiled into its own 3-dimensional configuration, with the amino terminal at one end and the carboxy terminal at the other.

Two Sickle Beta chains related to two Alpha chains end up a hemoglobin molecule known as sickle Hemoglobin (HbS) (Tripathy et.al. 2018). In hypoxia condition this HbS loss their elasticity and polymerizes, which leads to sickle RBCs. The life span of sickled RBCs is most effective approximately 10-20 days and the bone marrow cannot update them speedy enough. likewise, sickled RBCs lost their elasticity, turn out to be stiff and sticky, and generally tend to block the blood waft in microcapillaries. Blocked blood flow in microcapillaries reasons ischemia leading to severe ache and slow damage to organs (Aleluia et.al., 2017). The HbS gene is observed in overall populations of tropical African origin (which encompass most African-Americans). The occurrence of the gene in a few African populations is as excessive as 40%; in African-Americans, the

prevalence is 8%. The gene is likewise located in tribal and non-tribal people of India and in the Central East. Uncommon cases had been mentioned in Caucasians of Mediterranean descent.

Sickle cell anemia is an inherited sickness that existed in Africa for at the least 5000 years but there were no facts of its existence until it became observed in 1904. Lastly, it was observe that the deer is the most effective living creature apart from humans regarded to be afflicted by sickle cell disorder. Since, in 1840, the British Zoologist Gulliver discovered under the microscope sickle-formed red blood cells inside the blood of a variety of extraordinary deer belonging to the London Zoological garden. In North America, the white-tailed deer is from time to time encountered demise in sickle cell vaso-occlusive crisis inside the forests of Michigan. For one thing, the first description of sickle red blood cells in human beings was published in 1899 whilst Dr. Hayem, a French colonial medical doctor, mentioned his microscopic discovery from Africa. Therefore, he erroneously defined that the crescent shape of these red blood cells represented a regular variation of erythrocyte morphology. Previousely, The first document of the sickle red blood cells in North America was posted in 1904 through Dr. Dresbach. Subsequently, aggregates of hemoglobin in fish erythrocytes have been reported since 1865 (Perutz, 1950). Owsjannikow described the crystallization of Hb inside the red cells of fishes, leading to elongation of cells. Moreover, He also found crystals inside the red cells of rabbits, dogs, cats and guinea pigs. (Owsjannikow, 1865). Aboveall, the first scientific description of sickle cell anemia was recorded by means of James Bryan Herrick in 1910. This was posted within the November 1910 issue of "archives of internal medicine," while James B. Herrick of Chicago mentioned on "Peculiar Elongated and Sickle-Shaped Red Blood Corpuscles in a Case of Severe Anemia."

1910 is regarded as the year of the invention of sickle cell anemia. In 1922, Verne Rheem Mason an eminent internist named the disorder as "sickle cell anemia" primarily based on the description of Ernest Irons. In 1927, Hahn and Gillespie discovered that red blood cells from humans with the sickness might be made to sickle through eliminating oxygen. There have been additionally humans at that time, frequently relatives of the patients, whose red blood cells have been sickling when deprived of oxygen however who had no signs and symptoms. Those became known as "sickle trait." Hahn and Gillespie had been the first to accomplice the red cell sickling to low oxygen and acidic conditions.

The first splenectomy for the treatment of red blood cell anemia was reported by Hahn and Gillespie in 1927. In 1949, the famous Nobel Prize-winning chemist Dr. Linus Carl Pauling (February 28, 1901 – August 19, 1994) and his colleague Dr. Harvey Itano and Dr. William Castle were the first to point out that the reason behind the disease was an abnormality within the hemoglobin molecule. They demonstrated that sickle cell anemia occurs as a result of an abnormality within the hemoglobin molecule. They found that the red, oxygen-carrying protein called "hemoglobin" had a special chemical structure in persons with red blood cell anemia. They coined the term "molecular disease." Thus, sickle cell anemia became the first molecular disease ever described and Dr. Pauling received the first of his two Nobel Prizes. They published their findings in 1951 in the paper titled "Sickle Cell Anemia, a Molecular Disease."

Firstly, Dr. Ingram was able to isolate the abnormal beta globin chain, digest it with trypsin, and secondly, Dr. Harvey Akio Itano demonstrated it by two-dimensional electrophoresis that of the entire of 146 amino acids of the beta globin chain, just one amino acid differed from normal. Futhermore, at position 6, counting from the amino terminal, there was the amino acid valine in the place of glutaminic acid. Again, in 1956, Vernon Ingram, and J.A. Hunt sequenced sickle hemoglobin and showed that a glutamic acid at position 6 was replaced by a valine in sickle cell disease. Nowadays we all know quite clearly that the sickle mutation occurred within the beta globin gene inside chromosome 11 at the first exon where the triplet codon guanine- adenine-guanine has mutated to guanine-thymine-guanine. The result's replacement of glutamic acid at the sixth position of the 146 amino acids of the beta chain of hemoglobin by valine. This may result in alteration within the structure of hemoglobin and sickling of red blood cells. Dr. Makio Murayama, a biological chemist at the National Institute of Health, reported that since the amino acid valine is hydrophobic, it'll place itself in proximity to a different valine normally located at position 1 of the amino acid terminal. Moreover, this approximation of valine 1 and valine 6 results in the formation of a ring-like structure, a process called cyclization of the valine ring. In continuation to this ring structure is a chemical site for attachment to a complementary structure on the alpha chain of subsequent

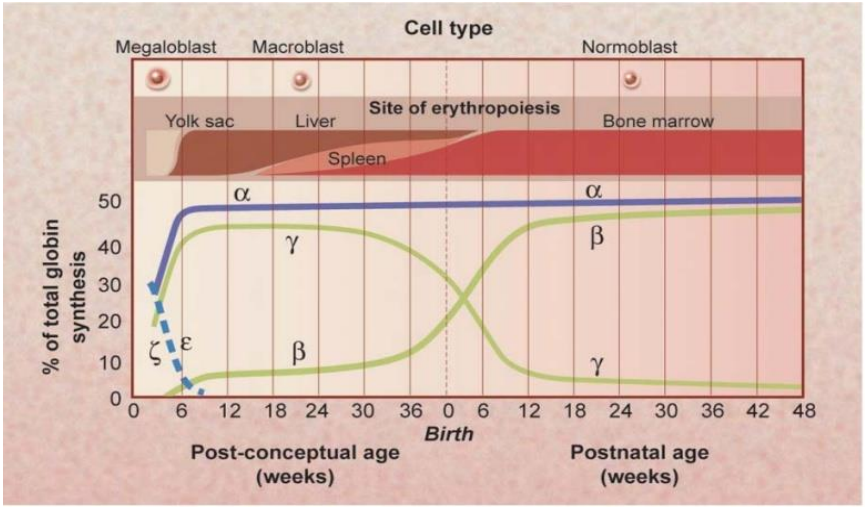
hemoglobin molecule, with the mutated valine 6 interacting with the beta globin of the adjacent hemoglobin S. Finally, this successively results in linear cord-like stacking of the hemoglobin S molecules and eventual sickling of the whole defective red blood corpuscle. Sickle cell disease (SCD) describes a bunch of inherited red blood cell disorders.

Structure of Hb

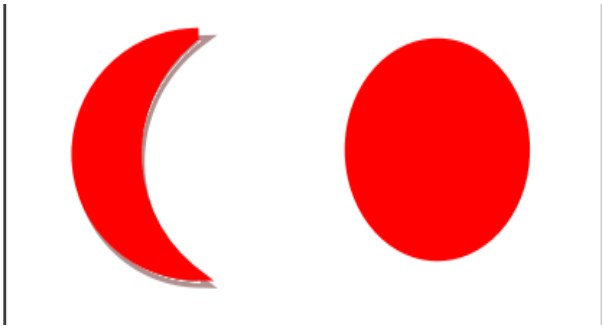
Hemoglobin (Hb) molecules are a collection of proteins shaped by using pairing of alpha (α) and beta (β) globin polypeptide chains right into a tetromeric unit. (Embury et. al., 1994) This unit, the α2β2 molecule, forms the foremost important adult hemoglobin responsible for the delivery of oxygen from the lungs to tissues. The α-globin gene is positioned on chromosome 16 and encodes the adult α-globin and therefore the ζ-globin chain, that's the embryonic shape of α-globin(Schechter, 2008). The β-globin gene is positioned on chromosome 11 which encodes four different globin molecules: embryonic ε-globin, fetal γ-globin, adult δ-globin and adult β-globin, all expressed during a different way at some stage specifically instances of 5 developments. (Schechter,2008)

Each of the 2 alpha chains are derived from 141 amino acids. Each of the 2 beta chains are derived from 146 amino acids. The 2 alpha chains are derived from genes within the alpha gene cluster on chromosome 16. The 2 beta chains are derived from genes within the beta gene cluster on chromosome 11.

Hemoglobin A — this is often the conventional hemoglobin that exists after birth. Hemoglobin A is a tetramer made from two alpha chains and two beta chains (α2 β2). Hemoglobin A2this is often a minor component of the hemoglobin found in red blood cells after birth. Hemoglobin A2 consists of two alpha chains and two delta chains (α2 d2). Hemoglobin A2 generally comprises less than 3 % the whole red cell hemoglobin. Hundreds of different structural variations of hemoglobin had been mentioned inside the literature. The most common clinically significant structural variants are HbA, HbA2, Hb S, HbC, HbF, HbD, and Hb O, (Serjeant,1992). SCD is used to elucidate a good form of hemoglobinopathies, which could be characterized via Hb S within the presence of another variation beta globin chain. Most of the people of SCD cases are composed of 4 primary subtypes: SS, SC, S βo, and Sβ+. (Serjeant, 1992).



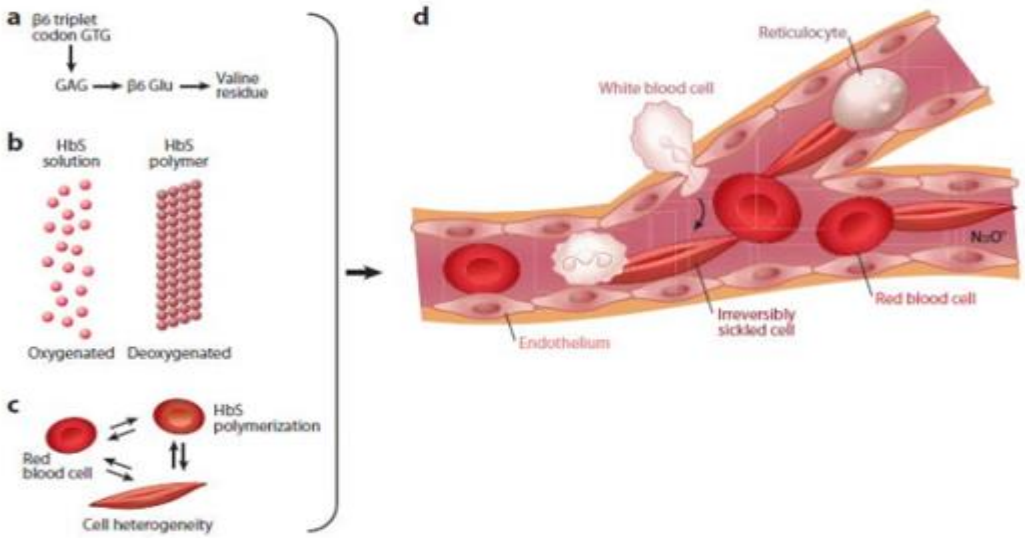
The timeline of human globin gene expression Adapted from Schechter, 2008



Hallmark Sickle-Shaped red blood cell shape (L) versus normal red blood cell shape(R)

Table 1. Different Structural Variants of Hemoglobin

Hb A	α2β2
Hb A2	α2δ 2
Hb S	Glutamic acid (GAG) valine (GTG) at position β6
Hb C	Glutamic acid (GAG) lysine (AAG) at position β6.
Hb F	α2γ 2
Hb D Los Angeles or Punjab	Glutamic acid (GAG) glutamine (CAG) at position β121
Hb O Arab	Glutamic acid (GAG) lysine (AAG) at position β121
Hb E	Glutamic acid (GAG) lysine (AAG) at position β26
βo Thalassemia	Absence of Hb A due to inability to produce normal β-chain
β + Thalassemia	Reduced amount of β-chain production leads to variable amount of Hb A.



Pathophysiology of SCD

(*a*) Point mutation in sickle cell disease. (*b*) Polymerization of hemoglobin S (HbS) under-deoxygenation. (*c*) Red blood cell shape change in response to HbS polymerization. (*d*) Reticulocyte adhered to endothelium initiates vaso-occlusion by trapping irreversibly sickled cells and forming aggregates with white blood cells (Barabino et. al.,2010).

INHERITANCE

The scd is inherited as an autosomal recessive pattern, subsequently, which takes the place while the individual inherits one of the abnormal hemoglobin genes from each parent. If an individual inherits one abnormal hemoglobin gene and one normal gene as a result he/she is considered to possess the sickle cell trait or a carrier for the scd. Certainly, If each parent is carriers for the scd then there may be 25% or 1 in four a chance in every pregnancy of getting a baby who will inherit each unusual hemoglobin genes and present with the scd (Serjeant & Serjeant, 2001). People with sickle cell trait are protected against malaria. On the other hand, this is often the result where Plasmodium falciparum is the causative organism.

CLINICAL SYMPTOMS

Scientific signs or manifestations of SCD contains hemolysis, anemia, ache disaster, contamination, acute chest syndrome swelling and vascular occlusion probably resulting in ischemic attacks and organ damage (long, 2011). Furthermore, in older children, periodic occurrences of acute episodes with diverse manifestations facilitate recognition of red blood cell anemia. Those episodes, recognized as "crises". Subsequently, while the erythrocytes or red blood cells start to sickle, this as a result cause pain episodes (Lin-Fu, ibid, 1965). On the other hand, under deoxy conditions, HbS undergoes several changes including:

- Marked decrease in solubility.
- Increased viscosity.
- Polymer formation.
- It forms a gel-like substance containing Hb crystals called tactoids.

The gel- like form of Hb is in equilibrium with its liquid-soluble form.

Variety of factors influence this equilibrium, including: • Oxygen tension • Concentration of Hb S • the presence of other hemoglobins – Oxygen tension is a crucial factor in the polymer

formation which occurs only within the deoxy state. If oxygen is present, the liquid state prevails.

Blood

Acute painful crises are the foremost common type of vaso-occlusion that happens in SCD and is that the commonest purpose for admission to hospital for both adults and kids. [Rees et. al.2010] The precise mechanism is resulting in a pain episode continues to be uncertain although it's concept to be a non-stop cycle wherein vaso-occlusion effects in tissue ischemia and damage, resulting in a secondary inflammatory response that triggers the discharge of nor epinephrine which in the long run ends up in further tissue ischemia(Rees,2005). The severity, frequency, region and duration of pain crises are variable among sufferers and should be prompted by means of variety of distinct activities or factors which include: severe temperatures or adjustments in humidity, dehydration, stress, alcohol intake, contamination, menses, obstructive sleep disorder and cardiac or pulmonary impairments. The reduced lifespan of the red blood cells (one hundred twenty days to**<20 days) is likewise responsible for sufferers to experience persistent anemia with various ranges of severity. (Amjad, et.al., 1974, Yoong et. al., 2002)**

Bone

However, because of a constrained blood supply to some areas of the body, SCD sufferers may additionally experience the bone and joint issues. Bone complications contain the bone infarction or osteomyelitis. Osteomyelitis, an acute or continual bone infection, is most typically caused by salmonella (Embury, et. al., 1994 & Costanzo, 2011).

Bone infarctions

Consist of the dactylitis, usually called the hand-foot syndrome that's characterized by the way of a painful swelling of the arms and feet that normally resolve inside per week. The hand-foot syndrome is usually the primary medical manifestation visible in babies with a sickle cell disease between the very long time of six months and two years. (Embury, et. al., 1994 & Costanzo, 2011).

Sickle Cell Trait

The trait is a kind of sickle cell disease has no clinical manifestations besides underneath excellent instances, at some stage during which the individual might enjoy a crisis almost like that of the anemia sufferers. As a result, symptoms in sickle cell trait could also be absolutely obliterated by means of averting those circumstances, which could precipitate a crisis condition.

Sickle cell trait individuals could also be incapacitated by way of (1) conditions of excessive hypoxia, beside flying in an unpressurized plane or with underwater swimming, (2) during anesthesia when anoxia has inadvertently to befall, (3) occurrence of occasional excessive pneumonia and (four) extreme bodily exercise.(Al-Salem, 2016)

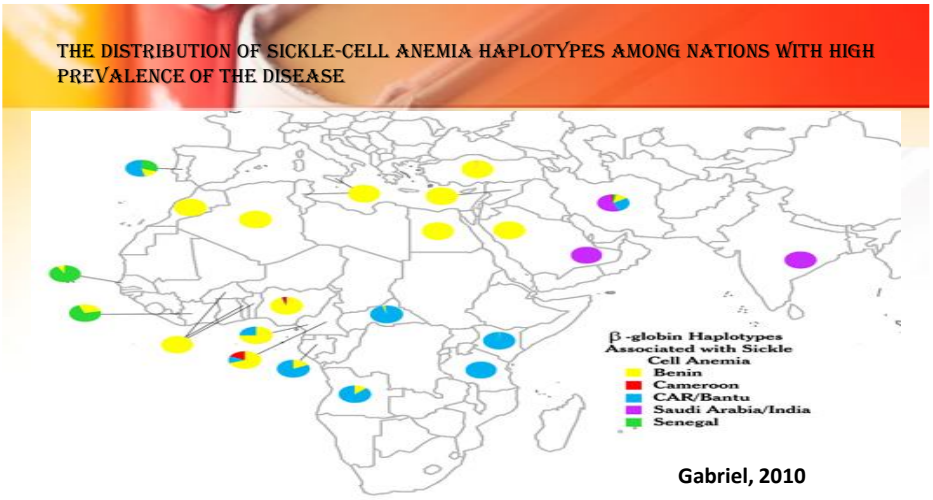
HAPLOTYPES

A haplotype is a collection of alleles in an organism, which are inherited collectively from a single parent. furthermore, in sickle cell disorder, beta S-globin haplotypes constitute the ethnic group or geographical area from which patients originated. According to the latest observations, a number of the Arabian/Indian and Senegal haplotypes recommend that they may be related to milder kinds of SCD, while the Bantu haplotype is related to an extra intense clinical direction. Moreover, this remark correlates with distinction within the average degree of fetal hemoglobin in sufferers with distinctive haplotypes. People with the Arabian/Indian haplotype had a mean 17% HbF, people with the Senegal haplotype had a mean 12.4 % HbF and people with Bantu and Benin haplotypes had even lower average HbF.
Haplotyopes means subtypes of genotypes (Dacie JV and Lewis SM, 1991).

There are 4 type of haplotypes are found such as,

1. The Senegal(SEN)
2. The Benin (BEN)
3. The Central African republic or Bantu (CAR)
4. The Asia/Arab-Indian haplotype

SEN is lesser sickle cell manifestation. So, SEN is the best type of haplotype with high HbF.CAR or Bantu is more clinical manifestation. So, this is the worst type of haplotype with least HbF. BEN haplotype is intermediate.(M H Steinberg, Chui, & Dover, 2014)



Data on File. Novartis Pharmaceuticals Corp; 2019.

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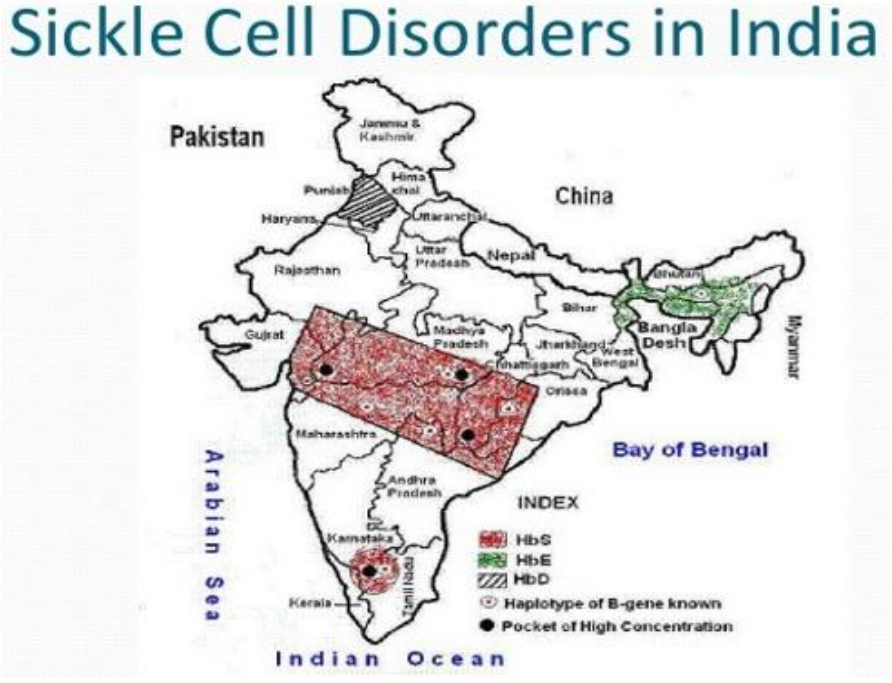
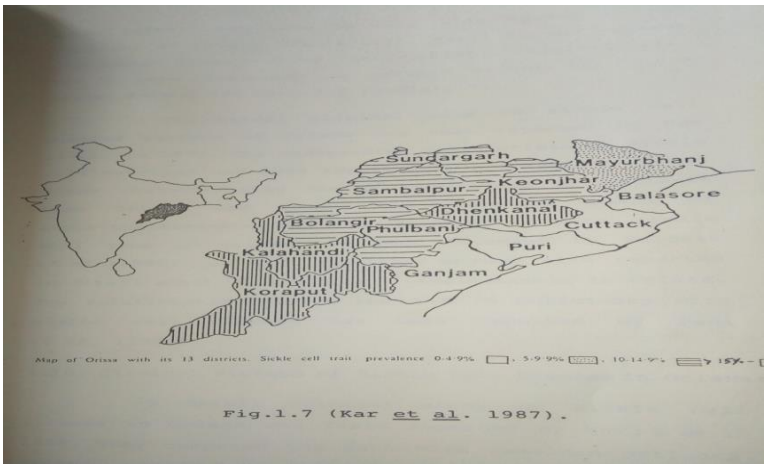


Epidemiology of Sickle patients in India (Gupta et al, 2009)



Epidemiology of Sickle patients in Odisha (Dash. B.P.,Ph.D Thesis,1994)

HAPLOTYPES

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Sickle gene in Orissa:

Sickle cell anaemia is a single-gene haemoglobin condition caused by a transversion mutation on the 6th codon (GAG>GTG) of the beta globin gene on the 11th chromosome q arm (Maske et al., 2015).Sickle cell disease (SCD) or sickle cell anaemia (SCA) is a haemoglobin (Hb) hereditary condition caused by the replacement of one nucleotide from thymine to adenine (GAG GTG) inside the -chain of haemoglobin, resulting in the amino acid valine instead of glutamic acid (Rees et al., 2010). Mutation is now responsible for changes in the characteristics of the haemoglobin tetramer, which has a proclivity to polymerize in the deoxygenated state (Ballas, 2002), transforming normal, flexible biconcave-shaped red blood cells (RBCs) into stiff, inflexible sickle cells. The rate of sickle cell haemoglobin (HbS) polymerization is closely related to the pathophysiology of hemolytic anaemia and vaso-occlusion (Samuel et al., 1990). Dunlop and Mazumder discovered sickle haemoglobin in Oriya people in 1952. Batabyal and Wilson (1958) proposed cases of sickle cell disease in Assam, each born to Orissa immigrant parents. Vella and Hart also cited a case from Malaya with Orissa ancestors in 1959. Das et al. (1967) and Roy and Roychaudhuri (1967) reported the existence of this gene in a few Koraput district tribes. Praharaj et al. reported the prevalence of sickle cell - thalassaemia The high prevalence of this gene in several Agharias of Orissa has been documented by Nanda et al. (1967), Samal et al. (1978), and Samal and Naik (1983). The prevalence of sickle cell disease. The HbS gene is widely distributed throughout Orissa and is prevalent in Hindu civilization (Kar et al. 1986). The S gene frequency ranges from 0.009 to 0.216 in several Orissa tribes. Kar et al. discussed the detailed medical facts on sickle cell disease patients in Orissa. In 1986. Kar et al. (1986) established the impartial Asian origin of red blood cell mutation after characterising the S - haplotypes of Orissa patients. Kulozik et al. (1988) discovered the molecular basis of alpha-thalasemia and its association with the sickle cell gene in Orissa. Anugul has the most cases, followed by Khurda, Nayagarh, Phulbani, Cuttack, Jajpur, Dhenkanal, Ganjam, Keonjhar, and Mayurbhanj.

Sickle cell disease is an inherited blood ailment that primarily affects persons of African, Arabian, and Indian descent. Sickle cell disease affects persons of African descent throughout North and South America, the Caribbean, and much of Europe.

This is predominantly of the Benin haplotype, and this form of the disease has been well characterised, is relatively severe, and successful therapies have been found to improve the disease's result.2016 (Serjeant et al.)

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Hypersplenism is rather prevalent. In Indian patients, painful crises and dactylitis are not uncommon. Indian patients had a higher prevalence of alpha thalassaemia, higher levels of HbF, total haemoglobin, and red cell counts, and lower levels of HbA2, MCHC, MCV, MCH, and reticulocyte. The greater the prevalence of alpha thalassaemia and high HbF levels may be expected to result in less intravascular sickling and a lower rate of haemolysis, as well as lower reticulocyte counts, lower haemoglobin levels, and higher haemoglobin levels (Kar et al.,1986). HbF levels are elevated in Orissa patients. Kulotzik and colleagues (1991). 2017; Mishra et al.Despite a typically milder course, moderate to severe anaemia, vaso-occlusive episodes (86.5-89.36%), splenic sequestration (8.43%-12.76%), crippling avascular bone necrosis (5.7%-35.08%), osteomyelitis (5/700), and epistaxis (28.92%-35.08%) are nevertheless clinical occurrences (Kar et al., 1991).

SCD is characterised by recurrent bouts of musculoskeletal discomfort, anaemia, jaundice, and splenomegaly in children.

Fever and anaemia, with or without jaundice or splenomegaly, are common in young children.

Regular administration of long-acting penicillin up to the age of 6-8 years can help prevent such attacks that can lead to a sickle crisis.

The majority of the children (77%) had total haemoglobin levels between 6 and 10 g/dl. In 12% of the children, it was greater than 10 g/dl. They thrived and grew well with such haemoglobin levels. Although they required extra folate supply on a regular basis, no attempt was made to raise their haemoglobin levels through blood transfusion or iron therapy. (Kar and colleagues, 1997).

This mean number was significant between controls and sickle cell homozygotes (p 0.01), as well as sickle cell trait and disease (p 0.01) mothers. This mean number was significant between controls and sickle cell homozygotes (p 0.01), and sickle cell trait and disease (p 0.01) mothers. Balgir and colleagues (1997).All tribals with sickle cell disease experienced painful crises, with jaundice evident in 57.5 percent of cases (Kaur et al., 1997). According to B.C. Kar, 50% of children experienced their first manifestation by the fifth year of life, and another 25% by the tenth year of life. Palpabale spleen was found below the left costal border in 13.73% of all children. Splenomegaly was discovered in 30.1% of sickling positive youngsters, which was much higher than expected.

Sickle cell disease in children is clinically and hemologically indistinguishable. They are seen in siblings and run in families. As a result, children with recurring episodes of abdominal discomfort, muculoskeletal pain, ARI, fever, splenomegaly, anaemia, and epistaxis should be suspected of having sickle cell disease (2003, Sahu et al) .High levels of Hb F were found in both sickle cell disease and sickle cell trait patients (considered to be the cause of the mild clinical presentation). This is consistent with the findings of previous investigations.

Hb E is frequently found in cases referred from the state's eastern coast (Balasore and Bhadrak districts), a location exposed to Southeast Asia and closer to parts of India (Assam and West Bengal) where this Hb is widespread. As a result, it is safe to assume that its presence in Orissa is primarily due to gene flow from these places. 2004; Chhotray et al.

Hepatomegaly was observed in 36 patients, with four patients having substantial hepatomegaly.

Gross hepatomegaly was observed in 8% of the individuals. This is consistent with the findings of Papadaki MG et al. (2003).Hepatomegaly has been linked to kupffer cell erythrophagocytosis and sickle cell aggregation engorgement of sinusoids. Patients with extensive hepatomegaly had high bilirubin levels, clinical jaundice, and concomitant

liver discomfort, indicating a painful crisis. Mild to severe splenomegaly was reported in patients in their second (64%), third (53%), and even fourth (60%) decade of life. In contrast, Sergeant et al. (1985) discovered that after 30 years of age, the spleen exhibits increasing fibrosis and atrophy only 6% had splenomegaly beyond 30 years. Kar et al in 1986, attributed this persistence of splenomegaly in later age (Mohanty et al., 2004)

Higher fetal hemoglobin concentration in patients with sickle cell disease in eastern India reduces frequency of painful crises without a threshold effect, is associated with splenomegaly and has no effect on the prevalence of avascular necrosis of the femoral head (Patel., 2010).

Sickle cell disease (SCD) is a common condition in India, with a normal or slightly subnormal mean corpuscular hemoglobin concentration (MCHC) and only 17% of cases of hemoglobinopathy having a low MCHC. Malaria is the leading cause of death in children with SCD, and bone pain crises are usually attributable to bone marrow necrosis in the juxta-articular areas of the long bones, spine, ribs, and sternum. Both SCD and P. vivax infection are common in central India (Shukla et al., 2017).

Sickle beta thalassemia patients have higher mean values of HbA, HbA2, and decreased levels of MCV, MCH and MCHC, suggesting hypochromic and microcytic anaemia. The persistence of splenomegaly is higher in SCD patients, and the intensity of weakness and pain is greater. This will help to alleviate psychosocial affliction (Bindhani & Nayak, 2018).

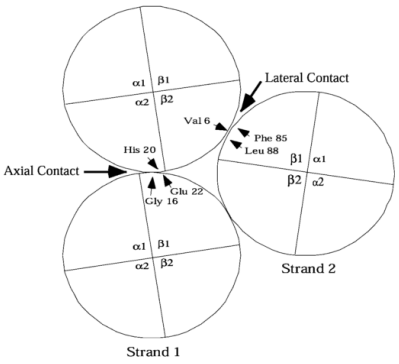
Hemoglobin polymerization, vaso-occlusion, and hemolytic anemia are key to the pathophysiology of sickle cell anemia, leading to a cascade of pathologic events and complications (Mishra & Chhabra, 2018)**.**

Pathophysiology of Sickle Cell Anemia

The concept of sickling and sickled cells is important to the pathophysiology of SCD. Oxygen tension, hydrogen ions concentration, temperature, intracellular HbS concentration, and non-HbS hemoglobin all play a role in in-vitro sickling. Oxygen tension is associated with an increase in sickled cells in SCD, while hydrogen ions concentration promotes cellular water loss through activation of the KCl co transport device. Temperature also affects the speed of sickling, with whole inhibition of sickling occurring at temperatures near freezing (1-4 0C). Intracellular HbS concentration is also important, as red cells from patients with SCD sickled more easily than those from AS. HbD Punjab was interacting strongly with HbS, while HbF inhibited sickling to a more extent (Charache et. al., 1987).

Sickle cell anemia is characterized by means of

- Chronic episodes of persistent haemolytic anemia
- The prevalence of acute episodes of sickling crises
- Repeated infections
- Acute haemolytic anemia
- Recurrent vaso-occlusive episodes
- Complications related to those episodes Red blood cells normally live for 90–120 days, but in patients with sickle cell anemia, they live for less than 10–20 days. This may cause persistent chronic anemia.



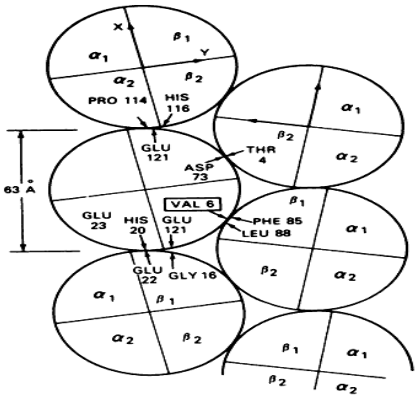


Figure-1A and 1B Structure of human HbS fibers. Two types of contacts occur between deoxy-HbS tetramers in the doublestranded fibers. Contacts along the long axis of the fiber are termed axial contacts, whereas contacts along the sides of tetramers are termed lateral contacts. The 6 valine plays a crucial role in the lateral contact by interacting with the hydrophobic 85 phenylalanine and 88 leucine on a neighboring tetramer. An important axial contact is the interaction of the 22 glutamic acid with an 20 histidine on an adjacent tetramer. (Levasseur et al. 2004)

Levasseur et. al (2004) make an recombinant hemoglobin HbAS3 contains amino acid changes at

positions 16, 22, and 87 of the -globin chain. The modification at 16, glycine to aspartic acid, is understood as HbJBaltimore and provides HbAS3 with an enhanced ability to interact with the -globin subunit to make AS3 dimers. The amino acid modification at 22, glutaminic acid to alanine, disrupts the axial contact interaction with the 20 histidine, and therefore the mutation at 87, threonine to glutamine, disrupts the lateral contact of the 6 with the hydrophobic pocket. (Levasseur et al 2004)

B chain contact sites in the hemoglobin S polymer by Nagel RL et.al. Nature (1980).

The two β chains of HbS behave unequally within the formation of the polymer. Nagel R.L et al (1980) give an evidence that one of the β 6 valine substitution sites of the HbS tetramer is actively involved in a part of contact. The symmetrical area within the other β S chain isn't involved in any area of contact. because of symmetry related relationship between the chains within the haemoglobin tetramer each β chain includes a different set of interactions with each of the α chain. With one among the α chain, a β chain forms a highly stable system of strong hydrophobic and polar interaction leading to a really stable α1β1 dimer. a similar β 1 chain forms weaker interaction with the α2 chain in order that the α1β 2 interface is broken when the tetramer dissociates in to dimers. Mutation sites β16, β17, β19, β83 and β95 had an inhibitory effect, where because the mutation at site β 66 facilitated polymerization, an impression similar to that found with substitution at β 121 (Hb O Arab and Hb D Los Angeles). Crystal structure of deoxy HbS and located that the basic unit is a double strand stabilized with side to side and up down interaction involving mainly the β chain. A salient feature of this structure is that just one of the β chain has 6-valine residue involve in a very contact area. Of the sites examined so far β66, β73, β87, which were found to be involved within the side to side contact of the crystal, were also found to possess strong effects on the polymerization of solutions of HbS . Note that these residues were shown to be a part of the receptor site located within the β 2 chain of the crystal (the β1 chain carries the active β 6 valine site. Although β126 valine participate in the interacting site of the crystal it's provided by a similar (β 1) chain carrying the active 6 valine site. Residue β 80 implicated by the inhibitory effect of Hb Gifu. The fact that residue β83 is involved additionally, within the HbF crystal, β80 is featured in this area of contact. The residue at β90 is included in the side to side contact within the crystal. Up down interaction along the crystal strand axis. Of the residue involve by the crystallography map of the deoxy HbS, β16, β17, β19, β22, and β121 are examined. All of them are found to be active in solution. Only five contact sites provided by α1 and two contact sites provided by α2 that stabilize the up down contact of the double strand within the crystal. On β chain β66,β73, β83 and β87 are involve in side to side contact position on crystal the polymerization . The hemoglobin polymer even in the thickly packed bundles, were always separated from one another by a distance of 40A to 90 A. These arrangements of parallel but equidistant fibre closely resemble the description offer by Harris (1950)

Inhibitory effects of hemoglobin F and hemoglobin A2 on the polymerization of hemoglobin S

Nagela et. al. 1979 found that hemoglobin F (Hb F) inhibits the polymerization of Hb S by forming asymmetrical hybrid tetramers of the kind 2s. Examination of the gelling properties of binary mixtures of Hb S and other Hb variants revealed that residues 80 (EF4) and 87 (F3) are a minimum of partly responsible for this inhibition. Mixing Hb A2 (22) with Hb S strongly inhibits gelling to an extent similar to that seen with Hb S/Hb F mixtures. These residues 22, 80, and 87 of the chain appear to be involved in intermolecular contact sites that stabilize the deoxy Hb S polymers.

ROLE OF GORDUS CHANNEL IN SICKLING POLYMERISATION IN PATIENT WITH SCA

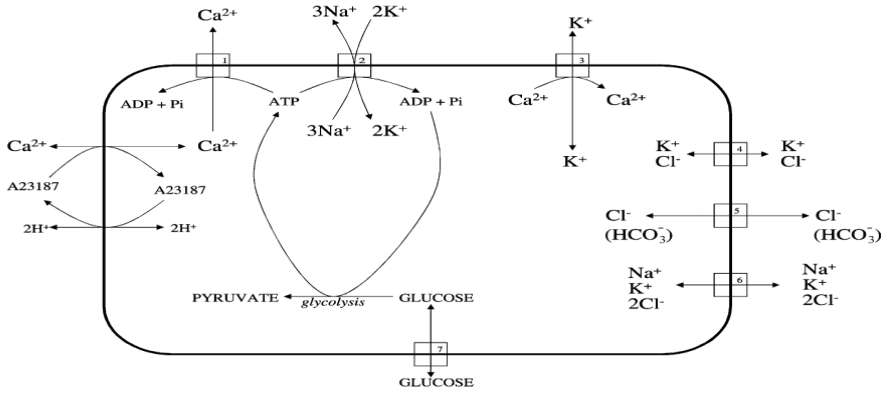


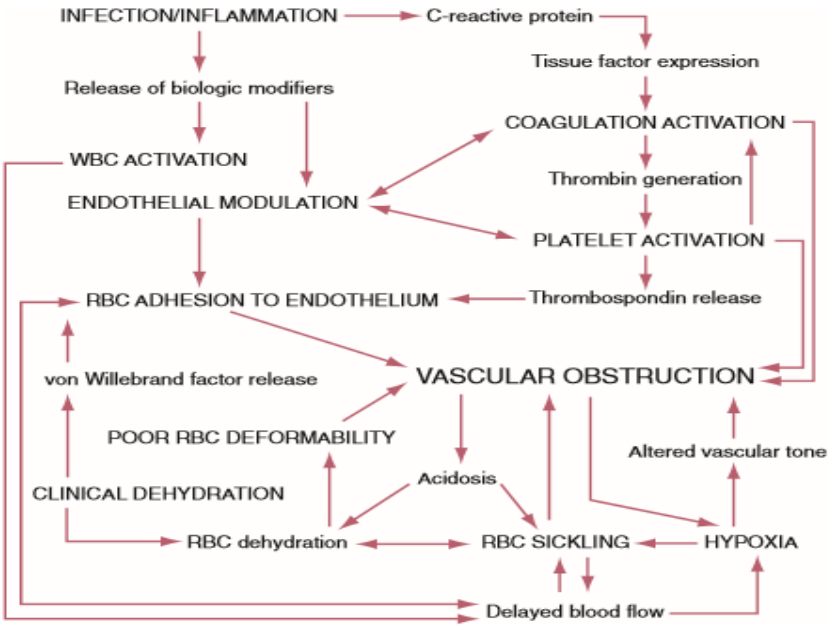
Figure-6 . Erythrocyte ion transport pathways relevant to transmembrane potassium distribution. Also included is Ca2 + transport across the membrane via A23187. The driving force for K+ efflux via the Gardos channel arises from the chemical potential set up by the Na´ +, K+-ATPase which, in turn, is 'powered' by the ATP produced in glycolysis. Note that energy from ATP hydrolysis also sets up a Ca2 + gradient across the membrane. The numbered transport pathways are: (1) Ca2 +-ATPase; (2) Na +, K+-ATPase; (3) the Gardos channel; (4)´ K+/Cl − co-transport; (5) Band 3 anion exchanger; (6) Na +/K+/2Cl − co-transporter; (7) glucose transporter. (Maher and Kuchel 2003)

Vaso – occlusion

SCD has two essential outcomes: hemolysis and vasoocclusion. Hemolysis is the breaking down of RBCs due to sickling and the presence of immature reticulocytes. Vasoocclusion occurs when RBCs adhere to postcapillary venules, narrowing the lumen and entrapping poorly deformable dehydrated cells. Free plasma hemoglobin (Hb) is a scavenger of NO, leading to functional NO depletion and a shift in vascular tone in the direction of vasoconstriction. This contributes to vasoocclusion and the development of pulmonary hypertension (Franco, 2006).

The pathogenesis of vasoocclusion in SCD is not fully understood, but Hb S polymerization and sickling of RBCs play a major role. Risk factors include decreased deformability, sickle cell-endothelial cell adherence, endothelial cell activation, WBC and platelet activation, hemostatic activation, and altered vascular tone (Bartolucci et al., 2012).

Increased WBCs, platelet activation, and clinical dehydration can trigger RBC adherence to endothelium, leading to vascular obstruction. Dense cells are at greatest risk for intracellular polymerization due to their higher Hb S concentration. Adherence to endothelium correlates with severity of painful episodes and intimal hyperplasia, which can slow blood flow (Embury, et. al.,1994)(Jandl et. al., 1996) (Randolph, 2020)



Numerous Risk Factors for Vasoocclusion Illustrating Physiological Interrelationships. (Embury, et. al., 1994).

The most pathophysiological defects of sickle cell anemia are:

– HbS polymerization

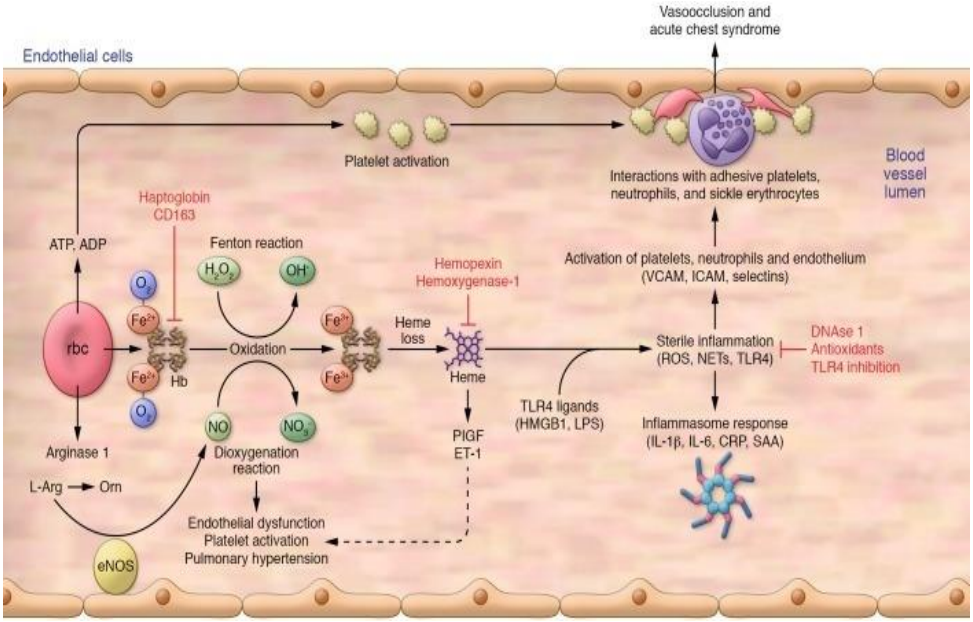
– Red cell sickling

Sickle cell anemia is characterized by vascular endothelium activation and improved blood cell-endothelium interactions. This abnormal adhesion of sickle red cells to the vascular endothelium is triggered through repeated sickling, expression of adhesion molecules, dense red cell formation, and up regulation of endothelial adhesion molecules. Ischemia and reperfusion within the microcirculation can also cause endothelial oxidant era, endothelial activation, and up regulation of adhesion molecules. Inflammatory activation of endothelium and increased leukocyte recruitment may also contribute to stasis. Sickle red cell

adhesion in postcapillary venules can cause elevated microvascular transit instances and provoke vaso-occlusion.
• Add to this, involvement of an array of adhesion molecules expressed on sickle red cells including:
– CD36
– A-4-β-1 integrin
– ICAM-4
– Basal cell adhesion molecule (B-CAM)
– Activated endothelium:
• P-selectin
• Vascular cell adhesion molecule-1 (VCAM-1)
• Alpha-V-beta-3 integrin
• Plasma factors and adhesive proteins:
– Thrombospondin (TSP)
– Von Willebrand factor (vWf)
– Laminin

Sickle cells express VLA-4, which interacts with the endothelial cell adhesive molecule, VCAM-1, which is upregulated by hypoxia and inhibited by nitric oxide. Additionally, a-V-ß-3 integrin is upregulated in activated endothelium in patients with sickle cell anemia. Intravascular hemolysis produces free hemoglobin, which drives fenton reactions to provide oxidants and scavenges NO. Oxidized Hb releases free heme, which activates release of placenta growth factor (PIGF) and endothelin -1 (ET – 1). Heme also primes the innate immune system to acute rises in endogenous (HMGBI) and exogenous (LPS) ligands of TLR4, which activates production of ROS, neutrophil extracellular traps (NETs) and downstream activation of the inflammasome.

Adenosine binds receptors on red cells, leading to decreased oxygen affinity of hemoglobin. Proteins on the surface of the activated endothelium interact with adhesive platelets, neutrophils and sickle erythrocytes, producing vasoocclusion and acute chest syndrome. Intravascular hemolysis also releases asymmetric dimethylarginine, which inhibits eNOS by Gladwin et. al.,2012.



Gladwin et. al., 2012
Hypoxia decreases nitric oxide production, which increases the adhesion of sickle cells to the vascular endothelium. Continuing active hemolysis of RBCs results in liberation of free Hb within the plasma, which is a dedicated scavenger of nitric oxide. Additionally, inflammatory activation of endothelium may have an essential role in enhanced sickle RBC-endothelium interactions. Sickle RBC adhesion in postcapillary venules can cause increased microvascular transit times and initiate vaso-occlusion. Sickle RBCs also adhere to macrophages, which may contribute to erythrophagocytosis and the hemolytic process.

Hemolysis may be a constant finding in sickle cell syndromes, with approximately one third of RBCs undergoing intravascular hemolysis and the rest hemolyze by erythrophagocytosis by macrophages. Sickle RBCs have increased immunoglobulin G (IgG) on the cell surface. Vasoocclusive crisis is commonly triggered by infection and is caused by elevated levels of fibrinogen, fibronectin, and D-dimer. Plasma clotting factors likely participate in the microthrombin in the pre arterioles. Increased leukocyte recruitment and adhesion of sickle RBCs may also contribute to stasis.

Chronic anemia is caused by premature destruction of the sickled red blood cells, occlusion of the fine

capillaries, and recurrent bone pain. Long-term, recurrent episodes of vaso-occlusive crisis result in damage to the inner organs, especially the kidneys, heart & lungs. Sickle cell anemia usually presents in infancy with repeated infections. Dactylitis is a painful swelling of the dorsum of the hands and feet, and splenomegaly occurs when sickle cells gain Na+ and lose K+.

Under deoxy conditions, HbS undergoes several changes including:
– Marked decrease in solubility.
– Increased viscosity.
– Polymer formation.
– It forms a gel-like substance containing Hb crystals called tactoids.

The gel type of Hb is in equilibrium with its liquid-soluble form. Variety of factors influence this equilibrium including:
• Oxygen tension
• Concentration of Hb S
• The presence of other hemoglobins

Oxygen tension is an important factor in the polymer formation of HbS, which occurs only in the deoxy state. Concentration of Hb S is another factor in the formation of the gel-like type of HbS, which occurs at concentrations greater than 20.8 g/dL. The presence of other hemoglobins can be a factor in why patients with high HbF have a milder disease, compared to those with low HbF. Affected patients with sickle cell anemia present with a wide range of clinical problems that result from vascular obstruction and ischemia. Although sickle cell anemia are often diagnosed at birth, clinical manifestations usually don't occur before age 6 months.

Hydroxyurea:
Hydroxyurea was the first approved drug for the treatment of sickle cell anemia in 1995. It was shown to be beneficial for patients with sickle cell anemia, reducing the number and severity of vaso-occlusive attacks, reducing the number of hospitalizations, reducing the frequency of acute chest syndrome, and reducing the need for blood transfusions. Blood and bone marrow stem cell transplant is the only known cure for sickle cell anemia, but it is expensive and has a high morbidity and mortality. Gene therapy is being researched to permanently increase fetal hemoglobin levels, and counseling and testing for sickle cell before marriage is important to dilute the gene within the communities and reduce the amount of affected siblings.

Recent development
ADAKVEO® (crizanlizumab) is indicated to reduce the frequency of vaso-occlusive crises (VOCs) in adults and pediatric patients, aged 16 years and older, with sickle cell disease.

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