

Review

Sickle Cell Disease, a Review

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Abstract: Sickle cell disease and its variants constitute the most common inherited blood disorders affecting millions of individuals worldwide. Significant information regarding the nature of the genetic mutations and modifier genes that result in increased or decreased severity of the disease are available. In recent years, detailed data regarding molecular genetics, pathophysiology, mechanisms for the development of symptoms and side effects of sickle cell disease have been published. The relationship of physiological changes, cellular interactions, coexisting coagulation disorders, effects of association with other genetic disorders and a number of intervening factors have been explored. New techniques for pre-conception, prenatal, in utero, and neonatal screening are available. Means for prediction of the severity of the disease, clinical course of the disorder, and prevention of some of its major complications have been developed. The effects of psychosocial and environmental factors have been explored. Various therapeutic strategies including bone marrow and stem cell transplantation are currently employed in the treatment of patients with sickle cell disease. Recent progress in understanding the molecular pathways controlling mammalian erythropoiesis and globin switching, as well as advances in genome engineering, particularly the gene-editing techniques, have opened a venue for genetic-based treatment of the disease. Currently, sickle cell disease is often associated with a high rate of complications and mortality. The development of new pharmacological agents, methods for gene therapy, and alterations and modification of the coexisting genetic factors and modifiers for treatment of the disease are encouraging.

Keywords: sickle cell disease; genetics; etiology; pathophysiology; symptoms; screening; diagnosis; complications; treatment; coagulation; inflammatory factors; modifiers; gene therapy; transplantation



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1. Introduction

The term sickle cell disease encompasses a group of common inherited genetic disorders characterized by a point mutation involving the gene which encodes the hemoglobin subunit β (HBB). Genetic changes include homozygous missense mutation [Glu6Val, rs334] in the β -globin gene which upon deoxygenation results in polymerization of hemoglobin S (Hb S). This alteration in a single DNA base leads to a cascade of physiological consequences that can affect multiple organs and systems [1,2]. Polymerization of the two-mutant sickle β -globin subunits leads to erythrocytes assuming a crescent or sickled shape, thus the designation of sickle cell disease (SCD). It should be noted that polymerization is equivalent to crystallization.

Worldwide, sickle cell disease constitutes the most common monogenic disorder [3,4]. There is a significant variation in symptomatology of the disease based on a number of variables, including coinheritance of genetic factors which can enhance or reduce the severity of the symptoms [2–9]. Even in homozygous SCD, which is the most severe form, there is a notable diversity in manifestation of the disease among patients with identical hemoglobin genotypes. These variations are in part due to the modulating effects of coexisting modifier genes and a number of social and environmental factors [5,10]. Thus, many features of SCD, including frequency of vaso-occlusive crisis, rate of hemolysis, occurrence and severity of complications cannot be entirely explained by the polymerization [2–9]. Higher levels of fetal hemoglobin, association with other hemoglobins, coinheritance with α -thalassemia,

and certain genetic factors can potentially result in a milder disease. Inheritance of genetic factors causing thrombophilia may enhance the hypercoagulable state and increase some complications of the SCD.

During the first several months of life, infants with SCD can be asymptomatic, however, in the long term, as a rule, the disease is fraught with complications throughout life, which worsens with age. Reoccurring pain, mainly in bones and joints, due to the intermittent vaso-occlusive crises, is common. Occurrence of this complication, however, can be extremely variable with some individuals having as many as 6 or more episodes annually, while others may have less frequent pain crises or not experience this symptom. Manifestation of the SCD can virtually involve all organs and systems [10–18]. Homozygote sickle cell disease is associated with anemia, vaso-occlusion and hemolysis, heightened by inflammatory response, hypercoagulability, defective arginine metabolism, and oxidative stress. The disease results in a number of major events, most, but not all of which originate from a coherent pathophysiological scheme based on the abnormal polymerization of deoxy-hemoglobin S. While polymerization results in erythrocyte injury and sickling, multiple other factors are also involved [19–84]. These in part include dysfunction of the vascular endothelium causing a pro-inflammatory environment, circulating activated endothelial cells, and an inflammatory response resulting in the activation of adhesion receptors. Signaling pathways in the erythrocytes can be modulated by hypoxia, stress, and inflammatory response which can influence activation of the adhesion receptors [6,19–24]. Clonal hematopoiesis (CH) can potentially increase risk of vascular occlusive events and development of leukemia [25]. Physiologic abnormalities in this disease can modify the incidence and distribution of genetic alterations in CH [25].

Hemolysis results in the release of free hemoglobin in the plasma which acts as a scavenger of nitric oxide [26,27]. This represents an intrinsic mechanism for inducing vascular disease, a common complication of sickle cell disorders [26,27]. With arginase-1 activity, which is necessary for production of nitric oxide (NO), being lower in the sickle cells than normal erythrocytes, de novo nitric oxide cannot be made. Repeated polymerization and depolymerization of hemoglobin can cause oxidative stress which has a role in the pathophysiology of hemolysis, vascular occlusion, and the resulting organ damage [26–30]. Hemolysis can also lead to a number of complications which are attributed to vascular NO depletion. This is as a result of direct scavenging reactions due to cell-free plasma hemoglobin, limiting nitric oxide bioavailability and defective generation of this agent which is related to enzymatic consumption of arginine by erythrocyte arginase [29,30]. Hemolysis can lead to the formation of reactive oxygen species by reactions involving free hemoglobin.

Normally, there is a significant microRNA expression in the human mature erythrocytes. This is significantly altered in the erythrocytes of patients with sickle cell disease and is associated with their defective downregulation during their terminal differentiation [31]. Other influencing factors include elevated polymorphonuclear count along with increased adhesiveness, plasma thrombospondin-1 (TSP-1) secreted by activated platelets complex, cellular interactions, and global inflammation-mediated cell activation [32]. In addition, erythrocytes in SCD are shown to be capable of pathologically interacting with leukocytes, especially with polymorphonuclear cells. Leukocytes, including neutrophils, monocytes, invariant natural killer T (iNKT) cells, and platelets are activated and participated in the process of vascular occlusion [84]. These, along with the adhesion of sickled cells to the vascular endothelium and fibrin deposition can cause microvascular occlusion. Abundance of the adherent leukocytes in the post-capillary venules may point to their participation in the vaso-occlusive crisis (VOC), and their volume may add to the circulatory slow down and the process of obstruction [84]. Fragility of the erythrocytes result in the hemolytic anemia. The high degree of clinical heterogeneity among patients with identical hemoglobin genotypes and variation in the rate of hemolysis further points to the complexity and multiplicity of factors in the process of VOC and hemolysis in patients with sickle cell disease [33].

Patients with SCD at 'steady-state' have chronic activation of coagulation compared to the healthy individuals with normal hemoglobin [34–41]. These patients often have enhanced production and increased plasma levels of the markers of thrombin and fibrin generation, including prothrombin fragment 1.2, fibrinopeptide A, thrombin-antithrombin complexes (TAT), D-dimers, and plasmin-antiplasmin complexes (PAP) [34–41]. While reports regarding the relation of an increase in the levels of coagulation activation markers, and frequency of painful crisis and their elevation during these episodes, as compared to the steady-state, are conflicting, a significant correlation between D-dimer levels during the time without any acute events and the frequency of pain crises in the following year have been reported [42]. The formation of polymer fibers in SCD triggers a cascade of seemingly unrelated events. Activation of some ion channels such as the K-Cl co-transport system and the Ca-dependent K-channel (Gardos channel) results in the loss of potassium and cellular dehydration [33]. This in turn leads to an increased intracellular hemoglobin concentration which can further trigger deoxy-HbS polymerization. These changes result in denatured hemoglobin and hemichromes accumulation at the internal side of the cell wall, next to the cytoskeleton protein, including band 3 of the membrane. These events, along with the loss of heme and the liberation of Fe^{3+} , create an oxidizing microenvironment. Disruption of the asymmetry of membrane phospholipids leads to exposure of anionic phosphatidylserine at the cell surface. Aggregation of the anti-band 3 IgGs on the protein band 3 induces erythrophagocytosis by macrophages. These destructive membrane changes result in the production of microparticles [43,44]. Other physiological changes, cellular interactions or modifying genes may also be involved. These include coexisting hypercoagulable states or coinheritance of genetic variants which increase thrombophilia. Certainly, intervening environmental factors, including climate, air quality, behavioral, and psychosocial factors have a role in the manifestation of the disease. Infections are another common event complicating sickle cell disease and can be a significant cause of morbidity and mortality in these patients [45].

Oxidative stress is one of the key factors in the pathophysiology of SCD. This disorder is known to be associated with prooxidant enzyme activation, the release of hemoglobin and heme due to the hemolysis, erythrocyte autooxidation, and decreased nitric oxide bioavailability [27,28]. These events result in oxidative stress and a proinflammatory state and various vasculopathy events. Normal pathways to reduce oxidative stress such as nonenzymatic and enzymatic antioxidant mechanisms are defective in SCD. Superoxide dismutase (SOD), a part of the enzymatic antioxidant mechanism, and its second isoform (SOD2) are located in the mitochondrial matrix [46]. The role of a variant of SOD2 (SOD2^{V16A}) on the mitochondrial function and vascular dysfunction in sickle cell disease has been explored [46]. The mitochondrial complex IV inhibition and higher reactive oxygen species (ROS) production has been detected. Prior investigations indicate the presence of the above variant reduces pulmonary and cardiovascular functions, increases anemia and hemolysis, and decreases the ability to exercise and participate in physical activities. Various antioxidant therapies such as l-glutamine, l-arginine, and N-acetylcysteine supplementations have been tried in SCD in order to reduce oxidative stress. These therapies are expected to have a greater effect in patients with the SOD2^{V16A} variant [45,46].

In a 2005 report, the median life expectancy for women and men with sickle cell disease in the United States (US) was approximately 42 and 38 years, respectively [49]. During the last 60 years, the survival rate of patients with sickle cell disease in high-income countries has steadily improved [48,49]. New treatments and better means of prevention and strategies for genetic and nongenetic therapies are needed to improve quality of life and reduce death in patients with sickle cell disease in the future. This review addresses the etiology and epidemiology of sickle cell disease, its course, major complications, treatment, and future research in this disease.

2. Genetic Background

Sickle cell disease and its variants are genetic disorders resulting from a single point mutation. The disease is the most common severe monogenic disorders in the world. The prevalent severe form of SCD in North America is homozygous Hemoglobin S disease (HbSS) which is an autosomal recessive disorder. Hemoglobin S results from a single base-pair mutation in the gene for the beta-globin chain of adult hemoglobin (HbA). Sickle hemoglobin (HbS) allele, β^S , is a hemoglobin subunit β allele, where adenine-thymine substitution in the sixth codon of beta-globin has resulted in the replacement of glutamic acid with valine at position 6 in the β -globin chain. Alteration of structure due to this single nucleotide substitution leads to the production of the sickle HbS allele β^S , the mutant protein generated from the β^S allele is the sickle β -globin subunit. Upon deoxygenation, hemoglobin tetramers, which include two of the mutant sickle β -globin subunits, can polymerize. This polymerization, and its repeat, results in erythrocytes assuming a sickled shape. These changes lead to erythrocyte rigidity, increased adhesion and vaso-occlusion, causing chronic anemia, hemolysis, and vasculopathy. The severity of the disease can vary based on a number of variables such as coinheritance of various genetic modifiers. These include those which result in higher levels of fetal hemoglobin, α -thalassemia, inherited coagulation factors, ancestry, nutrition, psychosocial environment, degree of care, and socioeconomic factors [50–55]. Over 90% of children with SCD are born in sub-Saharan African countries and India. While the disease is most prevalent in Africa, it has a worldwide distribution and internationally constitutes a major health concern [56–66].

3. Genetics and Origin

The manner of inheritance of sickle cell disease is that of Mendelian autosomal codominant trait with homozygous individuals for β^S allele having the disorder [1]. As noted before, sickle cell disease is caused by a mutation in the hemoglobin subunit beta gene located on the short arm of chromosome 11. This involves a single base change mutation (GAT→GTT) in the sixth codon of exon-1 of the beta-globin gene. Such a mutation results in the replacement of normal glutamic acid with valine ($\beta^6\text{Glu}\rightarrow\text{Val}$). Valine is nonpolar, and unlike glutamic acid which has a negative charge, is hydrophobic. Replacement of the hydrophilic glutamic acid with the hydrophobic valine residue is the basis for sickle cell formation. In a deoxygenated state, hemoglobins develop a hydrophobic pocket (notch) on the surface of the protein. The mutated sickle cell hemoglobin, however, also has the hydrophobic valine protruding from its surface. The exposed hydrophobic area of one hemoglobin S molecule interacts with the mutated region containing the valine in another HbS molecule. During this interaction, mutated HbS molecules have a tendency to attach together, polymerize, and form stiff fibers. These fibers cause the erythrocytes to develop the sickle form which is characteristic of the disease.

Studies of haplotypes linked to the sickle cell gene in Africa have provided evidence for three origins of the mutation in that region, i.e., Benin, Senegal, and the Central African Republic [67,68]. Structural analysis of the 5' flanking region of the beta-globin gene in African sickle cell anemia also points to three origins for the sickle cell mutation in Africa [67,68]. The data has resulted in a proposal for an evolutionary scheme for the polymorphisms in the 5' flanking region of the beta-globin gene, supporting the hypothesis that there are three origins for the sickle mutation. A separate and fourth occurrence of the sickle cell mutation is believed to have occurred in the Arabian Gulf and India and is designated the Arab-Indian or Asian haplotype [69]. Sickle cell gene is prevalent in the Deccan plateau of central India with a lesser affected population in the north of Kerala and Tamil Nadu. Occurrence of the disease in the region varies significantly with frequency of the heterozygotes ranging from 1–40 per cent [70–72]. The Asian haplotype is generally milder than those seen in Africa. Although various maps of β^S in India have been previously published, a model-based national map is currently lacking [73]. Severe and moderate forms of HbS/ β -thalassemia are prevalent in the eastern Mediterranean region and parts of India, while mild forms are more common in individuals with African ancestry.

The most severe SCD genotypes have two β^S alleles (β^S/β^S), however, there are other combinations of SCD genotypes (Table 1). These combinations include HbSC genotype with one β^S allele and one hemoglobin subunit β (HBB) allele with Glu6Lys substitution or β^C allele that generate hemoglobin C. The latter, as a rule, results in a milder form of the disease with higher hemoglobin and less hemolysis, and fewer complications, except retinopathy and osteonecrosis. The β^S allele combined with a null *HBB* allele (Hb β^0) and no protein translation results in HbS β^0 -thalassemia. Microcytosis is one of the features of this disorder. The β^S allele combined with a hypomorphic *HBB* allele (Hb β^+ with a decreased amount of normal β -globin protein) results in HbS β^+ -thalassemia disorder, which is generally milder than sickle cell disease. Less frequent compound heterozygous SCD genotypes include HbS in combination with HbD, HbE, HbO^{Arab} or Hb Lepore.

Table 1. Various hemoglobin concentrations in major Sickle Cell genotypes.

Genotype	Hb (g/dL)	% Hemoglobins A, A2, and F	%Hb S
Hb SS	~6–9	Hb A 0% Hb A2 < 3.5% Hb F < 10%	>90%
Hb S β^0 thal	~7–9	Hb A2 > 3.5% Hb F < 20%	>80%
Hb S β^+ thal	~9–12	Hb A 10–30% Hb A2 > 3.5% Hb F < 20%	>60%
Hb SC	~9–14	Hb C~45% Hb A2 < 3.5% HbF \leq 1.0	50%

Abbreviations: Hb (g/dL), Hemoglobin (gram/deciliter); Thal, Thalassemia; Hb, Hemoglobin.

4. Epidemiology, Distribution, Incidence, and Prevalence

Sickle cell disease and its variants constitute the most common inherited human blood disorders affecting millions of individuals worldwide [2,56]. While statistical reports vary [3,4,75,76], globally, approximately 4.4 million individuals have sickle cell disease [75,76]. Worldwide, each year, approximately 300,000 infants are born with this disorder [60,61], including an estimated 3000 in the United States, where it is estimated that 80,000 to over 100,000 individuals have this disease [57]. According to the Centers for Disease Control and Prevention (CDC), one in 365 Black and one in 13,600 Hispanic Americans have SCD [57]. The disease is most prevalent in Sub-Saharan Africa with approximately 75–80% of cases occurring in individuals with a background from that region [2,58–61]. Sickle cell disease is also prevalent in parts of India, the Middle East, Eastern Mediterranean, Arabian Peninsula, Sicily, Greece, southern Turkey, and in individuals with African ancestry living in North and South America and other parts of the world [77–80]. It is believed that due to the “protective effect” and the survival advantage of individuals heterozygote for sickle cell, the disease is prevalent in areas where malaria is endemic [62–64,77]. It is postulated that compared to the survival of noncarriers, carriers of the recessive SCD gene have a survival advantage if affected by malaria and are more likely to reach reproductive age [62–64]. Due to this fact, prevalence of SCD in Africa, where malaria is a major life-threatening disease, is high [62–64]. Migration of West and Central Africans within Africa has led to the introduction of SCD to South Africa [65]. While SCD has a worldwide distribution, in some countries, it constitutes a rare disorder, resulting in diagnostic and management problems. It is worthwhile to note that in 2006, the World Health Organization (WHO) recognized SCD as a public health priority [66].

5. Pathophysiology

As previously noted, hemoglobin S forms polymers under deoxygenation conditions and has marked decreased solubility and molecular stability. Upon deoxygenation, hemoglobin S forms “tactoids”, a gel-like substance containing hemoglobin crystals. A number of other factors including concentration of Hb S, and the presence of other hemoglobins, have the potential to influence in this phenomenon. The sickling process results in gradual development of a parallel array of filaments called polymer formation. As a result of changes in the hemoglobin structure, ultimately, with recurrent episodes of sickling and membrane damage, erythrocytes can assume the characteristic biconcave sickled shape with rigid membranes. In addition to change in shape, sickling results in loss of potassium, gain in intracellular sodium and calcium in the sickled erythrocytes. The latter is, at least partially, due to the membrane permeability to calcium, in part due to the impairment in the Ca^{++} pump that depends on adenosine triphosphatase (ATPase). Oxygen affinity of hemoglobin S is much less than that of the normal hemoglobin (Hemoglobin A). This affinity is further reduced by the high levels of 2,3-diphosphoglycerate (2,3-DPG), which is a glycolytic intermediate, and is known to be present in sickle cell erythrocytes and to interact with deoxygenated β -globin subunits, reducing the oxygen affinity of the hemoglobin. Reduced hemoglobin and low oxygen affinity further exacerbate hemoglobin S polymerization and sickle cell formation, and ultimately results in painful crises [80].

Mechanisms leading to sickling process are diverse and complicated. Kinetics of polymerization depend on erythrocyte dehydration causing increased hemoglobin concentration within the erythrocytes. As noted above, ion transport pathways including K-Cl cotransport and $\text{Ca}(2+)$ -activated K^+ channel play an important role in the process of sickle cell erythrocyte dehydration. Normally, Gardos channel can prevent erythrocyte dehydration. In SCD, the Gardos channel, alone or in conjunction with K-Cl cotransport, plays a major role in cell dehydration. The transport pathway regulating volume in reticulocytes, the potassium-chloride cotransporter (KCC), does not function normally in SCD, resulting in hemoglobin concentration, causing the reticulocyte to sickle. In a fraction of sickle erythrocytes, polymerization caused by deoxygenation of Hb S activates a nonselective cation leak pathway. Calcium entry by this sickling-induced pathway results in activation of the Gardos channel. This mediates rapid KCl and water loss. In the sickle reticulocytes, abnormal KCC activity can facilitate a cascade of events in which sickling and Gardos channel activation reinforce each other to dehydrate the erythrocytes.

The pathophysiology of the SCD is complex and multi-systemic. This, as noted previously, starts with polymerization of HbS under low oxygen tension, changing the shape, structure and function of the erythrocytes, and reducing their lifespan. Sickled erythrocytes are highly adhesive and along with other events which occur in SCD, cause occlusion of blood vessels in nearly every organ and chronic hemolytic anemia, the hallmarks of the disease. These, along with a cascade of other pro-inflammatory events set off a number of pathophysiological factors which, among others, involve neutrophils, platelets, and vascular endothelium. The sickle red blood cells interact with the vascular endothelium and trigger activation of neutrophils, monocytes, and platelets. Neutrophils interact with erythrocytes and endothelium upregulating expression of cytoadhering molecules such as P- and E-selectins. Activated platelets form aggregates with erythrocytes, neutrophils, and monocytes. In addition, hemolysis and release of cell-free hemoglobin on a continuous basis, depletes hemopexin and haptoglobin, resulting in reduced bioavailability of nitric oxide (NO) and vascular endothelial dysfunction. These, along with other events, terminate in chronic organ damage. Energy metabolism in erythrocytes is based on oxygen responsive variations in flux through the Embden Meyerhof (EMP) or the hexose monophosphate (HMP) pathways. Thus, the generation of ATP, NADH, and 2,3-DPG (EMP) or NADPH (HMP) shifts with the oxygen content of erythrocytes [81]. This is due to the constant competition between deoxyhemoglobin and key EMP enzymes for binding to the cytoplasmic domain of the Band 3 membrane protein (cdB3). Band 3 and protein 4.2 are the major components of the ankyrin complex. These link their associated proteins and the

lipid bilayer membrane to the spectrin cytoskeleton through ankyrin. It is known that enzyme inactivation by cdb3 sequestration in oxygenated erythrocytes favors HMP flux and NADPH generation. It has been postulated that sickle hemoglobin disrupts cdb3-based regulatory protein complex assembly, thus creating susceptibility to oxidative stress in erythrocytes of patients with sickle cell disease. In this disorder, it has been shown that there is constrained HMP flux, NADPH, and glutathione recycling, and reduced resilience to oxidative stress manifested by membrane protein oxidation and membrane fragility [81].

As noted previously, inflammatory events, common in sickle cell disease, trigger a series of events involving neutrophils, platelets, and vascular endothelium. Hemolysis and release of hemoglobin depletes hemopexin and haptoglobin, resulting in the decreased bioavailability of nitric oxide and dysfunction of vascular endothelium with consequent effects that underly the chronic organ damage.

Sickle cell disease is a complex genetic blood disorder that affects multiple organs and systems. Changes which result from hemoglobin S polymerization, alteration of blood flow due to the increased erythrocyte tendency for adhesion, which result in vaso-occlusion, endothelial dysfunction related to hemolysis, and iron-regulated gene expression associated with upregulation of inflammasome pathway gene expression, are major factors involved. The latter results in a 200-fold increase in Toll-like receptor 4 (TLR4) expression in the peripheral blood mononuclear cells. TLR4 is expressed on monocytes and macrophages and its ligand, lipopolysaccharide (LPS), promotes expression of the pro-inflammatory cytokine interleukin 6 (IL-6). It is hypothesized that intracellular iron is involved in this process [82–84]. A combination of these events and other processes promotes vaso-occlusion resulting in pain, end organ injuries, and failure in sickle cell disease. It is shown that high levels of proinflammatory cytokines IL-6 and interleukin 8 (IL-8) are associated with a poor clinical outcome in sickle cell disease [85].

Passage of erythrocytes in tissues with high oxygen demand results in the deoxygenation of hemoglobin S tetramers and their binding, resulting in polymerization. This is a major starting event in pathophysiology of SCD. Deoxygenation of hemoglobin S and production of long polymer fibers lead to deformity of erythrocytes resulting in sickled shape, rigidity, and premature hemolysis. Furthermore, cellular dehydration contributes to this phenomenon. The level of polymerization is proportional to that of the erythrocyte hemoglobin S concentration and has an inverse correlation with hemoglobin F content. Coinheritance of hereditary persistence of HbF or α -thalassemia or β^C -allele alongside β^S modulate these events [86,87]. High plasma levels of asymmetric dimethylarginine (ADMA) have been detected in children with SCD and appear to correlate with elevated tricuspid regurgitation, pulmonary hypertension, and sickle cell retinopathy [88,89].

The flow properties of blood or biorheology are altered by the level of hematocrit, viscosity of plasma and deformability of erythrocytes. As a rule, the level of hematocrit in sickle cell disease is reduced. Increased plasma viscosity, due to the chronic hemolysis and sickled-shape erythrocytes, impaired flow of blood in the microcirculation of high oxygen-demanding tissues can obstruct red cells passage through capillaries and postcapillary vessels in sickle cell disease. Furthermore, stiff and poorly deformable sickle erythrocytes can result in the production of damaged red cell membranes, promote exposure of adhesion molecules and an abnormal binding process. Adhesion molecules such as phosphatidyl serine (PS), basal cell adhesion molecule-1/Lutheran (B-CAM-1cfl/Lu), integrin-associated protein (IAP), and intercellular-adhesion-molecule-4 (ICAM-4), which interact with the inflammatory and endothelial cells, promote vaso-occlusion and can be involved in the process [83,90,91]. Reticulocytes are known to have disproportionately increased levels of adhesion molecules such as $\alpha 4\beta 1$ integrin (VLA-4) and CD36, as compared to the mature erythrocytes. Due to anemia, patients with sickle cell disease have increased reticulocytes which promotes the process of vaso-occlusion [83,90,91].

6. Disease Manifestations

Individuals with sickle cell disease, homozygous for the β^S , are most likely to have characteristic common symptoms and complications of the disease. During early infancy, sickle cell disease is basically asymptomatic. Manifestations of the disease begin with a decline in the fetal hemoglobin. Later in life, a number of intervening factors can alter the symptomatology of sickle cell disease. Some of the common manifestations of the disease include hemolytic anemia and chronic low-level pain, mainly in bones and joints. Intermittent vaso-occlusive crises are common. This disorder is also associated with several complications including hand-foot syndrome, acute chest syndrome, splenic sequestration, loss of vision, growth retardation, leg ulcers, deep vein thrombosis, infections, damage to various tissues and organs including liver and bones [10–16]. Disorders of the heart, kidneys, liver, gallstones, priapism, and most importantly, stroke and other central nervous system (CNS) complications are common [18]. Approximately 10 percent of children with SCD can have asymptomatic stroke [18]. Ischemic stroke, sinovenous thrombosis, posterior leukoencephalopathy, and acute demyelination can result in additional complications including seizures, learning problems, physical disabilities, and coma. Painful crisis due to vaso-occlusion and bone infarction frequently occurs [92–103]. While acute pain crisis is most often managed at home, it is the common reason for patients with this disease to seek medical attention and constitutes the most frequent cause for emergency room visits and hospitalization [97,104–110]. Dactylitis or bony infarction of digits, resulting in pain and swelling of fingers or toes, are often seen in infants. Recurrent vaso-occlusions and infarctions result in avascular necrosis of bones and articular surfaces, especially heads of long bones [10,13,14]. Osteopenia and osteoporosis are frequent findings in sickle cell disease and can cause vertebral collapse and chronic back pain [13]. Increased rates of bone infarction have been reported to be influenced by the higher levels of hematocrit and the concomitant presence of α -thalassemia trait [11]. The latter, however, is based on a small number of patients and not statistically significant data [11].

The most common symptoms and morbidities associated with SCD include pain crises, acute chest syndrome, cerebrovascular accidents, and splenic and renal dysfunction.

6.1. Pain Crises

Acute pain episodes are one of the principal symptoms of sickle cell disease [92–102]. In a study of children and adolescents with SCD which required keeping a daily diary, pain was reported on 2592 days of 18,377 days on the study [94]. In an unrelated investigation by the Cooperative Study of Sickle Cell Disease (CSSCD) of 3578 patients with various types of sickle cell disorders, there were 12,290 episodes of pain in 18,356 patient-years [92]. This averaged 0.8 episode per patient-year in the homozygous SCD, 1.0 episode per patient-year in sickle β^0 -thalassemia, and 0.4 episode per patient-year in hemoglobin SC disease and sickle β^+ -thalassemia. Alpha thalassemia had no effect on pain, except for its association with an increased hematocrit. While the most severe SCD genotypes have two β^S alleles (β^S/β^S), there are other combination SCD genotypes. These combinations include HbSC genotype with one β^S allele and one hemoglobin subunit β (*HBB*) allele with Glu6Lys substitution or β^C allele that generates hemoglobin C, which, as a rule, results in a milder form of the disease with higher hemoglobin and less hemolysis and fewer complications, except retinopathy and osteonecrosis [93].

The β^S allele combined with a null *HBB* allele ($Hb\beta^0$) and no protein translation results in $HbS\beta^0$ -thalassemia. Microcytosis is one of the features of this disorder. The β^S allele combined with a hypomorphic *HBB* allele ($Hb\beta^+$; with a decreased amount of normal β -globin protein) results in $HbS\beta^+$ -thalassemia disorder which is generally a milder disease than SC. Less frequent compound heterozygous SCD genotypes include HbS in combination with HbD, HbE, HbO^{Arab} or Hb Lepore.

It is of interest that the frequency of pain crisis is variable. As noted before, some individuals may have as many as six or more vaso-occlusive episodes annually, while others have less frequent pain crisis or do not experience this complication. In one study,

39 percent of patients with sickle cell disease had no episodes of pain, and 1 percent had more than six episodes per year [92]. Furthermore, 32.9 percent of all episodes in study patients occurred in 5.2 percent of patients with 3 to 10 episodes per year. In the above study, frequency of pain crisis was found to have a correlation with the survival of patients over age 20, with those who had high rates of pain episodes being more likely to die earlier than those with low rates. This is collaborated with other studies [48]. There is a significant interindividual variability in the type and combination of pain with some individuals having acute episodes of pain accompanied by hemolysis and other manifestations of active SCD [95,96]. The use of analgesics also varies among patients. In a multicenter study of hydroxyurea in SCD, at-home analgesics were used for pain during 40% of diary days and on 80% of two-week follow-ups. Oxycodone and codeine were used most frequently for the pain relief [97].

Pain in SCD has generally been divided into four groups, i.e., painful vaso-occlusive crises, neuropathic pain, chronic pain with or without an identifiable cause, and chronic pain without obvious pathology [96]. Painful vaso-occlusive crisis is defined as a sudden onset of severe and continuous pain. The pain can be confined to a certain area or be associated with other complications such as acute chest syndrome. Pathophysiology of VOC is complex and can be due to hemoglobin S polymerization, alteration of blood flow, increased erythrocyte tendency for adhesion, endothelial dysfunction, upregulation of inflammasome pathway, and other factors. Tissue injuries result in the release of inflammatory mediators, macrophages, mast cells, and platelets, which activate the peripheral afferent nerves and lead to nociceptive pain. Generally, the frequency of VOC resulting in hospitalizations is increased with increasing age, higher hematocrit, and lower fetal hemoglobin [92,102]. As described before, in infants, dactylitis and bone infarctions of finger along with pain is seen. Recurrence of infarctions in bones over time result in avascular necrosis [13,14,99,103]. Frequency of VOC and duration of pain-free periods between consecutive episodes vary but, as a rule, become shorter with increasing age [94,111]. Chronic pain in SCD generally is not that of continuation of the vaso-occlusion, but often secondary to avascular necrosis of the bones involved. While acute pain crisis is usually managed at home, it is the most common reason for patients to seek medical attention and constitutes the most frequent cause for emergency room visits and hospitalization.

Pain associated with cholelithiasis, and its complications are common in sickle cell disease. Most gallstones are produced from excess bilirubin, can be a source of secondary infection, and may require cholecystectomy.

When contrast imaging studies are needed, hyperosmolar contrast media can potentially induce erythrocyte dehydration, polymerization, and sickling resulting in sickle crisis. Therefore, low osmolar or isotonic contrast media should be used in SCD patients. Additionally, radiologic contrast should be avoided in patients with renal failure.

6.2. Acute Chest Syndrome

Acute chest syndrome (ACS) is a frequent complication of sickle cell disease with a frequency second only to the painful crisis [112–125]. This syndrome is defined as development of a new radiodensity on chest radiograph accompanied by fever and/or respiratory symptoms. It may be associated with pain crisis. Acute chest syndrome has the risk of respiratory failure, the development of chronic lung disease, and death in patients with sickle cell disease [112]. ACS accounts for the second most common reason for hospitalizations of patients with SCD. Recurrent episodes of acute chest syndrome and hypoxemia may lead to debilitating chronic pulmonary disease, chronic disability and death in these patients [48,113–117]. The mortality due to this syndrome is age dependent and much higher in adults than children, amounting to <1% in those less than 9 years of age to 2% for ages 10–19 and 9% for adults [124].

The pathogenesis of acute chest syndrome is not entirely clear. Pulmonary vascular occlusion can be a cause. Interaction of sickled erythrocyte with dysfunctional pulmonary microvasculature, increased expression of vascular adhesion molecules, activation of coag-

ulation factors, retention of sickled erythrocytes in the pulmonary circulation, activation of the transcription factor nuclear factor-kappa B (NF- κ B), upregulation of the expression of the adhesion molecule VCAM-1 and its effects on endothelium, as well as a number of other factors for pulmonary complications in SCD have been suggested [113–118]. Among other factors involved are pulmonary embolism, opiate narcosis, fluid overload, and hypoventilation. Infections can also have a role in the development of acute chest syndrome [119]. Vaso-occlusive crisis and acute chest syndrome due to coronavirus disease (COVID-19) have been reported [119].

Fever, cough, chest pain, dyspnea, tachypnea, tachycardia, wheezing, intercostal recession, nasal flaring, skeletal pain, hypoxia, and hemoptysis are main symptoms at presentation. The disease can manifest as a combination of fever, chest pain, symptoms of pulmonary disease, cough, dyspnea, pleuritic chest pain, and pleural effusion [120].

Laboratory investigations should include radiography, high-resolution computerized tomography of the chest, complete blood count, biochemistry, blood and nasal culture, bacterial and viral studies, blood gas measurements, and bronchoalveolar lavage, if needed. Leukocytosis, thrombocytopenia, and increased anemia can be seen. Radiological findings may include multiple lobe infiltrates and pleural effusion. Recurrent infiltrates are common. Duration of the clinical illness varies significantly and clearing of the radiological infiltrates may take several days to weeks [121–124]. If symptoms of acute chest syndrome exist, absence of abnormal findings in a chest X-ray does not necessarily exclude the diagnosis of ACS. Severe hypoxia can be a predictor of severity of the disease and outcome. It is important to monitor the patients for hypoxia, increased respiratory rate, reduced platelet count and hemoglobin levels, progress of the pulmonary involvement, and the development of any possible neurological complications. Early recognition and prevention of the progression to acute respiratory failure is essential [125].

Treatment of ACS is by and large those of the symptoms and related complications, with the aim of prevention or reversal of the acute respiratory failure. The treatments often include administration of fluids and electrolytes, oxygen, antibiotics, regular or exchange blood transfusion to reduce HbS levels, and respiratory therapy and ventilation, if necessary. Some recommend administration of antibiotic coverage for atypical respiratory organisms such as *Mycoplasma* and *Chlamydia* [125–127]. If the H1N1 subtype of the Influenza A virus is found, it must be aggressively treated.

6.3. Cardiac Complication

Cardiac complications are common in SCD and can cause significant morbidity and mortality. Chronic anemia results in increased cardiac output, but only a minimal elevation of heart rate. Anemia causes an increased left ventricular stroke volume with significant dilation of the left ventricle, and eventually, development of eccentric hypertrophy and myofibers resulting in an increased left ventricular mass and elongation, as well as diastolic dysfunction [128–134]. Pulmonary hypertension and right ventricular dysfunction have also been reported [135].

6.4. Genitourinary Complications

Patients with sickle cell disease may have a wide variety of renal dysfunctions [136–141]. Relative hypoxia in the renal medulla and decreased blood flow in the vasa recta can result in sickle formation of the erythrocytes causing veno-occlusion and infarction. The manifestations of sickle cell nephropathy can include impairment of urinary concentrating ability, some degree of hypophosphatemia and increased creatinine clearance, and impairment of urinary acidification and potassium excretion. Hematuria, proteinuria, tubular disturbances, and chronic kidney disease are common in sickle cell disease patients. Painless hematuria is often seen and is usually benign. Tubular functional defects and proteinuria may be signs of the development of chronic sickle cell nephropathy.

Priapism can be a very painful and serious complication of sickle cell disease [142–144]. The probability of having at least one episode of priapism by age 20 can be 89%. While conser-

vative therapy is often sufficient, treatment with hydroxyurea, etilefrine, pseudoephedrine, leuprolide, sildenafil, and other agents are deemed to be helpful. The role of other treatments such as nitric oxide is uncertain. Possible use of polyethylene glycol-modified ADA (PEG-ADA) enzyme therapy and A(2B)R antagonists as a future novel treatment for priapism have been suggested. If an episode, despite hydration and analgesic therapy persists, intracavernosal aspiration and instillation of an α -agonist and surgery may be considered.

6.5. Hepatobiliary Complications

Hepatobiliary system is commonly affected in SCD. The “sickle cell hepatopathy” can have a broad spectrum of manifestation ranging from benign hyperbilirubinemia to liver failure. Hyperbilirubinemia due to sickling process can lead to ischemia, sequestration and cholestasis. Cholelithiasis is common in sickle cell disease affecting 15% of children with this disorder before 10 years of age and 80% of patients over age 30 [145,146]. While the best option for asymptomatic cholelithiasis is not clear and should be decided on an individual patient’s basis, laparoscopic cholecystectomy appears to be the treatment of choice in patients with clinically symptomatic disease [145,146].

6.6. Infections

There is a high incidence of bacterial infections including pneumonia, urinary tract infection, osteomyelitis, meningitis, and septicemia in sickle cell disease. Osteomyelitis and septic arthritis, often due to *Salmonella*, *Staphylococcus aureus*, and Gram-negative enteric bacilli, are relatively common [16]. The pathological basis for susceptibility to infections in these patients is complex. Defective splenic function, opsonization, complement pathway, antibody production, leucocyte function, and cell-mediated immunity are among more common reasons for an increased rate of infections [147,148].

6.7. Neurological Complications

The neurological complications of sickle cell disease include silent cerebral infarcts (39% by age 18 years), headache (both acute and chronic: 36% in children), ischemic strokes (9 to ~11% in children with sickle cell anemia (SCA) without screening), hemorrhagic stroke in children and adults with SCA (3% and 10%, respectively), intracerebral hemorrhage, including subarachnoid hemorrhage (SAH), and cerebral infarction [149–153]. Aneurysms are not uncommon in these patients.

Sickle cell disease is associated with a high risk of stroke [149–153]. This is the case, especially in children, where it confers a risk of stroke more than any other disease. Silent cerebral infarcts producing no symptoms are common. However, while asymptomatic, these can be progressive and can result in neurocognitive impairments and reduced academic performance. The risk of first stroke is highest during the first decade of life at 1.02% per year between ages 2 and 5 years [153]. By age 20, approximately 11% and by age 45, approximately 24% of all patients with SCD can develop an overt stroke. A second stroke can develop in 75% of children with a history of first stroke within the first 2–3 years of the initial event [154]. The risk of stroke can be determined by using transcranial Doppler ultrasound and measurement of blood velocities in the middle cerebral and internal carotid arteries. The occurrence of the first stroke in children can be reduced by 70% by transfusion and keeping HbS concentrations at <30% [155]. Discontinuation of transfusion therapy, or its substitution, has been shown to result in further strokes [156]. Regular blood transfusions can reduce the incidence of silent cerebral infarctions and their consequences [152–158].

6.8. Splenic Sequestration

Splenic sequestration is a result of blood pooling, and in life-threatening instances, it can be associated with severe anemia and hypovolemic shock. Individuals with sickle cell disease become susceptible to bacterial infections due to functional asplenia and disordered humoral immunity.

7. Screening, Diagnosis, and Prevention

Sickle cell disease can be prevented prenatally, diagnosed in utero or in the newborn period by screening, or be detected at any time during life [159–164]. For the prenatal management, pre-embryonic diagnosis of sickle cell disease can be done by testing oocytes for the maternal sickle cell allele by PCR analysis of the first and second polar body, and selection of mutation-free oocytes detected by polar body analysis [165].

Preimplantation diagnosis of sickle cell disease in 8-cell embryos of heterozygous parents can be performed, and only those without sickle cell are selected. Discarding embryos that are found to be abnormal during preimplantation genetic diagnosis may be ethically unacceptable to some parents. In these cases, pre-embryonic diagnostic technique eliminates post-embryonic selection of a normal fetus in *in vitro* fertilization.

Fetal DNA is present in the plasma during pregnancy and can be used for noninvasive prenatal diagnosis. The detection of paternally inherited fetal mutations can be done in the maternal plasma. Advances in the single-molecule counting allows the mutation dosage of the fetus to be analyzed. With this technique, noninvasive prenatal diagnosis of several hemoglobinopathies is possible [163]. Prenatal diagnosis can be performed at 8–12 weeks of gestation to obtain DNA samples from chorionic villus (also called CVS). DNA also can be obtained by sampling amniotic fluid cells at 16 weeks' gestation. Extracted DNA is subjected to amplification refractory mutation system (ARMS-PCR) to detect sickle cell mutation (GAG→GTG) in the sixth codon of β globin gene. The laboratory procedures employed are fairly rapid and sensitive. Since traditional chronic villus biopsy, amniocentesis and cordocentesis are invasive, and can potentially be associated with a risk of fetal loss, isolation of fetal cells from maternal blood for DNA assay may be a preferred technique. The cell-free fetal DNA (cffDNA) in maternal plasma and serum provides an opportunity for noninvasive prenatal diagnosis (NIPD). The presence of paternally inherited alleles in maternal plasma of sickle cell disease may allow the diagnosis of the disorder or exclusion of autosomal recessive diseases in the fetus. Genotyping can be done using high-resolution melting (HRM) without utilizing labeled probes [166]. More complex regions can be analyzed with unlabeled hybridization probes.

Newborn screening at birth is used to detect sickle cell disease, prior to the development of any symptoms. This has been shown to be effective in reducing death. While two types of programs, *i.e.*, a selective program targeting high-risk parents and universal screening exist; the latter is preferable and generally, more cost-effective. Selective screening is used in certain areas of France [167], Spain [168], and India [169], where the targeted residents have a larger number of high-risk populations or are restricted to newborn babies whose parents both originate from SCD-endemic regions. Universal screening programs, predominantly using HPLC and isoelectric focusing with DNA analysis confirmation, have been in effect in the United States since 1975, starting with New York State in 1972. Several countries such as Brazil [170], England [171], Italy [172], and Germany [173] have also implemented systematic screening for sickle cell disease. In sub-Saharan Africa, generally, screening programs are on a small-scale or on pilot basis or are still lacking.

Currently, clinically utilized means for the diagnosis sickle cell disorders are based on the clinical picture of the disease, including chronic hemolytic anemia, vaso-occlusive crisis, and laboratory tests. Hemoglobin electrophoresis is used to confirm the diagnosis of homozygous sickle cell disease and to exclude other hemoglobin disorders such as hemoglobin S-beta+ thalassemia or hemoglobin SC disease. More detailed molecular genetic testing is also available.

7.1. Psychosocial Effects

Sickle cell disease and its complications have a very significant social and psychological effect on the patients and their families [174]. Management of the disease and its complications pose a challenge to patients, their families and medical care providers. Among the major problems causing psychological problems are coping with the symptoms of the disorder and its complications, restrictions in daily functioning imposed by the disease, anxiety and stress due to unforeseen events, and neurocognitive impairments. Effects of the disease and its treatments on the social functions, education, and occupation of the patients can be substantial. Attention to the effects of the psychological consequences of the disease, its complications, and treatment on the patients and their families is extremely important and needs attention. This requires a consistent management and involvement of the medical staff, social workers, and psychologists throughout the course of the disease, especially during hospitalizations. Frequent interruption of education during childhood and adolescence, and requirements for care can interrupt family dynamics and affect parents and siblings. The psychosocial effects of the disease, its complications and treatments, and interruption of a patient's activities and work can be considerable and need to be addressed. Psychological adaptation to SCD and its complications can be difficult. This depends on a number of factors, some of which include an individual's community, home, social and family environment, personality structure, and available resources. In communities where the patient belongs to a minority group, this can have a potential negative affect on a patient's adaptation to the disease. Educating patients about their disease, providing counseling, hope, encouragement, and participation in some group meetings and activities can potentially improve the patient's adaptation.

7.2. Cost of Care for SCD

Complications associated with sickle cell disease require frequent medical interventions resulting in significant inactivity, emotional, psychosocial, sleep, and educational disturbances which affects the quality of life for the patients and their families. These can cause a significant interruption of parents' activities and patient's work or school activities and loss of income. More complex future treatments likely will add to the cost of care [175]. The changes associated with SCD result in decreased life expectancy. While in recent years, the mortality rate for sickle cell disorders in the United States has decreased, still, it remains high with approximately 15,654 disease-related deaths per year. Complications of sickle cell disease result in significant utilization of medical resources and expenditures [176–178]. These require frequent emergency room visits and hospitalizations with high medical costs. For example, in the United States (US), for the year 2004, 113,000 patients with sickle cell disease were hospitalized with the cost of approximately USD 488 million. Available data dating back from 1989 through to 1993 reveals that in the US, an average of 75,000 hospitalizations related to SCD had occurred, costing approximately USD 475 million per year. Based on the US Centers for Disease Control and Prevention (CDC), during 2005, medical expenditures for children with SCD averaged USD 11,702 for those with Medicaid coverage and USD 14,772 for those with employer-sponsored insurance. About 40% of both groups had at least one hospitalization.

8. Bases of Treatment

Currently, in most cases, goals for the treatment of sickle cell disease include prevention and control of symptoms and complications (Table 2). Early intervention and treatment of the complications of sickle cell disease is essential. This includes obtaining timely and serial transcranial doppler ultrasounds for the prevention of stroke, detection and treatment of pulmonary hypertension, and detection and treatment of complications and damages to the various organs and systems which are associated with this disease. Preventive treatments with hydroxyurea, P-selectin inhibitors, e.g., crizanlizumab hemoglobin oxygen-affinity modulators, e.g., Voxelotor are commercially available [179–186]. Hydroxyurea increases total and fetal hemoglobin in SCD, thus reducing gelation and sickling of erythrocytes.

It also reduces the levels of circulating leukocytes, which decreases the adherence of neutrophils to the vascular endothelium [182]. Voxelotor is a hemoglobin S polymerization inhibitor designed to reversibly bind to hemoglobin in order to stabilize the oxygenated hemoglobin state [185–187].

Table 2. Treatment of sickle cell disease.

Prevention
<ul style="list-style-type: none"> • Prevention and treatment of infections • Prevention of the complications including various organ damages associated with the disease • Prevention of stroke • Early detection and treatment of pulmonary hypertension
Management
<ul style="list-style-type: none"> • Vaso-occlusive crisis • Chronic pain syndromes • Chronic hemolytic anemia
Transfusion
<ul style="list-style-type: none"> • Transfusion or exchange transfusion
Pharmacotherapy
<ul style="list-style-type: none"> • Vigorous hydration (plus analgesics): For vaso-occlusive crisis • Analgesics (aspirin, acetaminophen, ibuprofen, ketorolac) • Opioid analgesics (oxycodone, morphine sulfate, oxycodone/acetaminophen, fentanyl, nalbuphine, codeine, codeine, methadone) • Tricyclic antidepressants (amitriptyline) • Antibiotics (Prophylactic penicillin VK, cefuroxime, amoxicillin/clavulanate, ceftriaxone, azithromycin, cefaclor) • Vaccines (pneumococcal, meningococcal, influenza, COVID 19, and recommended childhood/adult vaccinations) • Endothelin-1 receptor antagonists (bosentan) • Phosphodiesterase inhibitors (sildenafil, tadalafil) • Vitamins particularly folic acid • L-glutamine • Ribonucleotide diphosphate reductase inhibitor (Hydroxyurea) • α-Globin reversible binding, Oxbryta (Voxelotor) • Poloxamer/Vepoloaxmer • Antiemetics (e.g., promethazine) • Other approaches to treatment of SCD
Modification of patient's genotype
<ul style="list-style-type: none"> • Allogeneic stem cell transplant • Autologous transplant
Gene therapy
<ul style="list-style-type: none"> • Lentiviral strategies • Induction of fetal hemoglobin • Gene editing

Newer treatments being considered in the treatment of SCD include agents that can reduce polymerization by various means including Pan histone deacetylase inhibitors [LBH589/Panobinostat (NCT01245179)], DNMT1 inhibitors [Decitabine/THU (NCT01685515)], agents targeting carbon monoxide delivery [Sanguinate (NCT02411708)],

and phosphodiesterase 9 inhibitors [IMR-687 (NCT04053803)]. For prevention of vaso-occlusion agents, nonionic block copolymer surfactant such as Poloxamer and Vepoloxamer are tried. For inflammation, which is a major player in complications of sickle cell disease, P2Y2 inhibitors [Prasugrel, ticagrelor (NCT02482298)], agents which affect neutrophils and monocytes activation [Intravenous immunoglobulin (NCT01783691), vascular endothelium [Simvastatin (NCT03599609)], anti-factor Xa [Rivaroxaban (NCT02072668)], and agents reducing effects of oxidative stress [N-Acetylcysteine (NCT01800526)] are considered [187].

In a steady state, generally, patients with sickle cell disease have normal values for platelets and white blood cells including neutrophils and monocytes. These values usually increase during acute events. Neutrophilia has been shown to correlate with the severity of sickle cell disease. Neutrophils interact with erythrocytes and endothelium resulting in the upregulation of the expression of cytoadhering molecules including P- and E-selectins. Pathogenesis of vaso-occlusion and sickle cell-related pain crises involve the up-regulation of P-selectin in endothelial cells and platelets. Platelet aggregation is shown to be dependent on P-selectin. Crizanlizumab, an antibody against the adhesion molecule P-selectin, can reduce the rate of sickle cell-related pain crises in sickle cell disease [188]. Chronic therapy with phosphodiesterase inhibitors such as sildenafil and tadalafil in sickle cell disease patients with mild-to-moderate pulmonary hypertension can improve pulmonary arterial systolic pressure and exercise capacity [189]. Tadalafil, a phosphodiesterase-5 inhibitor, has been used for chronic treatment of stuttering priapism, prevention of recurrent priapic episodes, and restoration of erectile function [190]. Oral therapy with pharmaceutical-grade L-glutamine (USAN, glutamine) has been shown to increase the proportion of the reduced form of nicotinamide adenine dinucleotides in sickle cell erythrocytes, and there is evidence that it reduces oxidative stress and could result in fewer episodes of sickle cell-related pain. Food and Drug Administration has approved hydroxyurea and L-glutamine for the treatment of sickle cell disease in the United States.

Vaccination with pneumococcal, meningococcal, and influenza, in addition to general recommended immunizations, reduces occurrence of major infections.

Surgery in sickle cell disease has a potential risk of vaso-occlusive crisis, acute chest syndrome, post-operative infections, and congestive heart and organ failures with a significant morbidity and mortality. Therefore, significant pre-operative assessment and management, including transfusion or exchange transfusion, are required.

8.1. Treatment Strategies

Major commonly available treatments for sickle cell disease currently include standard general patient care and therapy, blood transfusion or exchange transfusion, hydroxy urea, Voxelotor/GBT440 (OxbrytaTM), Crizanlizumab (Adakveo[®]), L-glutamine, and hematopoietic stem cell transplantation. Agents that target bases of complications seen in sickle cell disease such as inflammation, cellular adhesion, oxidant injury, platelets and coagulation, vascular tone, hemoglobin polymerization, and ultimately, gene therapy to correct the β^s point mutation are presently under active investigation. Studies to better understand mechanisms of switching from fetal to adult hemoglobin, and the role of transcriptional regulators such as BCL11A, can provide genomic-based methods for therapeutic reactivation of HbF as a treatment approach for this disease [175].

Transfusion: Currently, general patient care and blood transfusion or exchange transfusion remain essential parts and portions of management of care, acute or chronic complications of sickle cell disease, and prevention of primary and secondary strokes [125–192]. Blood transfusion decreases the HbS percentage and improves the oxygen-carrying capacity and microvascular perfusion. Transfusion, however, is associated with several limiting factors and complications. This includes availability of fully compatible blood, and many adverse events including risks of hemolytic transfusion reaction, erythrocyte alloimmunization and iron overload. These complications significantly increase with repeated transfusions. These are more evident in countries predominantly inhabited by

a white population, since the recipients are ethnically mostly of African descent [193,194]. A major cause of the mismatch is often the difference between serologic Rh phenotype and *RHD* or *RHCE* genotype due to variant *RH* alleles [193,194].

Hydroxyurea (HU): Hydroxyurea is a major current therapeutic agent for the treatment of sickle cell disease. The effect of this agent is via induction of fetal hemoglobin (HbF, $\alpha 2\gamma 2$), improvement of RBC hydration and reduction of neutrophil count, leucocyte adhesion, and pro-inflammatory markers. Hydroxy urea also serves as NO donor, thus inducing vasodilation [182,195,196]. While the effects of Hydroxyurea, due to its uneven and variable effects on hematopoietic cells and increase in fetal hemoglobin, is not uniform, currently, this agent is one of the most effective therapies available for sickle cell disease [197]. The effect of Hydroxyurea is found to be comparable to that of blood transfusion in preventing primary, but not secondary, stroke [198].

Hydroxycarbamide is reported to reduce episodes of pain and acute chest syndrome, hospitalization, and transfusions in adults and children with SCD [198,199]. Among mechanisms of the effects of Hydroxyurea is its stimulation of cyclic guanosine monophosphate (cGMP). Phosphodiesterase 9 (PDE9) is an enzyme in the neutrophils and RBCs of patients with SCD which degrades cGMP.

PDE9 inhibitors: A selective PDE9 inhibitor (IMR-687) is shown, in vitro, to increase levels of cGMP and fetal hemoglobin F, and is the subject of clinical investigation for the treatment of sickle cell disease [200], gene therapy, and genetic alteration.

Gene therapy: Recent progress made in understanding the molecular pathways controlling mammalian erythropoiesis and globin switching, as well as advances in genome engineering tools, particularly the gene-editing technique CRISPR/Cas9, has opened a venue for genetic base treatment of sickle cell disease [201–208]. Understanding the genetics and transcription factors which have a role in the production or suppression of fetal hemoglobin is important [209]. Progress in genetic engineering has made genetic correction in induced pluripotent stem cells and patient-derived hematopoietic stem and progenitor cells possible. Ex vivo gene therapy via globin gene addition can be a curative treatment of this disorder. This is currently being investigated in clinical trials with significant success and encouraging results. Gene therapy, along with the use of LentiGlobin for the treatment of sickle cell disease consists of autologous transplantation of hematopoietic stem and progenitor cells transduced with the BB305 lentiviral vector encoding a modified β -globin gene, which produces an anti-sickling hemoglobin, HbA^{T87Q}. One-time treatment with LentiGlobin is reported to result in sustained production of HbA^{T87Q} in most erythrocytes, thus a reduction in the hemolysis and complete resolution of severe vaso-occlusive events [201–203].

Gene-editing to correct defective DNA in its native location is another approach. An example is a clinical trial where a patient's own *BCL11A* gene is disrupted to induce fetal hemoglobin expression [210]. Genetic therapy using a patient's own stem cells has been pursued, however, this technique has the disadvantage of myeloablative conditioning. Additionally, gene therapy, while very promising, is costly and not free of side effects [211–214]. Acute myeloid leukemia after gene therapy for sickle cell disease has been reported [212,213].

8.2. Alteration of Coagulation Cascade and Other Therapies

As noted before, inflammatory state and coagulation cascade have a significant role in the development of the arterial and venous thrombotic events of SCD and can be used for therapeutic purposes [215–222]. P-selectin plays a role in the platelets and neutrophils aggregations which are a part of this cascade. These and other findings regarding pathogenesis of SCD are used to an advantage to explore new therapeutic strategies for this disease. In this regard, Crizanlizumab (Adakveo[®]), is a monoclonal antibody which binds to P-selectin molecule and was approved by the US Food and Drug Administration (FDA) for the treatment of vaso-occlusive crisis of SCD.

An approach for the prevention of VOD is increasing oxygen affinity of the hemoglobin molecule, to prevent sickle cell formation. Voxelotor/GBT440 (Oxbryta™), a polymerization inhibitor, was approved by the US FDA as an anti-sickling agent. Voxelotor binds to the N-terminal valine of the alpha chain of hemoglobin S, prevents its polymerization, thus reduces sickling, and increases the half-life of the erythrocytes [186,219,220].

To remedy inflammation which is one of the important factors in VOC, and other relevant causes involved in the development of complications in SCD, the effects of various means to control the process have been evaluated. These include Intravenous immunoglobulin (IVIG), statins, and *N*-Acetylcysteine (NAC). The use of other agents such as poloxamer that reduces blood viscosity and cell–cell interactions to lower the duration of vaso-occlusive episodes has been unsuccessful [223].

9. Transplantation

Currently, the “curative” treatment for SCD is transplantation with stem cells from an immunologically matched sibling [224]. However, this approach may be limited by the lack of availability of matched sibling donors. Of course, transplantation is costly and fraught with multiple short- and long-term complications, including graft versus host disease (GVHD), morbidity, and mortality [225].

Progress in gene editing and the use of genetically engineered autologous cells for transplantation eliminates the need to find a genetically matched donor and required immunosuppression and eliminates the risks of GVHD and graft rejection [225]. Ideally, therapeutic reactivation of HbF or prevention of switching from fetal to adult hemoglobin can be a treatment of choice for SCD. A better understanding of transcriptional regulators such as BCL11A, provides genomic-based approaches for prevention of switching or reactivation of fetal hemoglobin production [209,210]. Gene therapy of the beta-hemoglobinopathies by lentiviral transfer of the beta (A(T87Q))-globin gene is an approach in this regard [201–208].

10. Conclusions

Since the first description of sickle cell disease in 1910 and discovery of its genetic origin in 1945, there has been significant progress in understanding the complex pathophysiology, pathobiology, clinical manifestation, educational, psychological effects and complication of this disease. Until recently, however, progress in the treatment of sickle cell disease has been limited to addressing and prevention of its acute events and complications. With significant advances made in gene therapy, future treatment of sickle cell disease may be a genetic alteration which may permanently resolve the disease and prevent the significant known complications and long-term social, psychological, disabilities and mortality of this genetic disorder.

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