Sickle Cell Disease: An Overview

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Sickle cell disease evolved as a genetic mutation in areas of the world where malaria is endemic. The gene responsible for the disease is located on the short arm of chromosome 11. It is inherited as an autosomal recessive inheritance in which the abnormal gene product is an altered beta chain in the hemoglobin structure.

Sickle cell disease is an age-old disease which has been known for hundreds of years in Africa. It was known in Africa by onomatopoeic names denoting the recurrent, unrelenting, and painful nature of the disease.1 Translated, some of these names mean a child destined to die young or a child who brings sadness and pain to his parents.2 Since a heterozygous mating produces a 25% chance of bringing forth a child with the disease during each pregnancy, a couple could have a series of pregnancies resulting in babies with the disease. In some ethnic groups, elaborate ceremonies were performed to prevent future reincarnations of the child. It is still a feared disease in parts of Africa, although there is a greater understanding of the genetics and disease etiology.

Though once considered a disease of childhood with a mortality approaching 20% by age 3, diagnosis soon after birth and advances in clinical research have placed current life expectancies, on average, at more than 48 years.3,4 Sickle cell disease is an inherited disease in which defective sickle-shaped red cells fail to carry adequate oxygen to tissues in the body. As a result of the sickling, the cells tend to block and damage the smallest blood vessels in the body, resulting in damage to organs served by those vessels. Today, the prognosis is not as bleak for the patient with sickle cell disease as it was in the past. Various treatment options are now available to help the patient with this chronic disease cope with its many complicating aspects.

Although the symptoms of the disease could be traced to 1670 in a Ghanaian family, disorders of hemoglobin synthesis were unrecognized by the scientific community until 1910.5 The first article to describe the sickle cell phenomenon appeared in 1910, when Herrick,6 a Chicago cardiologist, wrote a case report titled “Peculiar elongation and sickle shaped corpuscles in a case of severe anemia.” The article contained a description of the symptoms and pictures of blood obtained from a 20-year-old patient from the West Indies. In 1927, Hahn and Gille-spie7 delineated the conditions affecting sickle cell formation in vitro, including pH, temperature, fixatives, toxicity, and others, and determined that loss of oxygen was responsible for the sickle shapes observed in the blood. They postulated that similar effects of hypoxia could occur in vivo, leading to cellular distortion with consequent hemolysis. In 1940, Sherman noted a birefringence of deoxygenated red cells suggesting that low oxygen altered the structure of the hemoglobin (Hb) molecule.8 In 1948, Watson suggested that the scantiness of sickle cells in the peripheral blood of newborns was due to the presence of fetal hemoglobin in the red cells.2 In 1949, Pauling and colleagues9 showed that hemoglobin from sickled cells had an abnormal mobility in an electric field. In 1949, Neel published a report establishing that sickle cell trait was the heterozygous state, and sickle cell anemia was the homozygous state for the same gene.10 In 1956, Ingram11 revealed that substitution of valine for the glutamic acid in the sixth position of the beta globulin molecule was responsible for the abnormal function of the molecule after deoxygenation.

The hemoglobin S (HbS) molecule is a protein whose quaternary structure is a tetramer consisting of 2 normal α-globulin chains and 2 abnormal β-globulin chains. The pathology that leads to the sickle shapes of the red blood cells involves this molecule. After deoxygenation of HbS molecules, polymers of HbS form through hydrophobic interactions between the β-6 valine of a tetramer and the β-85 phenylalanine and β-88 leucine of an adjacent tetramer.12 The Hbs molecules aggregate upon deoxygenation to form polymer nuclei that become seeds for further polymerization. The polymerization of the sickle red cells take place as the cells traverse the microvasculature.13 Factors that increase the intracellular concentration of hemoglobin, factors that increase time spent in the microvasculature, and deoxygenation of the hemoglobin all contribute to increased polymerization. Increased levels of non-S hemoglobin such as Hbf and HbA2 slow the rate of polymerization and reduce the intracellular polymer content at any oxygen saturation.13

Epidemiological Considerations

The sickle cell disease was first recognized among persons of western African ancestry. The sickle cell trait is seen in 10% to 30% of people in equatorial Africa but infrequent in northern and southern Africa.9 The Hbs gene is distributed worldwide, occurring around the Mediterranean in Sicily and other parts of southern Italy, northern Greece, Turkey along the south-east coast, the north African coast, in Saudi Arabia especially the eastern province, Iran, and throughout central India.14 Approximately 1 in every 400 to 500 African Americans has sickle cell disease.15 An estimated 80,000 Americans have the disease and about 9% of African Americans have the trait. One in
every 1,000 to 14,000 American Hispanic children are born with sickle cell disease. In India, the sickle cell gene is present in all tribal populations who inhabit hilly forest areas where falciparum malaria is common. It presents as a lethal disease in Africa but benign or mild in India and Saudi Arabia. It is the most prevalent inherited monogenic pathology in South America and it is estimated that 2% of the population of Brazil and 6% to 9% of Brazilians of African descent are heterozygous for the HbS gene, with 700 to 1,000 new cases yearly. The HbS gene is found at a frequency of 20% to 30% people in some villages in northern Greece, 25% in the Qatif oasis of eastern Saudi Arabia, and in 20% to 30% of many communities in the Indian states of Orissa, Madhya Pradesh, and Maharastra.

Genetics
The gene for HbS is located on the short arm of chromosome 11. It is a hemoglobinopathy with autosomal-recessive inheritance in which the abnormal gene product is an altered beta chain in the structure of hemoglobin. Most common at birth is the homozygous sickle cell disease in which the HbS gene is inherited from both parents. Next in frequency among people of West African ancestry is sickle cell/hemoglobin C disease which results from the inheritance of HbS gene from 1 parent and HbC gene from the other parent. The inheritance of HbS gene and a gene for the beta thalassemia may result in either HbS/β+ thalassemia with mild disease (9 to 12 g/dL hemoglobin, >3.5% HbA, and 20% to 30% HbA1) or HbS-βthalassemia with more severe disease (no HbA1, >3.5% HbA2, 6 to 10 g/dL hemoglobin). If both parents are carriers of the abnormal gene, there is a 1 in 4 chance of having a child that has sickle cell disease. If 1 parent has sickle cell disease and the other parent has the trait, the risk of an affected child is doubled. When a population is properly informed and educated about the genetics of sickle cell disease and their own genetic status, they can elect to avoid relationships between carriers or, when applicable, make informed decisions about the birth of a potentially sickle cell diseased child.

Pathophysiology
The pathogenesis of the disease is hypothesized to be due to the adherence of sickle cells to vascular endothelium, which initiates and contributes to microvascular occlusion and pain episodes. Adherence involves plasma proteins, endothelial cell adhesion molecules, and receptors on sickle erythrocytes and vascular tissue. It also involves interaction with leukocytes, increased levels of circulating inflammatory cytokines, enhanced microvascular thrombosis, and endothelial damage resulting in abnormal rheology due to an increase in blood viscosity. The viscosity of blood is augmented by several interrelated factors including membrane rigidity, hemoglobin polymerization, and increased intracellular hemoglobin concentration. The equilibrium of HbS between its liquid and solid phases is determined by 4 variables: oxygen concentration, HbS concentration, temperature, and hemoglobin other than HbS. Though patients share a defining point mutation of the beta globin gene, sickle cell disease is also characterized by extraordinary variability of the clinical expression.

Sickle cell disease is a systemic illness with manifestations that range over nearly all organ systems. It is characterized early in life by severe chronic hemolytic anemia caused by sickled hemoglobin and vaso-occlusion, bacterial infection, and organ infarctions including the brain. Other systemic effects include retardation of growth and cognitive development and chronic organ dysfunction. The etiology of growth deficits in children with sickle disease may be multifactorial and include inadequate energy and nutrient intake and/or increased metabolic rate. One of the complications of sickle cell disease is acute chest syndrome (ACS), which is defined by the occurrence of chest symptoms, new pulmonary infiltrate on chest radiograph, and in some cases fever, cough, dyspnea, and chest pain. There might be hypoxemia with hemoglobin concentration falling below steady state values which may require blood transfusion. Causes of ACS include infectious diseases, in situ thrombosis, hyperventilation secondary to chest pain, and fat embolism. Other factors that may precipitate ACS include hypoventilation after opioid analgesics, splinting due to rib infarction, and excessive intravenous hydration.

Another complication of sickle cell disease is priapism, in which the patient suffers erections that can last for hours or even days. Priapism often has its onset during sleep and may be experienced by many men and boys. It results from obstruction of venous blood by sickled red blood cells in the corpora cavernosa without apparent thrombus formation. Aggressive hydration and adequate analgesia are of primary importance in the treatment of priapism. Other complications of sickle cell disease include ocular manifestations, nephritic syndrome, leg ulcers, hepatobiliary manifestations, genitourinary manifestations, and hematuria. All of these complications require an adequate and aggressive approach in their management. Sickle cell disease in which 1 gene produces HbS and the other gene produces HbC is a milder form of the disease with less severe course and fewer neurological complications. Individuals with HbAS are usually asymptomatic.

Hemoglobinopathies
Hemoglobinopathies result from the production of abnormal hemoglobin proteins or from the production of reduced amounts of otherwise normal hemoglobin proteins. There are different types of hemoglobin, including HbA, HbS, HbC, HbE, HbF, and HbD. Hemoglobin A is considered the normal hemoglobin found in humans. Hemoglobin S is the gene product of the HbS gene and resulted from a genetic mutation. Hemoglobin C is also a product of genetic mutation of the normal HbA gene. The glutamic acid in the sixth position of the beta chain of HbC is replaced by lysine. This gives HbC a net positive charge. It is found predominantly in people of African ancestry.
Hemoglobin E is an amino acid substitution in the 26th position of the beta chain from glutamic acid to lysine. This gives HbE a net positive charge. The hemoglobin is unstable when subjected to oxidizing agents. It is found commonly in people of Asian descent and usually produces few or no problems in the patients. Hemoglobin E in combination with HbA is benign. People with homozygous E genes have a mild anemia and few other manifestations.26

Hemoglobin F is produced in fetuses before birth and arises from a gene different from the one that produces adult normal hemoglobin. The gene for HbF and HbA are closely related. The production of HbF falls dramatically after birth. Some people continue to produce small amounts of HbF for most of their lives.26 Hemoglobin D refers to any hemoglobin variant with an electrophoretic mobility on cellulose acetate similar to HbS but which has a negative dithionite solubility test. Hemoglobin D Punjab is the most common with the substitution being at the 121st position of the beta chain with glutamic acid being substituted by glutamine. It is found in northwest India and in some English, Portuguese, and French individuals.27

The Thalassemias

Thalassemias are a group of diseases in which normal hemoglobin molecules are produced in reduced amounts. They are characterized by decreased hemoglobin, hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) with a normal or increased mean corpuscular hemoglobin concentration (MCHC).28 Gene deletion is the most common cause. There are 2 main types of thalassemias, α-thalassemia resulting from defective production of alpha chains and β-thalassemia resulting from defective production of beta chains. Both are inherited as autosomal dominant disorder with heterogeneous expression of the disease. It is a common hereditary disorder and it has a worldwide distribution. Like the sickle cell gene, prevalence of the thalassemia gene has been attributed to the protection it offers against falciparum malaria.27 Alpha-thalassemia occurs with high frequency in Asian populations and also is seen in black, Indian, and Middle Eastern populations. HbS-α thalassemia is fairly common but it is usually clinically insignificant. HbS-β thalassemia is a common sickle cell syndrome seen in people of Mediterranean descent (Italian, North African, Greek, Turkish, and Romanian ancestry).26

Modulators of Sickle Cell Disease

Various factors have been found to modulate sickle cell disease. The clinical severity of the disease is influenced by a variety of factors including the presence of α-thalassemia, elevated HbF, and the haplotype that is linked to the β-globin gene. However, the presence of elevated HbF in children or adults with sickle cell disease should not lead to complacency, and a high index of suspicion should be maintained in such patients to facilitate early diagnosis of any possible complications.19

Hemoglobin F

Hemoglobin F has been found to ameliorate the effect of sickle cell disease. The level of HbF needed to benefit people with the disease varies. It has been found that high levels of HbF (5.4% to 39.8%) reduced the risk for early onset of dactylitis, pain crises, acute chest syndrome, and acute splenic sequestration. Fetal hemoglobin of 10% or more have been shown to be associated with fewer chronic leg ulcers in American children with sickle cell disease.12 Hemoglobin F ameliorates the sickle cell disease symptoms by not participating in polymerization between HbS molecules. The lack of valine at the sixth amino acid residue on the gamma chain prevents hydrophobic interaction with the HbS molecule. Increased concentration of HbF means decreased concentration of HbS, which reduces the number of sickle cells. The inheritance of genes determining high levels of HbF is not well understood. In most SS subjects with high HbF levels, either 1 or both parents show a modest elevation of HbF with normal range.29

Alpha-Thalassemia

Alpha-thalassemia is a genetically inherited disorder. It is due to the loss of 1 or more genes that encode the α-globin chain. Thalassemia is characterized by a quantitative reduction or total absence of a globin polypeptide chain, most often α or β chain. Hemoglobin S-α thalassemia is fairly common. Alpha-thalassemia is a modifier of sickle cell disease severity. In a study to determine the effect of α-thalassemia on sickle cell disease, Embury and colleagues30 evaluated the concurrence of sickle cell anemia and α-thalassemia and proposed that α-thalassemia reduces intraerythrocytic HbS concentration which reduces poly-
merization of deoxyHbS and hemolysis. They further stated that the data from their study indicated that the fundamental effect of α-thalassemia is to inhibit the generation of sickle red blood cells having high density and mean corpuscular hemoglobin concentration (MCHC), and that the other beneficial effects of sickle red blood cells are secondary to this process.

Hemoglobin S-β thalassemia is associated with clinical features similar to sickle cell disease. Homozygous β⁺ thalassemia and HbS-β⁺ thalassemia both demonstrate no β chain production and consequently no HbA. They are usually more severe than HbS-β⁺ thalassemia and similar in severity to sickle cell disease. The β⁺ thalassemia and HbS-β⁺ thalassemia both demonstrate a reduced beta chain production.28

**Hemoglobin Haplotype**

The hemoglobin haplotype also modulate sickle cell disease. The sickle cell gene has evolved 4 times in Africa and once in India/Saudi Arabia.19 The geographical areas in which they were first discovered identifies the haplotypes. The haplotypes include Senegal, Benin, Central African Republic (CAR), Cameroon, and Arabo-Indian.31 The Senegal haplotype is found in Senegal and in countries west and above the Niger River. The Benin haplotype is found in Nigeria, Ghana, and countries in the Bight of Benin. The Central African Republic haplotype is found in the Central African Republic and countries south of central Africa.12 The 3 most common haplotype in the Americas are the Senegal, Benin, and CAR haplotypes. The Cameroon haplotype is found in Cameroon, and the Arabo-Indian haplotype refers to those haplotypes found in the Persian Gulf and India.

The different haplotypes have varying degrees of severity. The Senegal haplotype has the least severe course, and the Central African Republic haplotype has the most severe course. The Senegal haplotype is associated with fetal hemoglobin levels of 20% or more, which is probably responsible for the milder course of the disease.12,32 A study by Diop and colleagues33 to evaluate disease severity in Senegalese patients confirmed the relative good tolerance of homozygous sickle cell disease in Senegal. The Benin haplotype presents with an intermediate level of fetal hemoglobin and thus is associated with an intermediate course of disease severity. The CAR haplotype presents with the most severe disease and also has the lowest level of fetal hemoglobin.34 Thus the severity of the disease presentation appears to correlate with the haplotype and fetal hemoglobin level. However, this correlation is not absolute.

The effects of heterozygous sickle haplotype on clinical severity have been studied. Nagel and colleagues32 found that the presence of 1 Senegal haplotype still results in high HbF levels, higher overall hemoglobin levels, lower reticulocyte counts, and lower bilirubin levels. It has also been shown that Benin/CAR heterozygotes tended to produce lower HbF levels as well as greater disease severity while Senegal/CAR haplotype tended to produce intermediate HbF levels.12,32 High HbF levels are associated with the Arabo-Indian haplotype in SS disease and even in Indian AS subjects, the HbF level increased with the number of copies of this haplotype.29

**Laboratory Diagnosis of Sickle Cell Disease**

Screening programs for the detection of HbS gained popularity in the 1970s. The goals of the programs were initially uncertain. Due to a lack of effective education of those screened, there was widespread misunderstanding about the critical difference between sickle cell disease and sickle cell trait. The stigma of having the disease was reinforced with inappropriate health, social, and economic restraints.23 Eventually, guidelines were established in 1987 by an expert panel convened by the National Heart Lung and Blood Institute (NHLBI) and comprehensive screening programs emerged.4,35 The screening programs were widely implemented after a double-blind randomized, placebo-controlled trial demonstrated an 84% reduction in the incidence of pneumococcal sepsis when prophylactic oral penicillin was initiated by the age of 3 months. Newborns are now screened for sickle cell disorders in 42 states and the District of Columbia.4,36

The most useful test for the diagnosis of hemoglobinopathies includes hemoglobin electrophoresis for variant identification by cellulose acetate at alkaline pH and citrate agar at acid pH [T1 and T2], the dithionite solubility tube test for sickling hemoglobin, and the metabisulfite slide test. The alkali denaturation test for fetal hemoglobin may be used to identify conditions in which HbF is elevated such as in HbS disease and

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Methods include isoelectric focusing, high-performance liquid chromatography (HPLC) or DNA analysis. Solubility methods and sickle cell preparations are inappropriate diagnostic techniques because these tests may miss hemoglobin C and other genetic variants. In addition, solubility testing is inaccurate in newborns, in which fetal hemoglobin is predominant. Solubility testing also fails to detect sickle hemoglobin in patients with severe anemia.

**Cellulose Acetate Hemoglobin Electrophoresis**

In this method, a small quantity of red cell hemolysate is placed on cellulose acetate membrane between the center and cathode of an electrophoretic chamber. An electrical field is created in the chamber containing a buffer at alkaline pH. Hemoglobin molecules have a net negative charge at alkaline pH and migrate on the membrane toward the anode. Due to the amino acid content of the different hemoglobins, the net charge of each of the hemoglobin molecules varies. This determines the rate of migration of the various hemoglobin types in the electric field. After electrophoresis, the membrane is stained and cleared. Comparing the hemoglobin migration with that of a control is what makes the interpretation. The fastest migrating molecule is HbA, followed by HbF, then HbS, and HbC is the slowest.

**Citrate Agar Hemoglobin Electrophoresis**

Citrate agar hemoglobin electrophoresis is the most important confirmatory test that can be done after abnormal hemoglobin is found on cellulose acetate electrophoresis. In this method, the different hemoglobin molecules are separated based on the interactions among the hemoglobin variants, agar, and citrate buffer ions, in addition to the altered electrical charge of the various hemoglobins at acid pH. The hemoglobin variants are identified by their migration toward the anode and the cathode in comparison with a known control sample. Hemoglobin F, which is the most soluble, has the fastest cathodal mobility. Adult hemoglobins with solubilities that are similar to HbA move with HbA. Hemoglobin S, which is relatively insoluble, moves behind hemoglobin A, while Hbc moves behind Hbs.

**Dithionite Tube Test (Sickledex)**

Sickling hemoglobin in the deoxygenated state is relatively insoluble and forms a precipitate when placed in a high-molarity phosphate buffer solution. Red cells are lysed by saponin allowing hemoglobin to escape. Sodium dithionite binds and removes oxygen from the test environment. Hemoglobin S polymerizes in the resulting deoxygenated state and forms a precipitate in the high-molarity phosphate buffer solution. The precipitate consists of tactoids, which are liquid crystals. The tactoids refract and deflect light, and make the solution turbid. It is recommended that packed red blood cells be used to avoid false positive and false negative results. Positive and negative control must be run with each test.

**Alkali Denaturation Test**

The alkali denaturation test is an accurate method for quantifying the percentage of Hbf in blood. It may be used when Hbf concentration is in the range of 2% to 40%. It is based on the fact that Hbf resists denaturation in 1.2 N NaOH for 1 minute. After the specified period, the reaction is stopped with ammonium sulfate, which lowers the pH and precipitates out the denatured Hba. The test solution is filtered and the Hbf in the clear solution can be quantitated spectrophotometrically at 540 nm as a percentage of total hemoglobin.

**Treatment Options**

Since infection, fever, dehydration, acidosis, hypoxemia, and cold exposure precipitate vaso-occlusive crises (VOC), measures to prevent or remedy these conditions are important. Optimal hydration is essential especially during febrile illnesses. Infections require prompt attention particularly in children. Treatment of the disease involves 1 of 3 ways: bone marrow transplantation (BMT), which can be curative; hydroxyurea which reduces the number of pain episodes but whose effects on organs are uncertain; and chronic transfusions used for primary and secondary stroke prevention. Patients with sickle cell disease require special transfusion consideration because they tend to have a higher rate of alloimmunization and often develop unusual and exotic antibodies. Nitric oxide treatment and gene replacement therapy are treatment modalities which are still in the experimental stages of development.

**Bone Marrow Transplant**

Bone marrow transplantation is the only known cure for sickle cell disease at this time. Other, the underlying disease recurs in about 10% of transplant patients. There is also concern about the short-term and long-term toxicity, lack of suitable stem cell donors, and limited access to this treatment which currently makes it an infrequently utilized treatment for sickle cell disease. By destroying the sickle cell patient’s diseased bone marrow and stem cells, and transplanting healthy bone marrow from a genetically matched or allogeneic donor, normal hemoglobin may be produced. Candidates for this procedure have included those with a history of stroke, recurrent ACS, sickle pulmonary disease, or VOC. Only patients who are under age 16 are candidates for this procedure. The procedure carries with it a high degree of risk and about 10% of patients die from the procedure. Complications may include graft versus host disease (GVHD), bleeding, pneumonia, and severe infections. Due to the fact that sickle cell disease patients are transfused on multiple visits, they usually develop alloimmunization to red cell and platelet antigens. The platelet antibodies produced cause platelet refractoriness in some of these patients.

In sickle cell disease, the loss of reticuloendothelial function results from vaso-occlusion events, which occur in the spleen. Such resultant asplenia may be corrected by bone marrow transplant. Ferster and colleagues found that bone marrow transplant can correct permanent asplenia in sickle cell disease patients. It remains to be seen if such treatment can also correct other sickle cell disease related organ dysfunction. A study by Wal-
and was halted to allow all patients to receive the treatment.

Immunologic phenomena, hemosiderosis, and risk for transmission of infectious agents are the 3 most noticeable risks associated with repetitive transfusion.40 Alloimmunization to red cell antigens is a significant risk in chronically transfused patients with sickle cell disease. A way of decreasing the likelihood of alloimmunization and facilitating long-term management is antigen matching, which may decrease morbidity.51-53 Ambruso and colleagues52 found that matching red cell antigens may diminish the incidence of alloimmunization in patients with sickle cell anemia, thus requiring transfusion. In a similar study, Tahhan and colleagues54 recommended that transfusion centers engaged in the management of chronically transfused sickle cell anemia patients consider providing antigen-matched units for such patients. It is thought that not only does such a program prevent alloimmunization but it also provides additional clinical benefits to the patient that may outweigh the higher cost of the process. Platelet refractoriness is a significant problem in chronically transfused sickle cell patients. It is estimated that 85% of heavily transfused sickle cell anemia patients are alloimmunized to HLA and/or platelet-specific antigens. These patients may be refractory to platelet transfusion, a condition that would increase their risk during BMT.54 Leukodepletion in the transfusion support of sickle cell anemia patients should be considered to prevent platelet alloimmunization.54,55

Hydroxyurea
Hydroxyurea inhibits ribonucleotide reductase, blocking DNA synthesis and cell division. Hydroxyurea can increase fetal hemoglobin and improve clinical course of sickle cell disease patients. Hydroxyurea increases production of fetal hemoglobin, increases red cell mean corpuscular volume, and reduces the number of dense cells and irreversibly sickled cells in the circulation.56 A multicenter study of hydroxyurea involving 299 patients ages 18 and older was suspended by the National Institutes of Health (NIH) in 1995 because patients on the hydroxyurea arm of the study had fewer painful crises than did the controls.4,57 Results of the Hug-Kids study,58 a phase I/II trial to determine the safety and efficacy of hydroxyurea in pediatric patients with sickle cell disease, showed that laboratory toxicities were mild, transient, and were reversible upon temporary discontinuation of hydroxyurea. No life-threatening clinical adverse events occurred and no child experienced growth failure.58

Thus, hydroxyurea is the first drug proven to prevent sickle cell crises in patients. Hydroxyurea is approved by the Food and Drug Administration (FDA) to treat certain types of leukemia and other cancers. It has been observed that more than 50% fewer episodes of ACS occurred in patients treated with hydroxyurea.49 Of concern are several issues of hydroxyurea therapy including differences in patients’ drug clearance, predictability of drug response, reversibility of sickle cell disease-related organ damage by hydroxyurea, and the efficacy of elevated HbF.59 Cluster and colleagues60 have shown that chronic hydroxyurea therapy may reverse splenic dysfunction in certain patients. For patients with no HbF elevation after treatment with hydroxyurea, 5-aza-2’-deoxycytidine (5-aza-CdR, decitabine) could serve as alternate mode of treatment.51,62 Arginine butyrate and similar compounds can also increase HbF production.47 However, the effect is not as dramatic as with hydroxyurea. The drug is usually given intravenously and has a half-life of about 5 minutes.

Nitric Oxide Treatment
Nitric oxide may be effective in the treatment of sickle cell disease. Nitric oxide has been known to cause the dilation of blood vessels, enabling more blood to flow through them. Nitric oxide, a gas produced in many parts of the body, relaxes the smooth muscle cells in blood vessels.63 It is sometimes used in respiratory therapy to open up or widen the blood vessels of the lungs and improve the flow of blood through the vessels.64 As the muscles relax, the vessels expand and allow more blood to pass through. Improving blood flow is a critical aspect
in treating the painful and potentially deadly complications associated with sickle cell anemia.65 Nitric oxide enables sickle hemoglobin to bind to oxygen molecules with greater affinity, which reduces the amount of sickle cells formed in the body.

Increased adherence of sickle red cells to the endothelium is implicated as an initiating event of vaso-occlusion event in sickle cell disease. In a study by Space and colleagues66 to determine whether nitric oxide inhibits red blood cell adherence to the endothelium, they found that nitric oxide inhibits both normal and sickle red blood cell adherence to the endothelium. They concluded that strategies that enhance nitric oxide activity may be therapeutic in sickle cell disease. The possible long-term effect of the use of this gas still has to be worked out before it becomes an approved therapy for sickle cell disease. Current trials involving sickle cell patients who have breathed low concentrations of nitric oxide have shown promising results.67 Another strategy to induce nitric oxide production involves L-arginine (L-Arg) supplementation which has been found to be effective in inducing an increase in nitric oxide metabolite production.68 Thus oral arginine may be beneficial to sickle cell disease patient as a way to induce nitric oxide production.

**Gene Therapy**

Effective gene therapy of sickle cell disease needs safe, efficient, and stable transfer of globin genes into human hematopoietic stem cells. An important requirement of this process is a high expression of these genes in the red cells derived from these stem cells. As clinical evidence indicates that relatively small amounts of HbF ameliorate the disorder and clinical course, gene therapy targeted at achieving expression of HbF might be a reasonable and achievable goal. Some researchers are focusing on therapies that transfer certain genes to bone marrow that might prevent the sickling process. One unique form of gene therapy for sickle-cell disease involves actually repairing existing defective genetic material to restore production of healthy hemoglobin. Blouin and colleagues69 assessed the in vivo potential curative threshold of HbF in the SAD transgenic mice expressing the human fetal Agamma-globin gene. They found that with increasing levels of HbF, Agamma-SAD mice showed considerable improvement in all hematologic parameters, morpho-pathologic features, and life span. According to Blau,70 the first generation gene therapy trials are unlikely to confer major therapeutic benefits but will provide the foundation upon which subsequent, more effective protocols will be based. Even if any of these therapies are successful, however, widely available treatments are still years away.

**Anti-Sickling Agents**

Several anti-sickling agents have been studied for their ability to prevent sickling. Sodium cromoglicate has been found to be effective in its anti-sickling activity in the treatment of sickle cell disease. Toppet and colleagues71 showed in their study that sodium cromoglicate is a good candidate for anti-sickling treatment and that its activity both in vitro and ex vivo is associated with very low toxicity. Furthermore, they concluded that the low cost of the drug makes it affordable for patients in developing countries. Guis and colleagues72 studied the effect of dimethyl adipimidate (DMA), an amino-reactive cross-linking reagent with anti-sickling properties in vitro on the survival of 51Cr-labeled autologous sickle cells. They found that in some patients, the survival of cells pretreated with 5 mmol/L DMA (pH 7.4) was normal, in others it was near normal, and in others it was much longer. The study showed that the effect of DMA treatment on survival of sickle cells in vivo equals or exceeds that of any other agent tested to date.72

Elevated level of 2,3-bisphosphoglycerate (2,3-DPG) has been found to be effective as an anti-sickling agent. Poiloin and colleagues73 studied the anti-sickling effect of 2,3-DPG depletion after activation of the 2,3-DPG phosphatase activity of bisphosphoglycerate mutase by glycolate-2-phosphate, leading to rapid loss of intracellular 2,3-DPG. The modest effect on solubility of removing intraerythrocytic 2,3-DPG was amplified into a much larger anti-sickling effect by interaction with 3 other cellular variables affecting solubility and polymer content.73 Potassium tellurite (K2TeO3) has been found to be a potent anti-sickling agent that inhibits red cell sickling at concentrations less than 10 mmol/L. The anti-sickling effect is thought to be due to the decreased mean cell hemoglobin concentration resulting from the swelling of the red cells.74

**Prognosis**

Prognosis of this chronic disease has undergone dramatic changes due to early diagnosis, patient education, and therapeutic interventions. The screening programs, which were introduced in the 1970s and implemented in many states in the late 1980s, have made it possible to identify patients early so as to place them on prophylactic therapeutics. Prophylactic use of penicillin in children with sickle cell disease has significantly reduced early childhood mortality from this disease.75 This has tended to reduce the number of crises and provided patients with better quality of life.4 The great improvement in the prognosis of sickle cell disease is directly related and attributable to newborn screening for the disease, vaccination for childhood illnesses, the use of prophylactic antibiotics, and aggressive diagnosis and treatment of febrile events.76 77 Today, approximately 50% of patients survive beyond the fifth decade. One third of deaths still occur during an acute crisis, even in patients clinically free of organ failure.78 However, as death due to acute complications of the disease are eradicated, the major issue becomes chronic complications of the disease and its consequence on quality of life.

**Conclusion**

There is greater worldwide awareness that sickle cell anemia is a chronic disease, which is the consequence of a genetic mutation. Since the observation made by James Herrick3 in 1910, great strides have been made in our knowledge and understanding of this inherited disease and how to best treat and manage it. Screening programs and early preventive measures have
improved health outcomes for most patients. Life expectancy of patients with the disease continues to rise, with better quality of life now a qualified possibility. With BMT, a cure is now possible for some patients when genetically matched donors are available. Hydroxyurea treatment to increase fetal hemoglobin levels is effective in ameliorating the disease symptoms, and various anti-sickling agents are under trial. New therapies such as nitric oxide treatment and gene replacement therapy are on the horizon. Unlike treatment and gene replacement trial. New therapies such as nitric oxide

5. Konotey-Ahulu FID. Clinical manifestations of sickle cell diseases including “the sickle cell crisis.” Arch Intern Med. 1974;133:611.


