REVIEW

SICKLE CELL DISEASE: MOLECULAR AND PATHOPHYSIOLOGICAL FEATURES

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ABSTRACT:

The present study was conducted to review the literature about sickle cell disease. Molecular and patho-physiological features were discussed. Furthermore, we discussed its prevalence, clinical picture, diagnosis, and therapeutic options. We also reviewed connective tissue diseases associated with sickle cell disease. Vascular changes associated with sickle cell disease were also discussed. We also discussed the impact of MTHFR polymorphism on the vascular complications of sickle cell disease.

KEYWORDS: Sickle cell disease, Diagnosis, Treatment, Prevalence

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1. INTRODUCTION

1.1 Overview of Sickle Cell Disease

Sickle cell disease (SCD) is considered the most common inherited blood disease, with a worldwide distribution[1].SCD is characterized by vaso-occlusive crises because of the morphology of the red blood cells resulting from the polymerization of one type of hemoglobin, called hemoglobin S, hemolytic anemia and increased susceptibility to infections[2, 3].

The cause of sickle cell hemoglobinopathies is due to defected genetic structure of hemoglobin[4].The structure of hemoglobin protein includes two α subunits and two ß subunits[5].

According to Pauling *et al*[6], sickle cell anemia results from the substitution of valine for glutamic acid in the sixth amino acid of the ß globin chains.

If homozygous inheritance of this hemoglobin is encountered, sickle cell anemia and clinical symptoms will develop. On the other hand, if heterozygous inheritance is encountered, sickle cell trait (HbSA) and generally few clinical complications will develop[7].If S one sickle ß geneand one ß thalassemia gene are inherited, sickle ß thalassemia disorders are likely to develop[8].

1.2 Clinical Picture of Sickle Cell Disease

Patients with SCD have several organs affected with alterations in the central nervous system, bone and joints, cardiovascular system, respiratory system, gastrointestinal tract, and kidneys, which increases morbidity and mortality in this group of patients[1].

The reasons standing beyond developing clinical manifestations of sickle cell anemia are increased blood viscosity and vascular obstruction by deformed, sickled red cells. It is thought that such clinical situations are attributed to the loss of oxygen to necessary tissue areas. Accordingly, the capacity of the red blood cells is lessened, causing a decrease in their ability to carry oxygen. Further, the disturbance of blood flow induces vascular occlusions, hemorrhages, infarctions, and ischemic necrosis of tissues and organs throughout the body[9]. Several complications such as recurrent vaso-occlusive crises, splenic sequestration crisis, aplastic crisis, infections, bone damage (i.e. hip necrosis), jaundice, leg ulcers, priapism, delayed growth, fatigue, and pain episodes[9, 10].

Various studies have targeted secondary crises related to pain since pain management requires cooperation between children and family in cases of having children affected by SCD. Childrenmay be subjected to frequent hospitalizations, school absences and limitation of activities, which is expected to have great effects on a child's quality of life and cooperation in pain management[11-13].

1.3 Prevalence of Sickle Cell Disease

As shown in table 1, the SCD is mostly prevalent among people from Africa, India, the Caribbean, the Middle East, and the Mediterranean[14]. It has been firstly reported to have abnormal Hbs (HbS) and thalassaemia in Egypt[15,16]. Lehmann[17]reported the presence of HbS in Eastern Saudi Arabia. The prevalence of SCD in Yemen was reported to be 0.95% [18].The prevalence of SCD is varied from country to another (table 1).

Table 1: Gene frequency and common disease pattern of sickle cell hemoglobin (HbS) in the Middle Eastern Arab countries

Country	HbS (%)	Common clinical pattern
Yemen	0.95	Severe
Saudi Arabia	<1-17.0	Benign - severe
Bahrain	HbAS 7-18.1	Benign
Qatar	HbAS 7.46	Benign
Kuwait	HbAS 6	Mainly benign
UAE	0.04- 4.6	Benign to severe
Oman	5.8	Benign to severe

Palestine	?	Severe
Syria	<1	?
Iraq	2.5-16	Mainly severe
Jordan	HbAS 0.44-4.45	Benign - severe
Lebanon	0.34	Mainly severe
Sudan	1.52-10.0 HbAS 24-29	Mainly severe
Egypt	<1-22.17	Mainly severe
Algeria	0.83-3.5	Mainly severe
Tunisia	0-6	Mainly severe
Libya	0.44-6.31	Mainly severe

Source: Mohsen et al. (2011).

In Brazil, 700 to 1000 infants with SCD are estimated to be born each year[19].

1.4 Diagnosis of Sickle Cell Disease

In case of HBSS, complete blood count (CBC) shows hemoglobin levels within the range of 6-8 g/dl accompanied with increased reticulocyte count. On the other hand, in other forms of sickle cell disease, hemoglobin levels are likely to be higher. Examining blood film could show target cells and Howell-Jolly bodies.The addition of sodium metabisulfite on red blood cells can induce sickling phenomenon. Sickle solubility test can also be used to demonstrate the availability of sickle hemoglobin, a test based on giving turbid color in mixture containing reducing solution, while normal hemoglobin gives clear solution. It is worth mentioning that abnormal hemoglobin forms can be demonstrated through using hemoglobin electrophoresis. For diagnosis confirmation, high performance liquid chromatography is used (HPLC) [20].

Due to the fact that acute sickle-cell crisis is usuallyassociated by infection; accordingly, it is advised to investigate an occult urinary tract infection as well as occult pneumonia[21].

1.5 Treatment of Sickle Cell Disease

The treatment philosophy of SCD rotates on two axes: preventive measures for some complications and three main therapeutic strategies: chronic blood transfusions, hydroxyurea and bone marrow transplantation[1]. Furthermore, it is advised to carry out periodic assessment of the organs and systems to detect any early alterations and advising patients and their families about the disease[22].

Due to the fact that the vaso-occlusive episodes could be induced by infection, fever, dehydration, acidosis, hypoxia, and exposure to cold, the proper measures to prevent these episodes are crucial in the management of SCD patients. The treatment of painful cases have to be performed using hydration and analgesia; dipyrone, paracetamol, non-steroidal anti-inflammatory drugs(NSAIDs) or opioid derivatives can be used and it should be aware that the latter should be used with caution due to renal impairment. It is extremely important to carry out proper hydration in the case of fever. It has been observed that administration of drugs that induce the production of Hb F to benefit SCD patients. It has also been indicated that the administration of hydroxyurea, a cytotoxic drug with no effect on DNA methylation, increases Hb F production and is associated with increased survival[23].

Other studies showed that hydroxyurea inhibits the maturation of erythroid precursors, resulting in the recruitment oferythroid progenitors with greater capacity for Hb F synthesis and even has a possible effect of 'reprogramming' the synthesis of hemoglobin in erythroid progenitors[1]. The use of hydroxyurea offers patients with SCD various effects such as reduced vaso-occlusive episodes and painful crises, increased interval between episodes of pain, fewer episodes of acute chest syndrome, and reduced need for blood transfusions and hospitalization[24, 25].

1.6 Pathophysiology of Sickle Cell Disease

Patients with SCD are associated with activated blood coagulation and fibrinolytic systems. There is also increased platelet activity as well as utilization of coagulation inhibitors during vaso-occlusive crises and the steady state of the disease[26-29].

Vascular complications have been shown as important and confusing aspects of the clinical spectrum of sickle cell anemia, although there is controversial evidence surrounding the role of thrombosis in this complication[30, 31].

1.7 Connective Tissue Diseases Associated with Sickle Disease

Several studies showed the rare occurrence of connective tissue diseases such as rheumatoid arthritis and systemic lupus erythematosus among SCD patients[32, 33].Other studies indicated that SCD patients are likely to be at greater risk to develop autoimmune diseases, and this idea was supported through activation disorders of the alternative pathway of complement cascade[34, 35].

In their study, Michel et al[36]showed that among 2000 adults with SCD, connective tissue diseases were diagnosed in 30 patients and the prevalence of rheumatoid arthritis was 0.75% and of lupus, 0.35%.

Studies in African population indicated that the prevalence of rheumatoid arthritis to be less than 0.5%, while the prevalence of lupus among Africans was higher eight times than in other populations[37-39].

1.8 The Relationship between Sickle Cell Disease and Vascular Changes

Solomon et al[40] (2012) investigated the number and function of angiogenic progenitor cells and growth factors among children within the age 5–18 years without acute illness. The study sample included 43 with Hemoglobin SS and 68 with normal hemoglobin. The results showed that hemoglobin SS subjects had doubled mononuclear cell colonies and more circulating progenitor cells compared with control subjects. Their results also indicated that plasma concentrations of erythropoietin, angiopoietin-2, and stromal-derived growth factor (SDF)-1α were significantly higher in children with hemoglobin SS compared to control subjects. Furthermore, SDF-1α concentration was associated with both circulating progenitor cells (CPC) number and total white blood cell count in the Hemoglobin SS group, which implies that SDF-1α produced by ischemic tissues plays a role in mobilizing these cells in children with Hemoglobin SS.

According to Kato[41], hemolytic anemia, vaso-occlusion, and abnormal flow dynamics in SCD participate to vessel injury. Furthermore, chronic intravascular hemolysis delivers free heme, which, in turn, binds avidly to nitric oxide (NO), causing NO depletion, and subsequent vasoconstriction and inflammation.

Woollard *et al* [42]clarified that reactive iron from eryrthrocyte as well as oxygen species directly harm endothelial cells. In another study, Wood and Granger[43] demonstrated that cyclic events of acute vaso-occlusion lead to tissue ischemia and reperfusion, which, in turn, induce inflammation and increased oxidative stress.

It is worth to mention that data reported by Krishnan *et al*[44] showed that episode of continuing inflammation and vascular injury to occur in people with sickle cell anemia even when asymptomatic, accompanied by elevated levels of high sensitivity C-reactive protein (hsCRP) and circulating endothelial cells.

Reendothelization is required after vascular injury to retain vascular homeostasis. Endothelial progenitor cells (EPCs) leave bone marrow to sites of vascular injury and this process is regulated by cytokines and growth factors released at the sites of vascular insult[40]. Several studies have found that reduced numbers of endothelial progenitor colonies in adults with cardiovascular risk factors[45], diabetes[46], and those with established cerebro-vascular disease[47]. It has also been reported to have association between cardiovascular diseases and functional impairments in EPC migration or angiogenesis[48]. Furthermore, EPC have been found to be elevated during acute myocardial infarction[49]. It has also been found that EPC to be stimulated by hematopoietic growth factors such as erythropoietin[50].

According to Solomon et al[40], data concerning the number and function of EPCs or the growth

factors involved in EPC recruitment and homing in people who have sickle cell disease remain limited. Van Beem[51]showed the presence of increased numbers of circulating EPCs (expressing CD34 and VEGFR2) in adults with Hemoglobin SS or Sβ0-thalassemia during painful crisis, but there was no difference between asymptomatic adults with sickle cell disease and healthy controls.

According to findings of Duits *et al* [52], it has been found that the higher number of circulating EPCs during painful crisis to be associated with increased serum levels of erythropoietin, soluble VCAM-1(sVCAM-1), and vascular endothelial growth factor (VEGF).It has also been reported that several angiogenic growth factors to be elevated in Hemoglobin SS. Angiopoietin (Ang)-2 and erythropoietin were higher in adults with Hemgoglobin SS compared to healthy controls and further elevated during acute painful crisis.

Some studies such as Solovey et al[53] and Niu et al[54]indicated that higher levels of vascular endothelial growth factor (VEGF) were found in subjects with Hemoglobin SS compared to controls.

According to Niu et al[54], the presence of higher VEGF levels was associated with reduced odds of elevated tricuspid valve regurgitant velocity by echocardiography in children with sickle cell disease, a noninvasive measure suggesting pulmonary artery hypertension.

Contradictory findings were reported by Landburg et al[55] in which children with sickle cell disease with elevated tricuspid regurgitant velocity had higher concentrations of platelet derived growth factor (PDGF)-BB. Furthermore, higher levels of SDF-1have been found in adults with Hemoglobin SS than controls, particularly in those who had pulmonary hypertension.

Case et al[56]. (2007) conducted a study and pointed to debate about the *in vitro* phenotype of EPCs. Circulating cells expressing hematopoietic stem cell marker CD34, vascular endothelial growth factor receptor (VEGFR)-2, and early progenitor marker CD133 were considered EPCs, but other observations pointed out to another point in which these cells were immature hematopoietic cells and did not differentiate into EPCs or form vessels.

Subramaniyam *et al.* (2009) investigated the effects of granulocytema crophage colony-stimulating factor (GM-CSF) on vascular function in adults with peripheral arterial disease. He found that treatment-induced increase in the number of circulating CD34 expressing cells correlated with clinical improvements inflow-mediated dilation and pain-free walking time which implies that undifferentiated hematopoietic cells have angiogenic potential or are a surrogate marker of vascular repair cells.

1.9 The Clinical Impact of MTHFR Polymorphism on the Vascular Complications of Sickle Cell Disease

Moreira et al[58] conducted a study to find out the possibility that the availability of the factor V gene G1691A mutation (factor V Leiden), the prothrombin gene G20210A variant, and methylene tetra hydrofolate reductase (MTHFR) C677T polymorphism could be be risk factors for vascular complications in individuals with SCD. Study sample included 53 patients with SCD, 29 with SS (sickle cell anemia; and 24 with SC (sicklehemoglobin C disease hemoglobinopathy). The data showed that one patient was heterozygous for factor V Leiden (1.8%) and there was no prothrombin G20210A variant. MTHFR 677TT polymorphism was detected in 1 patient (1.8%) and the heterozygous form 677TC was observed in 18 patients (34%, 9 with SS and 9 with SC disease). No association was detected between the presence of the MTHFR 677T allele and other genetic modulation factors, such as α-thalassemia, ß-globin gene haplo type and fetal hemoglobin, but the presence of the MTHFR 677T allele was associated with the occurrence of vascular complications in SCD. It can be concluded that MTHFR C677T polymorphism could be a risk factor for vascular complications in SCD.

1.10 Cardiovascular Autonomic Dysfunction in Sickle Cell Anemia

Wolney *et al*[59] conducted a study taking into considerations various aspects associated with sickle cell anemia (SCA) such as increased cardiac output, normal heart rate (HR), abnormal QT dispersion and lower diastolic blood pressure

(DBP). The mechanisms underlying these aspects are still unknown. The research team aimed to satisfy two objectives: the first objective is to examine the hypothesis that there is cardiovascular autonomic dysfunction (CAD) in SCA, while the second objective is to distinguish the roles of chronic anemia and hemoglobinopathy and to evaluate the predominance of the sympathetic or parasympathetic systems in the pathogenesis of CAD. Study sample included 16 subjects with SCA, 13 with sickle cell trait (SCT), 13 with iron deficiency anemia (IDA), and 13 healthy volunteers (HV). The following investigations were carried out for all subjects: 24 h-electrocardiogram (24h-ECG), plasmano-repinephrine (NE) measurement before and after isometric exercise (IE), and also Valsalva maneuver (VM), diving maneuver (DV), and tilt test (TT). The study findings did not reveal significant variations among groups for minimum, average and maximum HR as well as the percentage of bradycardia and tachycardia at 24-h ECG. No variations were also observed for NE at baseline and after IE between groups. However, the SCA group showed less bradycardia at phase IV of VM, less bradycardia during DV, and also less tachycardia and lower DBP during TT. Taken together, there is CAD in SCA, and it is characterized by the limitation of HR modulation mediated by the parasympathetic system, cardiovascular sympathetic activity is preserved in SCA and hemoglobinopathy is the preponderant ethiopathogenic factor.

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