INTRODUCTION

Sickle cell disease (SCD) is a hereditary hemoglobinopathy characterized by abnormal hemoglobin production, hemolytic anemia and intermittent occlusion of small vessels, leading to acute and chronic tissue ischemia, chronic organ damage, and organ dysfunction ***(Smiley et al., 2008)***.

Common manifestations include vaso-occlusive crises, the result of hypoxic injury or infarction, which may affect the brain, pulmonary vessels, spleen, bone marrow, kidney, retina, penis or other tissues resulting in sequelae such as acute chest syndrome with pulmonary infarction, stroke or splenic infarction which ultimately contribute to increased susceptibility to infections and increased risk of meningitis, the most common causes of death in children with SCD ***(Crane and Bennett, 2011)***.

The most common variations of SCD include sickle cell anemia (HbSS), hemoglobin S-beta thalassemia (HbSβ), sickle cell-hemoglobin C disease (HbSC) and sickle cell hemoglobin E disease (HbSE). Symptoms and signs of SCD usually begin in early childhood and its severity varies from mild symptoms to hospitalization with serious complications ***(Stuart and Nagel, 2004)***.

Sickle hemoglobin (hemoglobin S [HbS]) results from a single-nucleotide polymorphism (SNP; rs334) of the *HBB* gene encoding the β-globin chain, leading to an aminoacidic substitution from glutamic acid to valine (sixth codon: GAG → GTG). Individuals with the SS genotype have sickle cell anemia, a highly lethal condition ***(Rees, 2010)***.

Although the causal mutation of SCD is known, the sources of clinical variability of SCD remain poorly understood, with only a few highly

heritable traits associated with SCD having been identified ***(Quinlan et al., 2014)***. Inter-individual clinical variation likely reflects a combination of the effects of several factors including haplotypic variation in the β-globin locus region, the action of genetic modifiers elsewhere in the genome, and a wide range of environmental factors ***(Weatherall, 2001; Sankaran et al., 2010)***. Phenotypic heterogeneity in the clinical expression of SCD is problematic for follow-up, management, and treatment of patients ***(Quinlan et al., 2014)***.

Single nucleotide polymorphisms (SNPs) in several genes of the TGF-beta/BMP superfamily, and some other genes linked to the endothelial function, and nitric oxide biology are associated with the subphenotypes of stroke, osteonecrosis, priapism, leg ulcers, pulmonary hypertension, and a more general measure of overall disease severity. Genetic association studies can have immediate prognostic value;

they might also help to identify new pathophysiological pathways that could be susceptible to modulation. The relation between hemoglobin genotypic variants and vascular dysfunction in SCD has not been explored ***(Steinberg et al., 2009)***.

Carotid intima media thickness (CIMT) is a surrogate marker of atherosclerosis and provides a non-invasive method for the risk assessment of cardiovascular disease ***(Oren et al., 2003)***. CD163 and platelet microparticles are two established markers of vascular dysfunction among patients with SCD ***(Tantawy et al., 2012; Tantawy et al., 2013)***. CD163 is a member of the scavenger receptor cysteine-rich family of proteins. It is expressed on cells of monocyte–macrophage lineage and is the main hemoglobin–haptoglobin (HbHp) receptor. Increased sCD163 levels in children with SCD and their trait siblings may explain some of the clinical variability, which characterizes this disease and

are related to the clinical condition of the patient ***(Tantawy et al., 2012)***.

MPs are intact vesicles derived from blebbing and shedding from cell membrane surfaces following activation or apoptosis and vary in size from 0.2 to 2.0 mm. They are present in low concentrations in normal plasma. Platelet-derived MPs (PMPs) are the most abundant, representing about 70–90% of all circulating MPs ***(Owens and Mackman, 2011)***.

AIM OF THE WORK

The aim of this study was to assess the interrelation between different genotypes among children and adolescents with SCD and vascular markers (soluble CD163 and platelet microparticles) as well as vascular complications and carotid intima media thickness as an index for subclinical atherosclerosis.

Chapter 1
SICKLE CELL DISEASE

Definition

Sickle cell disease is a hereditary hemoglobinopathy characterized by abnormal hemoglobin production, hemolytic anemia and intermittent occlusion of small blood vessel leading to acute and chronic tissue ischemia, chronic organ damage and organ dysfunction ***(Smily et al., 2008).***

Incidence

Sickle cell disease affects millions of people worldwide. It is most common among people whose ancestors come from Africa, Mediterranean countries such as Greece, Turkey and Italy, the Arabian Peninsula, India and Spanish-speaking regions in South America, Central America and parts of the Caribbean (**Figure 1)** ***(Powars et al., 2005).***

Approximately 1 in every 400 to 500 African American has sickle cell disease ***(Miinauskas et al., 2000).*** An estimated 80, 000 Americans have the disease and about 9% of African Americans have the trait. 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 1000 new cases yearly ***(Cipolotti et al., 2000).***

Sickle cell disease (SCD) is not frequent in Egypt except in the Oases where the carrier rate varies from 9 to 22% ***(El-Beshlawy and Youssry, 2009).***

The sickle gene has a genetic advantage that, it protects heterozygous carriers from succumbing to endemic plasmodium falciparum malaria infection ***(Nagel and Steinberg, 2001a).***

Geographic distribution

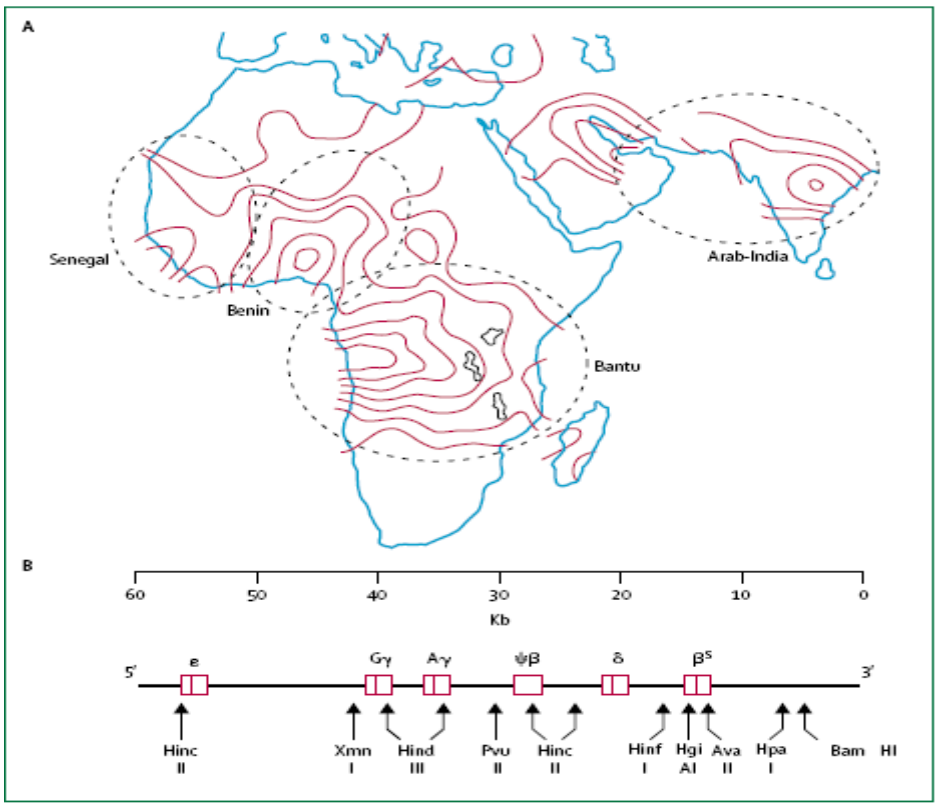

Figure (1): Geographical distribution and schematic representation of the sickle gene. (A) Map identifies the three distinct areas in Africa and one in the Arab- India region where the sickle gene is present (dotted lines). Numbers of individuals with sickle-cell disease (red lines) in Senegal, Benin and Bantu are higher near the coast and falls concentrically inland. (B) The β-globin gene cluster haplotype is determined by DNA polymorphic sites (boxes) that are identified by endonuclease enzymes. With this information, haplotypes are constructed as shown ***(Nagel and Steinberg, 2001b).***

Pathophysiology:

HbS is caused by a mutation in the B-globin in which the 17 th nucleotide is changed From thymine to adenine and the sixth amino acid in the B-globin chain becomes valine instead of glutamic acid. This mutation produces a hydrophobic motif in the deoxygenated Hbs tetramer that results in binding between B1 and B2 chains of two hemoglobin molecules. this crystallization produces a polymer nucleus, which grows and fills the erythrocytes, disrupting its architecture and flexibility and promoting cellular dehydration, with physical and oxidative cellular stress ***(Rees et al., 2010).***

The main determinants of disease severity is the rate and extent of Hbs polymerization, which is affected by co-inheritance of genetic factors of co-inherited Alpha-thalassemia or hereditary persistence of Hb F ***(Rees et al., 2010).***

The manifestations of SCD are driven by two processes vaso-occlusion with ischemia-reperfusion injury and hemolytic anemia.

First, actual vaso-occlusion is caused by entrapment of erythrocytes and leucocytes in the circulation causing vascular obstruction and tissue ischemia, Although this process reguires Hbs polymerization, the event that triggers the vascular obstruction by sickled erythrocytes is often inflammatory of Cycles of experimental hypoxia or treatment with inflammatory drugs increases endothelial leucocytes erythrocyte adhesive interactions in the post capillary venules and start vascular

occlusion in transgenic mice ***(Osarogiagbon et al., 2000; Frenette, 2002).***

In addition to inflammatory triggers, pre capillary obstruction by rigid, deformed erythrocytes with high HbS polymer content also contributes to micro vascular vaso - occlusion. Vascular occlusion is the result of a dynamic interaction between erythrocytes and the vascular endothelium, resulting in episodic micro vascular occlusion and ischemia, followed by restoration of blood flow which further promotes tissue injury mediated by reperfusion. These cycles of ischemia and reperfusion cause oxidant stress, with activation of vascular oxidases ***(Wood et al., 2005)*** and inflammatory stress, increasing synthesis of inflammatory cytokines, and can cause leucocytosis ***(Rees et al., 2010).***

Bone marrow infarction leading to fat embolization might also contribute to vascular occlusion, particularly in the lungs, where it causes acute chest syndrome ***(Rees et al., 2010).***

The second pathophysiological process in SCD is hemolysis caused by HbS polymerization. Hemolysis causes anemia, fatigue and cholelithiasis, moreover there is now evidence that it contributes to the development of progressive vasculopathy; as patients with SCD are at risk of vasculopathy that characterized by systemic and pulmonary hypertension, endothelial dysfunction and proliferative changes in the intima and smooth muscle of blood vessels ***(Juno et al., 2006).***

The release of Hb into the plasma during hemolysis inhibits endothelial nitric oxide (NO) signaling leading to endothelial cell dysfunction and NO resistance; also it generates reactive oxygen species (ROS) such as the hydroxy and superoxide radical which is a potent scavenger of NO that is normally produced by the endothelium and regulates basal vasodilator tone and inhibits platelet and hemostatic activation and transcriptional expression of nuclear factor RB (T KB) dependent adhesion molecules such as vascular cell adhesion molecule-l, intercellular cell adhesion molecule-I and the selectins ***(Rees et al., 2010).***

Hemolysis also releases erythrocyte arginase- 1 into plasma. Arginase metabolites plasma arginine into ornithine, decreasing the required substrate for NO synthesis and compounding the decreased bioavilability of it in patients with SCD ***(Monis et al., 2005).***

Clinical spectrum of SCD

The clinical spectrum of SCD ranges from acute episodes to chronic organ damage. It includes anemia, repeated infections and periodic episodes of pain. The severity of symptoms varies; some people have mild symptoms, while others are frequently hospitalized for more serious complications. Although SCD is present from birth, symptoms are rare before the age of three to six months, due to the persistence of HbF ***(Heeney and Dover, 2009).***

Forms of sickle cell disease:

The term 'sickle cell disease' is often used to include sickle cell anemia and other conditions in which a clinically significant disorder results from sickle cell formation and the associated pathological processes.

1- Sickle cell anemia (SS disease) is considered the prototype of the sickle cell diseases and it is the most severe of these disorders.

2- Heterozygosis for hemoglobin S (ββs), referred to as sickle cell trait, is usually asymptomatic.

3- Hemoglobin SC disease and sickle cell β-thalassemia tend to be milder.

4- Hemoglobin SD disease is the mildest of the group.

However, there is a great deal of overlap in the severity of the clinical manifestations of these disorders. A major difference among these diseases is in their laboratory diagnosis ***(Bain, 2000).***

Table (1): Causes of Sickle cell disease ***(Bain, 2000)***

Sickle cell anemia (homozygosity for hemoglobin S)
• ***Compound heterozygous states***
- *Sickle cell/ hemogolobin C disease*
- *Sickle cell/β thalassemia*
- *Sickle cell/ hemogolobin D-Punjab*
- *Sickle cell/ hemogolobin C-Harlem*
- *Sickle cell/ hemogolobin S-Antilles*
- *Sickle cell/ hemogolobin O-Arab*
- *Hemogolobin S-Antilles/ hemogolobin C*
- *Sickle cell/ hemogolobin Lepore*
- *Hemogolobin C/ Hemogolobin C- Harlem*

Hb SS disease (homozygous sickle cell disease):

Hb SS disease results from homozygosity for the βs gene. The red cells in sickle cell anemia contain large amount of Hb S, variable amount of Hb F and normal or slightly elevated level of Hb A2. The sickle cells are fragile, non deformable and rapidly destroyed. The clinical and hematological manifestations of sickle cell disease are due to these processes: severe hemolysis, vaso occlusion and infarction involving many tissues and organs. The development of hemolytic anemia parallels the declining level of Hb F postnataly and becomes obvious by 4 months of age ***(Lane et al., 2002).***

Clinical manifestations of sickle cell disease:

The newborn infant is protected by the high level of fetal hemoglobin in the red cells during the first 8 to 10 weeks of life. 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 ***(Fields, 2002)*.**

Low-percentage HbF is associated with a higher risk of developing vaso-occlusive complications organ damage, and early death ***(Russel and Ware, 2010)*.**

The clinical manifestations of sickle cell disease are quite variable but all are ultimately related to hemolytic anemia and vascular occlusion. Acute, painful vaso-occlusive crises are the most common and earliest clinical manifestations of sickle cell anemia. Half of all patients with sickle cell anemia experience a painful crisis by 4-9 years of age. The pain is usually described as bone pain, although crises may involve virtually any organ. They are presumed to be caused by microvascular occlusion with subsequent tissue ischemia. In young children vaso occlusive crises most commonly manifest as dactylitis, a painful swelling of the hands, fingers, feet and toes. Other problems in sickle cell anemia include osteomyelitis,

osteonecrosis, splenic infarct, splenic sequestration, acute chest syndrome, stroke, papillary necrosis and renal insufficiency ***(Yaster et al., 2000)***.

Clinical features may be divided into:

I- Chronic organ affection in sickle cell anemia:

a. Growth and development:

Impaired growth is common in homozygous SCD. There are early deficiencies in height and weight and delays in the onset of puberty ***(Thomas et al., 2000).***

Considerable growth occurs in late adolescence so that adults with sickle cell anemia are at least as tall as normal. Growth hormone (GH) deficiency may be associated with growth failure in some patients with SCD. These patients may benefit from treatment with GH ***(Nunlee-Bland et al., 2004).***

b. Bony abnormalities:

The skeletal system of patients with sickle cell anemia is remarkable for its life long preservation and frequent expansion of red (cellular) marrow throughout life. In patients with sickle cell anemia, most of their marrow space tends to be preserved as red marrow, sometimes even in their epiphyses. Expansion of the medullary space (due to increased hematopoietic demands from the anemia) may be especially evident in the skull, where a hair-on-end appearance may result from diploic space widening **(Figure 2).** Persistence of red marrow may make detection of marrow abnormality, such as infarction and