INTRODUCTION

Sickle cell disease (SCD) is a chronic hemolytic anemia is caused by a variant of the β-globin gene called sickle hemoglobin (*Hb S*). Individuals with sickle cell disease exhibit significant morbidity and mortality ***(Koch et al., 2000).***

The SCD is most common among people from Africa, India, the Caribbean, the Middle East, and the Mediterranean ***(El-Hazmi et al., 2011).***

Sickle cell disease may result from coinheritance of the *HbB* hemoglobin S mutation with a second *HbB* mutation associated with another abnormal hemoglobin variant including Hemoglobin C "sickle-hemoglobin C disease (Hb SC)",β-thalassemia mutations "$S\beta^{+}$-thalassemia and Sβ°-thalassemia", Hemoglobin D, Hemoglobin O ***(Bender, 2012).***

In sickle-cell disease, haemoglobin polymerisation, leading to erythrocyte rigidity and vaso-occlusion, is central to the pathophysiology of this disease, although the importance of chronic anaemia, haemolysis, and vasculopathy has been established ***(Rees et al., 2010)***

Recurrent episodes of vaso-occlusion and inflammation result in progressive damage to most organs, including the brain, kidneys, lungs, bones, and cardiovascular system, which becomes apparent with increasing age ***(Rees et al., 2010)***

Most patients with sickle cell anemia develop abnormal pulmonary function characterized by airway obstruction, restrictive lung disease, abnormal diffusing capacity, and hypoxemia ***(Koumbourlis et al., 2001).***

Sickle cell patients with pulmonary hypertension have a significantly increased mortality rate compared with sickle cell patients without pulmonary hypertension ***(Sutton et al., 1994).***

Previous studies of sibling pairs have demonstrated a genetic component to the development of cerebrovascular disease in SCD, but few candidate genetic modifiers have been validated as having a substantial effect on stroke risk ***(Flanagan et al., 2013)***.

There is a correlation between Genotype and Phenotype of SCD; individuals with Hb SS and Sβ°-thalassemia are generally more severely affected than individuals with Hb SC or Sβ+-thalassemia ***(Steinberg and Adewoye, 2006).***

α-thalassemia improves red cell survival and decreases hemolysis in the sickle cell disease syndromes. However, the clinical effect on SCD is unclear and can be variable including possible decreased complications arising from hemolysis and potentially increased complications from vaso-occlusive events ***(Steinberg, 2005).***

AIM OF THE WORK

The aim of the present study is to study the genotype of patients with sickle cell disease attending the Pediatric Hematology Unit in relation to the different clinical presentations of the disease, the episodes of vasocclusive crises and the occurrence of morbidties and mortality.

SICKLE CELL

Sickle cell disease (SCD) is a wide spread inherited hemolytic anemia that is due to a point mutation leading to a valine/glutamic acid substitution in the β globin chain, causing a spectrum of clinical manifestations in addition to hemolysis and anemia ***(Mousa and Qari, 2010).***

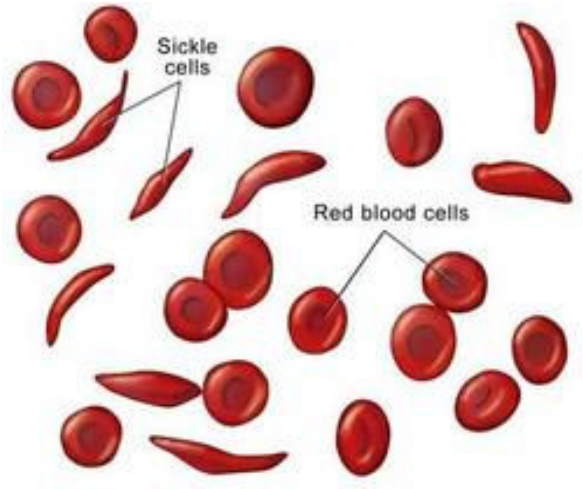

Figure (1): The difference between the sickle cell and the normal red blood cell ***(www.vanguardngr.com)***

The term sickle cell disease refers to all clinical and hematological significant sickling disorders; including homozygous sickle cell anemia (HbSS), HbSB-thalassemia, HbSC, HbSD, HbSO and HbSE. Most of the non (HbSS) produce less severe manifestations that HbSS ***(Quirolo and Vichisnsky, 2004).***

Incidence and geographic distribution:

The inherited disorders of hemoglobin are the most common gene disorders, and it is estimated that 7% of the

world's population are carriers. Approximately 300.000 children world wide are born with documented sickle cell disease every year. Sickling disorders are found frequently in the Afro-Caribbean populations and sporadically throughout the Mediterranean regions, India, and the middle East ***(Jeremiah, 2006).***

Four region-specific African haplotypes (the Senegal, Benign, Bantu, and Cameron haplotypes) and one Asian haplotype (the Arab-India haplotype) have been defined ***(Rees et al., 2010).***

There is a close geographic correlation between the frequency of the HbS gene in populations and the historic incidence of malaria ***(Rees et al., 2010)*** With evidence for the partial resistance of carriers to all forms of plasmodium falciparum malaria ***(Williams et al., 2005; May et al., 2007).*** The mechanism of this protection is probably due to both innate and immune mediated mechanisms ***(Wellems et al., 2009)***.

In Egypt, along the Nile Valley, the HbS gene is almost non existent, but in the western desert near the Libyan border variable rates of 0.38 per cent in the coastal areas to 9.0 per cent in the New Valley oases have been reported. HbS carrier rates vary from 9 to 22 per cent in some regions ***(El-Beshlawy et al., 2009)***.

Pathophysiology of SCD:

HbS is caused by a mutation in the β-globin gene in which the 17th nucleotide is changed from thymine to adenine and the sixth amino acid in the β-globin chain becomes valine instead of glutamic acid ***(Rees et al., 2010).***

This mutation produces a hydrophobic motif in the deoxygenated HbS tetramer that results in binding between Beta$_1$ and Beta $_2$ chains of two hemoglobin molecules. This crystallization produces a polymer nucleus, which grows and fills the erythrocyte, disrupting its architecture and flexibility and promoting cellular dehydration, with physical and oxidative cellular stress. The rate and extent of HbS polymerization is proportional to the extent and duration of hemoglobin deoxygenation ***(Schnog et al., 2001)***.

The manifestations of SCD are driven by two processes: vaso-occlusion with ischemia-reperfusion injury and hemolytic anemia. Acute vaso-occlusive pain is caused by entrapment of eythrocytes and leucocytes in the microcirculation, causing vascular obstruction and tissue ischemia. Although this process requires HbS polymerization, the event that triggers the vascular obstruction by sickle erythrocytes is often inflammatory ***(Osarogiagbon et al., 2000).***

Vascular occlusion is the result of a dynamic interaction between erythrocytes and the vascular endothelium, resulting in episodic microvascular occlusion and ischemia, followed by restoration of blood flow which further promotes tissue injury mediated by reperfusion. ***(Wood et al., 2005)***

These cycles of ischemia and reperfusion cause oxidant stress, with activation of vascular oxidases and inflammatory stress, increasing synthesis of inflammatory cytokines, and can cause leucocytosis ***(Frenette, 2002).***

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 sickle cell disease is hemolytic anemia, which is caused by HbS polyermization. Hemolysis has long been known to cause anemia, fatigue and cholelithiasis, but there is now evidence that it contributes to the development of progressive vasculopathy ***(Steinberg et al., 2003)***.

As RBCs are cycled through the spleen, the site of their ultimate destruction, they also injure the tissue of the spleen. Their rigidity impairs their ability to flow smoothly through the sinusoids, and their sharp edges causes them to be stuck, and to damage splenic tissue ***(Roseff, 2009).***

As patients with sickle cell disease age, they are at risk of vasculopathy, characterized by systemic and pulmonary hypertension, endothelial dysfunction and proliferative changes in the intima and smooth muscle of blood vessels ***(Kato et al., 2006).***

An important disease mechanism involves the release of hemoglobin into the circulation during intravascular hemolsyis. Free plasma hemoglobin generates reactive oxygen species, such as the hydroxy and superoxide radical, which is a potent scavenger of nitric oxide. Nitric oxide is normally produced by

the endothelium and regulates basal vasodilator tone, and inhibits platelet and hemostatic activation ***(Rees et al., 2010).***

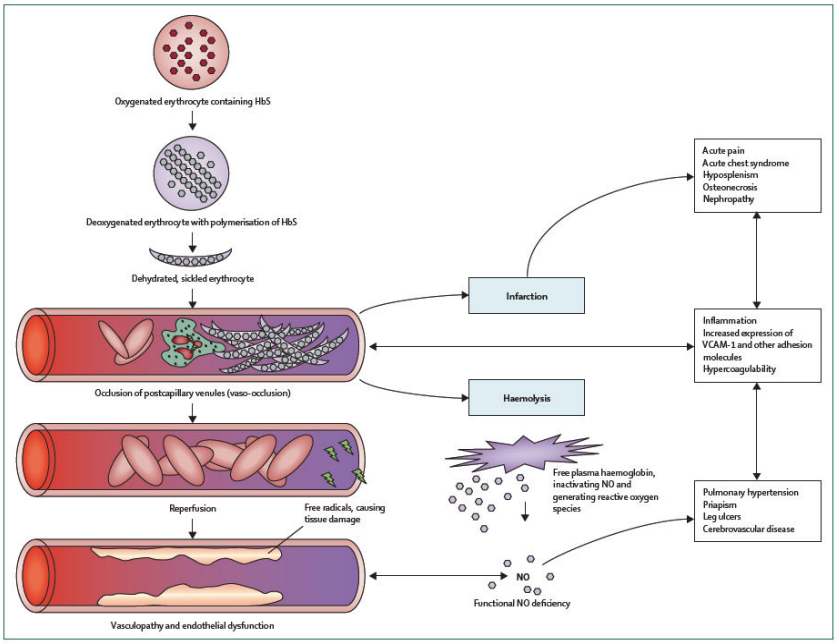

Figure (2): The pathophysiology of sickle-cell disease. The roles of HbS polymerisation, hyperviscosity, vaso-occlusion, hemolysis, and endothelial dysfunction are shown. Deoxygenation causes HbS to polymerise, leading to sickled erythrocytes. Vaso-occlusion results from the interaction of sickled erythrocytes with leucocytes and the vascular endothelium. Vaso-occlusion then leads to infarction, hemolysis, and inflammation; inflammation enhances the expression of adhesion molecules, further increasing the tendency of sickled erythrocytes to adhere to the vascular endothelium and to worsen vaso-occlusion. Reperfusion of the ischaemic tissue generates free radicals and oxidative damage. The damaged erythrocytes release free haemoglobin in to the plasma, which strongly bind to nitric oxide, causing functional nitric oxide deficiency and contributing to the development of vasculopathy. HbS= Sickle haemoglobin. NO= Nitric oxide. VCAM= Vascular cell-adhesion molecule ***(Rees et al., 2010)***.

Hemolysis also releases erythrocyte arginase-1 into plasma. Arginase metabolites plasma arginine into ornithine, decreasing the required substrate for nitric oxide synthesis and compounding the decreased bioavailability of nitric oxide in patients with SCD ***(Morris et al., 2005).***

Studies have shown correlations between the rate of hemolsyis and levels of platelets activation and procoagulant factor sin the blood ***(Ataga et al., 2008).***

Increased platelet activation has been described in patients with SCD, a phenomenon that may contribute to the hemostatic activation observed in SCD.It is the result of the proinflammatory and prothrombotic characteristics of the microvasculature in SCD ***(Tomer et al., 2001).***

The novel link between platelet activation and severity of pulmonary hypertension in SCD is identified. Pulmonary Hypertension is a chronic vasculopathy recognized as the greatest mortality risk factor in the adult population with SCD. Its origin is likely multifactorial ***(Gladwin et al., 2004).*** but its pathophysiology, characterized by increased vascular tone, vascular proliferation, and in situ thrombosis, is ascribed in part to a hemolysis-associated impairment of NO bioavailability ***(Ataga et al., 2008)***.

Activated platelets conceivably might play a direct role in the development of these thrombi, which worsen pulmonary hypertension. Furthermore, their degree of activation might be a marker for ongoing prothrombotic activity and risk of pulmonary thrombi ***(Tomer, 2004).***

Clinical manifestations:

The clinical manifestations of sickle cell disease (SCD) result from intermittent episodes of vascular occlusion and variable degrees of hemolysis. The severity of disease manifestations varies from severe to minimal, even in individuals with the same HBB mutation status ***(Wierenga et al., 2001).***

- ***Hemolytic anemia:***

The marrow is very active, as evidenced by immature RBCs or reticulocytes in the peripheral blood. As the marrow increases production of RBCs, platelet and WBCs production also increases ***(Serjeant et al., 2007; Telen, 2007).***

In HbSS, the full blood count reveals haemoglobin levels in the range of 6–8 g/dL with a high reticulocyte count. In other forms of sickle-cell disease, Hb levels tend to be higher. A blood film may show features of hyposplenism (target cells and Howell-Jolly bodies). ***(Clarke et al., 2000).***

The individuals are usually asymptomatic at Hb 6–8 g/dL levels because of a right shift in the oxygen dissociation curve due to HbS that enhances oxygen delivery to the tissues ***(McMahon, 2006).***

An acute precipitous drop in hemoglobin can occur in sickle cell disease and lead to cardiac failure and death. The main reasons for this are acute splenic sequestration, hyperhomolysis or parvovirus B19 infection ***(McMahon, 2006).***

- ***Vasooclusive events:***

Common manifestations of sickle cell disease 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 acute chest syndrome with pulmonary infarction, stroke or splenic infarction ***(Crane et al., 2011).***

Vasooclusive episodes account for over 90% of all emergency hospital admissions and will most likely result in the patient's death either because of acute organ failure or chronic organ damage. Factors that may precipitate sickling and vasooclusion include hypoxia, dehydration and cold weather ***(Bender et al., 2012).***

1. Bone and joints:

a. Dactylitis (hand-foot syndrome)

Dactylitis is caused by vasooclusion and necrosis affecting the bone marrow and inner third of the cortex of the small bones of the hands or feet, it occurs most frequently in children aged 6 months to 2 years old. It presents as an acute painful swelling of the dorsum of the hands or feet and extends into the fingers and toes. It is usually associated with fever. It can take at least 1 week to resolve and tends to recur ***(Serjeant, 2001).***

Radiologic bone abnormalities may take 2 weeks to appear and include periosteal reaction, translucency or opacity of the diaphysis, and occasional bone loss. Recurrent events can

lead to bone deformity or early fusion of the epiphysis resulting in shortened digits. It is suggested that an episode of dactylitis in the first 2 years of life may predict severe manifestations of sickle cell disease in later life ***(Miller et al., 2000).***

Infection should be considered if the symptoms and signs do not resolve with conservative management ***(McMahon, 2006).***

b. Chronic bone disease:

Osteonecrosis or avascular necrosis is painful and disabling. It can occur with all types of SCD but is most common in those with HbSS/α thalassemia. The incidence is 2.5 per 100 patients years for hip and shoulder joints and a vascular necrosis of the femoral head has been described in children as young as 5 years of age. The cause of a vascular necrosis is unclear but may be due, in part, to microinfarction of cancellous bone ***(Miller et al., 2000).***

2. Abdomen:

- **Abdominal painful crisis:**

It usually affects younger children. The pain may be mild and transient to severe and generalized, sometimes with features of intestinal obstruction. Radiologic investigation has, in some cases, found a localized non functioning segment of bowel that recovers function after 3-4 days of conservative management, but often there is no apparent causes ***(Serjeant et al., 2001).***

- **Acute hepatic necrosis:**

Acute hepatic necrosis and failure can occur in the absence of viral liver disease and in association with other sickle cell related vasooclusive events. Exchange transfusion can bring about a rapid improvement in clinical and biochemical parameter ***(McMahon, 2006).***

- **Hepatic sequestration**

Acute hepatic sequestration is a rarely recognized complication of VOC. This syndrome is characterized by a rapidly enlarging liver accompanied by a decrease in hemoglobin/ hematocrit and a rise in reticulocyte count. The liver is smooth and variably tender. The bilirubin may be as high as 24mg/dl with a predominance of the conjugated fraction. The alkaline phosphatase can be as high as 650 IU/L but can be normal. The transaminases are only minimally elevated and often are normal. Recurrence is common ***(Davies et al., 1987).***

Ultrasonography and CT scanning show only diffuse hepatomegaly. Liver biopsy shows massively dilated sinusoids with sickle erythrocytes and Kupffer cell erythrophagocytosis. Intrahepatic cholestasis with bile plugs in canaliculi can occur. The pathophysiology is believed to be obstruction of sinusoidal flow by the masses of sickled erythrocytes, trapping of red cells within the liver and compressing the biliary tree. On the other hand, the pathophysiology could be similar to that of in splenic

sequestration, postulating obstruction at the smaller hepatic veins and/or sinusoids ***(Roshkow et al., 1990).***

Successful resolution of hepatic sequestration has been seen with simple or exchange transfusion, as well as with supportive care alone ***(Lee et al., 1996).***

Acute hepatic failure has been reported in several cases where massive hepatic necrosis was seen in the absence of markers for viral hepatitis ***(McMahon, 2006)***

- **Splenic sequestration:**

Acute splenic sequestration is a major cause of mortality in children with SCD. It is caused by intrasplenic trapping of red cells and is defiend as a hemoglobin decrease of at least 2 gm/dl associated with markedly elevated reticulocyte count and acutely enlarging spleen. The attacks are often associated with viral or bacterial infection. Sequestration can occur in children as young as 5 weeks but is seen most often in children between the ages of 3 months and 5 years it is also reported in older children and adults with HbSC and HbSS/β^+-thalassemia and patients not receiving disease modifying therapy such as chronic blood transfusion possibly because these treatment modalities delay splenic fibrosis ***(Bender et al., 2012).***

- **Renal complications**

Chronic sickling underlies several mechanisms for kidney injury. The arterial side of the renal microvasculature has a low O_2 tension. The hypertonicity and low pH of the renal

medulla promote the formation of hemoglobin polymers in the red cells with deformation of the sickled cells, resulting in an increase in the blood viscosity, functional venous engorgement, and interstitial edema, predisposing the renal microcirculation to ischemia and infarction ***(Van and de Jong, 1997).***

a) Hematuria:

Asymptomatic hematuria is one of the most prevalent features of the disease and occurs either in heterozygous or homozygous patients at any age. Gross hematuria may also be observed in patients with sickle cell trait, either alone or in combination with von Willebrand disease, even in the absence of extrarenal bleeding ***(Ter Maaten et al., 2007)***

Hematuria is usually unilateral with the left kidney four times more frequently involved than the right. This may be explained by increased venous pressure due to the greater length of the left renal vein**.** Most of the episodes are self-limited, although dramatic and prolonged periods of gross hematuria may be seen ***(Van and de Jong, 1997).***

b) Proteinuria:

Proteinuria is a frequent finding in SCD, and is present in 30% of patients during long-term follow-up ***(Van and de Jong, 1997).*** Both proteinuria and renal insufficiency increase with age in a parallel pattern ***(Scheinman, 2009).***

Glomerular hyperfiltration and tubular dysfunction also occur, and are possibly associated with anemia and increased