INTRODUCTION

Sickle cell disease (SCD) is a class of hemoglobinopathy, which results from a single mutation in the B-globin chain inducing the substitution of valine for glutamic acid at the sixth amino acid position. This mutation leads to the production of abnormal hemoglobin (hemoglobin S [HbS]). In addition to homozygous sickle cell disease (HbSS), other forms such as HbSC and HbSb-thalassemia also exist ***(Stuart and Nagel, 2004)***.

Sickle cell anemia (SCA) is expressed as chronic hemolytic anemia and a large variety of vaso-occlusive phenomena and their consequences, proliferative vasculopathy, and a predisposition to infections, leading to very high early morbidity and mortality rates ***(Belcher et al., 2010)***.

Patients with sickle cell disease exhibit numerous kidney structural and functional abnormalities, changes that are seen along the entire length of the nephron. Changes are most marked in patients with homozygous sickle cell anemia, but are also seen in those with compound heterozygous states and the sickle cell trait. The renal features of sickle cell disease include some of the most common reasons for referral to nephrologists, such as hematuria, proteinuria, tubular disturbances and chronic kidney disease ***(Guasch et al., 2006)***.

Fas (also known as Apo-1 or CD95), is a member of the tumor necrosis factor (TNF) receptor/nerve growth factor receptor family and transduces an apoptotic signal by activating a cascade of interleukin-1B-converting enzyme (ICE)-like cysteine proteases (caspases) ***(Nagata, 1997)***. Fas is a 45-kDa type I transmembrane protein that also exists in soluble forms ***(Nagata and Goldstein, 1995)***.

Fas ligand (FasL) is a 40-kDa type II transmembrane protein that is expressed in activated T cells and natural killer cells ***(Hasegawa et al., 1998)*** and on inflammatory cells ***(Nagata and Goldstein, 1995)***. Fas ligand, which exists in two forms-membrane-bound or secreted- and binds to a surface receptor called Fas on target cells and induce apoptosis ***(Hasegawa et al., 1998)***. It can be shed in a soluble form by the action of metalloproteinases ***(Suda, 1997; Krammer, 1999)***.

Fas/FasL may be considered a new target for therapeutic intervention in renal injury ***(Fleck et al., 1998)***. Inflammation, endothelial dysfunction, accelerated atherosclerosis and enhanced apoptosis are features characteristic for chronic kidney disease (CKD). CKD patients suffer from chronic inflammation, facilitating both Fas and FasL overexpression. Thus, the enhanced Fas–FasL binding, activating the extrinsic death receptor pathway, leads to increased apoptosis in the course of CKD ***(Stoneman and Bennett, 2009; Kornmann et al., 2000)***.

AIM OF THE WORK

The aim of this work is to determine levels of Fas, Fas ligand and their ratio in young patients with sickle cell disease and assess their relation to sickle vasculopathy including kidney disease.

Chapter One

SICKLE CELL DISEASE

Sickle cell disease (SCD) is a wide spread hemolytic anemia that is due to a single base pair change, thymine for adenine, at the 6^{th} codon of the β-globin gene. These disorders include sickle cell anemia (SS), the sickle beta thalassemia syndromes (S β^{+} or S β^{0}), and hemoglobinopathies in which Hb S is present in combination with another variant hemoglobin causing a spectrum of clinical manifestations in addition to hemolysis and anemia. SCD may lead to various acute and chronic complications, several of which have a high mortality rate ***(Mousa and Qari, 2010; Yawn et al., 2014).***

SCD is one of the most prevalent genetic disorders. There are more than 200 million carriers of sickle cell trait worldwide ***(Inati et al., 2008).*** SCD is a serious and life-threatening disease that affects approximately 1 in 600 African Americans ***(Rees et al., 2010).***

Historical review

The first description of SCD, published in 1910, was followed by six decades of genetic, hematologic, pathologic, clinical and molecular observations. Since the mid-1970s, two longitudinal prospective studies of children with SCD have produced a large body of clinical data on the evolution of the disease from birth ***(DeBaun et al., 2012).***

Herrick was the first to discover sickle cell hemoglobin (Alpha2 Beta-S2) with sickle-shaped erythrocytes. In 1910, he described the case of a young black student from the West Indies with severe anemia characterized by "peculiar elongated and sickle-shaped red blood corpuscle (Figure1)." Herrick also noted a slightly increased volume of urine of low specific gravity and thus observed the most frequent feature of sickle cell nephropathy: inability of the kidney to concentrate urine normally ***(Herrick, 2001).***

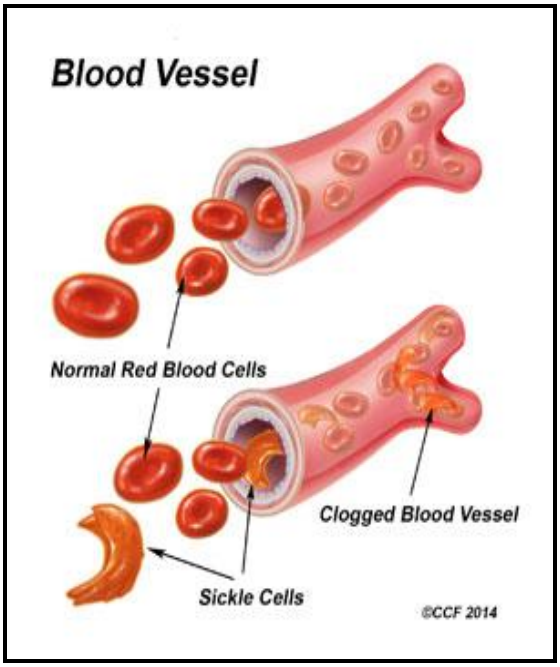

Figure (1): Shape of normal RBCs and sickled RBCs ***(Herrick, 2001).***

The chronological order of the important discoveries in SCD is shown in (Table 1).

Table (1): Important discoveries in the pathological and clinical features of sickle cell disease in chronological order

	Discovery	Importance
1910	Sickled erythrocytes in Grenadan dental student ***(Herrick, 1910).***	First description of disease linked to abnormal erythrocytes.
1924	Haemolysis in sickle-cell disease ***(Sydenstricker, 1924).***	Explanation for anaemia, jaundice, and cholelithiasis.
1924	Vaso-occlusion as cause of some pathological features ***(Graham, 1924).***	Explanation for ischemic tissue damage.
1948	No symptoms in infants noted ***(Watson et al., 1948).***	Beneficial effects of high concentrations of fetal hemoglobin identified.
1949	Abnormal electrophoretic mobility of sickle hemoglobin ***(Pauling et al., 1949).***	Identified pathophysiology to have a molecular basis.
1951	Characteristics of polymerization of deoxygenated HbS ***(Perutz et al., 1951).***	Primary molecular mechanism identified.
1980s	Value of penicillin in young children with sickle-cell anaemia ***(John et al., 1984; Gaston et al., 1986).***	Reduced mortality, role of neonatal screening.
1984	Bone marrow transplant in child with sickle-cell anemia and leukaemia ***(Johnson et al., 1984).***	Identified potential cure.
1995	Efficacy of hydroxycarbamide ***(Charache et al., 1995).***	Only disease-modifying drug identified.
1998	Reduced stroke incidence in children with abnormal transcranial dopplers who were given blood transfusion ***(Adams et al., 1998).***	Primary stroke prevention with fall in stroke occurrence.

(Rees et al., 2010)

Epidemiology of SCD:

No sex predilection exists, since sickle cell anemia (SCA) is not an X-linked disease. About 7% of the world's population carries genes responsible for hemoglobinopathies ***(WHO, 2010)***, about 2.3% for SCD ***(Barlow, 2007)***. In the Global Burden of Disease Study 2013, the incidence of deaths

due to SCD is 176, 000 deaths up from 113, 000 deaths in 1990 ***(GBD 2013 Mortality and Causes of Death Collaborators, 2015).***

The prevalence of SCD is highest in sub-Saharan Africa which is about 80% of the global total (more than 230000 affected children are born every year in sub-saharan region). In Egypt, along the Nile Valley, the sickle hemoglobin (HbS) gene is almost non existent, but in the western desert near the Libyan border variable rates of 0.38 percent in the coastal areas to 9.0 percent in the New Valley oases have been reported ***(Mohsen et al., 2011)***. HbS carrier rates vary from 9 to 22 percent in some regions ***(El-Beshlawy and Youssry, 2009).*** By comparison, the yearly estimate of affected births in North America is 2600 and 1300 in Europe ***(Modell and Darlison, 2008)*** (Figure 2).

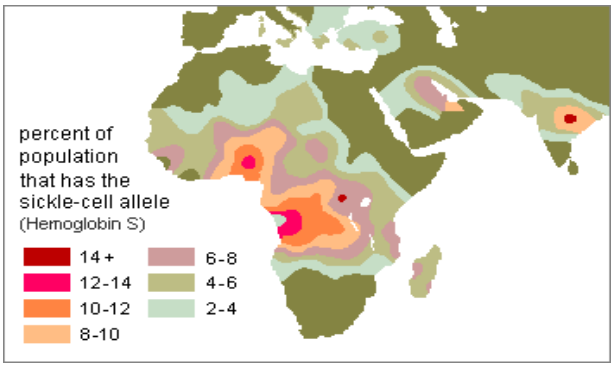

Figure (2): Percentage of population with SC allele ***(WHO, 2010).***

Four region-specific African haplotypes (the Senegal, Benign, Bantu, and Cameron haplotypes) and one Asian

haplotype (the Arab-India haplotype) have been defined, providing support for the hypothesis that the mutation causing HbS has occurred, and been locally amplified, on at least two, and possibly several separate occasions ***(Rees et al., 2010).***

This geographical distribution has probably arisen because sickle cell trait offers some protection against malaria, as sickle cell trait have an increased resistance to fatal malaria ***(Williams et al., 2005).*** Although the mechanism of this protection is yet to be fully understood, it probably includes both innate and immune mediated mechanisms ***(Wellems et al., 2009).***

Definition and genetic basis of SCD:

SCD was the first genetic disease to be characterized at the molecular level. The B-globin gene is located at the short arm of chromosome 11. The sole genetic problem in SCA is a mutation of adenine to thymine in position 2 of the 6 th Codon of β-globin gene, this change results in the substitution of glutamic acid in the 6 position of chain by valine (Figure 3) ***(Quirolo and Vichinsky, 2004; Rees et al., 2010).***

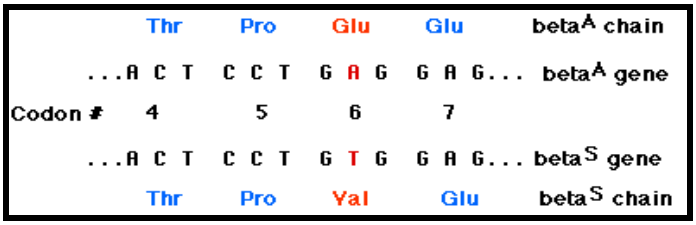

Figure (3): Amino acid position 6 substitution in beta hemoglobin chain ***(Quirolo and Vichinsky, 2004).***

The term sickle cell disease encompasses a group of symptomatic disorders associated with mutations in the hemoglobin β- globin gene (HBB gene) and defined by the presence of hemoglobin S (Hb S). SCA (homozygous Hb SS) accounts for 60%-70% of SCD in the US**.**Other forms of sickle cell disease result from coinheritance of Hb S with other abnormal β-globin chain variants, the most common forms being sickle-hemoglobin C disease (Hb SC) and two types of sickle β-thalassemia (Hb $S\beta^{+}$-thalassemia and Hb $S\beta^{\circ}$-thalassemia); rarer forms result from coinheritance of other Hb variants such as D-Punjab and O-Arab ***(Stuart and Nagel, 2004)***.

At least five different haplotypes are linked to the sickle gene, each of which can be identified based on mutation analysis in the promoter sequences of the ɣ-gamma and α-gamma globin genes. These haplotypes are named following the geographical locations where they most occur. Namely, the Arabia-Indian haplotype is characterized by significantly higher levels of fetal hemoglobin (HbF) levels and a milder course of the disease. In contrast, patients with the African haplotypes, Bantu and Bennin, express relatively lower levels of HbF with severe clinical presentations ***(Tadmouri et al., 2006).***

Heterozygous individuals are usually healthy, but they may suffer some symptoms of SCA under conditions of low blood oxygen, such as high attitude. When the co-inherited β-thalassemia gene is completely inactive (β°- thalassernia), the

resulting sickling disorder known as Sβ°-thalassernia tends to be of severity similar to that of homozygous HbSS disease. In contrast, when the co-inherited β-thalassemia gene is partially inactive active (β^+ thalassemic, the resulting sickling disorder known as S β^+ thalassemia can have a spectrum of clinical severity. If the β^+ thalassemia mutation is mild, commonly as in people of the African descent, the resulting Sβ^+ thalassemia tends to be clinically mild. In contrast, if the β^+ thalassemia mutation is severe, as commonly in the case of the Mediterranean populations, the clinical sickling disorder tends to be moderate ***(Paul and George, 2007).***

Variants of SCD:

The different types of SCD are shown in (Table 2).

Table (2): Different types of sickle-cell disease

Type	Characteristics
Severe sickle-cell disease:	
1- HbS/S (β6Glu>Val/β6Glu>Val); sickle-cell anaemia	• The most common form of sickle-cell disease
2- HbS/β^{o}thalassaemia	• Most prevalent in the eastern Mediterranean region and India.
3- Severe HbS/β^{+}thalassaemia	• Most prevalent in the eastern Mediterranean region and India; 1-5% HbA present.
4- HbS/Oarab (β6Glu>Val/β121Glu>Lys)	• Reported in north Africa, the Middle East, and the Balkans; relatively rare.
5- HbS/D Punjab (β6Glu>Val/β121Glu>Gln)	• Predominant in northern India but occurs worldwide.
6- HbS/C Harlem (β6Glu>Val/β6Glu> Val/β, β73Asp>Asn)	• Electrophoretically resembles HbSC, but clinically severe; double mutation in β-globin gene; very rare.
7- HbC/S Antilles (β6Glu>Lys/β6Glu>Val, β23Val–Ile)	• Double mutation in β-globin gene results in severe sickle-cell disease when co-inherited with HbC; very rare.
8- HbS/Quebec-CHORI (β6Glu>Val/β87Thr>Ile)	• Two cases described; resembles sickle-cell trait with standard analytical techniques.
Moderate sickle-cell disease	• 25–30% cases of sickle-cell disease in populations of African origin.
1- HbS/C (β6Glu>Val/β6Glu>Lys)	
2- Moderate HbS/β^{+}thalassaemia	• Most cases in the eastern Mediterranean region; 6–15% HbA present.
3- HbA/S Oman (βA/β6Glu>Val, β121Glu>Lys)	• Dominant form of sickle-cell disease caused by double mutation in β-globin gene; very rare.
Mild sickle-cell disease	• Mostly in populations of African origin; 16–30% HbA present.
1- Mild HbS/β^{++}thalassaemia	
2- HbS/E (β6Glu>Val/β26Glu>Lys)	• HbE predominates in southeast Asia and so HbSE uncommon, although frequency is increasing with population migration.
3- HbA/Jamaica Plain (Ba/β6Glu>Val, β68Leu/Phe)	• Dominant form of sickle-cell disease; double mutation results in Hb with low oxygen affinity; one case described.
Very mild sickle-cell disease	• Group of disorders caused by large deletions of the β-globin gene complex; typically 30% fetal haemoglobin.
1- HbS/HPFH	
2- HbS/other Hb variants	• HbS is co-inherited with many other Hb variants, and symptoms develop only in extreme hypoxia.

(Rees et al., 2010)

Pathophysiology of SCD:

1- Molecular pathogenesis:

SCA results from an A to T transversion at the sixth codon of the hemoglobin beta globin gene on chromosome 11p15.5. The mutation of a single DNA base leads to the substitution of a valine for a glutamic acid in the beta globin polypeptide of HbS. Deoxygenation of the heme moiety of HbS leads to dock of valine with complementary sites on adjacent globin chains (hydrophobic interactions) leading to aggregate of HbS molecules into larger polymers. The polymerization is dependent on intra-erythrocytic HbS concentration; degree of cell deoxygenation, pH, and the intracellular concentration of HbF. Inhibition of HbS polymerization by HbF requires the formation of asymmetrical HbS/ HbF hybrid forms ($\alpha_2\delta\beta^s$). Polymerization tendencies of mixtures of HbS and several Hb variants show that residues 22, 80, and especially 87 of the γ chain are implicated in intermolecular contact sites that stabilize the deoxygenated-HbS polymers. The polymer is a rope-like fiber that aligns with others to form a bundle, distorting the red cell into classic crescent or sickled forms. These shapes interfere with a critical erythrocyte feature; its deformability ***(Stuart and Nagel, 2004)***.

2- Biochemical pathology:

The biochemical pathology of SCD involves detoxication, reduced Glutathione (GSH), oxidative stress and inflammation. The first three are interrelated ***(Ilesanmi, 2010).*** *Detoxication* is the process of preventing toxic entities from entering the body. Hb S is more unstable than Hb A, contributing to increased generation of free radicals ***(Simoni et al., 2009),*** levels of both total and GSH are low ***(Gibson and Ellory, 2002),*** the pentose phosphate pathway is impaired, and oxidative damage is present in both proteins and lipids ***(Kiessling et al., 2000).***

The decreased intracellular GSH concentration is due to suppressed synthesis and/or increased consumption relative to synthetic capacity. In SCD there is indirect evidence for protein and energy deficiencies relative to metabolic demand ***(Singhal et al., 2002).***

Increased oxidative stress in Hb S cells, causing membrane damage, increased cell rigidity, altered cation permeability and cell dehydration, will all contribute to the chronic hemolysis and acute vaso-occlusive events of SCD. The oxygenation status of the red cell has other important consequences ***(Ilesanmi, 2010).***

The antioxidant defense systems include a complex of interrelated functions, each of which tends to buffer the effects

of the others. There is abundant evidence that the GSH concentration in erythrocytes of individuals with SCD is low and they also have increased oxidative stress ***(Manfredini et al., 2008).*** It has been proposed that oxidative damage of the membrane and ionic channels of the SCD erythrocyte alters its polymerization and depolymerization kinetics resulting in the formation of irreversibly sickled cells and microvascular occlusion ***(Gibson et al., 1998).***

3- Cellular pathology:

This refers to the platelets and coagulation factors. SCD is often referred to as a hypercoagulable state ***(De Franceschi et al., 2011a)*** because patients manifest increased thrombin and fibrin generation ***(Tomer et al., 2001; Schnog et al., 2004; Lee et al., 2006)***, increased tissue factor procoagulant activity ***(Bandyopadhyay et al., 2008),*** and increased platelet activation even when they are in a non-crisis, steady state ***(Inwald et al., 2000).***

Furthermore, thrombosis may contribute to the pathogenesis of several SCD-related complications. For example, stroke, caused by large vessel obstruction with superimposed thrombosis, often occurs in SCD patients ***(Switzer et al., 2006).*** Both pulmonary embolism and pregnancy-related venous thromboembolism appear to occur more commonly in SCD patients than in appropriate control patients ***(James et al., 2006; Stein et al., 2006).***

The coagulation system is in a state of activation in "steady state" sickle disorders as well as during painful crisis. It involves both a cellular (platelet) and a protein (coagulation factor) component ***(Ilesanmi, 2010).***

4- Vascular pathology:

The vascular pathology of SCD is influenced by many factors, including adhesiveness of red and white blood cells to endothelium, increased coagulation, and homeostatic perturbation. The vascular endothelium is central to disease pathogenesis because it displays adhesion molecules for blood cells, balances procoagulant and anticoagulant properties of the vessel wall, and regulates vascular homeostasis by synthesizing vasoconstricting and vasodilating substances. The occurrence of intermittent vascular occlusion in SCD leads to reperfusion injury associated with granulocyte accumulation and enhanced production of reactive oxygen species. The participation of nitric oxide (NO) in oxidative reactions causes a reduction in NO bioavailability and contributes to vascular dysfunction in SCD (Figure 4). Therapeutic strategies designed to counteract endothelial, inflammatory, and oxidative abnormalities may reduce the frequency of hospitalization and blood transfusion, the incidence of pain, and the occurrence of acute chest syndrome and pulmonary hypertension in patients with SCD ***(Ilesanmi, 2010)***.