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 and Bunn, 2008)***.

SCD is one of the most common severe monogenic disorders in the world. Haemoglobin polymerisation, leading to erythrocyte rigidity and vaso-occlusion, is central to the pathophysiology of this disease, although the importance of chronic anemia, haemolysis, and vasculopathy has been established ***(Rees et al., 2010)***.

SCD usually manifests early in childhood by "sickle-cell crisis" resulting in anemia and crises that could be of many types including the vaso-occlusive crisis, aplastic crisis, sequestration crisis, hemolytic crisis, and others. Most episodes of sickle-cell crises last between five and seven days. Although infection, dehydration, and acidosis can act as triggers favoring sickling, in most instances, no predisposing cause is identified ***(Kumar et al., 2009).***

With increased longevity, cardiovascular complications are increasingly evident, with the notable development of a progressive proliferative systemic vasculopathy, pulmonary hypertension, left ventricular diastolic dysfunction ***(Gladwin and Sachdev, 2012)***, right ventricular dysfunction, dysarrythmia,

cardiac iron load, myocardial infarction ***(Klings et al., 2014)***. Marked abnormalities in exercise capacity have consistently been seen in SCD patients. In addition to possible cardiac filling abnormalities, suggested mechanisms for this limitation in patients studied with cardiopulmonary testing include the anemia itself, pulmonary vascular disease, peripheral vascular disease, and/or a myopathy ***(Callahan et al., 2002).***

Pulmonary hypertension is a common complication in patients with SCD and is associated with an increased risk of death ***(Gladwin et al., 2004; De Castro et al., 2008)***. Although the pathophysiology of pulmonary hypertension in SCD is probably multifactorial, chronic intravascular hemolysis, with associated destruction of nitric oxide by cell-free plasma hemoglobin and reactive oxygen species, appears to play a central role ***(Aslan et al., 2001; Reiter et al., 2002)***. The development of pulmonary hypertension is associated with intravascular hemolysis, cutaneous leg ulceration, renal insufficiency, iron overload, and liver dysfunction.

The apelin receptor (also known as the APJ receptor) remained orphan until 1998, when Tatemoto and his coworkers isolated its endogenous ligand from bovine stomach extract. They isolated a 36-amino-acid peptide which was named apelin (from APJ endogenous ligand) ***(Tatemoto et al., 1998)***. Apelin exists in at least three forms, consisting of 13, 17, or 36 amino acids, all originating from a common 77-amino-acid Precursor ***(Rafa el al., 2006)***. Apelin has been shown to be involved in

vessel formation, where it exerts a pro-angiogenic role, and in the regulation of cardiovascular function by reducing arterial blood pressure via stimulation of nitric oxide-mediated vasorelaxation ***(Kasai et al., 2004; Ishida et al., 2004).*** Moreover, apelin has been added to the family of adipokines, which are adipocytokines mainly derived from adipose tissue as well as endothelial cells in various parts of the body ***(Rafael, 2006; Garica-Diaz et al., 2007)***.

Apelin is localized in vascular endothelial cells while the APLNR is localized in both endothelial and smooth muscle cells in vessels and in the heart ***(Kleinz et al., 2004)***. Apelin is regulated by hypoxia inducible factor -1α and bone morphogenetic protein receptor-2. Patients with PH have lower levels of plasma-apelin, and decreased apelin expression in pulmonary endothelial cells. Apelin has therefore been proposed as a potential biomarker for PH. Furthermore, apelin plays a role in angiogenesis and regulates endothelial and smooth muscle cell apoptosis and proliferation complementary and opposite to vascular endothelial growth factor ***(Andersen et al., 2011)***. To the best of our knowledge, apelin levels have not been explored in patients with SCD.

Aim of the Work

The aim of this study is to measure serum Apelin in children and adolescents with SCD and assess its relation to markers of hemolysis, iron overload as well as cardiopulmonary complications.

Chapter I
OVERVIEW OF SICKLE CELL DISEASE

Definition:

Sickle cell disease (SCD) is a wide spread 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. Acute painful crisis is common sequelae that can cause significant morbidity and negatively impact the patient's quality of life ***(Mousa and Qari, 2010).***

SCD is a multisystem disease, associated with episodes of acute illness and progressive organ damage, and is one of the most common severe monogenic disorders worldwide ***(Weatherall et al., 2005).***

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).*** Sickle cell disease 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 sickle cell disease have produced a large body of clinical data on the evolution of the disease from birth ***(De Baun 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 corpuscles ***(Herrick, 2001)*** **(Figure 1).**

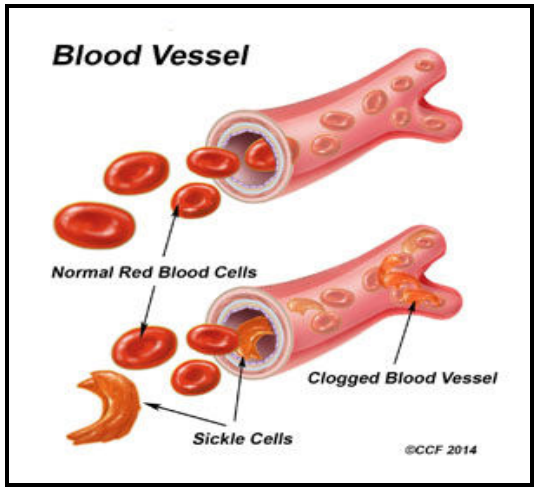

Figure (1): Shape of normal RBCs and Sickled cells ***(http://my.clevelandclinic.org/disorders/sickle_cell_anemia/hic_sickle_cell_anemia.aspx).***

In 1959, Ingram discovered the exact nature of the defect: substitution of valine for glutamic acid at the sixth residue of the beta chain, establishing sickle cell anemia as a disease of molecular structure, "a molecular disease" based on one point mutation. It is most fascinating that one substitution in the gene encoding, with the resulting replacement of Alpha 6 glutamic acid by valine, leads to the protean and devastating clinical manifestations of sickle cell disease ***(Ingram, 1957).*** 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 ischaemic 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 haemoglobin **(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)

Definition and genetic basis

Sickle cell disease is an autosomal recessive abnormality of the β-globin chain of hemoglobin (Hb) that changes the sixth amino acid from glutamic acid to valine. The resulting HbS polymerizes reversibly when deoxygenated to form a gelatinous network that stiffens the erythrocyte membrane and increases viscosity, producing the characteristic sickle shape. Such sickled cells lose flexibility needed to traverse small capillaries and have "sticky" membranes that adhere to endothelium of small venules ***(Rees et al., 2010).***

SCD was the first genetic disease to be characterized at the molecular level. The β-globin gene is located at the short arm of chromosome 11. The sole genetic problem in sickle cell anemia 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^{th} position of β chain by valine ***(Kutlar, 2007)*** (**Figure 2**)**.**

The term SCD 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). Sickle cell anemia (homozygous Hb SS) accounts for 60%-70% of sickle cell disease in the US ***(Stuart and Nagel, 2004).***

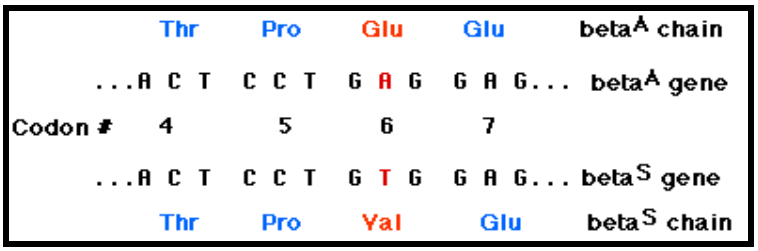

Figure (2): Amino acid position 6 substitution in beta-hemoglobin chain ***(http://www.sicklecellanemia2051.wordpress.com/structure-of-hemoglobin).***

Other forms of SCD 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 Sβ$^+$-thalassemia and Hb Sβ°-thalassemia); rarer forms result from coinheritance of other Hb variants such as D-Punjab and O-Arab ***(Tyagi et al., 2003)***.

SCD results from either the inheritance of two sickle beta globin genes or the inheritance of one sickle β gene in combination with another β globin chain defect. The most common form of SCD, hemoglobin SS (sickle cell anemia), is caused by the inheritance of hemoglobin S from both parents. Hemoglobin SC disease, hemoglobin S β$^+$ thalassemia, and hemoglobin S β° thalassemia are the other three common forms of SCD. Patients with hemoglobin S β$^+$ thalassemia and S β° thalassemia have a clinical picture that resembles SCD rather than thalassemia, because with the underproduction of normal β globin, the sickle β globin predominates. Sickle cell anemia (hemoglobin SS) and sickle β° thalassemia, conditions in which there is no normal β globin production, are generally more severe than hemoglobin SC and hemoglobin S β$^+$ thalassemia. SCD is a clinically heterogeneous disorder, however, and many

sickle cell anemia patients may have a clinical course that is milder than that of a hemoglobin SC patient ***(Tyagi et al., 2003)***.

Inheritance of SCD

The allele responsible for sickle-cell anaemia is autosomal recessive and can be found on the short arm of chromosome 11. In people heterozygous for HbS (carriers of sickling hemoglobin), the polymerisation problems are minor, because the normal allele is able to produce over 50% of the hemoglobin. In people homozygous for Hb S, the presence of long-chain polymers of HbS distort the shape of the red blood cell from a smooth doughnut-like shape to ragged and full of spikes, making it fragile and susceptible to breaking within capillaries. Carriers have symptoms only if they are deprived of oxygen (for example, while climbing a mountain) or while severely dehydrated. Under normal circumstances, these painful crises occur about 0.8 times per year per patient ***(Ballas and Lusardi, 2005).***

The abnormal haemoglobins and the thalassaemias are inherited as autosomal recessive (AR) disorders, where carrier parents transmit the abnormal genes to the offspring as shown in **(Figure 3)** ***(Imaga, 2012)***. If both parents are heterozygotes for HbS, there is a 25 % chance of having a homozygous HbSS (Sickle cell anaemia, SCA) child. If one parent is a carrier for HbS and the other is carrier for one of the abnormal HbS or thalassaemias, it results in a double heterozygote state. Heterozygotes are generally asymptomatic carriers (traits), while the SCD is expressed in the homozygotes and the double

heterozygotes for two abnormal haemoglobin genes or HbS and the thalassaemias ***(El-Hazmi et al., 2011).***

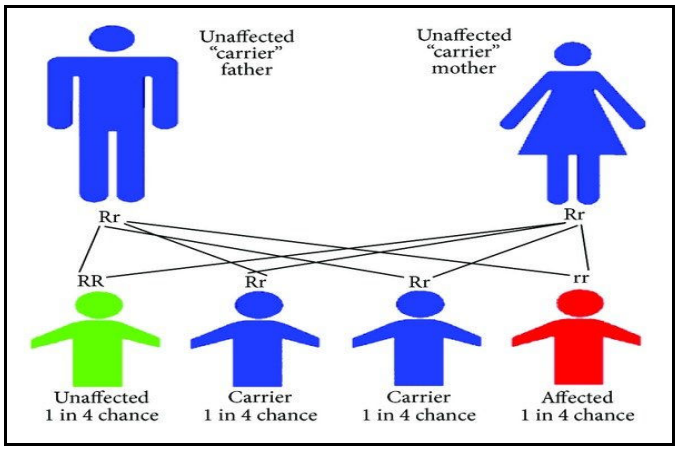

Figure (3): Sickle cell disorder inheritance pattern ***(Imaga, 2012).***

In the sickle β-thalassemias, mutation of the β^{A} gene results in a total inability to produce the normal β^{A} globin chain (β^{0}) or a reduction in its production (β^{+}). The child with sickle β -thalassemia inherits an S gene from one parent and a β -thalassemia gene from the other parent ***(Wethers, 2000).***

If the child inherits an S gene from one parent and abnormal hemoglobin, such as D, G or O, from the other parent, other rarer variants result. The normal hemoglobin present in the child with sickle β^{+} thalassemia (less than 30 percent hemoglobin A) ameliorates the clinical picture. In general, this is the mildest variant of sickle cell disease, followed in severity by sickle hemoglobin C disease. Homozygous sickle cell disease and sickle β^{0} thalassemia have a comparable spectrum of severity, and specific laboratory studies are needed to distinguish between the two conditions ***(Wethers, 2000).***

Types of sickle-cell disease

The different types of SCD are shown in **Table 2.**

Sickle Cell Trait

There is a long-standing controversy in the literature as to whether sickle-cell trait (SCT) should be viewed as a benign carrier state or as an intermediate disease phenotype. Because SCT is routinely detected by neonatal screening for sickle-cell disease, it becomes imperative that consensus on this issue be achieved in order to provide the best medical advice to affected individuals ***(Key and Derebail, 2010).***

SCT is the term used to describe the presence in an estimated 300 million individuals worldwide of a heterozygous glutamic acid-to-valine substitution in the β-globin gene on chromosome 11 (HbAS). In the United States, 6% to 9% of the African-American population and 0.01% to 0.05% of the remaining population (primarily those of Arab, Indian, Hispanic, and Mediterranean descent) are carriers of the β^s mutation ***(Key and Derebail, 2010).***

Overall, the evidence suggests that SCT may be neither a completely benign carrier state nor a true disease entity, but rather a risk factor for certain adverse outcomes that result from the interplay between genetic and environmental influences. Venous thrombosis and renal disease are among the manifestations under reevaluation ***(Key and Derebail, 2010).*** Until such time as these observations have been confirmed, expanding screening efforts must be considered to be of little benefit. Nonetheless, with ongoing newborn screening

identifying individuals with SCT, furthering research to better characterize the consequences of SCT is of paramount importance to providing better counseling on any associated health risks ***(Mitchell, 2007; Cavanaugh and Lanzkron, 2010).***

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^{0}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 (βA/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)

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 worldwide are born with documented sickle cell disease every year. Sickling disorders are found frequently in the Afro-Caribbean population 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, 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).***

The distribution of sickle-cell anemia haplotypes among nations with high prevalence of the disease are shown in **(Figure 4)** ***(Gabriel et al., 2010).***

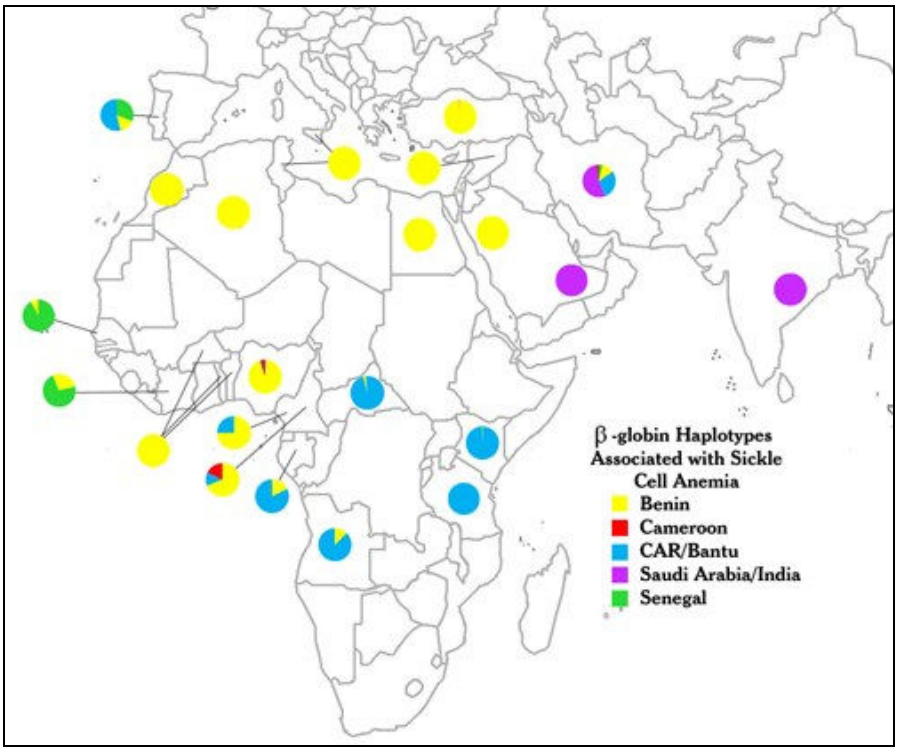

Figure (4): The distribution of sickle-cell anemia haplotypes among nations with high prevalence of the disease. Five distinct β-globin haplotypes (indicated by colors) are found in patients with sickle-cell anemia. Each color represents a different haplotype named after the country in which it was first discovered, not necessarily its genetic origin. Indeed, these haplotypes are not restricted to the eponymous nation, and they can be found broadly distributed (i.e., Benin haplotype in multiple nations, or multiple haplotypes within a single nation). The haplotype data represented in the image were summarized from genetic epidemiological studies of sickle-cell patients across different regions ***(Gabriel et al., 2010).***

In addition to the close geographic correlation between the frequency of the HbS gene in populations and the historic incidence of malaria, evidence for the partial resistance of carriers to all forms of plasmodium falciparum malaria has been reported in many populations ***(Williams et al., 2005).***