INTRODUCTION

Sickle cell disease (SCD) is caused by a mutation in the hemoglobin β chain, resulting in the production of an abnormal hemoglobin (HbS) in the erythrocyte. Under conditions of low oxygenation, HbS polymerizes leading the erythrocyte to adopt sickle-shaped morphology ***(Steinberg et al., 2009; Rees et al., 2010)***. SCD is a chronic and potentially, quite a debilitating disease. The disease is severe and may result in significant morbidity, as well as a shortened life span. There is considerable variability in the clinical course of sickle cell disease with some patients experiencing few to no complications and others having multiple organ involvement ***(Serjeant and Serjeant, 2001)***.

SCD is characterized by complex pathophysiological mechanisms that involve intravascular hemolysis and recurrent vaso-occlusion, in association with chronic vascular inflammation and endothelial activation, leading to painful vaso-occlusive episodes, auto-infarction of the spleen, acute chest syndrome, stroke, pulmonary hypertension, renal damage and a shortened lifespan ***(Steinberg et al., 2009; Rees et al., 2010)***.

Lung is one of the major organs involved in SCD. Two major forms of clinical lung involvement are acute chest syndrome and sickle cell chronic lung disease (SCCLD). The pathogenesis of acute chest syndrome includes hemoglobin S

polymerisation and red cell sickling, increased expression of adhesion molecules on sickle erythrocytes and endothelium, release of inflammatory mediators, interaction of sickle red cell with leucocytes, microvascular thrombosis, and endothelial damage, which may lead to microvasculature occlusion and tissue infarction. SCCLD is presumably related to recurring episodes of infarction and infection and is characterized by a decrease in radiolucency of the lungs and moderate to severe impairment of pulmonary function. Pulmonary manifestations of SCD remain under-diagnosed by physicians ***(Siddiqui and Ahmed, 2003; Purohit et al., 2016)***.

Interstitial pulmonary fibrosis (chronic scarring of the lung parenchyma) is occasionally seen in patients with recurrent episodes of acute chest syndrome with pulmonary infarction. The clinical manifestation of pulmonary fibrosis in SCD include dyspnea and scattered areas of honey combing on high resolution computed tomography (HRCT) of the chest ***(Vij and Machado, 2010).***

Pneumoproteins (proteins synthesized predominantly in the lungs) are promising blood biomarkers because they have high specificity for lung disease. One of them is surfactant protein-D (SP-D), which is a macromolecular lipoprotein complex synthesized by type II pneumocytes and Clara cells ***(Jaw and Sin, 2012*)**. SP-D is a member of a family of collageneous carbohydrate binding proteins known as collectins ***(Crouch, 1998)***.

Although surfactant protein was originally described for its essential role in reducing surface tension at the air–liquid interface of the lung, it is recognized also to have many other functions in innate immunity, regulation of cellular clearance as well as inflammatory and immune responses ***(Kishore et al., 2006)***. Elevation of SP-D in the serum reflects increased type II pneumocytes activity in the injured lung with resultant back leak into the blood ***(Herman and Bernard, 1999)***.

SP-D levels in serum may reflect disease activity and SPD has therefore been suggested as a potential biomarker for the epithelial integrity in chronic obstructive pulmonary diseases (COPD) ***(Kishore et al., 2006; Zaky et al., 2014)***. Increased SP-D serum levels have been reported for lung diseases such as idiopathic pulmonary fibrosis, pulmonary alveolar proteinosis, cystic fibrosis, COPD, and for infectious diseases like tuberculosis and bacterial pneumonia ***(Greene et al., 2002; Ohnishi et al., 2002; Lomas et al., 2009; Winkler et al., 2011)***.

AIM OF THE WORK

The aim of this study was to determine the level of surfactant protein D (SP-D) in children and adolescents with SCD and assess its possible relation to markers of hemolysis, iron overload and pulmonary complications.

SICKLE CELL DISEASE

Sickle cell disease (SCD) is a hereditary hemoglobinopathy characterized by abnormal hemoglobin (Hb) production, hemolytic anemia and intermittent occlusion of small vessels ***(Smiley et al., 2008)***

It causes acute and chronic illness, and median life expectancy is reduced by 30 years in all countries, with greater reduction in low income countries. There is a wide spectrum of severity, with some patients having no symptoms and others suffering frequent life changing complications ***(Tewari et al., 2015).***

Epidemiology:

It is estimated that around the world more than 312,000 infants are born with homozygous hemoglobin S (HbS), that is called SCA, each year with the majority in the developing countries, from them 230,000 annually in Sub-Saharan Africa ***(McGann et al., 2014)***.

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)***.

Genetics:

The β-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, that results in the substitution of glutamic acid in the 6 position of chain by valine ***(Quirolo and Vichinsky, 2004; Rees et al., 2010).***

Sickle cell disease is transmitted as incomplete autosomal dominant trait. Homozygotes (two abnormal genes) have red cells containing 90-100% hemoglobin S (HbS) while heterozygotes (one abnormal gene) have red cell containing 20-40% Hb arises as spontaneous mutation on chromosome 11 ***(Nathan et al., 2000).***

The role of genetic factors in SCD has been extensively investigated but only explain small amounts of the observed phenotypic variability ***(Tewari et al., 2015).***

Pathophysiology:

The main determinant of disease severity is the rate and extent of HbS polymerization, which is affected by co-inheritance of genetic factors that modulate the intracellular HbS or HbF concentration, such as the protective effects of co-inherited α-thalassemia or hereditary persistence of HbF ***(Rees et al., 2010).***

Clinical picture of SCD

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)***.

1- Infection:

It is the most immediate risk for infants diagnosed with SCD ***(Buchanan et al., 2010).*** The most frequent organisms responsible for pneumonia are Mycoplasma pneumonia, Chlamydia pneumonia, streptococcus pneumonia (S.pneumoniae) and Hemophalus influenza (H.influenza) ***(McMahon, 2006).*** Any infant or child who has SCD and fever (temperature more than 38.5oC) must be evaluated immediately for invasive bacterial infection ***(Quinn, 2013).***

2- Anemia:

All patients with SCD have some degree of anemia. Anemia is generally well tolerated throughout childhood ***(Serjeant, 2001)***.

3- Spleen:

It is the first organ injured in SCA with evidence of hyposplenism present before 12 months in the majority of children. Repeated splenic vaso-occlusion leads to fibrosis and progressive atrophy of the organ (autosplenectomy), which is generally complete by 5 years with increase susceptibility to

infection, which is notably reduced by penicillin prophylaxis and immunization. The precise sequence of pathogenic events leading to hyposplenism is unknown ***(Brousse et al., 2014).***

4- Crisis and complications:

1) Vasoocclusive Crises:

Vasooclusive episodes account for over 90% of all emergency hospital admissions ***(Heeney and Dover, 2009)***.

- ***Hand- foot syndrome:***

It is thought to be precipitated by cold-induced vasoconstriction. Sickle cell dactylitis is common between 6 months and 2 years of age, but is rare after the age of 6 years because of the regression of red marrow in these areas with increasing age ***(Resnick, 2002).***

- ***Pain:***

Some patients have occasional episodes of acute pain, but others have more frequent episodes of pain which may lead to chronic pain if not adequately managed ***(Preboth, 2000).*** Chronic pain often results from orthopedic problems, such as avascular necrosis or chronic arthritis ***(Quirolo and Vichinsky, 2004)***.

- ***Painful bony crisis:***

The most frequent complications requiring hospital admissions for patients with SCD are painful vaso-occlusive crises and osteomyelitis ***(Almeida and Roberts, 2005).***

- ***Acute abdominal pain:***

It is due to vaso-oclusion of mesenteric blood supply, and micro infarction in liver, spleen, or lymph nodes that results in capsular stretching and subsequently auto splenectomy. The characteristic form includes: generalized abdominal pain, distension, tenderness and may be vomiting and diminished or absent bowel sound ***(Serjeantet al., 2001)***.

- ***Stroke:***

SCA is one of the most common causes of stroke in children. Most cases are associated with vasculopathy affecting the distal internal carotid and middle cerebral arteries, although extracranial vasculopathy can also be present ***(Deane et al., 2010)*** with recurrence rate more than 60% ***(Verduzco and Nathan, 2009).***

- ***Priapism:***

Priapism is potentially a serious problem for young men with sickle cell disease which may lead to impotence. It is a form of vasoocclusive crisis affecting the penile circulation ***(Nolan et al., 2005).***

2- Acute splenic sequestration crisis:

It results from the acute entrapment of large amount of blood in the spleen. The manifestations are left upper quadrant pain, exacerbated anemia and often, hypotension. Circulatory collapse and death can occur in less than thirty minutes. Early diagnosis and immediate intervention with intravenous fluid and blood transfusion are life- saving. A child who suffers one episode of splenic sequestration crisis is at greater risk of a second attack. Surgical splenectomy to prevent recurrence is often recommended ***(Wethers, 2000).***

3- Aplastic crisis (Barvovirus B19):

When the marrow cannot keep up with production (i.e., a lack of folic acid) or has its production impaired by viral ill-ness, such as parvovirus B-19, an aplastic crisis may occur ***(Roseff, 2009).***

4- Hyperhemolytic crisis:

It is an anemic crisis characterized by a dramatic, rapid drop in Hb and Hct, anemia, jaundice, and markedly elevated reticulocytic count > 20 %. Increased hemolysis with rise in bilirubin can be also seen in patient with concurrent glucose-6-phosphate dehydrogenase (G6PD) deficiency or occurs as a reaction to multiple transfusions in SCD ***(Crain and Gershel, 2003).***

5- Hepatic sequestration crisis:

It is not common and it is caused by obstruction of the blood flow from the liver sinusoids by the sickled RBCs leading to compression of the bile ducts ***(Koullapis et al., 2005).***

5- Neurological complications:

Children with SCD, present with a wide variety of neurological syndromes, including ischemic and hemorrhagic stroke, transient ischemic attacks, 'soft neurological signs', seizures, headache, coma, visual loss, altered mental status, cognitive difficulties, and covert or 'silent' infarction ***(Krejza et al., 2011)***.

6- Liver and biliary system:

I- Heptomegaly and jaundice:

Sickle hepatopathy occur due to regional obstruction of sinusoidal blood flow by impacted masses of sickle cell leading to residual patches of ischemic necrosis, fibrosis and nodular regeneration ***(Serjeant, 2001).*** Early diagnosis and treatment should be considered to prevent irreversible liver injury ***(Issa and Al-Salem, 2010).***

II- Gall stones:

It is one of the common complications of SCA. These are usually pigment stones that result from chronic hemolysis with increased bilirubin production. Its frequency is 5-55% which increases with age ***(Issa and Al-Salem, 2010).***

8- Renal complications:

Table (1): Classification of renal manifestations of SCD based on described phenotypes

Cortex: hemolysis-endothelial dysfunction phenotype
▪ Hyperfiltration ▪ Glomerular hypertrophy ▪ Glomerulopathy ▪ Hypermetabolism ▪ CKD
Medulla: viscosity-vasoocclusion phenotype
▪ Hematuria ▪ Papillary necrosis ▪ Impaired concentrating ability ▪ Impaired potassium excretion ▪ Tubular acidosis

(Nath and Katusic, 2012)

9- Leg ulcers:

They occur in about 10-20% of SCD patients in the form of single or multiple painful indolent ulcers over lateral and medial malleoli or occasionally on the dorsum of the foot or over the tibia. They either heal quickly or persist for years ***(McMahon, 2006).***

10- Ocular complications:

Sickle retinopathy occurs as a result of vasooclusion in the peripheral retina and is a common complication. Progressive neo-vascularization and enlargement of pre-

existing capillaries occurs as a result of ischemia which occurs in retinal areas ***(Powars et al., 2002).***

11- Hypercoagulability and thrombotic complications:

The mechanism of coagulation activation in hemolytic anemias is likely multifactorial. Both SCD and thalassemia are characterized by RBC membrane abnormalities, with abnormal exposure of phosphatidylserine (PS) ***(Eldor and Rachmilewitz, 2002)***.

12- Growth and developmental disorders:

Children with SCD have decreased height, weight and body mass index, as well as delayed sexual maturation, Inadequate nutrition, abnormal endocrine function and, in particular, increased caloric requirements due to elevated energy expenditure may all be etiologic factors ***(Ballas et al., 2010).***

Plasma zinc levels are low and zinc deficiency reduces helper T cell and cell mediated immunity, so zinc supplementation has been reported to improve sexual development and growth. Also, it reduces the number of bacterial infections, hospitalization and vasoocclusive episodes ***(Mc Mahon, 2006).***

13- Psychological aspects

Most patients with SCA handle their illness very well. Problems of particular concern to patients are coping with chronic pain, inability to keep up with peers, fears of premature death, and delayed sexual maturity. Self-help groups for

patients and families are gaining effectiveness and popularity ***(Heeney and Dover, 2009).***

Diagnosis of SCD:

1) Prenatal diagnosis:

- **Fetal DNA analysis:**

It can be obtained from amniocytes or from chorionic villi. The most widely used procedure is chorionic villi sampling mainly ***(Traeger et al., 2008).*** Both techniques allow the diagnosis to be known early 1st trimester to enable a couple at risk to make an informed decision about potential termination of pregnancy ***(Paul et al., 2007).***

- **Newborn screening:**

It can be performed using a blood spot technique from a heel-prick, often when blood is taken for inherited metabolic disease screening. The early diagnosis facilitates parental education and the introduction of preventive vaccination and antibiotic programs ***(McMahon, 2006)***

- **Education and Genetic Counseling:**

Education services should be offered to all parents of infants who are identified with a hemoglobin abnormality ***(Klandy et al., 2011; Kaufmann et al., 2014).***

2) Laboratory diagnosis:

1) The hemoglobin is usually 6-9 g/dl low in comparison to symptoms of anemia ***(Heeney and Dover, 2009).***
2) Blood film shows Target cells, poikilocytes, Sickle red cells, hypochromasia, nucleated red blood cells and Howell- jolly bodies ***(Quirolo and Vichinsky, 2004).***
3) Screening tests for sickling are positive. They demonstrate increased turbidity of the blood after deoxygenation (e.g., dithionate or Na2HPO4) ***(Clark and Higgins, 2000).***
4) Definitive testing: hemoglobin electrophoresis, high performance liquid chromatography (HPLC), isoelectric focusing (IEF), or mass spectrometry, shows hemoglobin with an abnormal migration: in Hb SS, no Hb A is detected. The amount of Hb F is variable and is usually 5-15%, larger amount are normally associated with a milder disorder ***(Atul and Hoffbrand, 2009).***
5) DNA analysis is used if above methods failed e.g. patient in who SC trait is diagnosed but clinical and laboratory phenotype consistent with sickling disorder. It determines the type of mutation and the gene it affects (e.g., α-, β-globin genes ***(McMahon, 2006).***