Alloimmunization in Egyptian Children with Sickle Cell Disease

A Thesis

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By

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Fatima Zahraa Abdelkarim Saed

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List of Abbreviations

Abb.	Full term
ACS	Acute chest syndrome
CBC	Complete blood count
CNS	Central nervous system
DHTR	Delayed hemolytic transfusion reaction
GSH	Glutathione
Hb	Hemoglobin
HbC	Hemoglobin C
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
NADPH	Nicotinamide adenine dinucleotide phosphate
NF κB	Nuclear factor κB
RBCs	Red blood counts
SCA	Sickle cell anemia
SCD	Sickle cell disease
VOC's	Vaso-occlusive crises

INTRODUCTION

Sickle cell disease (SCD) is a group of typically inherited red blood cell disorders resulting from presence of mutated form of Hemoglobin (Hb); HbS, People with SCD have abnormal Hb, called *Hb S or* sickle Hb, in their red blood cells (RBCs). The most common form of SCD is homozygous HbS disease, an autosomal recessive disorder. ***(Strouse, 2016)***

Electrophoretic abnormalities in sickle hemoglobin (HbS) had been identified and specified the term "molecular disease" in 1949. ***(Pauling and Itano, 1949)*** Sickle hemoglobin form stiff rods within the red cell, changing it into a sickle shape that are not flexible and can stick to vessel walls, causing a blockage that slows or stops the flow of blood and consequently oxygen can't reach nearby tissues. ***(Derebail et al., 2010)***

Blood transfusion remains a cornerstone of treatment of patients with SCD. Despite improved patient outcomes with hydroxyurea administration, indications for chronic transfusions have increased in the last 10 years and are associated with considerable reduction in morbidity and mortality, most notably in preventing first stroke in children. ***(Yazdanbakhsh et al., 2012)***

However, transfusions can lead to erythrocyte alloimmunization with serious complications for the patient. These antibodies are often directed against antigens expressed on RBCs of white persons, which represent the majority of donors in Western countries. Finding compatible units lacking those antigens can sometimes be difficult and identifying and characterizing the antibodies can be time-consuming and laborious, causing transfusion delays. Genetic as well as acquired patient-related factors are likely to influence the process of alloimmunization. ***(Lee et al., 2006)***

The most serious consequence of alloimmunization in SCD patients is the risk of developing a delayed hemolytic transfusion reaction (DHTR), which can be life-threatening. In many cases of DHTR in SCD, the patient's hemoglobin level falls below the pre-transfusion level, suggesting that, in addition to hemolysis of the transfused RBCs, the patient's own RBCs are lysed, a condition known as hyperhemolysis. Additional transfusions may exacerbate the hemolysis and further worsen the degree of anemia. The destruction of the patient's own RBCs in DHTR of SCD is partly explained by the presence of autoantibodies because alloimmunization is known to trigger autoantibody production. However, DHTR/ hyperhemolysis cases have also been reported in the absence of detectable alloantibodies or autoantibodies. ***(Noizat-Pirenne et al., 2007)***

Aim of the Work

Our objective was to measure the frequency of the occurrence of the alloimmune markers in sickle cell disease and to investigate its predicators.

SICKLE CELL DISEASE

Sickle cell disease (SCD) is a group of typically inherited red blood cell disorders resulting from presence of mutated form of Hemoglobin (Hb); HbS. The most common form of SCD is homozygous HbS disease, an autosomal recessive disorder ***(Strouse, 2016).*** HbS is caused by a mutation in the β-globin gene in which the 17^{th} nucleotide is changed from thymine to adenine and the sixth aminoacid in the β-globin chain becomes valine instead of glutamic acid ***(Piel et al., 2013).***

Human cells need a steady supply of oxygen to work well. Normally, Hb in red blood cells takes up oxygen in the lungs and carries it to all the tissues of the body. Red blood cells that contain normal Hb are concave disc shaped. This shape allows the cells to be flexible so that they can move through large and small blood vessels to deliver oxygen ***(Shatat et al., 2013).***

Sickle Hb (HbS) is not like normal Hb. It can form stiff rods within the red cell, changing it into a crescent, or *sickle* shape. Sickle-shaped cells are not flexible and can stick to vessel walls, causing a blockage that slows or stops the flow of blood. When this happens, oxygen can't reach nearby tissues ***(Derebail et al., 2010)***.

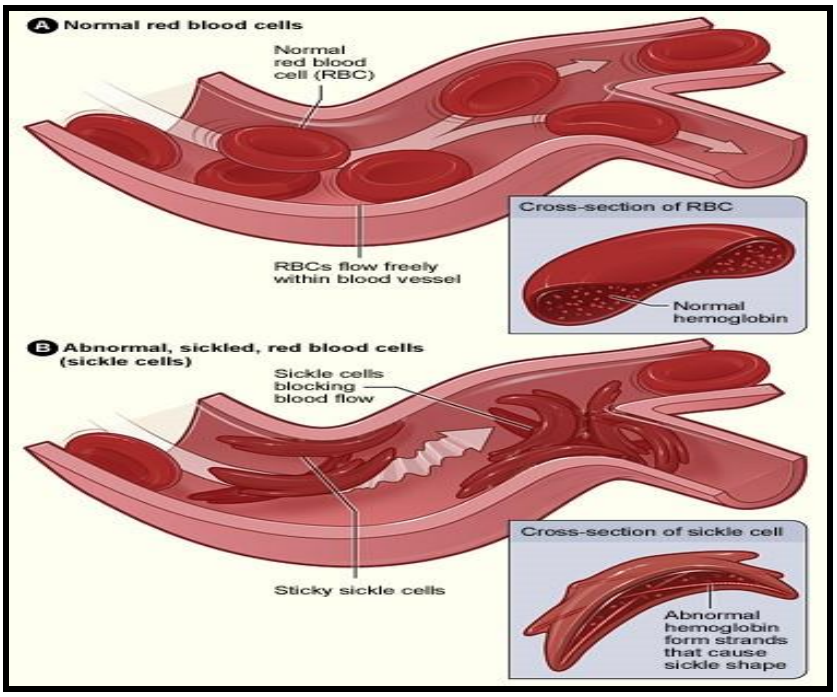

Figure (1): Normal RBCs and abnormal, sickled red blood cells. Picture (A) shows normal RBCs flowing freely in a blood vessel. The inset image shows a cross-section of a normal red blood cell with normal Hb. Picture (B) shows abnormal, sickled red blood cells blocking blood flow in a blood vessel. The inset image shows a cross-section of a sickle cell with abnormal (sickle) Hb forming abnormal stiff rods ***(Gary and Gibbons, 2016).***

Pathophysiology:

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. HbS is caused by a mutation in the β-globin gene this mutation produces a hydrophobic reason in the deoxygenated HbS tetramer that results in binding between β1 and β2 chains of two Hb 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 ***(Tewari et al., 2015).***

The rate and extent of HbS polymerisation is proportional to the extent and duration of Hb de-oxygenation, the intracellular HbS concentration, and the presence of fetal Hb in the erythrocyte, which effectively reduces the concentration of HbS. The main determinant of disease severity is the rate and extent of HbS polymerization, which is exemplified by co-inheritance of genetic factors that modulate the intracellular HbS or fetal Hb concentration, such as the protective effects of co-inherited α-thalassemia or hereditary persistence of fetal Hb ***(Westerman et al., 2008).***

The SCD manifestations are driven by two major pathophysiological processes: vaso-occlusion with ischaemia-reperfusion injury and haemolytic anaemia. Acute vaso-occlusive pain is thought to be caused by entrapment of erythrocytes and leucocytes in the microcirculation, causing vascular obstruction and tissue ischaemia. Although this process requires HbS polymerisation, the event that triggers the vascular obstruction by sickle erythrocytes is often inflammatory ***(Mittal et al., 2010).***

The second pathophysiological process in SCD is hemolytic anaemia, which is also driven by HbS polymerization. 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. As patients with SCD 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 ***(Bain et al., 2009).***

Data from epidemiological studies suggest that several complications are associated with increased rates of hemolysis; cholelithiasis, cutaneous leg ulceration, priapism, and pulmonary hypertension are associated with low steady state Hb concentrations and an increased rate of intravascular hemolysis ***(Ryan et al., 2010).***

An association between the development of pulmonary hypertension and the intensity of haemolytic anaemia was noted in three prospective screening studies of adults with SCD and in pediatric studies. Pulmonary hypertension has also been reported in other forms of chronic hereditary and acquired haemolytic anemia ***(Ballas et al., 2009).***

Therefore, patients with low Hb concentrations and high haemolytic rates seem to form a subphenotype of patients who are more likely to develop vasculopathy than are those with higher Hb concentrations who seem more prone to episodes of acute pain and, possibly, acute chest syndrome ***(Voskaridou et al., 2010).***

Although vaso-occlusion is important in all patients, the role of haemolysis as a pathophysiological mechanism in SCD is more controversial and is the focus of much research. An important disease mechanism involves the release of Hb into

the circulation during intravascular haemolysis. Free plasma Hb generates reactive oxygen species, such as the hydroxyl and superoxide radical, which is a potent scavenger of nitric oxide ***(Zimmerman et al., 2007).***

Sickling process:

All complications of SCD can he traced to changes in the make-up of the RBC. Normal RBCs are smooth surfaced, enabling them to change their shape to flow through small blood vessels. Under certain conditions (i.e., acidosis, dehydration, infection, and low oxygen) RBC's containing HbS become rigid, elongated, and sickle shaped. Some RBCs sickle immediately, while others remain normal for hours before sickling. Most RBCs containing HbS can sickle and then unsickle. After repeated cycles of sickling and unsickling, the RBC's become irreversibly sickled ***(Colombatti and Sainati, 2016).***

It is markedly accelerated when intracellular concentration of Hb S is increased. Sickle cells are short lived and can interact with endothelial cells, leukocytes, platelets, and other plasma components. It normally carries oxygen but begins to form semi-solid aggregate structures once oxygen is unloaded to the tissues. These Hb S aggregates distort RBCs and decrease the cells' flexibility. Repeated deoxygenation cycles cause permanent RBCs damage ***(Padayachee et al., 2012).*** It was suggested that any condition that caused a "primary increase in the plasma viscosity" would delay passage of the erythrocytes through the capillaries, producing an increase in sickling and a "vicious cycle"