INTRODUCTION

Sickle cell anemia (SCA) is an inherited autosomal recessive form of anemia in which there aren't enough normal red blood cells to carry enough oxygen throughout the body ***(McCavit, 2012)***.

In SCA, the red blood cells are rigid, sticky and sickles or crescent moons in shape. These irregularly shaped cells can lodge in small blood vessels, which leading to slow or block in the blood flow and decrease oxygen delivery to body tissues ***(McCavit, 2012)***.

Cerebrovascular accident is one of the most common critical complications of children with sickle cell disease (SCD). The incidence of stroke in SCD is 0.61 to 0.76 per 100 patients per year in the first 20 years of life. Approximately, this rate is 300 times higher than that observed in children without SCD (0.0023 per 100 patients per year) ***(King et al., 2014)***.

Currently there are no known genetic or molecular risk factors, that can be predicted the increased risk of stroke in these patients. However; there are candidate gene and genetic modifiers for increasing susceptibility to stroke include, those implicated in endothelial cell inflammation and adhesion ***(Switzer et al., 2006, Wang, 2007).*** Other studies showed that the presence of relative hypertension is considering a risk factor for stroke in patients with SCD ***(Paternoster et al., 2009)***.

The renin angiotensin system plays an important role in blood pressure regulation as a central regulator of sodium homeostasis ***(Lynch et al., 2007)***.

Angiotensinogen (*AGT*) is a key component of the renin angiotensin system, which is an important regulatory system with a powerful influence on salt and water absorption and blood pressure ***(Choi et al., 2004)***.

The M235T *AGT* mutation is a single base pair substitution of thymine (T) with cytosine (C) in the nucleotide 704 (T704C) at exon 2 of the angiotensinogen gene localized at chromosome 1q42- 43, resulting the substitution of methionine with threonine in amino acid position 235 at the pre pro-angiotensinogen molecule (M235T). Also, T235 alleles consider the mutant allele, while M235 alleles consider the wild type ***(Mignini et al., 2006)***.

AIM OF THE WORK

To investigate the potential associations between angiotensinogen M235T polymorphism and susceptibility to stroke in Egyptian patients with SCA.

SICKLE CELL ANEMIA

Sickle cell anemia (SCA) is an autosomal recessive disease due to β globin gene mutation ***(Pagon et al., 2003),*** with an A to T transversion in the codon of amino acid position 6. Consequently; a valine residue replaces the glutamic acid residue (glu6val) and HbS β globin chains are substituted for normal HbAβ globin chains ***(Ingram, 1956)***. SCA is the most widespread genetic disease in the world ***(Ebakisse-Badassou, 2010),*** with highest prevalence and incidence in populations of African origin ***(Labie and Elion, 2010).*** Pathophysiology of SCA is illustrated in Figure (1) ***(Steinberg, 2008).***

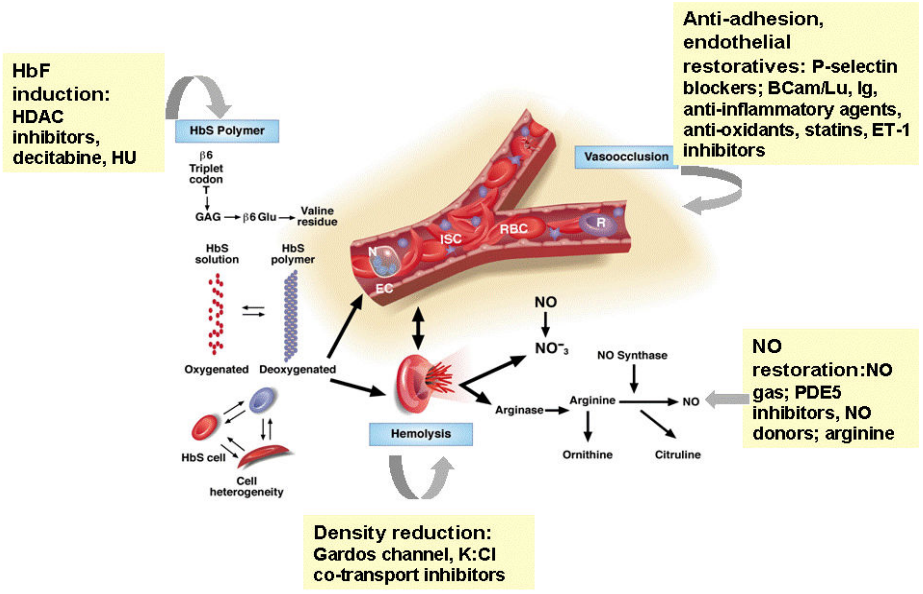

Figure (1): The pathophysiology of SCD and sites where drug treatment could be focused. HBS can undergo reversible polymerization when deoxygenated. The sickle polymer injures the erythrocyte and eventually produces irreversible membrane damage. These cells have a shortened life span (hemolysis), and also lead to vasoocclusion ***(Steinberg, 2008).***

Sickle Vasoocclusion

Sickle erythrocytes interrupt normal tissue perfusion, this process is complex, and it is called sickle vasoocclusion ***(Hebbel et al., 2004)***. Obstruction occur first in the small post-capillary venules via the characters of sickle cells (entrapment and adhesion to the vascular endothelium) and the lodgment of dense sickle erythrocytes, platelets and leukocytes ***(Kaul et al., 1996)***. Large arteries to the lungs and brain may be occluded; perhaps due to activation of inflammatory pathways and injury to their endothelium ***(Milbauer et al., 2008)***.

Cellular damage produces adhesive interactions between endothelial cells and sickle cells ***(Hebbel et al., 2001)***. This association interactions delay cellular passage, so that, sickling, vaso-occlusion and polymerization occur during microvasculature transit. Sickle cells move with platelets, leukocytes and "stress" reticulocytes. Stress reticulocytes are immature erythrocytes that are presented in populations with hemolytic disease. These cells present adhesive ligands that encourage erythrocyte-endothelial interactions ***(Rother et al., 2005)***. Intravascular hemolysis consequence is illustrated in Figure (2) ***(Kato et al., 2007)***.

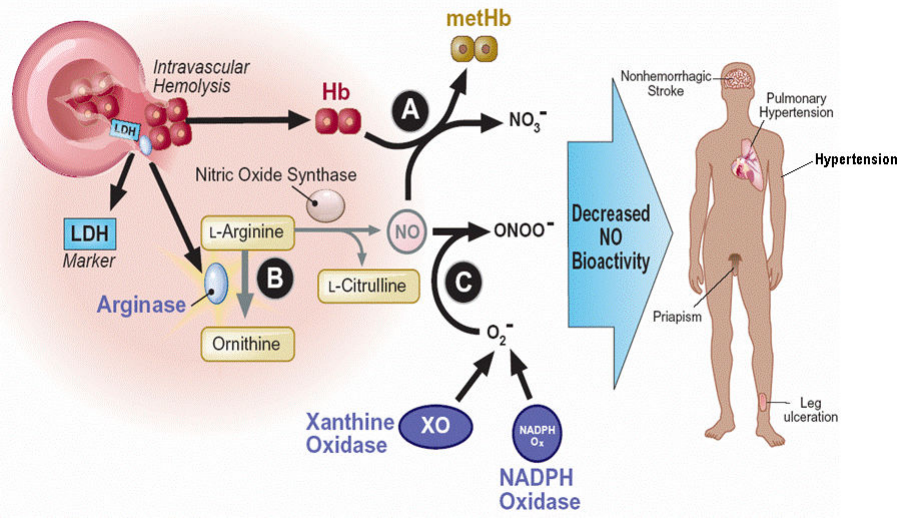

Figure (2): Intravascular hemolysis and NO bioactivity in sickle cell disease ***(Kato et al., 2007).***

Clinical Features of Sickle Cell Disease

Most sickle cell anemia (SCA) patients have a moderate degree of stable anemia and their packed cell volume between 25 -30 ***(Zennadi et al., 2004).*** In their early years, the disease is presented by painful episodes ***(Smith et al., 2008)***, stroke ***(Ebakisse-Badassou, 2010)*** and acute chest syndrome ***(Vichinsky et al., 2000)***. In their middle years, they may presented by osteonecrosis and bone diseases ***(Frempong et al., 2001)***, leg ulcers, priapism ***(Burnett et al., 2006)***, digestive disease and gall stones ***(Haberkern et al., 1997)***. While in their old years, the disease is presented by pulmonary hypertension, eye disease, cardiovascular complications ***(Taylor et al., 2008)*** and nephropathy ***(Powars et al., 2005).***

Diagnosis

Generally diagnosis of SCA is proved by finding significant quantities of HbS using high-performance liquid chromatography (HPLC), isoelectric focusing, cellulose acetate electrophoresis or DNA analysis ***(Paixão et al., 2001)***.

Targeted mutation analysis can identify the common mutations in the HBβ gene linked to HbS. HPLC or gel electrophoresis can differentiate related disorders as homozygous carriers (HbSS) or the heterozygous carriers (HbAS) ***(Wajcman and Moradkhani, 2011).***

Management of SCD:

A multidisciplinary team led by a hematologist with other many specialists, such as nephrologists, orthopedic surgeons and pain management experts is highly recommended for SCD patient care ***(Halasa et al., 2007)***.

Hydroxyurea; a ribonucleotide reductase inhibitor, is the only drug with allover regulatory approval for treating patients with SCA ***(Charache et al., 1995)***. Really, how hydroxyurea produce it's therapeutic effect isn't complete understood. However, the drug primary effect is likely to be owing to its ability to induce high levels of HbF. Some studies showed that the reduction in reticulocytes, neutrophils and monocytes give a clinical benefit of hydroxyurea. Other mechanisms of action include; its effects on the membrane of sickle cell, adherence

molecule expression and vascular reactivity ***(Lapoumeroulie et al., 2005)***, NO generation, erythrocyte cation transport ***(Cokic et al., 2008)***, production of erythropoietin and red cell ***(Papassotiriou et al., 2000)***. In a pivotal efficacy trial in SCA adults, hydroxyurea decreased the incidence of acute painful events, acute chest syndrome and the frequency of hospitalization, and blood transfusion by more than 40% ***(Steinberg et al., 2003; Elalfy et al., 2006)***.

Treatment by polymerization inhibition via increasing HbF concentration; reducing reperfusion injury, interrupting intercellular interactions, reducing the density of sickle erythrocyte and increasing nitric oxide (NO) bioavailability. So, provide a basis for the approaches of combination chemotherapeutic which illustrated in Figure (1) ***(Ilesanmi, 2010)***.

Blood Transfusion and Iron Chelation

Blood Transfusion can at times be patient lifesaving and is otherwise useful in treating some critical complications occurring with SCA patients. Although chronic transfusion reduces most disease complications, this approach produces iron overload, alloimmunization, loss of venous access, viral infection, and is expensive. Blood Transfusions can use as a preventive stroke measure in high risk children by estimation and monitoring of their cerebral blood flow and also, after a stroke, transfusion can prevent recurrent stroke ***(Scothorn et al., 2002)***.

Exchange transfusion is better than simple transfusion in stroke, acute chest syndrome and other acute complications hasn't been tested in clinical trials. The benefits of exchange transfusions able to better control blood viscosity and volume and decrease iron accumulation, but also it has many disadvantages including the exposure to more blood units, difficult venous access, lack of timeliness if transfusion is urgently needed and expense ***(Chou and Fasano, 2016)***.

SCA patients with continued transfusion showed iron overload develops and can result in liver and heart failure and also, multiple other complications, although these subsequent seem less common in patients with sickle cell disease than in patients with β thalassemia ***(Fung et al., 2006)***.

Three chelators have been widespread used: desferrioxamine, deferiprone and deferasirox ***(Neufeld, 2006)***. However, desferrioxamine might be the most efficacious chelation agent, its need require to be given parenterally via prolonged infusion and every day nearly has limited its benefit in many patients. Deferasirox have the same capacity to chelate iron as desferrioxamine but can be given orally. The limiting factor in its use is the renal toxicity produce from its long use. Deferiprone can be given orally and removes cardiac iron selectively ***(Porter and Garbowski, 2013)***.

STROKE

Sickle cell disease-associated stroke is one of the most devastating SCD complications ***(Prengler et al., 2002)***. Stroke is manifested by rapid loss of brain function(s) due to blood supply disturbance to the brain which is considered one of the most leading neurologic causes of long-term disability and death (>15% of cases) ***(Wierenga et al., 2001; World Health Organization, 2002)***.

Epidemiology of sickle cell disease-associated stroke

Each year, in general population, over five million people died as a consequence of stroke; and at least 1 of 6 patients who survive from stroke will suffer from another stroke within five years ***(Hankey and Warlow, 1999)***. Globally, stroke affect up to 17% of patients with SCD, and in this population; recurrent stroke can reach up to 80% within 3 years following the first stroke if therapy isn´t engaged ***(Debaun et al., 2012)***.

Pathogenesis of stroke in SCA patients:

Ischemic stroke may occur by many mechanisms in SCA patients. Several studies suggest a synergistic effect of different factors; leading to critical, episodic and regional oxygen delivery reductions to the brain that cause tissue infarction ***(DeBaun et al., 2006).***

1- *Increased Tissue Factor (TF) expression in SCA:*

Sickle cell patients show elevated blood TF procoagulant activity ***(Pawlinski et al., 2004)***, and increased levels of mRNA, TF antigen and activity in their circulating endothelial cells ***(Solovey et al., 1998)***.

In addition, sickle cell patient's blood contains endothelial cell-derived TF positive microparticles and monocyte ***(Shet et al., 2003)***. TF expression was similarly increased in whole circulating endothelial cells and blood with patients with steady-state disease and those in pain crisis. In contrast; higher numbers of TF positive microparticles were found during pain crisis episodes more than those in steady-state disease ***(Shet et al., 2003).***

2- *Increased thrombin generation and thrombosis in SCA*

Thrombin anti-thrombin complexes and levels of prothrombin fragment in plasma are increased in SCA patients. In addition, plasma levels of fibrinopeptide E, D-dimers, plasmin-antiplasmin and fibrin-fibrinogen peptide E complexes are elevated indicating that clot formation and fibrin degradation develops in sickle cell patients ***(Ataga, 2009; De Franceschi et al., 2011)***.

In contrast, there were not changed in plasma levels of TF pathway inhibitor; a natural inhibitor of TF in the sickle cell

patients ***(Key et al., 1998)***. Further, a state of hypercoagulable in SCA is demonstrated by the presence of multiple thrombotic complications in sickle cell patients, as in situ pulmonary embolism, venous thromboembolism and stroke ***(Stein et al., 2006)***. Also, pulmonary microthrombi have been found during acute chest syndrome episodes in SCA ***(de Franceschi et al., 2003; Guo et al., 2008).***

3- Large Artery Vasculopathy

The pathogenesis of vasculopathy in SCA is still unclear; however, a specific pathology can be obseved in these patients. Damage of the endothelium in the middle or large sized arteries in the brain; especially at branch points is the most common pathology which producing fibrin deposition and thrombus formation ***(Rothman and Fulling, 1986)***. The most common site of occlusion is the bifurcation of internal carotid artery into and the middle cerebral artery (MCA) and the anterior cerebral artery (ACA) ***(Serjeant and Serjeant, 2001)***. This arterial narrowing or occlusion may lead to formation a "Moyamoya" pattern in the collateral, which could be diagnosed by either magnetic resonance angiography (MRA) and magnetic resonance imaging (MRI) or with cerebral angiography ***(Tarasów et al., 2011).***

4- Chronic and Acute Anemia

Both chronic anemia and relative acute anemia consider risk factors for strokes with SCA patients. A contributing factor

is the further decrease in oxygen content [oxygen content ~ (1.34 × arterial oxygen saturation × hemoglobin concentration). Among SCA patients, the hemoglobin level was an independent risk factor for occurrence of strokes ***(Ohene- Frempong, 2001)***. SCA patients need a high reticulocyte count to maintain state hemoglobin between 6 and 10 g/dl ***(DeBaun et al., 2006).*** In addition, SCA patients have acute decline in the level of hemoglobin due to infection ***(Wierenga and Serjeant, 2001)*** or other causes which temporally related to the acute illness ***(Scothorn et al., 2002)***.

Hemoglobin is one of the physiological factors associated with arterial oxygen content and considers a major determinant of cerebral blood flow (CBF). In patients with a chronic anemia; low arterial oxygen content initially lead to increase CBF to maintain oxygen delivery to the brain by autoregulatory dilation. This cerebral vasodilation produce limitation in the ability of the cerebrovasculature in patients with SCA to further respond to other vasodilatory stimuli; such as increased cerebral metabolic demands, hypoxia and reduced cerebral perfusion pressure (CPP), leading to increase the risk of strokes ***(Naranjo et al., 2013)***. Reducing the concentration of hemoglobin reduces the oxygen content. As a consequence, either oxygen extraction fraction (OEF) or CBF or both must increase to support normal oxygen delivery ***(Kuwabara et al., 1990)***.

5- Low Nocturnal Oxygen Saturation

A low level of either oxygen saturation or hemoglobin or both together increase the risk of strokes in patients with SCA. Nocturnal pulse oximeter readings in SCA patients less than 95% were at risk for a further cerebrovascular accident in comparison to those whose oxygen saturation was more than 95% ***(Kirkham et al., 2001)***.

6- Blood Viscosity

In SCA patients, blood viscosity is a risk factor for strokes. Increased blood viscosity lead to further impair the blood flow, and in SCA patients, blood viscosity is dependent on a group of risk factors including, the hemoglobin S concentration, the total hemoglobin concentration, and the red blood cell deformability in deoxygenated and oxygenated states ***(Itoh et al., 1995)***.

7- Acute Medical Events Temporal Associated with Stroke

Acute medical conditions such as acute chest syndrome, systemic illness or acute infection are temporally associated with increasing the incidence of strokes in SCA patients. The cause of this association is still unknown. Presumably they may either decrease the oxygen delivery to the brain and/or increase the metabolic demands of the brain ***(DeBaun et al., 2006)***.

8- Abnormal Red Cell Adhesion in SCA

Patients with SCA have abnormal red blood cells which may contribute to the stroke pathogenesis. In situ thrombosis occur in small vessels due to sickle red blood cells which increased adherence to endothelial cells leading to formation in situ thrombosis ***(Laurance et al., 2011)***.

Patterns of Infarction in SCA

Cerebral infarcts in SCA patients are commonly occur in both deep white matter and cortex, while silent lesions usually were involved deep white matter only ***(Moser et al., 1996)***. The cerebral infarction pattern is strongly associated with impairment of the hemodynamics in other patient populations and supports poor oxygen delivery over a thromboembolic or local thombotic mechanism ***(Solomou et al., 2013)***.

Types of stroke

Stroke is classified into three major categories, and varies regarding to the age:

1- Ischemic or infarctive stroke:

It is the most common type (about 87%) caused via interruption of the blood supply that may be due to arterial stenosis / thrombosis, embolism or ischemia ***(Roach et al., 2008).***

In the SCD patients, primarily the clinical manifestation (i.e. signs and symptoms) of ischemic stroke depends on the