INTRODUCTION

Sickle cell disease (SCD) is one of the most prevalent genetic disorders. There are more than 200 million carriers of sickle cell trait worldwide ***(Bunn et al., 1997).***

Upon exposure to low oxygen tension the mutant haemoglobin S becomes less soluble and aggregates into large polymers. This results in a distorted erylhrocyte with marked decrease in its deformability contributing to the vaso-occlusive and haemolytic aspects of the disease ***(Embury, 2000)***.

Sickle cell disease (SCD) is characterized by an increased susceptibility to infections and vaso-occlusive complications. Patients with SCD can develop specific and sometimes life-threatening complications, as well as extensive organ damage reducing both their quality of life and their life expectancy ***(Schhnog et al., 2004)***.

Over many years, different methods for managing this disease have been proposed and studied. As a result, over the last three decades there has been some overall improvement in the understanding and care of sickle cell anemia patients. During recent years, some progress and developments in attempts to curtail the basic pathophysiology of sickle cell disease including hydroxyurea, bone marrow transplantation, and gene therapy had

been encountered. Unfortunately, there are major limitations with each of these modalities ***(Steinberg, 1996)***.

L-glutamine is a precursor for NAD and there is increase membrane transport and affinity for glutamine by sickle RBCs ***(Niihara et al., 2000)***.

Nicotinamide adenine dinucleolide (NAD) is essential for normal RBCs. In sickle cell disease there is over consumption of glutamine as evidenced by increased level of disease there is over consumption of glutamine as evidenced by increased level of glutamate a by – product of glutamine this result in decreased level of NAD which is responsible for the oxidative phenomenon that play an important role in the pathophysiology of sickle cell anemia ***(Chin et al., 1994)***.

L-glutamine is an amino acid that is readily available and has been shown to be safe for oral administration.

Glutamine supplementation may help promote synthesis of NAD and there were subjective reports of improvement in the patient chronic pain level and energy level with no evidence of major side effects in a dose of 0.6gm/kg/day ***(Sharbert et al., 2003)***.

AIM OF THE STUDY

The current study was undertaken to explore the value of L-glutamine therapy as a modifier in Sickle cell anemia and Sickle beta thalassemia patients as regard clinical and laboratory response after 4 weeks of administration of glutamine.

SICKLE CELL DISEASE

Definition:

Sickle cell disease (SCD) is a genetic disorder of hemoglobin synthesis. It's characterized by severe chronic hemolytic anemia resulting from premature destruction of the sickle red cells and presented by a special clinical course attributed to the ischemic changes, which result from the vascular occlusion ***(Powars et al., 2002).***

The disease usually occurs in periodic painful attacks eventually leading to damage of some internal organs, stroke or anemia and usually resulting in decreased life span ***(Charache et al.,* 1995)**.

Pathophysioloy:

HbS polymerization:

HbS arises from a mutation substituting thymine for adenine in the sixth codon of the beta-chain gene. This causes coding of valine instead of glutamine in position 6 of the Hb beta chain. The resulting Hb has the physical properties of forming polymers under deoxy conditions. It also exhibits changes in solubility and molecular stability. These properties are responsible for the profound clinical expressions of the sickling syndromes ***(Steinberg, 1999)***.

Under deoxy conditions, Hb S undergoes marked decrease in solubility, increased viscosity and polymer formation at concentrations exceeding 30 g/dL. It forms a gel-like substance containing Hb crystals called tactoids. The gel-like form of Hb is in equilibrium with its liquid-soluble form. A number of factors influence this equilibrium, including the following:

- Oxygen tension
    - Polymer formation occurs only in the deoxy state.
    - If oxygen is present, the liquid state prevails.
- Concentration of hemoglobin S
    - The normal cellular Hb concentration is 30 g/dL.
    - Gelation of Hb S occurs at concentrations greater than 20.8 g/dL.
- The presence of other hemoglobins
    - Normal adult hemoglobin (Hb A) and fetal hemoglobin (Hb F) have an inhibitory effect on gelation.
    - These and other Hb interactions affect the severity of clinical syndromes. Hb SS produces a more severe disease than sickle cell Hb C (Hb SC, and Hb with one normal and one sickle allele (Hb SA) ***(Bookchin and Lew, 1996).***

After recurrent episodes of sickling, membrane damage occurs and the cells are no longer capable of resuming the

biconcave shape upon reoxygenation. Thus, they become irreversibly sickled cells (ISCs). From 5-50% of RBCs permanently remain in the sickled shape ***(Goodman, 2004).***

Hemolysis is a constant finding in sickle cell syndromes. Approximately one third of RBCs undergo intravascular hemolysis. Sickle-shaped RBCs are rapidly hemolyzed and have a life span of only 10-20 days ***(Rodgers, 1997)***.

These physiological changes result in a disease with the following cardinal signs: (1) hemolytic anemia, (2) painful vasoocclusive crisis, and (3) multiple organ damage with microinfarcts, including heart, skeleton, spleen, and central nervous system ***(Burnt, 1997)***.

Sickle cell adhesion:

Vasoocclusion of small and some times large vessels is the hallmark of sickle cell disease (SCD), accounting for much of its morbidity and mortality. The hemoglobin polymerization itself is not sufficient to account for the episodic nature of vascular occlusion, there is emerging consensus that a key-contributor to vasoocclusion may be the increased tendency of sickle red cells to adhere to vascular endothelium ***(Rosse et al., 2000)***.

Vasoocclusion can occur when transient time of red cells through the capillaries is longer than the delay time for deoxygenation. Hemoglobin polymerization of sickle hemoglobin,

as adherence of sickle red cells to vascular endothelium will impede blood flow thereby increase capillary transient time, it has been suggested that increased cell adherence can initiate and propagate vasoocclusion ***(Rosse et al., 2000; El Alfy et al., 2002)***.

Factors such as inflammatory mediators that active endothelial cells and thereby enhance endothelial adhesivity sickle red cells thus have the potential to trigger vasoocclusive episodes. A partial list of agonists that may alter endothelium and play a role in sickle cell disease includes tumor necrotic factor (TNF), interferon, interleukin-lB (IL-1B), vascular endothelial growth factor (VEGF), thrombin, and histamine. and the effects of hypoxia and reperfusion ***(Rosse et al., 2000)***.

Sickle cells express very late antigen (VLA)-4 on the surface. VLA-4 interacts with the endothelial cell adhesive molecule, vascular cell adhesive molecule (VCAM)-l- VCAM-1 is upregulated by hypoxia and inhibited by nitric oxide(NO) ***(Wick and Eckman, 1996)***. Recently, the important role of NO in the pathophysiology of sickle cell disease has been recognized such as increasing the oxygen affinity of HbS, diminishing the expression and activity of adhesion molecules and relieving vasoconstriction ***(El Alfy et al., 2002)***.

Recent studies have begun to delineate the molecular interactions responsible for the adhesion of SS red cells to endothelium (Figure 1). Reticulocytes, especially those from

patients with SS disease, have on their surface the integrin complex $\alpha_4\beta_1$which binds to both fibronectin and VCAM- 1, a molecule expressed on the surface of endothelial cells, particularly after activation by inflammatory cytokines such as tumor necrosis factor α. In addition, both microvascular endothelial cells and a subpopulation of sickle reticulocyte have CD36, which binds to thrombospondin secreted activated platelets. Thrombospondin also binds to sulfated glycans on SS red cells ***(Bunn, 1997).*** In addition to thrombospondin, several other plasma proteins, including very-high-molecular-weight forms of von Willebrand factor ***(Kaul el al, 1993),*** may make an important contribution to adhesion. During inflammatory stress, the adhesion of SS red cells to endothelial cells may be increased as a result of increases in the above-mentioned plasma proteins as well as increased expression of VCAM-1 on endothelial cells. Increased binding of SS neutrophils to fibronectin may also contribute to vasoocclusive episodes ***(Kasschau et al., 1996)***.

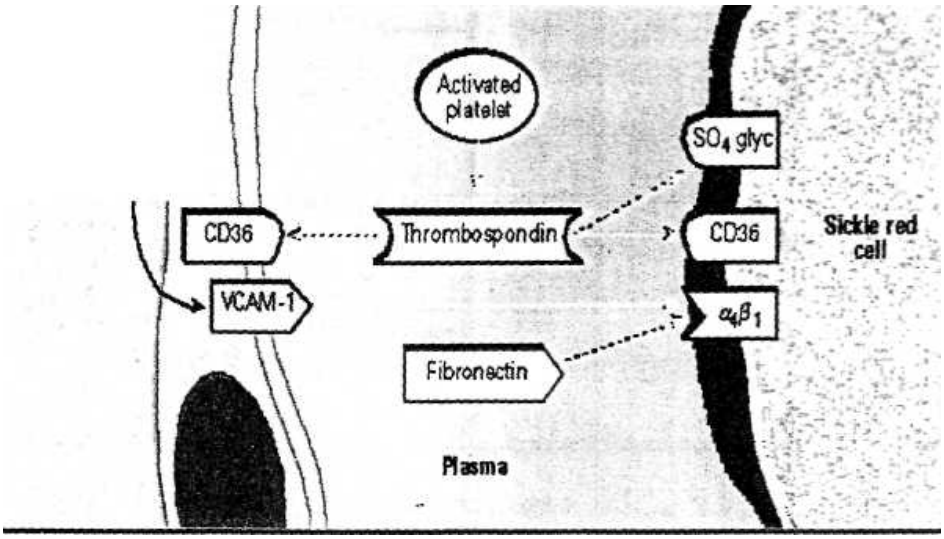

Fig. (1): Principal interactions responsible for the adhesion of a sickle red cell to the microvascular endothelium. Activation of platelets releases thrombospondin, which can act as a bridging molecule by binding to a surface molecule, CD36, on an endothelial cell and to CD36 or sulfated glycans (SO_4 glyc) on a sickle reticulocyte. Inflammatory cytokines induce the expression of vascular-cell adhesion molecule 1 (VCAM-1) on endothelial cells. This adhesive molecule can bind directly to the 04 Pi integrin on the sickle reticulocyte ***(Bunn, 1997).***

The different forms of sickle cell disease:

I-Hb SS –SCD (homozygous sickle cell disease)

Hb SS disease results from Homozygosity for the β_S-gene. The red cells in sickle cell anemia contain large amount of Hb S, variable amount of Hb F and normal level of Hb A_2.

The sickle cells are fragile, non-deformable and rapidly destroyed. The clinical and hematological manifestations of sickle cell disease are due to these two processes:

1- Severe hemolysis and the compensatory mechanisms evoked by hemolytic anemia.

2- Vaso-occlusion and infarction involving many tissues and organs.

The development of hemolytic anemia parallels the declining level of HB-F postnatal and becomes obvious by 4 months of age ***(Serjeant et al., 2001).***

The laboratory finding of Hb-SS including; moderate to severe normocytic normochromic anemia with morphologic abnormalities (target cells and irreversibly sickled cells). The mean corpuscular volume (MCV) is normal but the mean corpuscular hemoglobin concentration (MCHC) and the reticulocytic count are increased.

The white blood cell count is elevated, platelet count is usually increased and the bone marrow shows hypercellularity

with erythroid hyperplasia. Serum ferritin and iron stores are increased due to blood transfusion ***(O'Brein, 1978).***

II. Hb Sβ^0-Thalassemia:

Individuals with Hb S^{0}β-thalassemia are clinically identical to Hb SS disease. In Hb Sβ^0-thalassemia, the MCV and MCH usually low, and Hb A_2 is elevated. The electrophoretic pattern of HB SB^0-thalassemia is similar to the Hb SS.

III. Hb Sβ$^+$ -thalassemia:

Individuals with Hb Sβ$^+$-thalassemia have 10-30% Hb A In the red cells. Their hemoglobin level slightly decreased. MCV and MCH are usually low and Hb A_2 is elevated. Most of the clinical complications of Hb SS also occur in Hb Sβ$^+$-thalassemia, but less frequently.

IV. Hb Sγβ^0-thalassemia:

Its electrophoretic pattern is similar to that of Hb SS disease, but Hb F levels are 15-25%. There is mild anemia, and the MCV may be decreased, Hb A_2 is usually low to normal because of the presence of only one γ globulin gene and it has mild clinical course.

V. Hb SC disease:

It is due to inheritance of β^S globin gene from one parent and β^C globin gene from the other. It is characterized by a moderate chronic anemia. Hemoglobin ranges between 9-12g/dl.

Reticulocytic count 3-10%, blood smears contains about 50% target cells and occasionally cells with Hb C crystals. Clinically all symptoms found in Hb SS may occur in Hb SC. Usually less frequent and less severe. Hb SC is more prone to sickle cell retinopathy, gross hematuria and fat embolism ***(Chiniel et al., 1975).***

Other variants of SCD:

Hb S/HPFH, Hb SD disease, Hb SA, Hb SO arab disease and Hb SE disease are other variants of scd.

Table (1): Showing different forms and variants of sickle cell disease

o ***Hb SS disease:*** or sickle cell anemia homozygous for βS globin usually severe or moderately severe phenotype.
o ***HbS/β^{0}thalassemia:*** severe double heterozygous for HbS and β^0 thalassemia, and almost indistinguishable from sickle cell anemia phenotypically.
o ***Hb SC disease:*** double heterozygous for Hb S and Hb C with Intermediate clinical severity.
o ***HbS/β thalassemia:*** mild to moderate severity, but variable in different ethinic groups.
o ***Hb S/hereditary persistence of fetal Hb(HPFH):*** very mild phenotype or symptom free.
o ***HbS/HbE syndrome****:* very rare and very mild clinical course.
o Rare combination of HbS with ***HbD Los Angelos, HbO Arab, G-philadelphia***, among others.

(Chui and Dover, 2001)

COMPLICATIONS OF SICKLE CELL DISEASE IN CHILDHOOD

Complications of sickle cell disease are numerous, may occur suddenly and can rapidly become severe. Vasoocclusive crisis, pain, acute splenic sequestration crisis, aplastic crisis, acute chest syndrome, stroke, infection, cholelithiasis and renal disease are the major complications of this disease in children. Some complications lend themselves to simple management, whereas others, including aseptic necrosis of the hip, priapism and leg ulcers, require prompt referral for specialized treatment ***(Eckman, 1996)***.

Vasoocclusive Crisis:

Vascular occlusion may involve both the macro-circulation that leads to the end organs damage and the micro-circulation that underlies the acute painful crises. The sequential steps that occur in the micro-circulation culminating in the sickle cell painful crisis include: polymerization of Hb S, decreased red cell deformability, micro-vascular occlusion, hypoxia of the tissue supplied by the occluded micro-vascular network and tissue damage that triggers painful stimuli, by releasing secondary inflammatory mediators that precipitate pain in these episodes ***(Embury et al., 2004).***

When it involves abdominal organs, vasoocclusive crisis can mimic an acute abdomen. With repeated episodes, the spleen autoinfarcts, and becomes fibrotic and functionless. The liver also may infarct and progress to failure with time. Papillary necrosis is a common renal manifestation of vasoocclusion, leading to isosthenuria (ie, inability to concentrate urine). Vasoocclusive crises can involve the lungs e.g. acute chest syndrome ***(Lane, 1996)***.

Central nervous system manifestations of vasoocclusive crises including cerebral infarction (children), hemorrhage (adults), seizures, transient ischemic attacks, cranial nerve palsies, meningitis, sensory deficits, and acute coma. Cerebrovascular accidents are not uncommon in children, and they tend to be recurrent ***(Kirkham and DeBaun, 2004)***.

Skin ulceration, and retinal hemorrhages are frequent complications of sickle cell vasoocclusive crises. Finally, vasoocclusion may involve the corpus cavernosum, preventing blood return from the penis and leading to priapism ***(Okpala, 1998)***.

Acute Splenic Sequestration Crisis

Acute splenic sequestration crisis is most common in children with homozygous sickle cell disease (hemoglobin SS disease) who are less than three years of age, but it can occur in any type of sickle cell disease. Children with homozygous sickle

cell disease usually infarct their spleens before four years of age. However, children with variant disease can have acute splenic sequestration crisis at any time during childhood ***(Lane, 1996)***.

Splenic sequestration crisis results from the acute entrapment of large amounts of blood in the spleen. The manifestations are left upper quadrant pain, exacerbated anemia and, often, hypotension. Enlargement of the spleen from entrapped blood can be so rapid and sever that it causes circulatory compromise. Circulatory collapse and death can occur in less than thirty minutes. Early diagnosis and immediate intervention with intravenously administrated fluids and judicious transfusion can be life-saving ***(Wethers, 2000b)***.

A child who suffers one episode of splenic sequestration crisis is at greater risk of a second attack ***(Kinney et al., 1990)***. Surgical splenectomy to prevent recurrence is often recommended after recovery from life-threatening or recurrent episodes of sequestration ***(AAP, 2002)***.

Aplastic Crisis

Erythrocyte production by the bone marrow may pause temporarily in children with sickle cell disease. Human Parvo virus B19 infection is responsible for 80 % of aplastic crises. The infection is self-limited and may cause only mild, nonspecific symptoms ***(Serjeant et al., 1993)***.