INTRODUCTION

Transfusion-related iron overload in thalassemia has been associated with the onset of cardiovascular complications, inclu-ding cardiac dysfunction. Thalassaemic are known to have poor growth, altered puberty and immune dysfunctions , this is exagerated as thalassemic patients have reduced intake of many key nutrients, as well as the vitamins and minerals are being consumed as a result of oxidative stress, and found that 40-75%were deficient of vitamin A ,C, D and selenium. There is alot of comorbidities is typically described to the toxic effect of iron overload ***(Stoyanova et al., 2012)***.

Thalassemia major (TM) is the clinical diagnosis applied to patients who are homozygous for the beta thalassemia genes and who present early with symptoms and signs of anemia and bone marrow expansion. Without transfusion therapy these patients are unlikely to survive beyond the age of 10 years ***(Camaschella & Cappellini, 1995).***

Complications are still common and include heart disease (heart failure and arrhythmias), chronic liver hepatitis, which can evolve in cirrhosis and, rarely, in hepatocellular carcinoma, endocrine problems (hypogonadism, hypothyroidism, diabetes, hypoparathyroidism), stunted growth, nutrional deficiencies. The incidence of complications is decreasing in younger patients who have been transfused with blood that has been screened for viruses and thanks to the introduction of new

oral iron chelators and imaging methods. The accurate measurement of iron deposits allows better management of iron overload. In addition, therapy for several complications is available. Also, it has proven that Vitamin C is very important for thalassemia patients as most of them are having scurvy. As vitamin C has a protective antioxidant state, so protects against the chronic oxidative stress which occurs in thalassemia patients due to consumption of tecopherol, a chain breaking antioxidant. Morover, vitamin C helps the excretion of iron when using the iron chelators, if given 100mg/day up to 200mg/day ***(Cappellini et al., 2001).***

AIM OF THE WORK

Prospective study to asses the efficacy and safety of administration of vitamin C on different iron chelation therapy in children and adolescent with β-Thalassemia major

THALASSEMIA

Introduction:

Thalassemia is one of the most common genetic diseases worldwide, with at least 60,000 severely affected individuals born every year. Individuals originating from tropical and subtropical regions are most at risk. Disorders of hemoglobin synthesis (thalassemia) was among the first molecular diseases to be identified, and has been investigated and characterized in details over the past 40 years ***(Hamamy and Al-Allawi, 2013).***

Beta-thalassemias are a group of hereditary blood disorders characterized by anomalies in the synthesis of the beta chains of hemoglobin resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals. Three main forms have been described: thalassemia major, thalassemia intermedia and thalassemia minor ***(Lilleyman et al., 2000).***

Individuals with thalassemia major usually present within the first two years of life with severe anemia, requiring regular red blood cell (RBC) transfusions ***(El Beshlawy et al., 2007)***.

Historical background:

The first definitive descriptions of Thalassemia were published independently in the United States and Italy in 1925. In the United States, Cooley, a pediatrician from Detroit, identified a group of children of Mediterranean origin with profound anemia, enlargement of the spleen and peculiar bone changes ***(Weatherall, 2004)***. Following Cooley's epochal descriptions, other similar conditions were reported in North America and Europe. In 1932, Whipple and Bardfod in Rochester

Apparently wishing to avoid the eponym "Cooley's anemia," they coined the term "thalassemia" from the Greek word ***(thalss)***, meaning "the sea" (i.e., the Mediterranean). Thus, thalassemia means "the sea in the blood" ***(Whipple and Bradford, 1932).***

It was shortly thereafter that Gatto in Italy and Valentine and Neel in the United States clearly pointed out the relationship of these mild microcytic anemias to the severe Cooley's anemia and suggested the clinical terms thalassemia "minor" and "major" for the heterozygous and homozygous conditions ***(Gatto, 1942)***.

In 1925, Thomas Cooley and Pearl Lee described a form of severe anemia, occurring in children of Italian origin and associated with splenomegaly and characteristic bone changes

(Cooley and Lee, 1925). In the past 20 years, the two important forms of the disorder, α and β-thalassemia, resulting from the defective synthesis of α and β globin chains of hemoglobin, respectively, have become recognized as the most common monogenic diseases in humans ***(Efremov, 2007)*** and globally it is estimated that there are 270 million carriers of which 80 million are carriers of βthalassemia ***(Cunningham et al., 2004b).***

Epidemiology

The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world and 1 in 10,000 people in the European. It is estimated that more than 56,000 infants born annually are affected by thalassemia major, including 30,000 who will require lifelong regular blood transfusions to survive ***(Forget, 2000).***

β-thalassemia is encountered in polymorphic frequencies in almost all Arab countries with carrier rates of 1-11 % and a varying number of mutations. The most widespread mutation in Lebanon, Egypt, Syria, Jordan, Tunisia and Algeria is the IVS-I-110 (G>A). In the Eastern Arabian Peninsula, the Asian Indian mutations (IVS-I-5 (G>C), codons 8/9 (+G) and IVS-I (-25 bp del)) are more common ***(Olivieri, 1999).***

The α-thalassemias are encountered in the majority of Arab countries in frequencies ranging from 1 to 58 % with the

highest frequencies reported from Gulf countries. The (-α(3.7)) mutation is the most frequent followed by the non-deletional α2 polyadenylation signal mutation (1) ***(Hershko et al., 2004).***

Distribution and population at risk

The β thalassemias are widespread throughout the mideiterranean region with estimates of gene frequencies range from 3 to 10 percent in some areas ***(Weatherall, 1998).*** Within each population at risk for β thalassemia a small number of common mutations are found, as well as rarer ones ***(Flint et al., 1993).*** The epidemiology of the disease, however, is changing due to a fall in total birth rate, prevention programs, recent population movements and recent population migration ***(Thein, 2005a).***

Genetic background

Beta-thalassemias are caused by point mutations or, more rarely, deletions in the beta globin gene on chromosome 11, leading to reduced (beta+) or absent (beta0) synthesis of the beta chains of hemoglobin (Hb). Transmission is autosomal recessive; however, dominant mutations have also been report (2) ***(Kati and Melelis, 2007)***.

The thalassemias refer to a diverse group of hemoglobin disorders characterized by a reduced synthesis of one or more of the globin chains ***(Weatherall, 2001).*** As more than 200

mutations affecting the β globin gene are now known to result in a phenotype of β thalassemia ***(Olivieri, 1999).***

The β globin gene cluster

β globin is encoded by a structural gene found in a cluster with the other (3-like genes spanning 70 Kb on the short arm of chromosome 11 (11pl5.4) (Figure 3A). The cluster contains five functional genes, 5ε Γγ-Aγ-ψβ, δ-β3, which are arranged in the order of their developmental expression ***(Thein, 2005b).***

Upstream of the entire (3 globin complex is the locus control region (LCR), which is essential for the expression of all the genes in the complex ***(Kanavakis and traeger-Synodinos, 2006).***

The genomic sequence spans 1600 bp and codes for 146 amino acids; the transcribed region is contained in three exons separated by two introns or intervening sequences (IVS) ***(Weatherall, 2004; Marini et al., 2004).***

As an adaptation to changing oxygen requirements, different hemoglobins are synthesized in the embryo, fetus, and adult ***(Wood, 2001).*** The p-like genes undergo two switches (embryonic → fetal → adult). At 6 months after birth, Hb F comprises less than 5% of the total hemoglobin and continues to fall until reaching the adult level of <1% at 2 years of age ***(Olivieri, 1999).*** It is at this stage that mutations affecting the (3

gene become clinically apparent. The switch from fetal (γ) to adult (β) hemoglobin production is not complete since small amounts of (3expression persist in adult life. The residual amount of fetal hemoglobin (α2β2) is present in a sub-set of erythrocytes called F cells which also contain adult (α2β2) hemoglobin ***(Carter et al. 2002) (Tolhuis et al, 2002).***

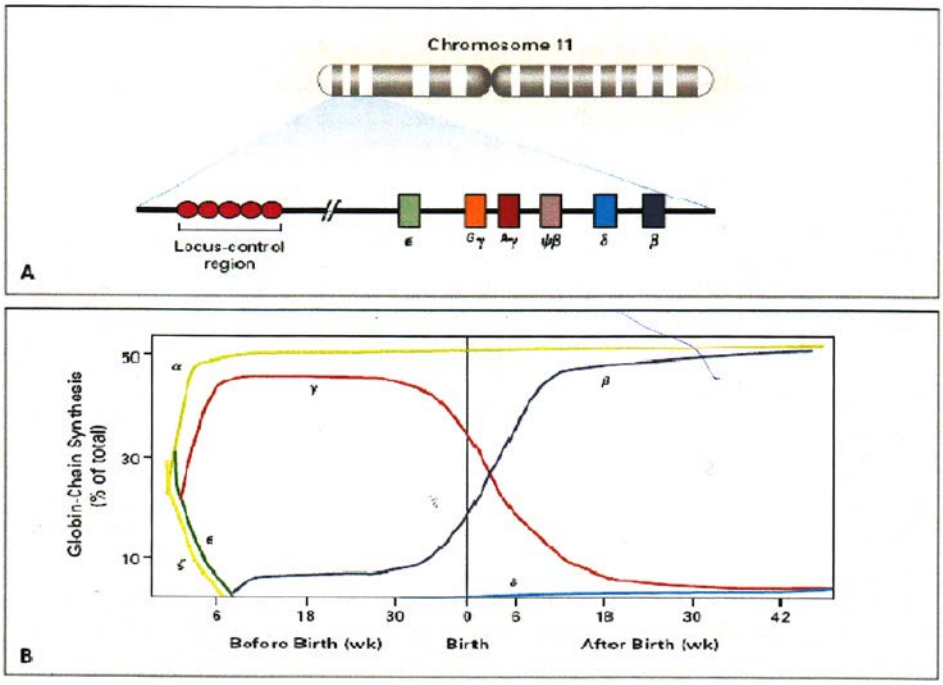

Fig. (1): The β globin gene cluster. Panel A: the β globin like genes arranged in order in which they are expressed during development. Panel B: shows the timing of normal development switching of human hemoglobin ***(Olivieri, 1999).***

Epidemiology:

- ***Race:***

Before the twentieth century, thalassemia tracked with areas of malarial prevalence. β-thalassemia arose in the Mediterranean, Middle East, South and Southeast Asia and Southern China. Patients of Mediterranean origin are more likely to be anemic with thalassemia trait than Africans because they have beta-zero thalassemia rather than beta-plus thalassemia ***(Kati and Farmakis, 2007)***

In Egypt, β-thalssemia is the most common genetically determined, chronic hemolytic anemia. The actual number of patients surviving to date is not, however, available ***(Adam et al., 1998).***

- ***Sex:***

This genetic disorder is caused by abnormalities in the beta-globin gene, located on chromosome 11. It is not a sex-linked genetic trait ***(Cohen et al., 2000).***

- ***Age:***

Patients with severe β-thalassemia are usually diagnosed between 6 months and 2 years of age when the normal physiologic anemia of the newborn fails to improve. Occasionally, the disease is not recognized until the child is 3 to 5 years of age because the infant is able to partially

compensate for the marrow's inability to produce hemoglobin A by prolonged production of HbF. On presentation affected infants usually have pallor, poor growth and development, and abdominal enlargement ***(Rati et al., 1997).***

Pathophysiology

β thalassemia occurs when there is a deficiency of functional β globin chains ***(Weatherall, 2001).*** This leads to an imbalanced globin chain production and an excess of β globin chains. The latter aggregate in red cell precursors forming inclusion bodies interferes with most stages of normal erythroid maturation, both intramedullary death of red-cell precursors through arrest in the G1 phase of the cell cycle and accelerated intramedullary apoptosis of late erythroblasts ***(Angelucci et al., 2002).***

The red cells that survive to reach the peripheral circulation are prematurely destroyed in the spleen which becomes enlarged, eventually leading to hypersplenism ***(Testa, 2004; Them, 2005c).*** Anemia in Beta thalassemia thus results from a combination of ineffective erythropoiesis, peripheral hemolysis, and an overall reduction in hemoglobin synthesis ***(Centis et al., 2000).***

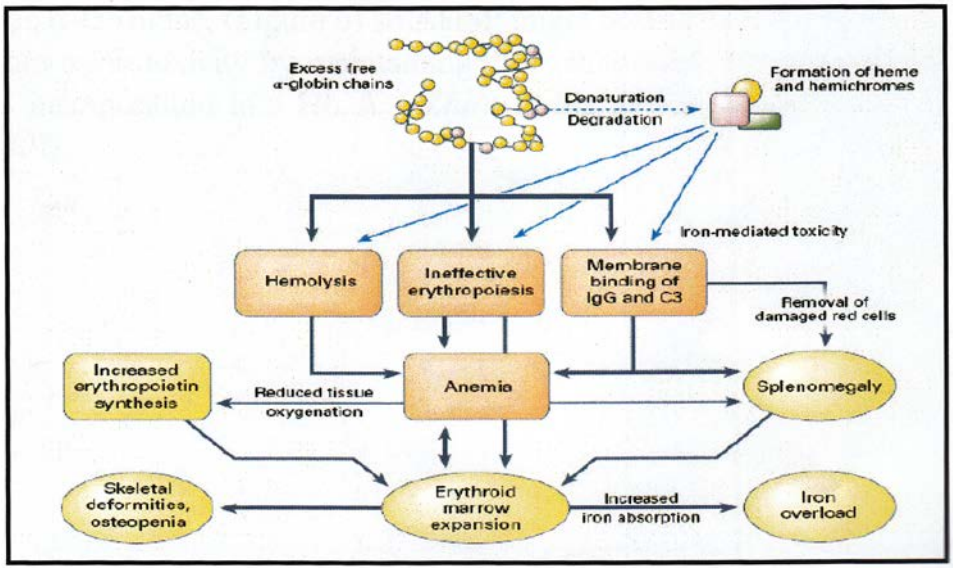

Fig. (2): Effect of excess production of free α globin chains ***(Olivieri, 1999).***

Late-stage erythroid development is largely dedicated to the production of the oxygen carrier hemoglobin (Hb) A, a tetramer consisting of two pairs of α-globin and β-globin protein and to minimize the accumulation of free α- or β-Hb subunits, which are cytotoxic. Excessive α-Hb is particularly damaging as intact monomeric α-Hb generates ROS that damage cellular proteins, lipids, and nucleic acids ***(Rachmilewitz and Schrier, 2001).***

Most cells contain compensatory mechanisms to cope with unstable proteins ***(Wickner et al., 1999)***. These include molecular chaperones that stabilize proteins and in some cases facilitate their folding into native structures ***(Viprakasit et al., 2004; Kong et al., 2004).*** In addition, there are degradation pathways that recognize and eliminate improperly folded

polypeptides. Accordingly, tissues typically tolerate some protein instability, with disease ensuing only when the compensatory mechanisms become overwhelmed ***(Weatherall, 2001).***

The alpha Hb-stabilizing protein (AHSP) heterodimerizes with α-Hb; but does not bind β-Hb or Hb A. Moreover, α-Hb bound to AHSP is more resistant to oxidant-induced precipitation than α-Hb alone, so AHSP might protect erythroid cells from α-Hb toxicity by maintaining α-Hb in a stable state prior to its incorporation into Hb A ***(Zhou et al., 2004; Xaing et al., 2007).***

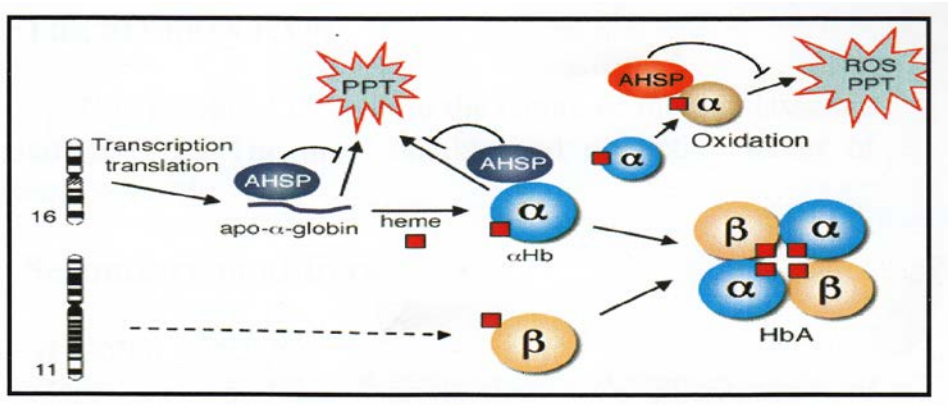

Fig. (3): The role of AHSP in stabilizing multiple forms of a globin at different stages of HbA synthesis and homeostasis. The AHSP (shown in red) inhibits ROS production and precipitation from excess αHb that accumulates in β thalassemia. The AHSP (shown in blue) acts as a molecular chaperone to promote native folding and stability of apo-αglobin and aHb en route to HbA synthesis. The brown circle indicates oxidized αHb ***(Xaing et al., 2007).***

Complications

Although most clinical manifestations of iron loading do not appear until the second decade of life in patients with inadequate chelation, evidence indicates that the effects of iron are initiated much earlier ***(Perifanis et al., 2006).***

Cardiovascular involvement represents a primary cause of mortality in thalassemic patients. Several factors have been reported affecting left and right heart, therefore, leading to heart failure. Beta-thalassemia patients are characterized by defective immunity determined by increased activity of CD8+ lymphocytes and reduced activity of the CD4+ subset. Also thalassemia patients have an increased risk of infections because of splenectomy, iron load, blood-borne infections, and iron chelation. Endocrine abnormalities are among the common complications of thalassemia despite good chelation therapy. Delayed puberty occurs in 56% of thalassemic patients. The main causes of liver injury in beta-thalassemia patients are hepatitis C virus infection and iron overload ***(Voskaridou et al., 2006)***.

The possible role of other factors including infectious agents, glucose intolerance, and iron chelation therapy was suggested. Renal involvement in patients with beta-thalassemia can be attributed to chronic anemia, iron overload, and chelation therapy ***(Mokhtar et al., 2013)***.

The most common morbidities observed were endocrinologic (44.7%) followed by cardiovascular (41.3%) and hepatic (40.5%), among the studied beta- thalassemia patients, proteinuria was the main manifestation (1.3%) of renal morbidities and around 94.4% were above 10 years. A total of 20.6% had elevated liver enzymes (serum ALT >3times the upper limit for normal), most of them above the age of 10 years(79.5%) with 1.1% having hepatitis B,16.8% having hepatitis C, and 2% having both ***(Abdulla et al., 2011)***.

Then renal (4%) and bone mineral density (BMD) affection (3.4%) Hypogonadism was the most frequent endocrinologic morbidity (40.9%), next in frequency was diabetes mellitus (2.7%), followed by hypothyroidism (0.4%) and hypoparathyroidism (0.4%). Impaired left ventricular contractility was the most frequent cardiovascular morbidity (37.4%), followed by arrhythmia (1.6%) (all had supraventricular tachycardia, one of them experienced also premature ventricular contractions) and pulmonary hypertension (0.4%). Most of the patients with impaired left ventricular contractility or arrythmia were above the age of 10 years (65.8%), and all the cases with pulmonary hypertension were among younger than 10 years ***(Tantawy et al., 2013)***.

Cardiac involvement in thalassemia major is generally characterized by iron-induced ventricular dysfunction cardiac uptake by Cell and animal studies have indicated that cardiac entry of iron is mediated by the divalent metal transporter 1