1INTRODUCTION

β-thalassemia is an inherited hemoglobin disorder resulting from impaired production of β-globin chains of the hemoglobin tetramer ***(Olivieri, 1999)***. The resultant phenotype is chronic hemolytic anemia of varying severity, depending on the level of β-globin chain deficiency and subsequent α-globin chain accumulation. Depending on clinical severity, two forms of β-thalassemia have been classified: thalassemia major and thalassemia intermedia. B-thalassemia major is characterized by severe transfusion-dependent anemia, starting from the first year of life ***(Aessopos et al., 2005)***.

In the absence of an iron chelating agent, patients with beta-thalassemia on regular transfusions present complications of transfusion-related iron overload ***(Veríssimo et al., 2013)***. Excess iron is deposited in major organs, resulting in organ damage. The organs that are at risk of damage due to iron overload include the liver, heart, pancreas, thyroid, pituitary gland and other endocrine organs ***(Olivieri, 1999)***. Common iron overload-related complications can be observed, including endocrine disturbances, dilated cardiomyopathy, liver fibrosis and cirrhosis ***(Taher et al., 2011)***.

The primary goal of iron chelation therapy is to prevent the accumulation of iron reaching harmful levels by matching iron intake from blood transfusion, with iron excreted by iron chelation ***(Porter, 2007).***

Currently there are three iron chelating agents available for continuous use in patients with thalassemia on regular transfusions (desferrioxamine, deferiprone, and deferasirox) providing good results in reducing cardiac, hepatic and endocrine toxicity ***(Veríssimo et al., 2013)***.

Deferoxamine (DFO) has been used for over 30 years for iron chelation. However, it is a parenteral drug that requires subcutaneous or intravenous infusions, owing to its short half-life and poor oral bioavailability, making compliance an issue ***(Cappellini et al., 2007).***

Two novel oral iron chelators; deferiprone and deferasirox provide potentially useful treatments for iron overload. A new enthusiastic era for iron chelation with less burdensome oral treatments is dawning, but long-term follow-up is required before pumps and needles can be thrown away ***(Rose, 2006).***

Essential trace elements such as copper, zinc and iron are antioxidant trace elements that are crucial for growth, carbohydrate and protein metabolism, gene transcription, endocrine function and nutrient transport in humans ***(Keen et al., 2003)***. Zinc is one of the essential micronutrients in human and acts as a cofactor for more than 300 enzymes and plays a particular role in human growth and development ***(Mahyar, 2005)***.

Likewise, the copper is considered an important micronutrient with highest amount in liver, brain, heart, and kidneys. Copper is also an essential structural coparticipant of many enzymes acting as cofactor in majority of enzymatic reactions including those of cytochrome C oxidase, lysyl oxidase, superoxide dismutase, and thyrosinase ***(Anderson, 2004)***.

Changes in serum zinc levels are still a subject for debate. Some studies have reported that patients with β-thalassemia major suffer from zinc deficiency which could be seen as one of the causes of delayed maturity in thalassemic Patients. Zinc deficiency causes growth retardation, alopecia, diarrhea and weight loss in human ***(De Sanctis, 2002)***. Evidence also indicates changes in serum level of copper in those patients ***(Mansi et al., 2009; Banihashem et al., 2013).***

Aim of the Work

The aim of this study was to determine the levels of essential trace elements; copper and zinc in children and adolescents with β-thalssemia major and to assess the effect of different iron chelating modalities on parameters of iron overload and serum levels of copper and zinc among those patients.

BETA THALASSEMIA

Production of red blood cells

First, a transient cohort of embryonic red blood cells originates in the blood islands of the yolk sac. Definitive haemapoietic stem cells (HSCs), which persist throughout fetal and adult life, emerge from the ventral wall of the dorsal aorta. These cells migrate from the ventral wall to the fetal liver and, by about 60 days of gestation, the first fetal red blood cells are released into the circulation to replace embryonic red blood cells. During fetal development, HSCs migrate to the bone marrow, which is the site of erythropoiesis for the rest of normal adult life. In early postnatal life, adult red blood cells from the marrow replace the fetal cells ***(Palis, 2008).***

At all stages of development, senescent red blood cells are continually replaced with new blood cells. These new cells are derived from HSCs, which differentiate into mature red blood cells via erythroid progenitors and precursors (erythroblasts). For an adult to maintain a normal red blood cell count, about 2 million to 3 million new cells must be produced every second. For severe forms of thalassemia, in which many erythroblasts and mature red blood cells are damaged, erythropoiesis can be increased by 20-30 times ***(Weatherall, 2010).***

Hemoglobin structure

Hemoglobin is the oxygen-carrying moiety of erythrocytes. It is a polypeptide tetramer, globular in structure, and consisting of two pairs of unlike globin chains (i.e., α plus β, δ, or γ), which form a shell around a central cavity containing four oxygen-binding heme groups each covalently linked to α globin chain. In healthy adults, 95% of the Hb is Hb A ($\alpha_2\beta_2$) with small amounts (3.5%) of Hb A_2 ($\alpha_2\delta_2$) and Hb F ($\alpha_2\gamma_2$) present. During embryonic development, "pre alpha" ξ globin chains contribute to embryonic Hb (Figure 1). During fetal development, β-like globin chains ε and γ contribute to the Hb ***(Kutlar, 2007)***.

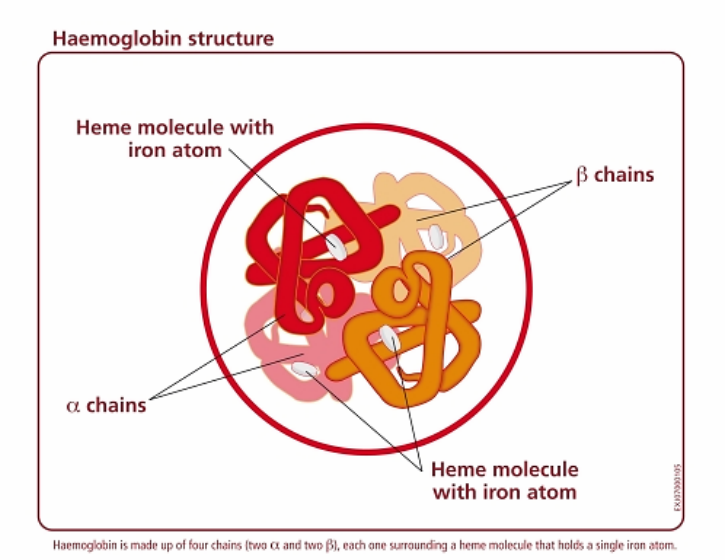

Figure (1): The structure of haemoglobin (2α chains and 2β chains). ***(http://164.109.71.105/Thalassaemia/General/Blood.html)***

Disease name and synonyms

The term thalassemia is derived from the Greek, thalassa (sea) and haima (blood). Beta-thalassemia includes three main forms: Thalassemia Major variably referred to as "Cooley's Anemia" and "Mediterranean Anemia", Thalassemia Intermediate and Thalassemia Minor also called "beta-thalassemia carrier", "beta-thalassemia trait" or "heterozygous beta-thalassemia". Apart from the rare dominant forms, subjects with thalassemia major are homozygotes or compound heterozygotes for $beta^0$ or $beta^+$genes, subjects with thalassemia intermediate are mostly homozygotes or compound heterozygotes and subjects with thalassemia minor are mostly heterozygotes ***(Galanello and Origa, 2010)***.

Historical review:

Thalassemia was first described by Dr Thomas Cooley in 1925 when he observed the disorder in patients of Mediterranean ancestry. He named the condition Cooley's anemia until doctors at the University of Rochester penned the name thalassemia, which means "sea in the blood", a reference to the fact that thalassemia is prevalent in the Mediterranean Sea area, although its genes are found in people worldwide. Although Cooley first described thalassemia in 1925, it wasn't until 1938 that it was recognized as a genetic disease. Bone marrow transplantation was first used as a treatment in 1982

and gene therapy was first used with a patient in 2007 ***(http://discovery.yukozimo.com/who-discovered-thalassemia/)***.

Definition and geographical distribution of β-thalassemia syndrome:

Beta-thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis. The resulting relative excess of unbound α globin chains precipitate in erythroid precursors in the bone marrow, leading to their premature death and, hence, to ineffective erythropoiesis resulting in reduced hemoglobin in red blood cells (RBC), decreased RBC production and anemia. Most thalassemias are inherited as recessive traits ***(Galanello and Origa, 2010)***.

Beta-thalassemias can be classified ***(Galanello and Origa, 2010)***:

- Beta-thalassemia:
  - Thalassemia major
  - Thalassemia intermediate
  - Thalassemia minor
- Beta-thalassemia with associated Hb anomalies:
  - HbC/Beta-thalassemia
  - HbE/Beta-thalassemia

  - HbS/Beta-thalassemia (clinical condition more similar to sickle cell disease than to thalassemia major or intermediate)
- Hereditary persistence of fetal Hb and beta-thalassemia
- Autosomal dominant forms
- Beta-thalassemia associated with other manifestations:
  - Beta-thalassemia-tricothiodystrophy
  - X-linked thrombocytopenia with thalassemia

The thalassemias are distributed across Africa, the Mediterranean region, the Middle East, the Indian subcontinent, and China and throughout southeast Asia in a line stretching from Southern China down the Malaysian peninsula to the Indonesian islands ***(Bernini et al., 2001)***. In these populations, the carrier frequency is greater than 1%, in contrast to a carrier frequency of approximately 0.1% in individuals of Northern European ancestry ***(Weatherall and Clegg, 2001).***

B-Thalassemia is the most frequent hemoglobinopathy in Egypt. The carrier rate of this disease varies between 5.3 and ≥ 9% and the gene frequency is 0.03, so it was estimated that 1,000/1.5 million per year live birth born with thalassemia disease (total live birth 1,936,205 in 2006) ***(El-Beshlawy et al., 2007).***

Epidemiology

β-thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia ***(Weatherall et al., 2010).*** The high gene frequency of β-thalassemia in these regions is most likely related to the selective pressure from Plasmodium falciparum malaria ***(Taylor et al., 2012).***

Population migration and intermarriage between different ethnic groups has introduced thalassemia in almost every country of the world, including Northern Europe where thalassemia was previously absent ***(Vichinsky, 2005).***

According to Thalassemia International Federation, only about 200,000 patients with thalassemia major are alive and registered as receiving regular treatment around the world ***(Thalassemia International Federation, 2008).*** The most common combination of beta-thalassemia with abnormal Hb or structural Hb variant with thalassemic properties is HbE/beta-thalassemia which is most prevalent in Southeast Asia where the carrier frequency is around 50% ***(Galanello and Origa, 2010)***.

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per year live births will suffer from thalassemia disease in Egypt (total live births 1,936,205 in 2006) ***(El-Beshlawy and Youssry, 2009)***.

Etiology and genetics

More than 200 mutations have been so far reported; the large majority are point mutations in functionally important regions of the beta globin gene. Deletions of the beta globin gene are uncommon. The beta globin gene mutations cause a reduced or absent production of beta globin chains. A list of common mutations according to the severity and ethnic distribution is reported in (Table 1) ***(Giardine et al., 2007).***

Three of the most common mutations characteristic for Mediterranean populations, IVS-II-745, codon 39 and IVS-I-110, account for more than 50% of all affected β-globin alleles ***(Hardison et al., 2002).***

Although most β-thalassaemias are caused by point mutations in the gene or its immediate flanking region, small deletions removing the β gene can also occur. When expression of β globin is abolished by the mutation it is referred to as β^0-thalassaemia, whereas reduced output of normal β chains produces β^+-thalassaemia. Some structural variants (particularly the β^Emutation) might also lead to a thalassaemic effect because they are produced at reduced levels, and their interactions with β^0 and β^+ thalassaemia (eg, β^E/β^T) lead to many forms of clinically severe β thalassaemia ***(Olivieri et al., 2010).***

Some rare forms of β thalassaemia result from deletions removing the upstream regulatory elements, but leaving all of the globin genes intact ***(Driscoll et al., 1989)***. These deletions result in a substantial reduction in expression of all of the linked globin genes, and first indicated the importance of long range regulatory elements in controlling expression of the β-like globin genes. Other rare cis-acting mutations (ie, mutations linked on the same chromosome) have been important in the development of our understanding of how the switch from γ-globin to β-globin gene expression is regulated. All these mutations result in variable levels of increased γ-globin expression and increased levels of fetal haemaglobin so called hereditary persistence of fetal haemoglobin (HPFH). HPFH can arise from mutations in the γ-globin promoters affecting the binding of activating or repressive complexes. Other forms of HPFH result from deletions removing the adult δ-globin and β-globin genes, but leaving at least one γ-globin gene intact. These deletions can lead to a moderate (δβ-thalassaemia) or considerable (HPFH) increase in γ-globin expression ***(Trachoo et al., 2003).***

Two new regulatory pathways that lead to increased γ-globin expression have been identified (BCL11A and HBS1L-MYB), and both seem to act by directly or indirectly affecting the production of repressor proteins that specifically target the γ-globin genes. These findings suggest that one or both of these pathways might provide targets that are useful for therapeutic

intervention because patients with haemoglobinopathies who co-inherit particular DNA sequence variants in these pathways, or mutations in the regulatory protein-binding sites, have high concentrations of hemoglobin F and mild disease phenotypes ***(Lettre et al., 2008; Nuinoon et al., 2010).***

Table (1): Common types of beta-thalassemia: severity and ethnic distribution

Severity	β-gene mutation	Population
β°	-619 del	**Indian**
β^{++}	-101 C→T	**Mediterranean**
β^{++}	-88 C→T	**Black**
β^{++}	-87 C→G	**Mediterranean; African**
β^{++}	-31 A→G	**Japanese**
β^{++}	-29 A→G	**African**
β^{++}	-28 A→C	**Southeast Asian**
β°	IVS1-nt1 G→A	**Mediterranean; Asian Indian**
β°	IVS1-nt5 G→C	**East Asian; Asian Indian**
$\beta^{+/++}$	IVS1-nt6 T→C	**Mediterranean**
β^{+}	IVS1-nt110 G→A	**Mediterranean**
β+	IVS2-nt654 C→T	**Chinese**
β+	IVS2-nt745 C→G	**Mediterranean**
β°	codon 39 C→T	**Mediterranean**
β°	codon 5 –CT	**Mediterranean**
β°	codon 6 –A	**Mediterranean; African-American**
β°	cod**on** 41/42 –TTCT	**Southeast Asian**
β^{++}	AATAAA to AACAAA	**African-American**
β^{++}	AATAAA to AATGAA	**Mediterranean**
β^{++}	codon 27 G→T Hb (Hb Knossos)	**Mediterranean**
β^{++}	codon 79 G>A (Hb E)	**Southeast Asian**

β^{0}: complete absence of beta globin on the affected allele.
β^{+}: residual production of beta globin (around 10%).
β^{++}: very mild reduction in beta globin production.

(Galanello and Origa, 2010)

Pathology of thalassemia syndromes:

The central mechanism underlying the pathophysiology of the beta thalassemias can be related to the deleterious effects of imbalanced globin chain synthesis on erythroid maturation and survival. An imbalance of the alpha/non-alpha globin chains leads to an excess of unmatched alpha globin which precipitates out, damaging membrane structures leading to accelerated apoptosis and premature destruction of the erythroid precursors in the bone marrow (ineffective erythropoiesis) ***(Thein, 2005)***.

(I) Evidences for an Ineffective Erythropoiesis in β-Thalassemia:

Dyserythropoiesis in β-thalassemic patients was suspected for a long time since it is largely recognized that many patients with an inadequate transfusional regimen have a dramatic expansion of the hematopoietic marrow and extramedullary hematopoiesis, which can lead to extensive bone deformity and/or bone marrow mass and splenomegaly ***(Pootrakul et al., 2000).*** Ferrokinetic studies done in the 70′s, studying incorporation of Fe into newly formed RBC, suggested that probably 60%–80% of erythroid progenitors were arrested in proliferation and/or underwent death ***(Ribeil et al., 2013)***.

Moreover, it has been shown that β-thalassemic bone marrow erythroblasts contain electron-dense alpha-globin inclusion (aggregates) beginning at early polychromatophilic stages, which increase in size and frequency during subsequent maturation ***(Ribeil et al., 2013)***.

Ineffective erythropoiesis defines the suboptimal production of mature erythrocytes from a proliferating pool of immature erythroblasts. It is thus characterized by (1) accelerated erythroid differentiation, (2) maturation blockade at the polychromatophilic stage, and (3) death of erythroid precursors ***(De Franceschi et al., 2011)*** (Figure 2).

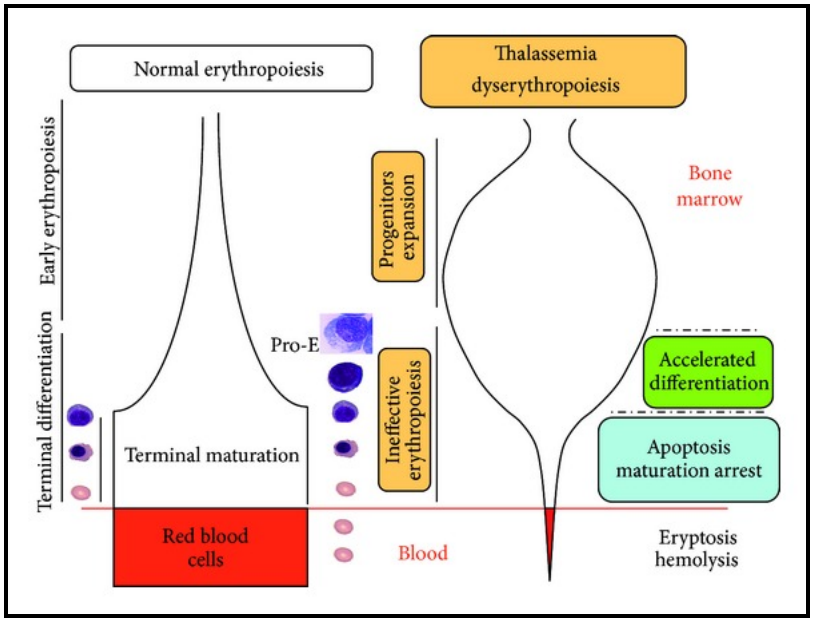

Figure (2): Difference between normal and β-thalassemia ineffective erythropoiesis ***(Ribeil et al., 2013).***