Introduction

Beta-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 **(Galanello and Origa, 2010).**

In these patients, complications are generally associated with iron deposition resulting from frequent blood transfusions, hemolysis, and increased intestinal iron absorption, and thus pose a risk for parenchymal organ injuries.

Iron deposition mainly affects the liver, heart, pancreas, gonads, parathyroid and thyroid glands, bones, lungs, peripheral and central nervous systems (CNS) **(Duman et al., 2010).**

CNS complications generally present as cognitive dysfunction, which usually results from iron deposition and neurotoxicity of deferoxamine (DFO), which is commonly used as a chelating agent **(Duman et al., 2010).**

Furthermore, hypoxemia and thromboemboli may cause CNS complications and cognitive dysfunction **(Duman et al., 2010).**

The side effects due to the disease itself or its treatment, being unable to attend school, frequent hospitalizations, and the physical and social restrictions as a consequence of chronic disease and its treatment also lead to cognitive dysfunction **(Duman et al., 2010).**

Aim of the Work

The aim of this study is to assess neurocognitive functions in patients with beta Thalassemia and its relation to serum ferritin and different iron chelator therapy.

Chapter (I)

Thalassemia

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 **(Galanello and Origa, 2010).**

Thalassemia is one of the most common single gene disorders and is widely distributed in the Mediterranean region **(Barragan et al., 2006).**

In 1925, Thomas Cooley and Pearl Lee described homozygous β-thalassemia. They recognized similarities in the disease entity and clinical course of severe anemia, splenomegaly, severe growth retardation, and bone changes affecting four children of Greek and Italian origin. As all early cases were reported in children with Mediterranean background the disease was termed "Thalassemia" from the Greek word "Thalassos" meaning "sea" and "emia" which means "related to blood". Over the years, the disease proved to be widely occurring throughout tropical countries ***(Cooley et al., 1925).***

Individuals with thalassemia major usually present within the first two years of life with severe anemia, requiring regular

red blood cell (RBC) transfusions **(Galanello *and* Origa, *2010).***

It represents a serious health problem, with a predicted 1000 new patients born each year ***(Hussein et al., 1993).***

The first case of β-thalassemia in Egypt was reported in the 1940s by Professor El Diwany, ever since there has been increasing interest to reveal the extent of occurrence of this problematic disease in Egypt ***(Selim et al.,1974).***

Incidence and carriage rate:

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 Union **(Galanello and Origa, 2010).**

In Egypt, beta-thalassemia is the most common type with a carrier rate varying from 5.3 to > or =9% and a gene frequency of 0.03. So, it was estimated that 1,000/1.5 million per year live births will suffer from thalassemia disease in Egypt (total live births 1,936,205 in 2006). Beta -Thalassemia creates a social and financial burden for the patients' family and the Egyptian government. The high frequency of beta-thalassemia carriers with increasing rate of newly born cases is a pressing reason for the importance to develop prevention program for beta-thalassemia in Egypt **(El-Beshlawy and Youssry, 2009).**

It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of beta Thalassemia, with about 60,000 symptomatic individuals born annually, the great majority in the developing world (**Vichinsky, 2005).**

Prevalence and Geographical Distribution:

Thalassemia is considered the most common genetic disorder worldwide, about 3% of the world population (150 million people) carry β-thalassemia genes and in Southeast Asia, 5-10% of the population carries genes for α-thalassemia **(Honig, 2004).**

According to ethnic group, α-thalassemia trait is most prevalent in south East Asia, affects 2.7% of American black newborns and less common in the Mediterranean region. B-thalassemia occurs in 5% in certain areas of Italy, Greece, Sardinia, India and 8% in American blacks **(Weatherall and Clegg, 2001).**

Normal human hemoglobin:

1- Function of hemoglobin:

Hemoglobin is a two-way respiratory carrier, transporting oxygen from the lungs to the tissues and facilitating the return transport of carbon dioxide. In the arterial circulation, hemoglobin has a high affinity for oxygen and a low affinity for

carbon dioxide, organic phosphates, and hydrogen and chloride ions. In the venous circulation, these relative affinities are reversed **(Marengo, 2006).**

2. Genetics:

The genes for the globin chains occur in two clusters ε, δ, γ and β on chromosome 11 and ξ on chromosome 16. Two types of γ chain, G γ and Aγ occur depending on whether there is a glycine or alanine amino acid at position 136 in polypeptide chain. The α chain gene is duplicated and both α genes (αl and α2) on each chromosome are active **(Hoffbrand et al., 2001).**

3. Ontogeny of human hemoglobin:

Humans switch from embryonic ε-globin to fetal _΄Y-globin gene expression when the site of erythropoiesis shifts from the yolk sac to the fetal liver **(Gale et al., 1979).** Importantly, expression of embryonic globin genes is restricted to the primitive erythroid lineage the ΄Y-globin genes are the major β-like globin genes expressed during definitive fetal liver erythropoiesis (**Stamatoyannopoulos et al., 2001).**

Adult hemoglobin, HbA (α2 β2), gradually replaces HbF to become the dominant hemoglobin during adult life **(McConnell et al., 2011).**

a) Embryonic hemoglobins:

The three common embryonic hemoglobins, Hb Portland-1 (ξ2 ΄Y2), Hb Gower-1 (ξ2 ε2), and Hb Gower-2 (α 2 ε2), are normally present during the first few months of life

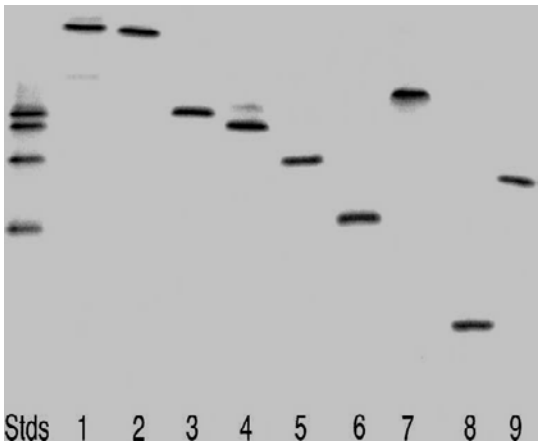

Figure 1: Isoelectric focusing gel of hemoglobins. The standard hemoglobins are A, F, S, and C from top to bottom. Lane 1, Hb Portland-1; lane 2, Hb Portland-2; lane 3, HbA; lane 4, HbF; lane 5, HbS; lane 6, HbA2; lane 7, Hb Gower-1; lane 8, Hb Gower-2; lane 9, Hb-Rothschild. About 5 mg of each protein was applied to the Hb Resolve gel (PerkinElmer).The anode is at the top and the cathode at the bottom **(MANNING et al., 2007).**

b) Fetal Hb:

Hemoglobin F is made up of 2 alpha chains and 2 delta (fetal) chains. These delta chains are polypeptide chains, referred to as gamma subunits that are homologous to the beta chains of Hemoglobin A. Hemoglobin F appears a few weeks post-conception and exists for a few months post-birth **(Michael, 1999).**

c) Adult Hb:

Some HbA (α2, β2) can be detected in even the smallest embryo. Accordingly, it is possible as early as 16-20 weeks gestation to make a prenatal diagnosis a major β-chain hemoglobinopathies, such as thalassemia major ***(Inatli et al., 2006).***

Table 1: The composition of embryonic, fetal and adult Hbs

Embryonic	Gower 1 Gower 2 Gower 3	ξ2 ε2 α2 ε2 ξ2 γ2
Fetal	Hemoglobin F	α2 γ2
Adult	Hemoglobin A 1 HemoglobinA2	α2 β2 α2 δ2

(Nelson, 2007).

Classification of Thalassemias:

β -Thalassemia:

B-thalassemia syndromes *(Table 2) are* the result of insufficient (β+) or absent (β0) production of β-globin chains **(Kohn, 2011).**

Their molecular causes are β-globin gene mutations. Most patients come from Mediterranean countries, South-East Europe, Arab nations, and Asia. Hematological changes become manifest from between the ages of three months and six months onwards **(Kohne and Kleihauer, 2010).**

1- β -thalassemia minor (thalassemia trait): (heterozygous β-thalassemia) Carriers of thalassemia minor are usually clinically asymptomatic but sometimes have a mild anemia. When both parents are carriers there is a 25% risk at each pregnancy of having children with homozygous thalassemia **(Galanello *and* Origa, *2010).***

2-β-thalassemia major (βTM):

(Severe homozygous or mixed heterozygous β-thalassemia) with long-term, transfusion-dependent anemia untreated children die before the age of 10 **(Olivieri, 1999).**

3- β-thalassemia intermedia:

(Mild homozygous or mixed heterozygous β-thalassemia) of patients present between the ages of 2 and 6 years and although they are capable of surviving without regular blood transfusion, growth and development are retarded. Hypertrophy of erythroid marrow with the possibility of extramedullary erythropoiesis is common. Its consequences are characteristic deformities of the bone and face, osteoporosis with pathologic fractures of long bones and formation of erythropoietic masses that primarily affect the spleen, liver, lymph nodes, chest and spine. Patients with thalassemia intermedia frequently develop leg ulcers and have an increased predisposition to thrombosis as compared to thalassemia major, especially if splenectomised **(Galanello and Origa, 2010).**

Beta-thalassemia associated with other Hb anomalies:

1-*The interaction of HbE and beta-thalassemia*: results in thalassemia phenotypes ranging from a condition indistinguishable from thalassemia major to a mild form of thalassemia intermedia. Depending on the severity of symptoms three categories may be identified:

a- Mild HbE/beta-thalassemia: It is observed in about 15% of all cases in Southeast Asia. This group of patients maintains Hb levels between 9 and 12 g/dl and usually does not develop clinically significant problems. No treatment is required.

b- Moderately severe HbE/beta-thalassemia: The majority of HbE/beta-thalassemia cases fall into this category.

The Hb levels remain at 6-7 g/dl and the clinical symptoms

are similar to thalassemia intermedia. Transfusions are not required unless infections precipitate further anemia. Iron overload may occur.

c- Severe HbE/beta-thalassemia*:* The Hb level can be as low as 4-5 g/dl. Patients in this group manifest symptoms similar to thalassemia major and are treated as thalassemia major patients.

***2-Patients with HbC/beta-thalassemia*:** May live free of symptoms and be diagnosed during routine tests. When present, clinical manifestations are anemia and enlargement of the spleen. Blood transfusions are seldom required. Microcytosis and hypochromia are found in every case. The blood film shows distinctive Hb C crystals with straight parallel edges, target cells, and irregularly contracted cells with features of thalassemia such as microcytosis. The association of hereditary persistence of fetal Hb (HPFH) with beta-thalassemia mitigates the clinical manifestations which vary from normal to thalassemia intermedia.

3-Individuals with HbS/beta-thalassemia: have a clinical course similar to that of Hb SS **(Galanello and Origa, 2010).**

Hereditary persistence of fetal HB (HPFH):

No β or δ-chain synthesis. It is a group of rare conditions characterized by continued synthesis of high levels of HbF in adult life **(Honig, 2000).**

α-Thalassemia:

α-thalassemias are caused by an α-globin chain synthesis defect. At the molecular level, they result from partial (α+) or total (α0) deletions, or more rarely mutations, of one or more of the four α-globin genes (αα/αα). They occur mainly in Africa,

Arab nations, and, more frequently, South-East Asia **(Kohn and Kleihauer, 2011)**

Age of Presentation:

Clinical presentation of thalassemia major occurs between 6 and 24 months **(Galanello and Origa, 2010).**

Sex: Both sexes are equally affected.

The Molecular Aspects of Thalassemia:

The β-like globin genes, a linked cluster on chromosome 11, are arranged over approximately 60,000 nucleotide bases (figure 2). Promoter elements upstream from the initiation codon of each active gene are involved in the initiation of transcription. The cluster also contains other regulatory elements that interact to promote erythroid-specific gene expression and to coordinate the developmental regulation of each gene **(Olivieri, 1999).**

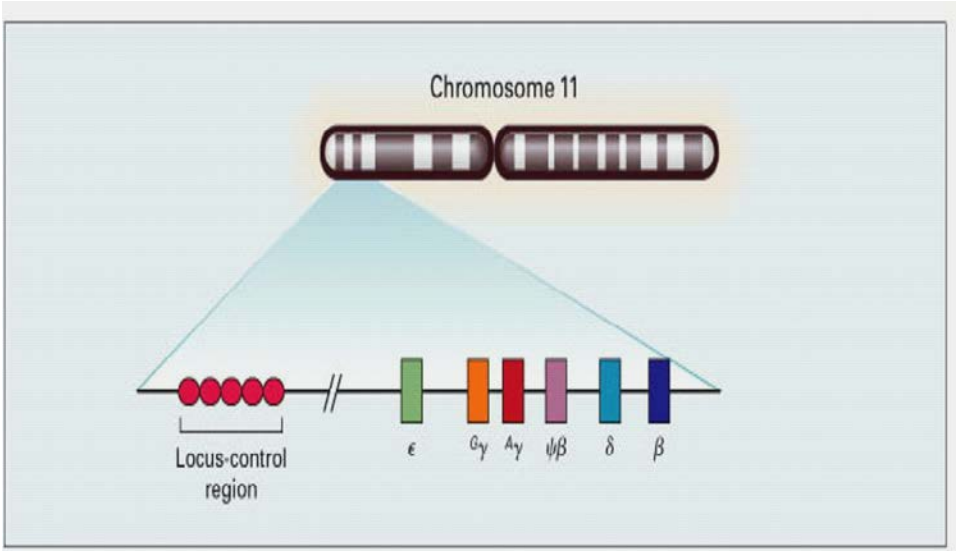

Figure 2: The β-globin gene cluster on the short arm of chromosome 11 **(Olivieri, 1999).**

The b -globin–like genes are arranged in the order in which they are expressed during development. The GY and AY genes are both active genes that produce G -globin chains that differ only at position 136 (glycine is the product of the AY gene, and alanine is the product of the AY gene). The cluster also contains regulatory elements that interact to promote erythroid- specific gene expression and to coordinate the developmental regulation of each gene, including hemoglobin switching **(Olivieri, 1999).**

These include enhancers, distant regulatory elements that increase gene expression, and a master sequence, the cluster's essential distal regulatory element, the β-globin locus-control

region. This is a region that lies 20 kb upstream from the ε-globin gene **(Olivieri, 1999).**

Mutations causing ß-Thalassemia:

Nearly 200 different mutations have been described in patients with *β*-thalassemia and related disorders.

Although most are small nucleotide substitutions within the cluster, deletions may also cause *β*-thalassemia.

All the mutations result in either the absence of the synthesis of *β*-globin chains (*β*0-thalassemia) or a reduction in synthesis (*β*+-thalassemia) (Fig. 3) **(Olivieri, 1999).**

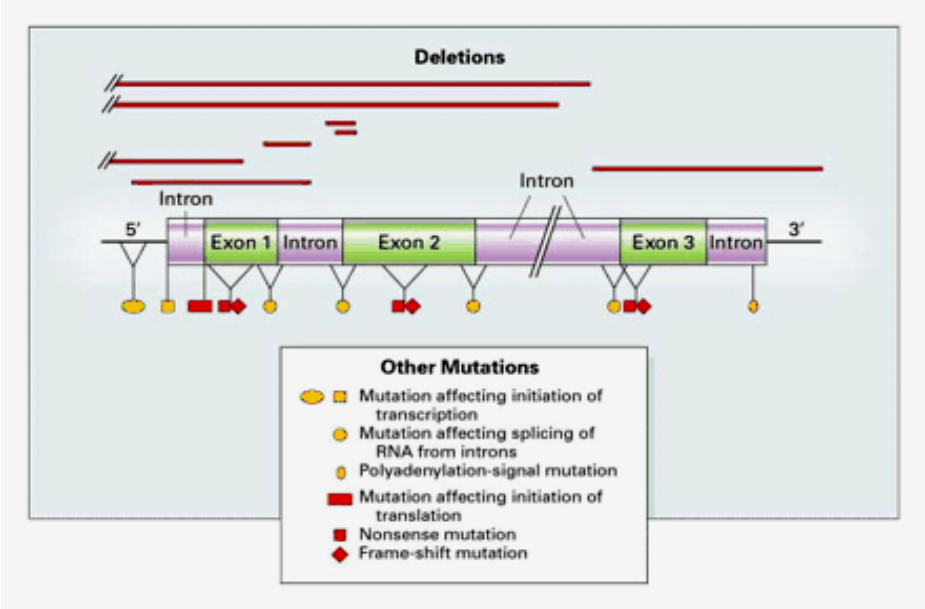

Figure 3: The normal structure of the ß-globin gene, the locations and types of mutations resulting in ß-thalassemia **(Olivieri, 1999).**