Introduction

The thalassemias are a group of inherited disorders that are caused by altered or absent hemoglobin chain synthesis leading to ineffective erythropoiesis and subsequent anemia ***(Cappellini et al 2013).***

Beta thalassemia is a group of hereditary blood disorders characterized by anomalies in the synthesis of beta chains of hemoglobin resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals. Three main clinical forms have been described: thalassemia major, thalassemia intermedia and thalassemia minor **(*Galanello and origa 2010).*** It is the most common genetic disorder worldwide and occurs in approximately 4.4 of every 10, 000 live births. ***(Nadeem et al., 2012).***

Tissue iron overload is the most important complication of beta thalassemia and is a major focus of therapeutic management . Blood transfusion is a comprehensive source of iron loading for beta thalassemia patients. Nevertheless, iron overload occurs also in patients who have not received frequent transfusions such as patients suffering from thalassemia intermedia ***(Ribeil et al., 2013).***

Iron homeostasis is regulated by the hepatic peptide hormone hepcidin. Hepcidin mediates this function by impairing the export of iron from macrophages, duodenal enterocytes, and hepatocytes by binding to ferroportin, thereby driving internalization and degradation of this key transmembrane iron exporter. Consequently, hepcidin regulates both intestinal absorption of dietary iron and reutilization of iron derived from recycling of the hemoglobin of senescent red blood cells ***(Cooke et al., 2014).***

Hepcidin deficiency is one of the main contributing factors of iron overload in iron-loading anemias such as β-thalassemia. Hepcidin deficiency results from a strong suppressive effect of the high erythropoietic activity on hepcidin expression. Although in thalassemia major patients iron absorption contributes less to the total iron load than transfusions, in non-transfused thalassemia, low hepcidin and the consequent hyperabsorption of dietary iron is the major cause of systemic iron overload. Hepcidin diagnostics and future therapeutic agonists may help in management of patients with β-thalassemia ***(Nemeth,2010).***

Aim of the work

The aim of this study is to measure the level of serum hepcidin in patients with beta thalassemia and to correlate its level with iron profile and determine any possible correlations with disease severity or complications.

Beta Thalassemia

Introduction

The term “thalassemia” is derived from the Greek words “Thalassa” (sea) and “Haema” (blood) and refers to disorders associated with defective synthesis of α- or β-globin subunits of hemoglobin (Hb) A (α_2; β_2), inherited as pathologic alleles of one or more of the globin genes located on chromosomes 11 (β) and 16 (α). More than 200 deletions or point mutations that impair transcription, processing, or translation of α- or β-globin mRNA have been identified. The clinical manifestations are diverse, ranging from absence of symptoms to profound fatal anemias in utero, or, if untreated, in early childhood. The thalassemia syndrome is classified according to which of the globin chains, α or β, is affected. These 2 major groups, α- and β-thalassemia, are subclassified according to absent (α° and β°) or reduced (α^+ or β^+) globin chain synthesis. In addition, where γ-chains together with α-chains compose fetal hemoglobin (HbF) in the fetus and δ chains in combination with α-chains compose hemoglobin A_2 in adults, impaired synthesis of γ-globin or δ-globin chains can occur ***(Rachmilewitz and Giardina,2011)***.

Geographic distribution and epidemiology

Approximately 5 percent of the world's population has a globin variant, but only 1.7 percent has alpha or beta thalassemia trait. Thalassemia affects men and women equally and occurs in approximately 4.4 of every 10,000 live births. Alpha thalassemia occurs most often in persons of African and Southeast Asian descent, and beta thalassemia is most common in persons of Mediterranean, African, and Southeast Asian descent. Thalassemia trait affects 5 to 30 percent of persons in these ethnic groups ***(Rund and Rachmilewitz , 2005).***

Figure (1) : Geographic distribution of β-thalassemia**(the green shaded areas)**
(Weatherall and Clegg, 2001b).

Classification of Thalassemia

Thalassemia is classified according to which globin chain is produced at a reduced rate. Theoretically, there are as many types of thalassemias as there are types of globin chains ***(Weatherall, 2005b).*** Most thalassemias are inherited in a Mendelian recessive fashion. The terminology "autosomal codominant" is preferred by some authors, because being heterozygous carries discernible but minor hematological findings ***(Sarnaik, 2005).***

1. Alpha (α) thalassemias:

The most common causes of alpha-thalassemia are deletions that remove one or both of the functional alpha-globin genes. In addition, more than 30 different point mutations and small deletions/insertions have been reported for the alpha-globin genes **(*Waye et al., 2001).***

2. Beta (β) thalassemias:

β-thalassemia is one of the genetic diseases which can be broadly defined as a syndrome of inherited hemoglobin disorders characterized by a quantitative deficiency of functional β-globin chains **(*Weatherall and Clegg, 2001a).***

Etiology

Mutations in globin genes cause thalassemias. Beta thalassemia affects one or both of the beta-globin genes. (Alpha thalassemia affects the alpha-globin gene[s].) These mutations, by causing impaired synthesis of the beta globin protein portion, a component of Hb, result in anemia ***(Tan et al., 2009).***

Although more than 200 causative mutations have been reported for β thalassemia, the spectrum of mutations and their frequencies in most populations consist of limited number of common mutations and a slightly larger number of rare mutations ***(Hardison et al., 2002)***. In the countries bordering the Mediterranean basin, the major mutations of the β-globin gene are CD-39, IVSI-110, IVSI-6, IVSI-1 and CD-37 ***(Moreno et al., 2002).*** 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 ($beta^{0}$) synthesis of the beta chains of hemoglobin (Hb). Transmission is autosomal recessive ***(Galanello and Origa, 2010).***

Clinical Classification of β – Thalassemia

- β-thalassemia major:

Homozygous β-thalassemia in which there is defective formation of β chain ***(Honig, 2004).***

There are 2 types:

1- β 0 thalassemia: Complete absence of β chain

2- β + thalassemia: β chain synthesis is reduced

- β-thalassemia minor:

Heterozygous β- thalassemia is associated with no clinical abnormalities and may be mistaken for iron deficiency anemia ***(Honig, 2004)***.

- β-thalassemia intermedia:

About 10% of heterozygous β- thalassemia have a syndrome of intermediate hematological severity. Those patients usually have onset of anemia after 2 years of age and they do not require regular blood transfusion.

- Other β-thalassemia syndromes:

- Hb-S-β-thalassemia,

- Hb-C- β-thalassemia

- Hb-D-β- thalassemia ***(Yaish, 2007)***.

Pathophysiology

Erythropoiesis in individuals with β-thalassemia reflects the consequences of excess, unpaired α-globin ***(Cao and Galanello 2010***). Indeed, the degree of imbalance in the α-globin versus β + γ-globin biosynthetic ratio is the major determinate of disease severity rather than the underproduction of hemoglobin ***(Rund and Rachmilewitz 2005).***

Because of this imbalance in chain synthesis, an excess of freed α-globin chains accumulates within erythroid cells. Aggregation, denaturation, and degradation of these chains leads to the formation of insoluble precipitates as well as hemichromes, which damage cell membranes. Membrane damage leads to ineffective erythropoiesis within the bone marrow, hemolysis of red cells within the circulation, and binding of immunoglobulin and complement components to red cell membranes, triggering loss of red cells in the spleen. The resulting anemia leads to diminished tissue oxygenation, an increase in erythropoietin levels, and further stimulation of the bone marrow. Bone marrow expansion causes skeletal deformities and osteopenia. Substances released from degenerating red cells increase iron absorption, which contributes to iron overload ***(Nienhuis and Nathan 2012).***

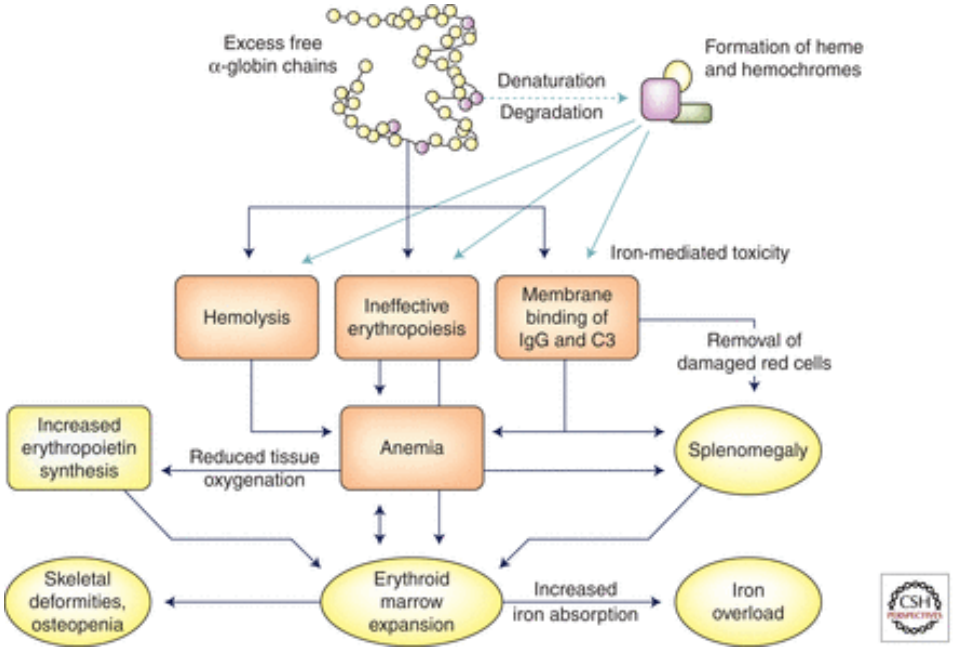

Figure (2): Pathophysiology of Beta Thalassemia ***(Nienhuis and Nathan 2012)***

Molecular Pathology of β-Thalassemia:

B-thalassemias are extremely heterogenous at the molecular level ***(Higgs et al., 2001)***. The mutations affect every step in the pathway of globin gene expression: transcription, processing of the mRNA precursor, translation of mature mRNA, and post-translational integrity of the β-polypeptide chain ***(Giardina and Forget, 2008)***.

- Transcription:

Point mutations alter the promoter region upstream of the β-globin mRNA-encoding sequence and tend to reduce binding of RNA polymerase, impairing mRNA synthesis, so they reduce the rate of mRNA transcription to 20-30% of normal. This results in a moderate decrease of β-globin chain output and hence, in a mild phenotype (β^+- thalassemia) ***(Thein, 2005).***

- Processing:

A wide variety of mutations interfere with processing of the primary mRNA transcript. Mutations within introns or exons, or at their junctions, interfere with the mechanism of splicing the exons together after the introns have been removed ***(Thein, 2005).***

-Translation:

Mutations that involve translation of β-globin mRNA fall into two groups. First, there are nonsense mutations that are single-base changes that produce stop codon in the middle of the coding part of mRNA. Mutations of this type cause premature termination of globin chain synthesis. It appears that message which contains mutation of this type is not transferred to the cell cytoplasm, a phenomenon called nonsense-mediated decay (Figure 5) ***(Weatherall, 2006).*** Other groups of exon mutations result in framshifts in which one or more bases are

lost or inserted which disturbs the normal reading frame ***(Weatherall and Clegg, 2001)***.

- Post-translational stability:

Some forms of thalassemia result from instability of the β-globin gene product. For example, it appears that nonsense mutations in the exon 3 are not subjected to nonsense–mediated decay and hence abnormal mRNA is transported to the cytoplasm and translated. The result may be long, unstable β-globin gene products that form inclusion bodies in the red cell precursor. This is the basis for dominant β-thalassemia ***(Weatherall, 2005a).***

Variants of β-Thalassemia:

a)Dominantly Inherited β-Thalassemia:

Most thalassemias are inherited in a Mendelian recessive fashion. Some forms of β-thalassemia alleles which produce the thalassemia intermedia rather than the thalassemia minor, were found to be transmitted in an autosomal dominant fashion. They are characterized by the creation of highly unstable β-globin which precipitate within red cell precursors as large inclusion bodies, so it has been called inclusion body β-thalassemia but since all severe forms of β-thalassemia have inclusions in the

red cell precursors, the term dominantly inherited β-thalassemia is preferred ***(Thein, 2004)*.**

b)**β-thalassemia trait with normal HbA2 levels:**

This form of β-thalassemia in which heterozygotes have normal or minimally increased HbA2 has been classified into two types ***(Weatherall and Clegg, 2001b).***

Type (1): In which there are minimal or no hematological abnormalities (silent carriers).

Type (2): In which the blood picture is typical of heterozygous β-thalassemia except for the normal level of HbA2.

Most cases result from coinheritance of δ thalassemia in *cis* or *trans* to the β0 or β+ type ***(Thein, 2005a).***

c)β-Thalassemia Trait with Unusually High HbA2

The increased levels of HbA2 observed in β-thalassemia alleles are remarkably uniform, usually 3.5 - 5.5% and rarely exceeds 6%. Unusual high levels of HbA2 characterize a subgroup of β-thalassemia caused by deletions of regulatory elements in the β promoter. This is often accompanied by modest increase in HbF ***(Thein, 2005b).***

d)β-Thalassemia due to Trans-acting Determinants

About 1% of the β-thalassemia remains uncharacterized despite extensive sequence analysis including the flanking regions of the β- globin genes. In several families, linkage studies demonstrated that the β-thalassemia phenotype aggregates independently of the β- globin complex implying that the genetic determinant is trans-acting ***(Giordano et al., 1998).***

Clinical Picture

A - Thalassemia major

Clinical presentation of thalassemia major occurs between 6 and 24 months. Affected infants fail to thrive and become progressively pale. Feeding problems, diarrhea, irritability, recurrent bouts of fever, and progressive enlargement of the abdomen caused by spleen and liver enlargement may occur. In some developing countries, where due to the lack of resources patients are untreated or poorly transfused, the clinical picture of thalassemia major is characterized by growth retardation, pallor, jaundice, poor musculature, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal

changes resulting from expansion of the bone marrow. Skeletal changes include deformities in the long bones of the legs and typical craniofacial changes (bossing of the skull, prominent malar eminence, depression of the bridge of the nose, tendency to a mongoloid slant of the eye, and hypertrophy of the maxillae, which tends to expose the upper teeth. ***(Galanello and Origa, 2010).***

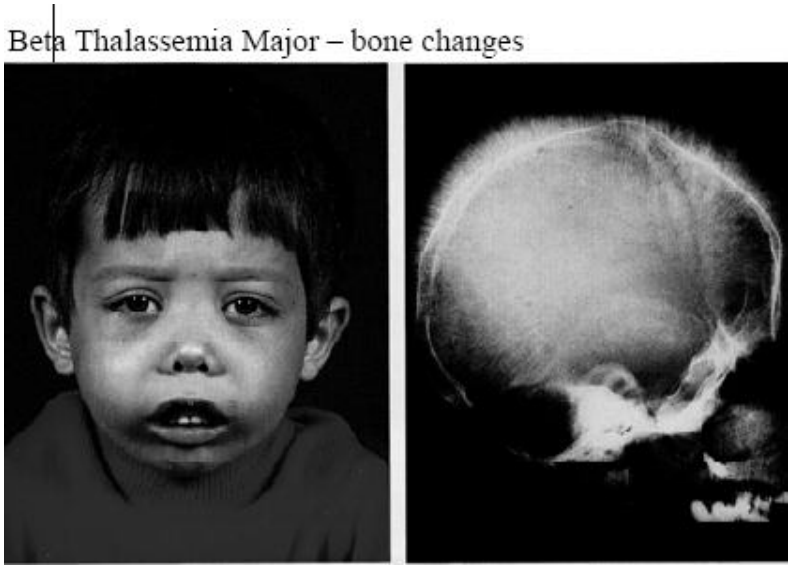

Figure (3): Bone changes in beta thalassemia