INTRODUCTION

Thalassemia is the most common mono genetic disease worldwide. It is a group of inherited disorders of hemoglobin synthesis. In β thalassemia, mutations of the β globin gene lead to various degrees of defective β chain production, an imbalance in globin chain synthesis, ineffective erythropoiesis and anemia ***(Weatherall et al., 2001)*.**

Bthalassemia major (TM) refers to those patients characterized by profound anemia and present to medical attention in the first year of life and subsequently require regular blood transfusions for survival. However, blood transfusions don't come without their own side effect as iron overload inevitably manifests resulting in multiple organ damage involving the heart, liver, and endocrine glands ***(Skordis et al., 2011)*.**

The introduction of safe blood transfusion practices and iron chelation therapy surely translated into prolonged survival and enhanced quality of life. The increased life span for patients with TM allowed for several morbidities of the disease and its treatment to appear, amongst which is bone disease ***(Borgna et al., 2004)*.**

Spectrum of bone abnormalities in B TM patients including osteopenia, osteoporosis, pain, fractures and spinal complications represent prominent causes of morbidity in

children and adults of both genders with TM. Despite normalization of hemoglobin levels, adequate hormone replacement and effective iron chelation therapy, patients continue to show an unbalanced bone turnover with an increased resorptive phase resulting in seriously diminished bone mineral density (BMD). In well treated TM patients the frequency of osteoporosisis about 40-50% ***(Vogiatzi et al., 2005; Mshirsaz et al., 2007 and Karimi et al., 2007)*.**

The pathogenesis of osteoporosis in thalassaemia major (TM) is complicated and several factors are implicated . Delayed sexual maturation, growth hormone (GH) and insulin growth factor-(IGF)-1 deficiency, parathyroid gland dysfunction, diabetes, hypothyroidism, ineffective haemopoiesis with progressive marrow expansion, direct iron toxicity on osteoblasts, as well as liver disease ***(Voskaridouet al., 2004 and Skordis et al., 2008)***. Furthermore, iron chelation has correlated with growth failure and bone abnormalities, and high desferrioxamine dosage has been associated with cartilage alterations ***(Bekheirnia et al., 2003)***. Vitamin D plays a critical role in global calcium homeostasis and bone metabolism ***(Bhattacharya et al., 2006)***. Vitamin D deficiency is quite common in TM patients due to increased metabolic demands, chronic medical care, and iron overload ***(Napoli et al, 2006)***.

Impaired calcium homeostasis is thought to be a consequence of iron overload seen in β-thalassemic transfused patients and defective synthesis of 25 OH vitamin D (25OH D) have been described in these patients and negatively affect their bone metabolism ***(Wattanap et al., 2005)*.**

The major circulating metabolite of vitamin D is serum 25 OHD, which has a half-life of between 10 and 19 days.. It is the best indicator of vitamin D statusIn thalassemia patients ***(Condamine et al., 1994)*.** Serum levels of 25-OHD are directly related to bone mineral density with a maximum density achieved when the 25-OH-D level reaches 40 ng/ml or more. Serum levels below 30 ng/ml are associated with a significant decrease in intestinal calcium absorption ***(Ferrari et al., 2006)*.**

Treatment of patients with thalassemia major consists of regular blood transfusions and iron chelation therapy, which is vital to prevent excess iron buildup in the body. There are three iron chelating agents available: deferoxamine (DFO, Desferal), an iron chelator given by infusion, and two oral chelatorsdeferiprone (DFP, Ferriprox) and deferasirox (DFX, Exjade). Treatment with iron chelators has significantly increased the life expectancy of affected individuals into the third to fifth decade ***(Modell et al., 2000)***. Studies have correlated Chelation therapy and bone metabolism markers in thalassemia major ***(Pratelli et al., 2006; DiStefano et al., 2004).***

AIM OF THE WORK

To assess the level of 25 hydroxy vitamin D and bone mineralization status in β-thalassemic patients and its relation to different types of chelation therapies to compare the effect of conventional chelators versus recent oral chelators on bone mineralization status.

β THALASSEMIA MAJOR

Definition:

Thalassemia is an inherited blood disorder, caused by mutations in regulatory genes transmitted autosomal recessively which results in a reduced rate of synthesis of one of the globin chains that make up haemoglobin. The disruption in the synthesis of either the α- or β-chains of haemoglobin, classified in α- and β-thalassaemia ***(Rund and Rachmilewitz 2005).***

In ß-thalassaemia major (TM) there is an underproduction of ß-globin chains combined with excess of free α-globin chains. The excess free α-globin chains damage the red blood cell (RBC) membranes leading to their destruction and ineffective erythropoiesis, which is the hallmark of this condition ***(Weatherall et al., 2002).***

Distribution and Population at Risk

Internationally:

- Thalassaemia syndromes are the most common single-gene disorder worldwide: about 3% of the world population (150 million) carries the β-thalassemia genes ***(El-Beshlawy et al., 2007).***
- The β-thalassemias are widespread throughout the Mediterraneanregion, Africa, the Middle East, the Indian subcontinent andBurma, Southeast Asia

including southern China, the Malay, Peninsula,and Indonesia. Estimates of gene frequencies range from 3 to10 percent in some areas ***(Weatherall et al., 1994).*** Because of increased population mobility, the disease is found today throughout the world, even in places far from the tropical areas in which it arose ***(Rund and Rachmilewitz, 2000).***

In Egypt:

- In Egypt, β-thalassaemia is the most common genetically-determined, chronic, haemolytic anaemia. The actual number of patients surviving to date is not, however, available ***(El-Beshlawy et al., 2007).***

Pathophysiology

β-**thalassemia** occurs when there is a quantitative reduction of β- globin chains that are usually structurallynormal ***(Weatherall and Clegg, 1999).*** They are caused by mutations that nearly all affectthe β-globin locus and are extremely heterogeneous ***(Forget et al., 2001).***

These genetic defectslead to a variable reduction in β-globin output ranging from a minimal deficit (mild β^{+}**thalassemia** alleles)to complete absence (β° **thalassemia)**. In β-**thalassemia,** the synthesis of normal α-globinchains from the unaffected α-globin genes continues as normal,resulting in

the accumulation within the erythroid precursors of excess unmatched α-globin chains. The free α-globin chains are notable to form viable tetramers and instead precipitate in the red cell precursors in the bone marrow forming inclusion bodies ***(Thein et al ., 2005).***

Although β thalassemia results from reduced synthesis of the βglobin chains of adult hemoglobin (α2β2), the pathophysiology is predominantly determined by excess free α-globin chains which precipitate and cause oxidative damage in developing red cells(causing dyserythropoiesis) and in mature red cells (causing hemolysis) ***(Weatherall and Clegg, 1999).***

Despite discoveries concerning the molecular abnormalities that led to the thalassemic syndromes, it is still not known how accumulation of excess unmatched α-globin in β thalassemia and β-globin in α- thalassemia leads to red blood cell hemolysis in the peripheral blood, and in the β thalassemias particularly, premature destruction of erythroid precursors in marrow (ineffective erythropoiesis) ***(Schrier et al., 2007).***

The severity of disease in β-**thalassemia** correlates well with the degree of imbalance between αand non-α globin chains and the size of the free α-chain pool. Thus, factors that reduce the degree of chain imbalance and the magnitude of α-chain excess in the red cellprecursors will have an impact on the phenotype ***(Thein et al., 2005).***

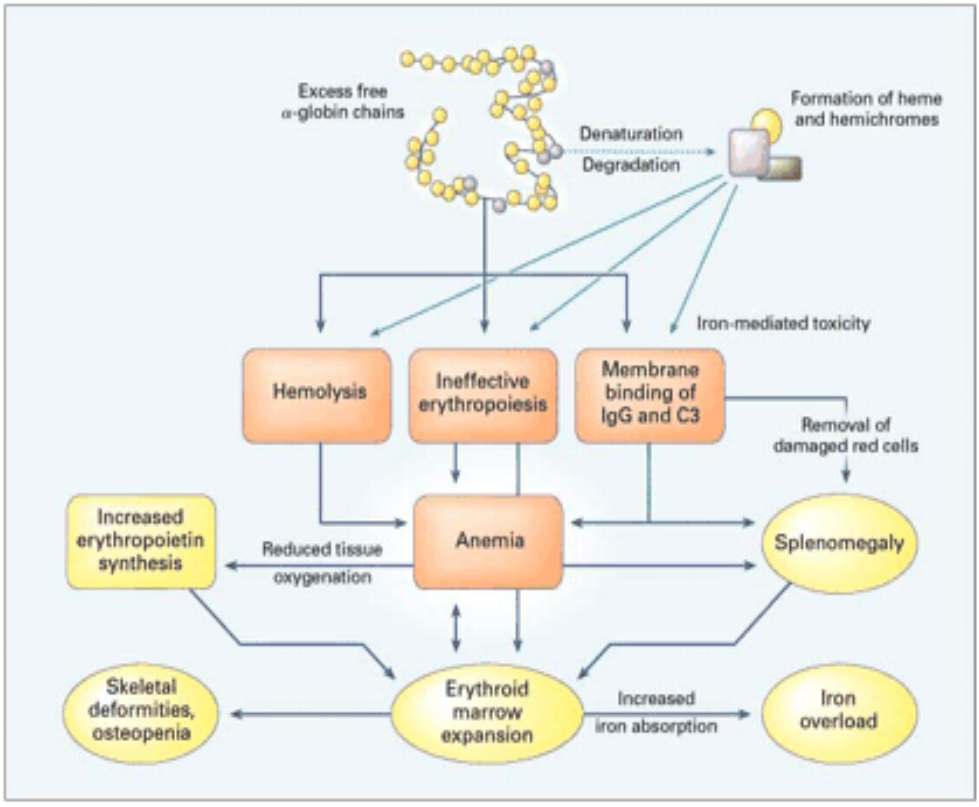

Figure (1): Pathophysiology of β thalassemia ***(Nienhuis and Nathan, 2012)***.

Anemia in β**thalassemia** thus results from a combinationof ineffective erythropoiesis, peripheral hemolysis, and anoverall reduction in hemoglobin synthesis ***(Thein et al., 2005).*** Oxidant injury may cause hemolysis, but there is no evidence that it causes ineffective erythropoiesis ***(Schrier et al., 2007).***

The constant exposure of the spleen to red cells with inclusions, consisting of precipitated globin chains, gives rise to the phenomenon of **"work hypertrophy".** A large spleen may sequester a considerable proportion of the red cell mass.

Furthermore, splenomegaly may also cause plasma volume expansion, a complication that may be exacerbated by massive expansion of the erythroid marrow ***(Weatherall et al., 2001).***

Both an increasein plasma volume as a result of shunting through expanded marrow and progressive splenomegaly exacerbate anemia ***(Olivieri et al., 1999)***.

Increased erythropoietin synthesis may stimulate the formation of extramedullary erythropoietic tissue, primarily in the thorax and paraspinal region. Marrow expansion also results in characteristic deformities of the skull and face, as well as osteopenia and focal defects in bone mineralization and may aggravate a painful periarticular syndrome characterized histologically by microfractures andosteomalacia ***(Olivieri et al., 1999).*** Iron overload is a potential complication of thalassemia, even in patients not requiring red blood cell transfusions. It results from excessive absorption of dietary iron, a consequence of ineffective erythropoiesis and rapid turnover of plasma iron ***(Yaish et al., 2005).***

Classification:

Beta-thalassemias can be classified into:

- Beta-thalassemia
    - Thalassemia major
    - Thalassemia intermedia (TI)
    - 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 intermedia)
  - Hereditary persistence of fetal Hb and beta-thalassemia
  - Autosomal dominant forms

(Galanello and Origa, 2010)

Clinical picture of B-thalassemia:

Classically, individuals with severe β-thalassemia have presented with variable but often very severe degrees of anemia, expansion of the bone marrow spaces secondary to erythroid hyperplasia, hepatosplenomegaly, and extramedullary hematopoiesis in the chest and abdomen. The external appearance is characterized by pallor and slight jaundice, frontal bossing and other abnormalities of the facies secondary to marrow expansion, and abdominal enlargement due to hepatosplenomegaly. Usually, these manifestations are absent or minimally present in patients with thalassemia major if transfusion therapy is initiated early during the first year of life provided that the hemoglobin levels are maintained at 9–10 g/dL. Where transfusion is readily available, the classical physical findings of thalassemia are now more commonly found ***(Rachmilewitz and Giardina, 2011)***.

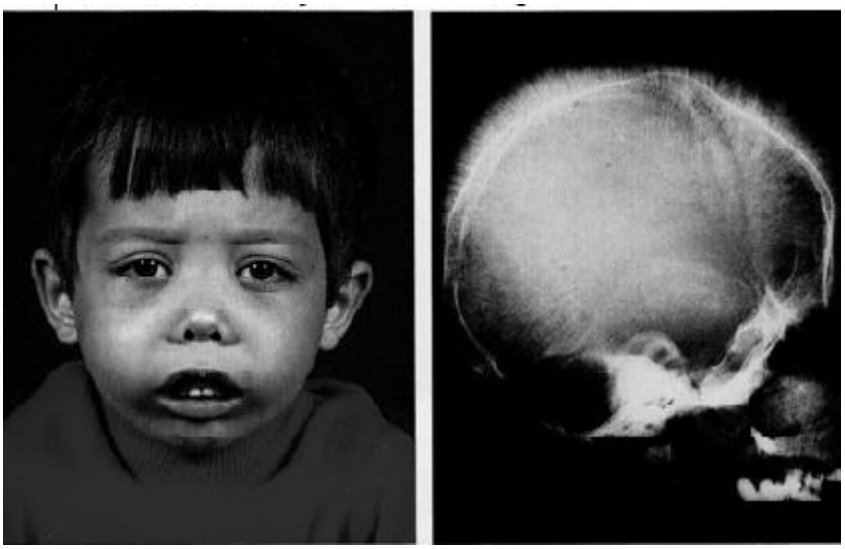

Figure (2): Bone changes and facies in beta thalassemia major-bone changes

Complications of Beta-thalassemia:

- **Iron Overload (IO):**

Iron overload results principally from the regular blood transfusions. Each milliliter of transfused blood contains ~1 mg of iron, thus receipt of a unit of packed red cells typically results in the deposition of 200 mg of iron ultimately in the tissues following red cell senescence ***(Ganz and Nemeth, 2012)*.**

The average daily losses are less than 1 mg in males and 2 mg in females ***(Nemeth et al., 2006),*** largely through shedding of the epithelial cells from the intestine, urinary tract, skin, and other mucosal organs.. Lacking excretory mechanisms, individuals with thalassemia who receive blood

transfusion inevitably experience significant iron overload ***(Ganz and Nemeth, 2012)*.**

In addition, TM patients absorb more iron than normal individuals. This paradoxical increase in iron absorption despite systemic iron overload probably reflects release of erythroid factors during the cellular apoptosis associated with ineffective erythropoiesis that inhibit hepcidin production by the liver. The end result of suppression of hepcidin expression is to enhance iron absorption from the intestine and to allow iron release from macrophages ***(Nemeth et al., 2010)*.** Iron deposition occurs within the reticuloendothelial system. As iron overload progresses, deposited iron also appears within the hepatic parenchyma, various endocrine tissues, and, more slowly, in the myocardium ***(Ganz and Nemeth, 2012)*.**

The clinical manifestations of iron overload have come to dominate the clinical phenotype of individuals with severe β-thalassemia. Cardiac dysfunction is the main clinical problem that may lead to early death. Endocrine abnormalities, particularly hypogonadism, low growth hormone, hypothyroidism, and diabetes mellitus, are also significant problems. Iron deposition of the liver may be substantial, although functional abnormalities are usually mild unless iron overload is very severe. Fortunately chelation therapy prevents and, when given in intensive combination therapy, may reverse the complications of iron overload ***(Farmaki et al. 2010)***.

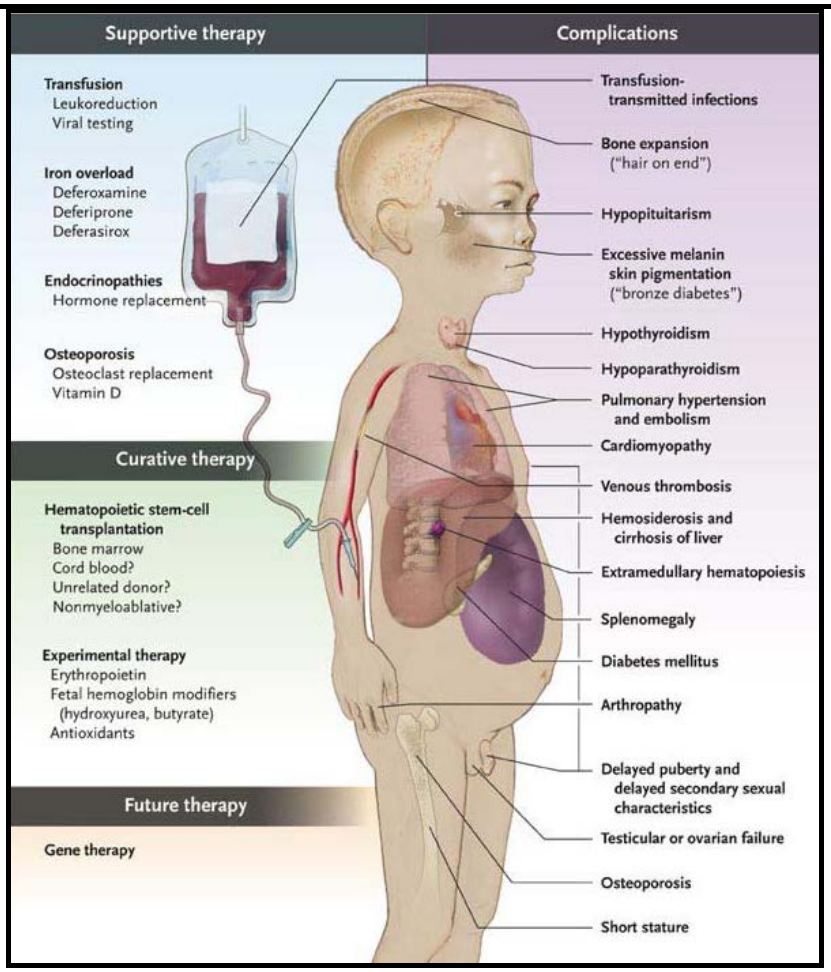

Figure (3): Organ systems susceptible to iron overload and management of thalassemia ***(Rund and Rachmilewitz, 2005).***

- **Endocrine Abnormalities:**

Growth retardation secondary in part to growth hormone deficiency and hypogonadism are typically the initial manifestations of iron overload in β-thalassemic patients ***(Chatterjee and Bajoria, 2010)***. In unchelated patients, failure to develop secondary sex characteristics during the teenage years was very common. Regular chelation therapy has reduced the incidence

of hypogonadism, although hormone replacement is often required ***(Wood et al., 2011).*** Well-chelated young men with thalassemia are fertile, although potentially requiring hormonal administration, and women with severe β-thalassemia may achieve motherhood either with or without obstetrical intervention. Other endocrine complications include impaired glucose tolerance and diabetes ***(Noetzli et al., 2011),*** hypothyroidism and hypoparathyroidism ***(Borgna-Pignatti et al., 2004).***

- **Hepatic Manifestations:**

Progressive iron deposition is characteristic in patients with severe β-thalassemia. Invasive liver biopsies and now non-invasive methodologies have established the correlation between liver iron concentration and histological and functional abnormalities. Iron accumulation to the point of 7 mg/g liver (dry weight) seems well tolerated, but as the liver iron concentration increases with regular transfusion and no or inadequate chelation, fibrosis in the periportal areas and ultimately frank cirrhosis occur. Liver functional abnormalities remain mild until iron overload is severe. Hepatitis C infection was common before the development of effective screening and potentially accelerated the development of cirrhosis and increased the risk for development of hepatocellular carcinoma ***(Mancuso et al., 2010).*** Chelation therapy prevents iron accumulation, although the increased risk of hepatocellular

carcinoma as a consequence of hepatic iron overload is not completely eliminated.

- **Bony Complications:**

Osteoporosis often occurs in patients with thalassemia, reflecting marrow expansion, endocrine deficiencies, iron toxicity, and the potential toxicity of chelators ***(Terpos and Voskaridou 2010).*** Cortical thinning and subclinical fractures as well as problematic clinical fractures may occur, the latter with minimal trauma.Additional genetic factors seem to play an important role in the development of low bone mass in these patients ***(Voskaridou et al., 2004).*** **Thrombosis** A hypercoagulable state, which increases the risk for thromboembolism, has also been described in patients with thalassemia secondary to platelet activation, red cell membrane damage, and endothelial cell activation ***(Cappellini et al., 2010)***. Pulmonary hypertension is an increasingly recognized complication in patients with severe β-thalassemia ***(Morris and Vichinsky, 2010).*** Advancing age and history of splenectomy are the major risk factors that have been identified. Among the pathophysiological factors are red cell membrane pathology, coagulation abnormalities, platelet activation, oxidative stress, and chronic hemolysis with release of thrombogenic vesicles ***(Morris and Vichinsky 2010)***.

- **Other Complications:**