INTRODUCTION

βThalassemia Major (TM) is a hereditary anemia resulting from defects in hemoglobin production. β thalassemia which is caused by decrease in production of β-globin chains affects multiple organs and is associated with considerable morbidity and mortality ***(Rund and Rachmilewitz., 2005)***.

In Patients with β thalassemia major life-long blood transfusions, extravascular hemolysis and increased gastrointestinal iron absorption lead to iron overload and toxicity in many organs including the heart ***(Oliveri, 1999)***.

Heart complications represent the leading cause of mortality in thalassemia major, even though following the introduction of chelating therapies, an important and progressive increase of life expectancy mainly due to reduction in mortality due to cardiac dysfunction has been demonstrated ***(Borgna-Pignatti et al, 2005)***.

Arterial and venous thromboembolic episodes in beta-thalassemia major patients have been reported. Endothelial cell activation and impaired flow-mediated dilation in the brachial arteries of beta-thalassemic patients, as shown in previous in vivo studies, implicate endothelial dysfunction in the pathogenesis of vascular complications. Endothelial dysfunction generally leads to vascular remodeling and potential changes in mechanical properties ***(Stoyanova et al., 2012).***

Endothelial cell proliferation plays a role in vascular injury repair and blood vessels formations. It is affected by plasma derived and blood cell derived component ***(Shitrit et al., 2008).***

Angiogenesis, or the growth of new blood vessels, is important for wound healing and for restoring blood flow to tissues after injury or insult. In normal physiology, inhibitors and angiogenic growth factors, such as vascular endothelial growth factor (VEGF), regulate angiogenesis. When regulation fails, blood vessels are formed excessively or insufficiently ***(Birk et al., 2008).***

Tissue hypoxia is a major stimulus for the up-regulation of VEGF and anemic patients have elevated levels of VEGF. This suggests that anemia might impact on the progression of angeiogenesis in malignant and benign diseases ***(Dunst et al., 2002).***

Pulmonary hypertension and left ventricular diastolic dysfunction are complications of β thalassemia. Pulmonary hypertension is associated with hemolysis and hypoxia, but other unidentified factors are likely involved in pathogenesis as well ***(Niu et al., 2009)***.

AIM OF THE WORK

The aim of the study is to assess the level of vascular endothelial growth factor (VEGF) as a marker of angiogenesis in patients with β-thalassemia major and to correlate it with echocardiographic finding.

BETA THALASSEMIA

Background:

Thalassaemia, first described by Cooley and Lee in 1925 ***(Cooley, 1925)***, is a hereditary anaemia resulting from a defect in haemoglobin production ***(Weatherall 2000).***

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).***

Due to various mutations in the different genes for the α- and β-chain genes and other modifying factors, there is a broad spectrum of clinical symptoms ranging from intrauterine death, through to severe anaemia with the need for regular red blood cell transfusions to asymptomatic anaemia ***(Olivieri 1999)***.

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 2002)***.

Pathophysiology:

The types of thalassemia usually carries the name of the underproduced chain or chains. The reduction varies from a slight decrease to a complete absence of production. For example, when β chains are produced at a lower rate, the thalassemia is termed β+, whereas β-0 thalassemia indicates a complete absence of production of β chains from the involved allele.

The consequences of impaired production of globin chains ultimately result in the deposition of less Hb into each RBC, leading to hypochromasia. The Hb deficiency causes RBCs to be smaller, leading to the classic hypochromic and microcytic picture of thalassemia. This is true in almost all anemias caused by impairment in production of either of the 2 main components of Hb: heme or globin. However, this does not occur in the silent carrier state, since both Hb level and RBC indices remain normal.

In the most common type of β thalassemia trait, the level of Hb A_2 (δ2/α2) is usually elevated. This is due to the increased use of δ chains by the excessive free α chains, which results from a lack of adequate β chains with which to pair. The δ gene, unlike β and α genes, is known to have a physiologic limitation in its ability to produce adequate δ chains; by pairing with the α chains, δ chains produce Hb A_2 (approximately 2.5-3% of the total Hb). Some, but not all, of the excessive α chains are used to form Hb A_2 with the δ chains, excess of freed α-globin chains accumulates within erythroid cells. Aggregation, denaturation, and degradation of these

chains lead to the formation of insoluble precipitates as well as hemochromes, which damage cell membranes. Membrane damage leads to ineffective erythropoiesis (IE) 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 as shown in figure 1 ***(Nienhuis and Nathan 2012).***

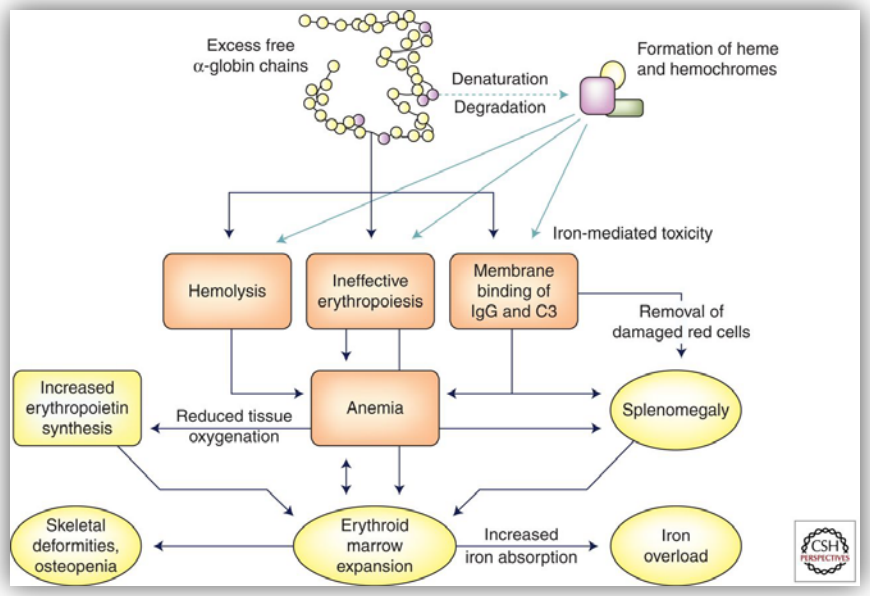

Fig. (1): Pathophysiology of β thalassemia ***(Nienhuis and Nathan, 2012).***

Hereditary transmission:

The beta-thalassemias are inherited in an autosomal recessive manner. The parents of an affected child are obligate heterozygotes and carry a single copy of a disease-causing beta globin gene mutation. At conception, each child of heterozygotes parents has 25% chance of being affected, 50% chance of being an asymptomatic carrier, and 25% chance of being unaffected and not carrier. The parents of the proband have a 1 in 4 (25%) risk of having further affected children in each pregnancy.Dominant forms of beta-thalassemia, associated with mutations that result in the production of highly unstable beta globulin variants and leading to a clinically manifesting phenotype of beta-thalassemia in heterozygotes ***(Galanello and Origa, 2010).***

Classification:

Beta-thalassemias can be classified into:

- Beta-thalassemia
  - Thalassemia major
  - Thalassemia intermedia (T I)
  - 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 manifestations and complications:

- **Anemia**

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)***.

- **Iron Overload (I O)**

Patients with the more severe forms of β-thalassemia, both intermedia and major, have increased tissue deposition of iron. Senescence of transfused red cells in patients with transfusion-dependent β-thalassemia results in iron deposition 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.

In normal humans, iron homeostasis is achieved by controlling absorption. Only ~1 mg is lost from the body each day, largely through shedding of the epithelial cells from the intestine, urinary tract, skin, and other mucosal organs. 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. Lacking excretory mechanisms, individuals with thalassemia who receive blood transfusion inevitably experience significant iron overload ***(Ganz and Nemeth 2012)***.

In individuals with thalassemia intermedia, increased tissue iron occurs as a consequence of occasional transfusions but mainly reflects increased iron absorption ***(Ginzburg and Rivella 2011)***. 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 2010)***.

Iron released from cells is bound to transferrin and transported to the bone marrow and other tissues, where iron is taken up by the transferrin receptors. Less than 1% of the total body iron is found in blood at any one time, although up to 25 mg may circulate as transferrin-bound iron throughout the 24-h cycle. The expanded erythropoiesis in individuals with β-thalassemia results in a dramatic increase in plasma iron turnover of 10-fold to 15-fold over normal ***(Hershko et al. 2005)***.

Normally, the transferrin saturation is maintained in a relatively low range of ~10%–50%. Iron is stored in tissue in the form of ferritin, a multicomponent protein shell that internalizes iron, thereby protecting cellular constituents from potential oxidative damage. Intracellular ferritin iron is in equilibrium with the cytosolic soluble iron pool. Hemosiderin is an insoluble aggregate of iron that forms in lysosomes when ferritin is degraded ***(Kim et al. 2011)***.

Both ferritin and hemosiderin accumulate in the cells of individuals with severe β-thalassemia. Accumulation of iron in reticuloendothelial cells is relatively harmless, whereas accumulation of iron in parenchymal tissues may damage critical cells in the heart, endocrine glands, and liver ***(Hershko et al. 2005)***.

As a consequence of iron overload in patients with thalassemia either from blood transfusion or excessive absorption or a combination of the two, transferrin saturation increases to

75%–100% and non-transferrin-bound iron (NTBI) is found in the blood. NTBI is very heterogeneous and exists in various forms including in complex with citrate and proteins ***(Evans et al., 2008)***.

Strategies have been devised to detect labile plasma iron (LPI), a fraction of the NTBI pool that is metabolically active in interacting with membrane constituents, leading to membrane damage via the formation of reactive oxygen species (ROS) ***(Hershko 2010).*** Rather than using the transferrin receptor, NTBI enters cells by various cellular channels in forms that have the potential to damage cells ***(Liuzzi et al., 2006)***.

The liver and myocardium clear NTBI at a rate 200-fold that of transferrin-bound iron . A portion of the NTBI is accessible to iron chelators directly ***(Breuer et al. 2001)***, whereas additional iron may be chelated in the presence of iron-mobilizing agents ***(Esposito et al. 2003)***.

Chelators administered to patients with thalassemia have the potential to reduce NTBI. Intravenous administration is more effective at reducing NTBI than subcutaneous administration. The reduction and reemergence of NTBI have rather complex kinetics, although these studies support the use of continuous rather than intermittent chelation in high-risk patients ***(Glickstein et al. 2006)***.

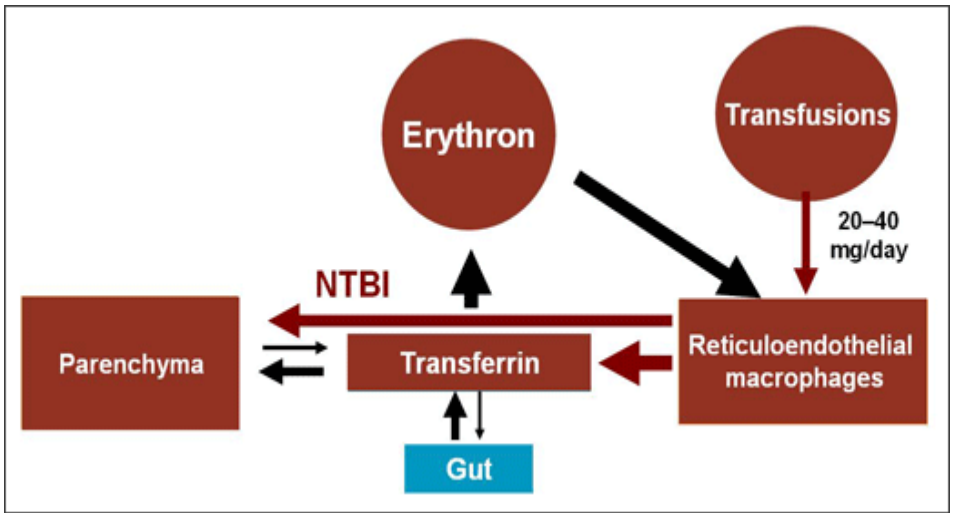

Fig. (2): Iron metabolism in transfusion-dependent thalassemia ***(Porter, 2005)***

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)***.

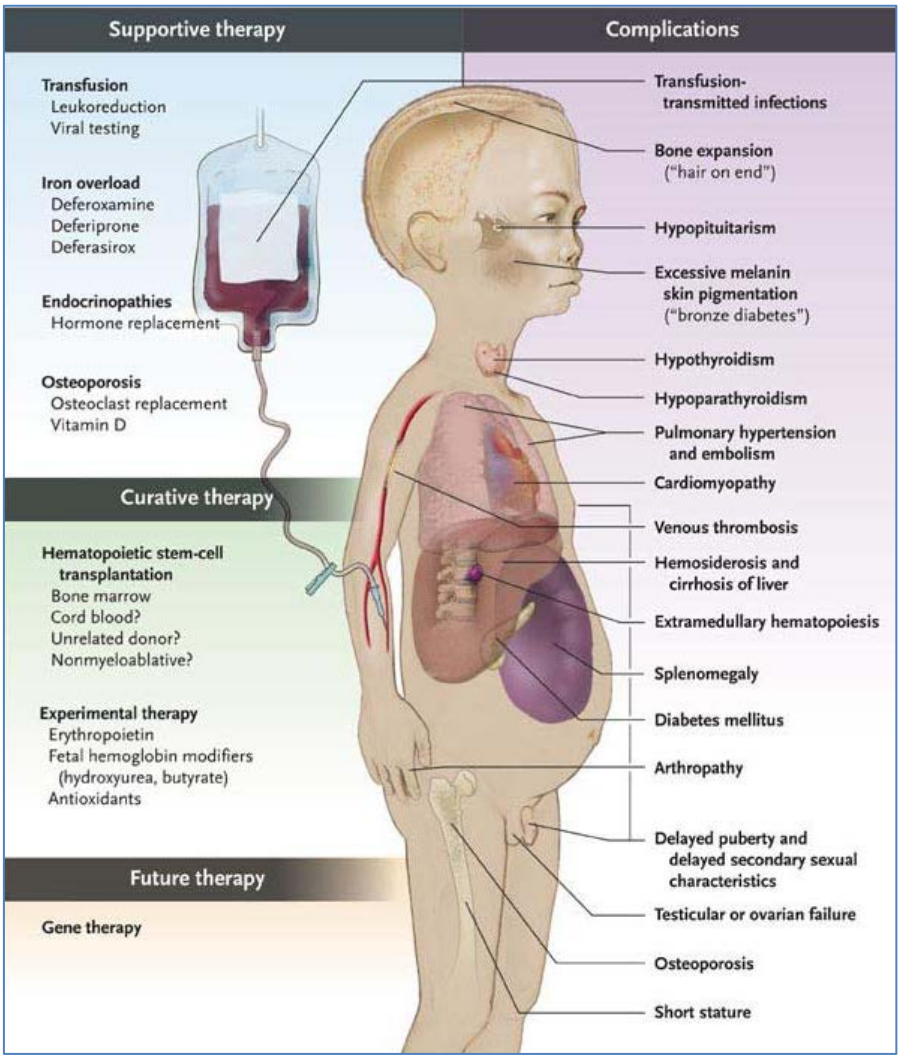

Fig. (3): Organ systems susceptible to iron overloadand management of thalassemia ***(Rund D and Rachmilewitz E., 2005).***

- **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 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 2010).*** Chelation therapy prevents iron accumulation, although the increased risk of hepatocellular carcinoma as a consequence of hepatic iron overload is not completely eliminated.

- **Other 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. 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)***. Another clinical manifestation is the occurrence of chronic skin ulceration about the ankles secondary to chronic anemia. All of these complications are more common in untransfused patients with thalassemia intermedia than in frequently transfused patients with thalassemia intermedia or patients with thalassemia major who receive regular transfusions ***(Borgna-Pignatti et al., 2010)***. Nutritional deficiencies are common in individuals with severe β-thalassemia and may contribute to other complications such as osteoporosis and diabetes mellitus ***(Fung 2010)***.

Investigations:

Laboratory findings:

- **Complete blood picture:**

Complete blood picture shows microcytic anemia. Thalassemia major is characterized by reduced Hb level (<7gm/dl), mean corpuscular volume (MCV) > 50 < 70 fl and mean