INTRODUCTION

Thalassemia is the most common hereditary disease in the world, which has an autosomal recessive inheritance ***(Detterich, 2012; Ujjwal, 2013).***

Thalassemia is defect in the synthesis of haemoglobin. The patients suffer ineffective bone marrow erythropoesis and increased red cell haemolysis resulting in anaemia ***(Bandyopadhyay, 2013).***

It is usually diagnosed in the first year after birth, and thereafter, patients need regular blood transfusions for survival ***(Kayrak et al., 2012).***

Iron induced cardiomyopathy is still major cause of morbidity and mortality in transfusion dependent β-TM and it can be reversible only if early intensive chelation therapy has been initiated. Also, the iron induced cardiomyopathy is silent in young patients with β-TM and conventional echocardiography is not sensitive for early diagnosis of the preclinical stage of cardiac involvement ***(Koonrungsesomboon et al., 2013).***

The outpouring of catabolic iron that exceeds the iron-carrying capacity of transferrin results in the emergence of nontransferrin-bound iron (NTBI), which catalyzes the formation of free radicals, resulting in oxidative stress (OS) and

damage to mitochondria, lysosomes, lipid membranes, proteins, and DNA ***(Hershko, 2010).***

It has also been demonstrated that such patients experience decreased antioxidant capacity and increased products of peroxidative damage. Collectively, these findings demonstrate that patients with β--thalassemia are under significant iron-driven oxidative stress ***(Koren et al., 2010).***

The N-terminus residues of human serum albumin tend to bind with transition metals such as cobalt, copper and nickel and alterations in this region of albumin hinder its binding capacity to such elements. Reactive oxygen species (ROS) resulting from conditions such as ischemia, hypoxia, acidosis, free radicals, and free iron can decrease the ability of the N-terminus to bind with transition metals. Human serum albumin with a decreased binding capacity as a result of ischemic events is referred to as ischemia modified albumin (IMA) ***(Roy et al., 2006).***

Oxidative stress, redox active forms of iron, and generation of ROS are well documented in patients with β-thalassemia major. It is therefore possible that under such conditions, the structure of human serum albumin is modified in such a way that permits for excessive production of IMA ***(Koren et al., 2010).***

AIM OF THE STUDY

To measure the level of Ischemia modified albumin (IMA) in patients with β--thalassemia major as a marker of oxidative stress and its relation to various clinical parameters, laboratory data and cardiac complications.

THALASSEMIA

Introduction:

Thalassemia is the most common hereditary disease in the world, which has an autosomal recessive inheritance ***(Detterich, 2012; Ujjwal, 2013).***

In this condition, since the globin chain synthesis is affected, the result is anaemia ***(Ansari and Shamsi, 2010; Behzad and Abbasi, 2012).***

Abnormalities in the structure and synthesis of the α-like and β-like globin chains that form tetramers of haemoglobin (α2β2) lead to the most common forms of inherited anaemias ***(Weatherall, 2010).***

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The β-thalassemia major (β-TM) is a hereditary chronic hemolytic anemia that typically requires lifelong regular transfusion therapy to keep hemoglobin levels close to normal

and allow adequate tissue oxygenation. The chronic administration of large amount of blood together with extravascular hemolysis and increased iron absorption in the intestinal system leads to iron deposition into the heart, liver, lung etc. In patients with β-TM ***(Delaporta et al., 2013).***

Iron induced cardiomyopathy is still major cause of morbidity and mortality in transfusion dependent β-TM and it can be reversible only if early intensive chelation therapy has been initiated. Also, the iron induced cardiomyopathy is silent in young patients with β-TM and conventional echocardiography is not sensitive for early diagnosis of the preclinical stage of cardiac involvement ***(Koonrungsesomboon et al., 2013).*** The outpouring of catabolic iron that exceeds the iron-carrying capacity of transferrin results in the emergence of nontransferrin-bound iron (NTBI), which catalyzes the formation of free radicals, resulting in oxidative stress (OS) and damage to mitochondria, lysosomes, lipid membranes, proteins, and DNA ***(Hershko, 2010).***

Thus, thalassemics are in a state of enhanced OS ***(Waseem et al., 2011).***

Classification:

The thalassaemias are classified according to which chain of the globin molecule is affected. In α thalassaemia, the

production of α globin is deficient and in β thalassaemia the production of β globin is defective.

There are two α genes on each chromosome 16, giving α thalassaemia the unique feature of gene duplication. There is only one β-globin gene on chromosome 11.

α thalassaemia

Normal: genotype α,α/α,α.

α+ thalassaemia heterozygous (genotype α,-/α,α): borderline haemoglobin level and mean corpuscular volume (MCV), low mean corpuscular haemoglobin (MCH); clinically asymptomatic.

α+ thalassaemia homozygous (genotype α,-/α,-): slightly anaemic, low MCV and MCH; clinically asymptomatic.

αo thalassaemia heterozygous (genotype α,α/,--): slightly anaemic, low MCV and MCH; clinically asymptomatic.

HbH disease (genotype α,-/-,-): HbH. Anaemic, very low MCV and MCH; splenomegaly, variable bone changes.

α thalassaemia major (genotype -,-/-,-): Hb Bart's. Severe non-immune intrauterine haemolytic anaemia. Hb Bart's hydrops fetalis, usually fatal.

β thalassaemia:

Normal: genotype β2/β2.

β-thalassaemia trait (genotype -/β2): HbA2 >4%. Slightly anaemic, low MCV and MCH; clinically asymptomatic.

β thalassaemia intermedia (genotype -/βo or β+/β+): high HbF, variable. Anaemic (symptoms usually develop when the haemoglobin level remains below 7.0 g/dL), very low MCV and MCH; splenomegaly, variable bone changes, variable transfusion dependency.

β thalassaemia major (genotype -o/-o): HbF >90% (untransfused). Severe haemolytic anaemia, very low MCVand MCH; hepatosplenomegaly, chronic transfusion dependency ***(Peters et al., 2012).***

Epidemiology: The word "thalassemia" comes FROM the Greek word"thalassa" (sea) because the disease thalassemia had been traditionally prevalent in and confined to the Mediterranean basin.

Beta thalassemia has a widespread occurrence in the Mediterranean region, Africa, the Middle East, India, Pakistan, Southeast Asia and the Indochina peninsula. However, immigration of those populations to USA., Canada, and Western European countries has resulted in a more universal

distribution of the disease ***(Modell et al., 2000; kremastinos et al., 2007; Kremastinos et al., 2010)***. ***Fig.(1)***

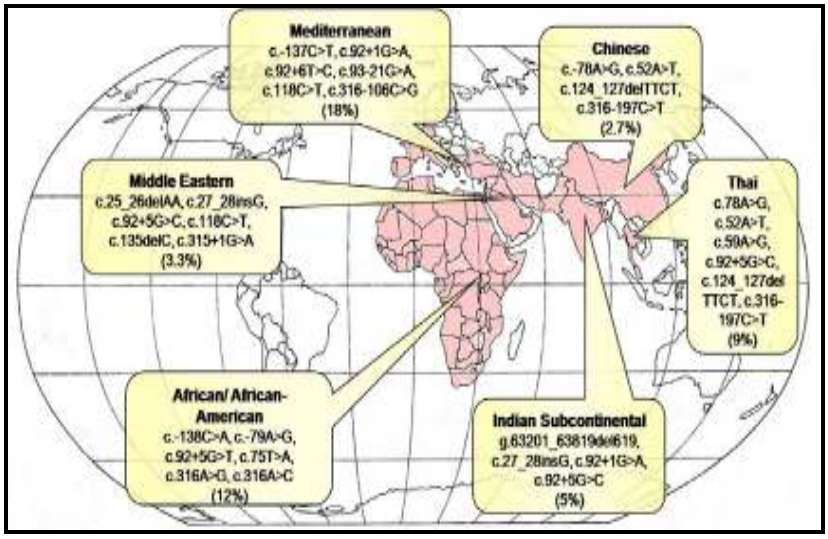

Figure (1): Most common beta-thalassemia mutations in at-risk populations ***(Livingstone, 1985; Weatherall and Clegg, 2001; Weatherall, 2003; Kham et al., 2004; Wong et al., 2006).***

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

Pathophysiology:

Normal human adult hemoglobin (Hb) A (HbA) consists of two pairs of globin chains, $\alpha 2\beta 2$, of which synthesis is normally tightly coordinated to ensure equal production. Mutation in the gene encoding β-globin which induce an absence or low-level synthesis of this protein in erythropoietic cells ***(Weatherall, 2001).***

The consequence of these mutations is an imbalance of α/β-globin chain synthesis, mostly evident in the homozygous forms, leading to the accumulation of free α-globin chains forming highly toxic aggregates ***(Khandros et al., 2012).***

The reduced amount (ß+) or absence (ß0) of beta globin chains result in a relative excess of unbound alpha globin chains that precipitate in erythroid precursors in the bone marrow, leading to their premature death and hence to ineffective erythropoiesis. The degree of globin chain reduction is determined by the nature of the mutation at the beta globin gene located on chromosome 11. Peripheral hemolysis contributing to anemia is less prominent in thalassemia major than in thalassemia intermedia, and occurs when insoluble alpha globin chains induce membrane damage to the peripheral erythrocytes. Anemia stimulates the production of erythropoietin with consequent intensive but ineffective

expansion of the bone marrow (up 25 to 30 times normal), which in turn causes the typical bone deformities.

Prolonged and severe anemia and increased erythropoietic drive also result in hepatosplenomegaly and extramedullary erythropoiesis ***(Galanello and Origa, 2010). Fig(2)(3).***

Clinical and hematological characteristics of beta-thalassemia are determined by several factors resulting in a wide spectrum of severity from no need to dependence on regular blood transfusions; they mainly include the type of disease causing mutation and the capacity production of alpha and gamma-globin chains It ensues severe anemia due to low red blood cell survival from ineffective erythropoiesis and hemolysis. The clinical presentation is widely variable because the amount of unbound alpha-globin chains can be modified by both the capacity to produce alpha-globin chains (HBA genes variants) and the capacity to produce gamma-globin chains (HBG2 gene modulators), that can bind available alpha-globin chains to form effective fetal hemoglobin (HBF) ***(Cao et Galanello, 2010; Danjou et al., 2011).***

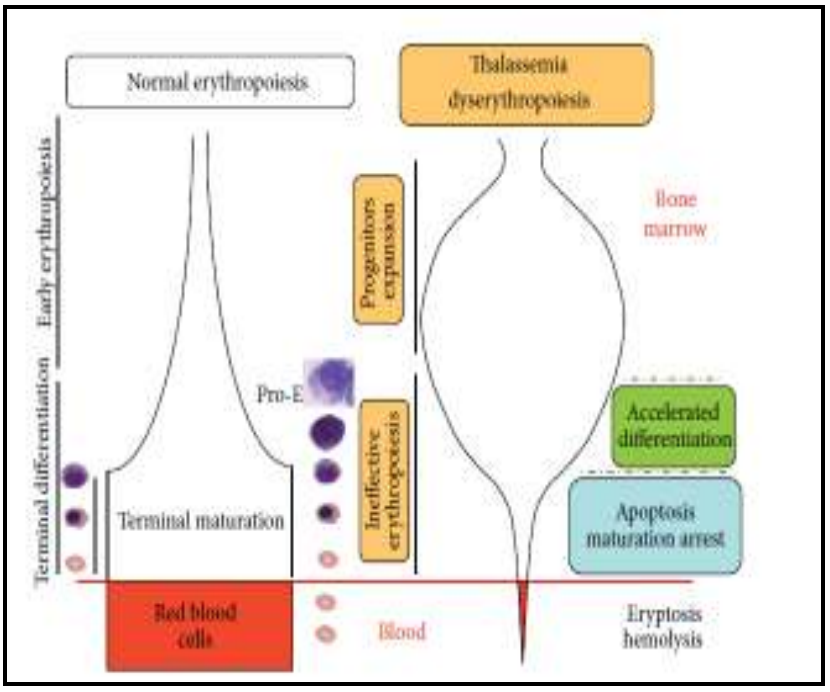

Figure (2): Difference between normal and β-thalassemia infective erythropoiesis.

Erythropoiesis is the pathway producing mature RBCs from hematopoietic stem cells, including several proliferation and differentiation steps. Erythroid differentiation is accompanied by temporally regulated changes in cell surface protein expression, reduction in cell size, progressive hemoglobinization, and nuclear condensation and extrusion. β-thalassemia dyserythropoiesis in human is characterized by expansion of very early erythroid precursors (proerythroblasts and earlier stages) and then ineffective erythropoiesis.

Ineffective erythropoiesis defines the suboptimal production of mature erythrocytes from a proliferating pool of

immature erythroblasts characterized by (1) accelerated erythroid differentiation, (2) maturation blockade at the polychromatophilic stage, and (3) death of erythroid precursors ***(Ribeil et al., 2013).***

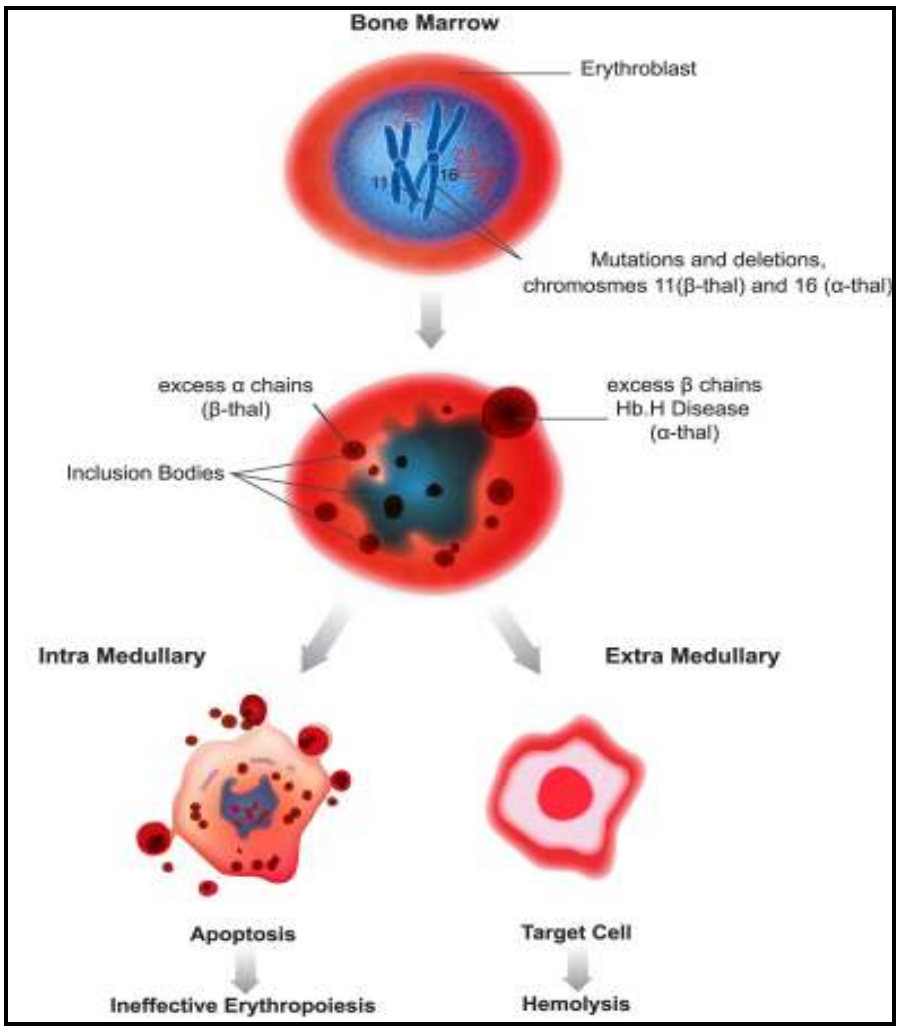

Figure (3): Show mechanism of ineffective erythropoiesis and hemolysis in thalassemia ***(Rachmilewitz and Giardina, 2011).***

Pathophysiology of iron overload:

The most common treatment for thalassemia is blood transfusion which is necessary to provide the patients with healthy red blood cells containing normal hemoglobin.

Repeated blood transfusion leads to iron overload ***(Pathare et al., 2010).***

Excess iron is deposited in body organs as liver, heart and endocrine glands causing organ damage ***(Kohgo et al., 2008).***

Iron loading in thalassemia depends on volume of transfused blood and amount accumulated from gut absorption. In β-thalassemia increased gastrointestinal iron absorption is mediated by downregulation of hepcidin and up-regulation of ferroportin ***(Gardenghi et al., 2007).***

Mechanism of Iron Toxicity:

Iron is highly reactive and easily alternating between ferrous (Fe2+) and ferric (Fe3+) states in a process which results in the gain and loss of electrons generating harmful free radicals (atoms or molecules with unpaired electrons). These can damage lipid membranes, organelles and DNA causing cell death and the generation of fibrosis ***(Kohgo et al., 2008).***

In healthy human, iron is absorbed by duodenal enterocytes and circulates in the plasma bound to transferrin.

Then iron is stored in monocyte-macrophage system and parenchymal cells of liver ***(Kohgo et al., 2008).***

So iron is 'kept safe' by binding to molecules as transferrin in blood and storage in the form of ferritin inside cells ***(Cappellini et al., 2008).***

In iron overload, excess iron is sequestered in the cells of the monocyte-macrophage system and then the liver ***(Rachmilewitz and Giardina, 2011).***

But when these organs get filled up and have no ability to store more iron, it is bound to transferrin and when the capacity of transferrin is saturated, iron circulates in the bloodstream as extracellular non-transferrin-bound iron (NTBI) which is very toxic ***(Wood, 2007).***

There is labile plasma iron (LPI) or 'free iron' which is a directly chelatable component of NTBI. It is highly toxic as it can catalyze and form harmful free hydroxyl radicals (***Kruszewski, 2004).***

LPI is detected in patients with transfusional iron overload. It isn't detected in healthy person ***(Papanikolaou et al., 2005).***

LPI is thought to be the iron that loads cells via a mechanism other than the transferrin receptor. Voltage dependent calcium channels have been hypothesized as the route of entry of LPI. LPI is taken up excessively by cells leading to iron-overload pathologies.

This raises the cell labile iron pool (LIP) ***(Porter, 2007).***

With sustained iron loading, the iron deposits can exceed the storage and detoxification capacity of ferritin and might eventually also transform into hemosiderin.

When LIP levels exceed the cell antioxidant capacity they evoke the formation of reactive oxygen species (ROS) that lead to cell damage by affecting lipids, proteins and nucleic acids.

The resulting 'free iron' damages many tissues in the body and is fatal unless treated by iron chelation therapy ***(Poggiali et al., 2012) Fig(4)***

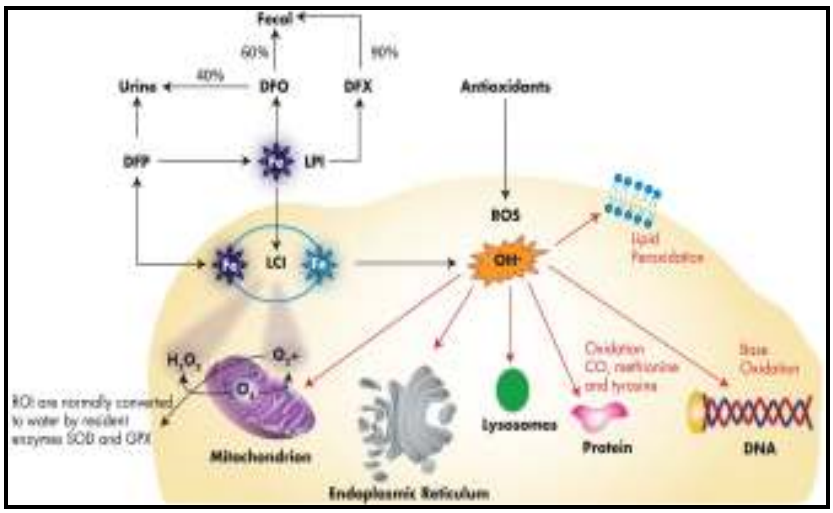

Figure (4): Amelioration of free iron species (LPI and LCI) by iron chelators and antioxidants. Labile plasma iron (LPI) is penetrating through the cell membrane with a consequent accumulation of labile cell iron (LCI). Both LPI and LCI react with reactive oxygen intermediate (ROI) producing noxious reactive oxygen species (ROS), for example, OH' radicals, which are highly reactive and oxidize DNA, proteins and lipid components of the cell. Deferiprone (DFP) chelates LCI alone or in combination with LPI by Deferiozamine (DFO). Deferasirox (DFX) mainly removes LPI ***(Rachmilewitz and Giardina, 2011).***