INTRODUCTION

Thalassemia is a hereditary anemia resulting from defects in hemoglobin production ***(Higgs et al., 2001)***. A mutation of β-globin gene leads to a defective β-chain production. This defect leads to an imbalance in α/β globin synthesis causing ineffective erythropoiesis, chronic hemolytic anemia and iron overload ***(Taher et al., 2011).*** Furthermore, patients with β-thalassemia major require continuous blood transfusion which leads to iron overload with subsequent organ and tissue damage ***(Koren et al., 2010).***

Increased iron accumulation due to lifelong chronic transfusion and mildly increase gastrointestinal iron absorption as a consequence of hepcidin suppression result in iron overload and hemosiderosis ***(Origa et al., 2007)***. High levels of local iron in the kidney con cause kidney injury that can eventually progress to end stage renal disease. Renal damage may develop through tubulointerstitial and/or glomerular injuries ***(Deveci et al., 2016)***. Unlike in the other organs, it is unclear whether kidney iron results solely from intravascular hemolysis, chronic transfusion therapy, or both ***(Tracz et al., 2007)***.

The success that has been made in the care of patients with thalassemia has led to the emergence of unrecognized complications including several renal abnormalities ***(Bakr et al., 2014)***. Renal tubular and glomerular damage is frequent in

adult patients with thalassemia intermedia and major ***(Elbedewy et al., 2015; Deveci et al., 2016)***.

Some published studies demonstrated proteinuria, aminoaciduria, low urine osmolarity, and excess secretion of the proximal tubule damage markers in pediatric patients with thalassemia ***(Aldudak et al., 2000; Hamed and ElMelegy, 2010)***. Shortened red blood cell (RBCs) life span, rapid iron turnover, and tissue deposition of excess iron are major factors responsible for functional and physiological abnormalities found in various forms of thalassemia ***(Hamed and Elmlegy, 2010)***.

On the other hand, chelation therapy by deferoxamine (DFO), deferiprone (DFP) or deferasirox (DFX) have been found to affect renal function in thalassemia patients ***(Economou et al., 2010; Hamed and Elmlegy, 2010; Dee et al., 2014)***. Thus, the necessity of monitoring renal damage in thalassemic patients receiving chelation therapy has been well-recognized ***(Hamed and Elmlegy, 2010)***.

β2-microglobulin (β2-M) is a low molecular weight protein that is freely filtered by glomeruli, reabsorbed by renal tubule and destroyed. The amount of β2-M is very low in the healthy individuals. Its level increases in case of inflammatory, immunologic, and neoplastic events ***(Kacar et al., 2015).***

Cystatin C (Cys C) is a non-glycosylated protein that belongs to the cystatin superfamily of cysteine protease inhibitors. It has been suggested as a sensitive marker of glomerular filtration rate (GFR) providing an early indication of renal impairment ***(Papassotiriou et al., 2010).*** Cys C is a better parameter on revealing important of renal function in comparison with creatinine clearance. The advantage of Cys C measurement is that it is not affected from height, sex, diet and muscle mass ***(Kacar et al., 2015).***

The use of early markers such as Cys C is useful for the early detection of small changes in GFR in β-thalassemia major ***(Mahmoud and Ali, 2012)***. A significant correlation was found between iron overload and glomerular filtration rate (GFR) estimated by Cys C based glomerular filtration rate (GFR) (Cys C eGFR) in patients with β-thalassemia major ***(Al-Khabori et al., 2014)***.

Magnetic resonance imaging (MRI) gradient echo (T2*), the reciprocal of T2* (known as R*), has been developed to quantify tissue iron in the liver, the heart as well as the kidneys. The pathophysiologic significance of kidney R2* is unclear. It may have a value as a long-term measure of hemolysis and could be a surrogate for renal iron accumulation in chronically transfused patients ***(Schein et al., 2008; Hashemieh et al., 2012).***

AIM OF THE WORK

The aim of this work was to assess levels of serum cystatin C and urinary β2-microglobulin to albumin ratio as markers of renal functions (glomerular and tubular) among transfusion dependent β-thalassemia patients without symptomatic renal disease and to evaluate their relation to tissue iron overload and renal MRI (R2*).

Chapter 1

BETA-THALASSEMIA

Thalassemia syndromes are the most common inherited hemoglobinopathies in the world caused by a genetic defciency in β-globin chain synthesis ***(Yesilipek, 2007; Hashemieh et al., 2012).*** This defect leads to an imbalance in α/β globin synthesis causing ineffective erythropoiesis, chronic hemolytic anemia and iron overload ***(Taher et al., 2011).***

The reduced amount (beta+) or absence (beta0) 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 ***(Galanello and Origa, 2010).***

Beta thalassemias are caused by mutations in the HbB gene on chromosome 11, inherited as autosomal recessive traits ***(Goldman et al., 2015)*** (Table 1)**.**

Table (1): Beta-thalassemias can be classified into**:**

▪ Beta-thalassemia - Thalassemia major - Thalassemia intermedia - 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
▪ Beta-thalassemia associated with other manifestations - Beta-thalassemia-tricothiodystrophy - X-linked thrombocytopenia with thalassemia

(Galanello and Origa, 2010)

Patients with beta-thalassemia major (β-TM) require regular transfusions of red blood cells to survive ***(Rachmilewitz and Giardina, 2011; Elalfy et al., 2014).*** Iron overload is a consequence of both the transfused iron and increased intestinal absorption of iron from ineffective erythropoiesis ***(Quinn et al., 2011).*** It was recently shown that hepcidin levels are decreased with β-thalassemia syndromes ***(Tanno et al., 2007).***

Transfused patients may develop complications related to iron overload. Complications of iron overload in children include growth retardation and failure or delay of sexual maturation. Later iron overload related complications include involvement of the heart (dilated myocardiopathy or rarely arrythmias), and endocrine glands (diabetes mellitus,

hypogonadism and insufficiency of the parathyroid, thyroid, pituitary, and, less commonly, adrenal glands) ***(Borgna-Pignatti and Galanello, 2004).*** Hepatitis virus C infection is the main risk factor for liver fibrosis in transfusion-dependent thalassemics ***(Elalfy et al., 2013).***

Epidemiology:

The disease is found most commonly in Mediterranean region, Africa, and Southeast Asia, presumably as an adaptive association to endemic malaria. The incidence may be as high as 10% in these areas ***(Advani et al., 2015).***

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. However, accurate data on carrier rates in many populations are lacking, particularly in areas of the world known or expected to be heavily affected ***(Vichinsky, 2008).*** According to Thalassemia International Federation, only about 200,000 patients with thalassemia major are alive and registered as receiving regular treatment around the world ***(Thalassemia International Federation, 2008).***

β-Thalassaemia represents a major public health problem in Egypt. The carrier rate varies between 5.5% to ≥9%; it is estimated that there are 1000/1.5 million per year live births born with β-thalassaemia ***(El-Beshlawy et al., 2012).***

Renal affection in β-thalassemia:

Renal dysfunction may occur in β-TM patients showing no clinical symptoms and before the manifestations of any other complications ***(Mohkam et al., 2008).***

Renal dysfunction in these patients seems to be multifactorial; attributed mainly to long-standing anemia, chronic hypoxia, iron overload and toxicity of iron chelators ***(Koliakos et al., 2000)*** (Figure 1)**.**

Increased mesengial matrix, focal global glomerulosclerosis, tubular atrophy, interstitial fibrosis were found on glomeruli in the kidney histopathological study of beta thalassemia patients Hemosiderin accumulation was found in glomerular visceral epithelial cells, but a bit less in parietal epithelial cells of mezengium and bowman capsule ***(Sumboonnanonda et al., 2003)*** (Table 2)**.**

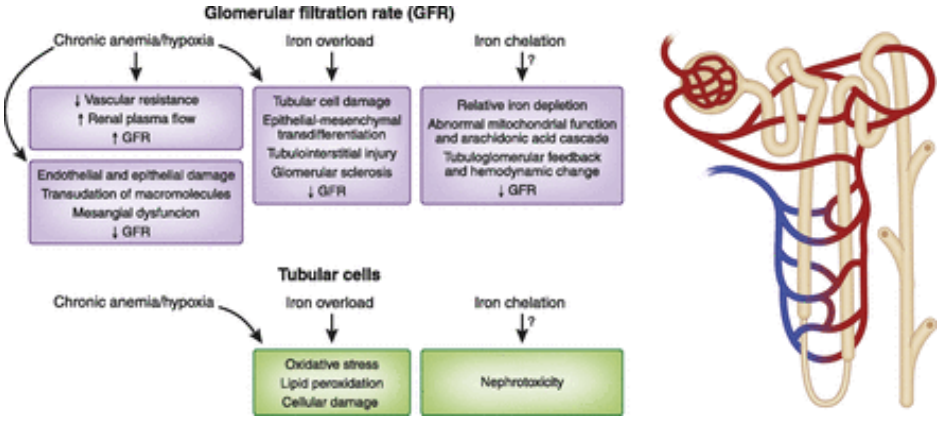

Figure (1): Mechanisms of renal disease in patients with β-thalassemia ***(Musallam and Taher, 2012)***.

Table (2): Tubular and glomerular abnormalities in beta-thalassemia ***(Mallat et al., 2013)***

1) Tubular dysfunction : • Hypercalciuria • Hyperuricosuria • Hyperphosphaturia • Elevated urinary β2-microglobulin • Hypermagnesuria • Aminoaciduria • Tubular proteinuria • Nephrolithiasis
2) Glomerular dysfunction
• Increased glomerular permeability • Overt proteinuria • Long-term decrease in glomerular filtration rate

Table (3): Clinical consequences of chronic anemia and hypoxia in the kidney ***(Mallat et al., 2013)***

1) Chronic hypoxia
• Proximal epithelial dysfunction • Interstitial fibrosis • Tubular atrophy
2) Chronic anemia
• Early glomerular hyperfiltration • Increased glomerular permeability

Pathophysiology:

1- Anemia and chronic hypoxia: (Table 3)

Anemia causes a change in renal hemodynamics affecting renal plasma flow and GFR by activating hypoxia-inducible factor (HIF), a known adaptive transcription factor for hypoxia. However, HIF may also have profibrotic effects and can promote interstitial fibrosis ***(Kimura et al., 2008).***

Anemia may reduce systemic vascular resistance, leading to a hyperdynamic circulation that increases renal plasmaflow and GFR. These changes can eventually lead to stretching of the glomerular capillary wall and subsequent endothelial and epithelial injury, together with transudation of macromolecules into the mesangium associated with glomerular dysfunction ***(Musallam and Taher, 2012).***

It is theoretically possible that persistent anemia, such as that seen in β-TM patients, may contribute to a progressive decrease in the glomerular filtration rate (GFR) ***(Ponticelli et al., 2010).*** A recent study showed that β-TM patients show a significant decline in estimated GFR with time ***(Lai et al., 2012).***

Moreover, chronic hypoxia of tubular cells with increased metabolic demand causes apoptosis or epithelial mesenchymal transition, leading to the development of

tubulointerstitial injury and consequent glomerulosclerosis and kidney fibrosis ***(Nangaku, 2006).***

Similarly, patients with iron-induced cardiac failure may have severe renal hypoperfusion causing a remarkable fall in GFR ***(Reinglas et al., 2010).***

2- Iron overload:

Iron is a source of oxidative stress in biological systems. In thalassemic patients, the increased intracellular content of nonhemoglobin iron generates free oxygen radicals that bind to different membrane proteins, altering the morphology, function and structure of membrane proteins ***(Sumboonnanonda et al., 2003).*** Free iron in the tubular lumen can also directly catalyze lipid peroxidation, by removing hydrogen atoms from the fatty acids that constitute the lipid bilayer of organelles ***(Mallat et al., 2013).***

There is ample evidence that free iron in the tubular lumen can catalyze the formation of toxic oxygen species, causing lipid peroxidation and generation of free oxygen radicals. This in turn can lead to organelle membrane dysfunction and subsequent cell injury/death ***(Kassab-Chekir et al., 2003, King et al., 2008).***

The proximal tubular dysfunction observed in patients with β-thalassemia may be caused by iron overload, as suggested by the positive correlation between serum ferritin

levels and urinary N-acetyl- β-D-glucosaminidase (NAG) ***(Koliakos et al., 2003)*** and by the correlation between the NAG changes and the duration and levels of blood transfusions ***(Jalali et al., 2011).***

Iron overloaded β-TM patients will probably have tubular cell damage may allow injured cells to migrate into the interstitium, releasing cytokines and growth factors that can cause tubulointerstitial scarring and glomerular sclerosis and leading to further decrease in GFR ***(Musallam and Taher, 2012).***

3- Oxidative stress:

Among several cellular mechanisms underlying acute tubular necrosis, oxidative stress plays an important role in progression to acute tubular necrosis by activation of inflammatory response via proinflammatory cytokine release and inflammatory cell accumulation in tissues ***(Hosohata, 2016).***

Proximal tubular toxicity develops due to direct nephrotoxic effects such as mitochondrial dysfunction, lysosomal hydrolase inhibition, phospholipid damage, and increased intracellular calcium concentration, leading to formation of reactive oxygen species (ROS) with injurious oxidative stress ***(Arany, 2003).***

Oxidative stress occurs as a result of the increased activity of free radical-producing enzymes, the decreased activity of free radical-removing enzymes, and insufficient levels of antioxidants. Oxidative damage leads to mitochondrial dysfunction and a loss of mitochondrial membrane, triggering mitochondrial permeability transition (MPT) and/or the release of proapoptotic proteins like cytochrome c to induce cell death ***(Orrenius et al., 2007).***

Reactive oxygen species (ROS) also stimulate tumor necrosis factor alpha with consequent cell death, increase in cytotoxicity, glomerular damage and a decrease in renal cell life span ***(Wajant et al., 2003; Akcay et al., 2010).***

4- Iron chelation therapy and nephrotoxicity

It has been reported that the iron chelator deferoxamine causes tubular dysfunction. They observed an elevated urinary excretion of β2-M, which was related to the duration of treatment, dosage and mode of administration of the drug ***(Mallat et al., 2013).***

Tubular dysfunction was reported in patients under deferasirox. Acquired proximal tubular dysfunction manifested by Fanconi syndrome was reported, that completely resolved after drug discontinuation ***(Rafat et al., 2009).***

A pathologic study in rats showed that deferasirox can cause vacuolization of the renal proximal tubular epithelium. It