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Early Detection of Renal Dysfunction in β Thalassemia with Focus on Novel Biomarkers

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Abstract
Improved survival among transfusion dependent thalassemia patients in recent years has led to the manifestation of morbidities such as renal dysfunction. Renal injury is still an underestimated complication in β thalassemia major patients. Chronic anemia, iron overload due to repeated transfusion, and specific iron chelators are the main factors in pathogenesis of renal dysfunction in β thalassemia. Early identification of this morbidity allows us to delay the progression of kidney damage and therefore reduce renal impairment. In recent decades , novel biomarkers for early recognition of renal dysfunction have been studied in thalassemic patients, such as cystatin C, beta 2 microglobulin , alpha 1 microglobulin, N-acetyl beta-D-glucosaminidase (NAG), neutrophil gelatinase associated lipocaline (NGAL) , kidney injury molecule 1 (KIM-1) , liver type fatty acid binding protein (L-FABP), and retinol binding protein (RBP). In this review, renal aspects of thalassemia with focus on novel biomarkers were discussed.

Key words: β thalassemia , Biomarkers, Renal Insufficiency

Introduction

β -thalassemia is considered as one of the most common genetic disorders in the world, caused by reduction or absence of β globin chain synthesis. Approximately, 1.5% of the global population are heterozygotes (carriers) of the β-thalassemia gene (1). It occurs in a high frequency in a broad belt and Iran is located on thalassemia belt. In Iran, the thalassemia gene prevalence rate is 4% to 10% in different parts (2), but fortunately the thalassemia prevention program in this country was formulated in 1995 and started to be implemented across Iran in 1997. Hereafter, the prevalence of the thalassemia has been reduced dramatically (3). The survival of patients has significantly improved in recent decades; however, complications of this disease in different organs can affect the quality of life among sufferers (4). Clinical manifestation of thalassemia syndromes appears in young infancy. β Thalassemia major and some of intermediate (β-TI) patients become transfusion dependent. The consequence of these repeated and regular transfusions is hemosiderosis or iron accumulation in different organs of body. Therefore, these patients need iron chelator drugs in order to survive. Without adequate and appropriate chelation therapy, the survival of transfusion dependent thalassemia (TDT) patients is limited (5) .With increasing the survival of thalassemia patients, some previously unrecognized complications have been detected. One of these morbidities is renal injury in thalassemia patients (6).

Chronic anemia and hypoxia may result in oxidative stress and lipid peroxidation and finally impairment in tubular cells function (7). In addition, iron overload due to repeated blood transfusions is a critical factor in the pathogenesis of kidney injury in thalassemic patients(8). Toxicity of iron chelators (deferoxamine, deferiprone, and

deferasirox) can lead to glomerular dysfunction (7).

The detection of renal involvement in thalassemia by traditional markers, such as blood urea nitrogen (BUN) and serum creatinine, has remained without any change for a few decades. However, novel biomarkers are needed for early recognition of nephropathy in these patients (6). In this review, the renal aspects of β thalassemia major, the mechanism of renal complications, types of renal dysfunction, tubular impairment, glomerular dysfunction, and novel biomarkers for early recognition of renal involvement in thalassemic patients were discussed.

Mechanism of renal injury in β-thalassemia:

Chronic anemia, iron overload due to repeated transfusion, and specific iron chelators are the main etiologic factors in the renal dysfunction in β thalassemia. Moreover, viral agents such as hepatitis B or C and human immunodeficiency virus (HIV) infection can cause a decrease in glomerular filtration rate (GFR) of thalassemia patients. In addition, iron-induced hepatic and cardiac dysfunction can lead to renal impairment (9). The mechanism of renal injury is discussed in more details under chronic anemia, iron overload, and iron chelation therapy subtitles.

Chronic anemia:

Chronic anemia and hypoxia can lead to oxidative stress and lipid peroxidation that are correlated to tubular cells dysfunction. Increased metabolic demand in association with chronic hypoxia in tubular cells may lead to apoptosis and then development of tubulo-interestitial injury and consequent glomerulosclerosis and kidney fibrosis (7). Moreover, in some studies, hyperfiltration was discovered in β-thalassemia. At first, this hyperdynamic circulation leads to increased plasma flow and glomerular filtration rate, but eventually stretching of glomerular capillary wall with subsequent endothelial and epithelial injury may result in glomerular dysfunction and a progressive decline in GFR (10).

Iron overload:

Excess free iron is known to be a catalyst of lipid peroxidation which damages cells (11). Blood transfusions can dump both non-transferrin bound iron (NTBI) and heme into nephrons. Hemosiderin deposits in proximal and distal tubules can result in tubular necrosis, cortical atrophy, and interestitial fibrosis. These factors play an important role in the pathogenesis of acute and chronic kidney injury in thalassemia patients. Injured tubular cells can also release growth factors and cytokines, leading to tubulo-interestitial fibrosis and glomerular sclerosis (8). ElAlfy et al., revealed that kidney iron deposition impaired renal glomerular and tubular functions in pediatric patients with β thalassemia (12). However, in another study on 120 transfusion dependent thalassemia (TDT) patients, a moderate correlation between kidney magnetic resonance imaging (MRI) T2* relaxation time and serum ferritin was found. These authors demonstrated a weak correlation between kidney MRI T2* with liver and heart T2* relaxation time and therefore suggested the use of kidney MRI T2* as a non-invasive method for evaluation of renal hemosiderosis in TDT (13). In a large study on 202 patients, Hashemieh et al., found that 77.7% of patients had kidney iron overload (14). In addition, cystatin C, which is a marker for glomerulopathy, has a strong positive correlation with serum ferritin in β-thalassemia patients (15)

Iron chelation therapy:

Consumption of all three available iron chelators (deferoxamine, deferiprone, and deferasirox) may result in glomerular dysfunction. This glomerulopathy ranges from mild increase in serum creatinine up to acute kidney injury (7). Deferasirox and

deferoxamine can cause renal injury in thalassemia patients more than deferiprone, especially when appropriate dosage monitoring is absent (8). Annayev et al., found that β2-microglobulin levels significantly increased in patients receiving high-dose deferasirox compared to those who were receiving a daily dose of 15-20 mg/kg or controls. (16). Iron depletion due to higher doses of iron chelators can play a critical role in pathogenesis of kidney injury in thalassemia (8). The most probable mechanism of GFR reduction in iron deprived nephrons is mitochondrial dysfunction and consequent production of adenosine and adenosine triphosphate. This phenomenon may lead to activation of the tubulo-glomerular feedback and vasoconstriction of the afferent preglomerular arterioles (7).

Types of renal injury:

Kidney injury in thalassemia increases with age and duration of blood transfusions (17). These patients can also manifest both tubular and glomerular dysfunction (18).

Tubular dysfunction:

Urinary markers of tubulopathy were increased in several studies. These markers include N-acetyl-β-D-glucosaminidase (NAG), β2 microglobulin, calcium, phophate, magnesium, uric acid, amino acids, and malondialdehyde (8). Tantawy et al., in a study on 66 β thalassemia major (β-TM) and 26 β thalassemia intermedia (β-TI) patients, found that proteinuria (71%) is associated with increased urinary level of RBP (69.4%), NAG (58.1%), α-1 microglobulin (54.8%), and microalbuminuria (29%) and also decreased urinary osmolality (58.1%) (19). Sadeghi -Bojd et al., in a study on 166 children with β thalassemia reported hypercalciuria (12.9%), proteinuria (8.6%), phosphaturia (9.2%), magnesiumuria (8.6%), and hyperuricosuria (38%) in these patients. (20). Mohkam et al., in another study on 103 children with β thalassemia reported abnormal level of urinary NAG in 35.9% of patients. Abnormal levels of fractional excretion of sodium, potassium, and uric acid were present in 29.1%, 7.8%, and 52.4% of patients, respectively (21). Hashemieh et al., in a study on adults with β thalassemia showed abnormal urinary level of NAG in 50% of patients (14). In addition, increased urinary excretion of NAG was demonstrated in other studies (6, 11, 12, 22). Moreover, many of thalassemia patients experienced hypercalciuria (14, 21, 23, 24). Higher transfusion intensity was associated with lower creatinine clearance and increased incidence of hypercalciuria(23).

Glomerular injury:

Glomerular hyperfiltration was reported in 20 to 40% of thalassemic patients. Regular blood transfusions can affect this phenomenon, but may be associated with increased hypercalciuria (8). Although reduction in GFR rarely occurs in pediatric patients with β thalassemia major , gradual decrease in GFR may happen with increasing age and progressive kidney damage. Lai et al., in a study on 81 adult patients with TM for 10 years revealed a mild decline in estimated glomerular filtration (e -GFR) in 66 patients and a significant reduction in 15 patients (e-GFR <90 ml/min). These authors concluded that tubular damage in childhood may result in abnormal e-GFR in adulthood (25). In pediatric TDT patients, proteinuria ranged from 24 to 47% (8). Hamed and El-Melegy in a study on 69 children with β Thalassemia documented significant higher levels of microalbuminuria and cystatin C in these patients (26). Shlipak et al., demonstrated that the use of cystatin C alone or in combination with creatinine improved the risk assessment in end stage renal disease (27).

Novel biomarkers of renal dysfunction in thalassemia:

Although the early identification of tubular and glomerular dysfunction in β thalassemia is of great importance, there are limited published data on novel biomarkers (6). Delay in diagnosis of renal impairment in these patients may result in progressive GFR decline (7).

N-acetyl β-D-glucosaminidase (NAG):

NAG is a lysosomal enzyme, which is found within the renal proximal tubules. An abnormal urinary NAG excretion was reported in different kinds of renal disorders, such as acute kidney injury, urinary tract infection, nephrotic syndrome, glomerulonephritis, drug nephrotoxicity, and renal allograft rejection (28). In a study by Voskaridou et al., on 87 sickle cell β thalassemia (Hb S/β Thal) patients, urinary NAG secretion was increased in 74.4% of the patients (29). In another study from Egypt, which was performed on 66 β thalassemia major, 26 β thalassemia intermedia, and 40 healthy controls, 58.1% of patients had increased urinary level of NAG (19).

Smolkin et al., demonstrated elevation of urinary N-acetyl-β-D-glucosaminidase (uNAG) and uNAG to creatinine ratio in children with β thalassemia major and intermedia (30). In other studies, elevation of urinary NAG was also reported (11,12,14, 21, 31).

Cystatin C:

Cystatin C is a cystein protease inhibitor that is produced by all nucleated cells. Cystatin C is an ideal marker of GFR and in contrast to creatinine, cystatin C levels are not affected by age, gender, race, or muscle mass. In addition, cystatin C is a useful marker for acute kidney injury (32). Murty et al., revealed that serum cystatin C was superior to creatinine for detection of impaired kidney function (33). Additionally, β Thalassemia patients had a high frequency of glomerular dysfunction , and cystatin C was a promising marker for monitoring of glomerular dysfunction in these patients (15). Behairy et al., in a study on 70 children with β -TM and 20 healthy controls found that thalassemic children had significantly higher cystatin C level compared with control (34). In another study on 202 adult β-TM, 33.2% of patients had also elevation of serum cystatin C and 104 patients (51.5%) had reduced e-GFR (14). Papassotiriou et al., in a study on 150 β TM patients found that slight changes of cystatin C during deferasirox treatment might not reflect renal injury. No correlation was found between cystatin C concentration and neutrophil gelatinase associated lipocaline (NGAL) (35).

β2 -Microglobulin:

β2 -Microglobulin (β2-M) is a urinary marker for the evaluation of proximal tubular function (36). β2-M is a low molecular weight protein that is freely filtered by glomeruli, and then reabsorbed by kidney tubules. The amount of this marker is very low in healthy individuals, but its level increases in conditions such as neoplastic, inflammatory, and immunologic conditions. Behairy et al., demonstrated that serum β2 microglobulin was higher in β thalassemia patients compared with controls. Moreover, these authors found a significant positive correlation between β2 microglobulin and serum ferritin, cystatin C, albumin / creatinine ratio, duration of chelation therapy, and frequency of blood transfusion / year (34). Kacar et al. found that β2-M and urea levels were increased in thalassemia patients compared to healthy control group (37). Voskaridou et al., in a published article demonstrated elevation of urinary β2 microglobulin in 70% of patients (29). Economou et al., in a study in Greece on 42 patients with β TM found increased urinary excretion of β2-M in 33.5% of patients (24). Behairy et al. reported that β2-M was positively correlated with urea, creatinine, serum ferritin, albumin/creatinine ratio, duration

of chelation therapy, and frequency of blood transfusion/year but negatively correlated with creatinine clearance, hemoglobin, and e-GFR in children with β thalassemia major (38). However, Sadeghi-Bojd et al. demonstrated evidence of tubular dysfunction, such as increased urinary excretion of β2-M, even in thalassemia minor patients (39).

Alpha- 1 microglobulin:

Urinary alpha-1 microglobulin is a marker for proximal tubular function (36). The source of this biomarker is liver (40 - 41). In addition, this urinary biomarker can be used for early detection of acute kidney injury (42). In patients with acute kidney injury, increased urinary level of alpha-1 microglobulin is associated with poor prognosis (43). Tantawy et al., showed that 54.8% of thalassemic patients had increased urinary excretion of alpha-1 microglobulin. Furthermore, they found that splenectomized β-Thalassemic patients had more elevation of urinary alpha-1microglobulin and lower urinary osmolality. Moreover, in β-TM, urinary alpha-1 microglobulin was significantly higher than β-thalassemia intermedia (β – TI). This biomarker had positive correlation with serum ferritin in thalassamic patients (19).

Neutrophil gelatinase associated lipocaline (NGAL):

The main function of NGAL is related to its capacity to bind iron-siderophore complexes, leading to a bacteriostatic property by preventing iron uptake with bacteria. NGAL is detected at very low level in various cell types. In vivo data revealed that the primary site of NGAL production in kidneys was the ascending loop of Henle and collecting duct cells (44). Kidney injury may result in NGAL secretion from the epithelial cells of kidney. NGAL is filtered by glomeruli and then reabsorbed by proximal tubule. After acute kidney injury, the reabsorption of NGAL in tubular cells decreases and therefore urinary NGAL concentration increases (44). Unlike NAG, there are limited data about NGAL in thalassemia patients. After ischemic or nephrotoxic injury, intra renal NGAL is upregulated. In the urine as early as 3 hours after injury, elevation of NGAL is detectable and 6 hours after injury, the concentration of urine NGAL reaches to peak level. Moreover, after acute kidney injury (AKI), hepatic production of NGAL increases. Thus, both urine and plasma NGAL can be used to predict the onset and course of kidney injury (45). Furthermore, Barrera – Chimal et al., found that NGAL had the ability to predict AKI before elevation of serum creatinine in ICU patients (32). Moreover, elevation of urinary NGAL was observed in chronic kidney disease (46).One of the limitations of this biomarker is that NGAL may be elevated in urinary tract infection or sepsis without acute kidney injury (47). Moreover, NGAL in lupus nephritis and IgA nephropathy increases (48). Another limitation for NGAL is the lack of specific cut value (49). Pastaoura et al., in a study on 35 β thalassemia intermedia and 20 controls found that NGAL levels were significantly higher in patients with thalassemia intermedia compared to controls. Additionally, these authors demonstrated that splenectomy and hydroxyurea did not have any effects on the NGAL level. No correlation was found between NGAL level and either parameters of erythropoiesis such as hemoglobin, Hb F, reticulocyte, and soluble transferrin receptor, either. In addition, no correlation was detected between NGAL level and ferritin or non-transferrin-bound iron (NTBI) (50). Şen et al., in a study in Turkey on 52 β thalassemia major, found that urinary NGAL to creatinine ratio (UNGAL / Cr) was significantly higher in β -TM compared to control group. These authors concluded that urinary NGAL might be a reliable and specific marker for monitoring of kidney injury in β –TM (6). However,

Nishida et al. found a significant correlation between the level of urinary NGAL and the degree of proteinuria in pediatric patients with chronic kidney disease from different etiologies (51). Roudkenar et al., performed a study on 25 adult β TM and 9 pediatric patients. These authors assessed NGAL expression by semi-quantitative RT-PCR, real time RT-PCR, and Elisa. Adult β –TM patients experienced upregulation of NGAL expression compared with the normal samples but no upregulation was reported in pediatric patients. In this study, authors concluded that the etiology of NGAL upregulation was due to the oxidative stress (52).

Kidney injury molecule (KIM-1):

KIM-1, a transmembrane glycoprotein, is recognized as a novel biomarker for recognition of tubular injury in the renal disease. The level of this biomarker is associated with the degree of tubular injury, interstitial fibrosis and inflammation in the injured kidney. KIM-1 is specific for the early detection of renal function impairment. This biomarker expressed at low levels in a healthy kidney, but after the tubular injury, the urinary level of KIM-1 rapidly increases. KIM-1 increases in chronic kidney disease (CKD) and the level of this biomarker is associated with the stage of CKD. Moreover the level of KIM-1 increases in diabetic nephropathy and poor glycemic control (53). In acute kidney injury, the increase of urinary KIM-1 may occur before the elevation of serum creatinine. Urinary KIM-1 increases a few hours after tubular injury and it allows early detection of kidney injury within 24 hours of injury (54). Also urinary KIM-1 increases in sepsis within 6 hours and remained elevated up to 48 hours (55). Urinary KIM-1 was significantly elevated in urinary tract infection, obstructive nephropathy, IgA nephropathy and cardiovascular disorders (53). Şen and their colleagues in a study on 52 β -TM and 29 healthy control (3-17 years) have reported no difference in urinary KIM-1 to creatinine ratio between patients and controls (6).

Liver type fatty acid binding protein (L-FABP):

L-FABP is a biomarker that has been found to be effective for the early detection and prognosis of acute kidney disease. Then expression of the L-FABP gene in the kidney is upregulated by stress, such as renal ischemia, hypertriglyceridemia, toxins and tubulo-interestitial damage (56). L-FABP can be used as a predictor for early and late stage of chronic kidney disease especially diabetic nephropathy (57). In a study from Turkey, there was no correlation between urinary L-FABP to creatinine ratio between thalassemic patients and healthy control group (6).

Urinary retinol binding protein (RBP) :

Retinol binding protein (RBP) is a low molecular weight protein that synthesizes in the liver. Its main function is to transport vitamin A. RBP acts as a biomarker for diagnosis of proximal tubular dysfunction. Also this marker is related to the progression of chronic kidney disease (58). Demosthenous et al. in a review article have displayed that urinary RBP is a sensitive marker for tubulopathy in β Thalassemia (41). Tantawy et al. in a study from Egypt on 66 β -TM, 26 β- TI and 40 healthy control found that 69.4% of patients had increased urinary level of RBP. Also RBP has negative correlation with creatinine clearance and positive correlation with serum ferritin and urinary total protein. These authors have demonstrated that RBP may be cost – effective for early detection of kidney involvement in thalassemic patients (19). Urine retinol binding protein 4 (RBP 4) is a sensitive marker for detection of tubulopathy, but the reduction of GFR affects the sensitivity of this biomarker. Some authors have found that

urinary RBP 4 has a prognostic value in kidney transplantation. Elevation of this biomarker in urine is a risk factor for loss of allograft in long term in recipients of kidney transplantation (56). Uzun et al. in a study on 118 β thalassemia patients (49 thalassemia major, 18 thalassemia intermedia and 51 thalassemia minor) and 51 healthy control have shown that urinary RBP was only higher in thalassemia major group when compared to controls. Also there was a significant positive correlation between ferritin and RBP, beta 2 microglobulin, protein creatinine ratio and cystatin C (59).

Other novel biomarkers:

Recently authors have found that other biomakers such as interleukin 18 (IL – 18), insulin like growth factor binding protein 7 (IGFBP 7), tissue inhibitor metalloproteinase 2 (TIMP – 2) and calprotectin play a role in the diagnosis of acute kidney injury (44). However these biomarkers have not been evaluated in the thalassemic patients.

Discussion:

Despite the increase of life span in thalassemic patients, kidney involvement has received little attention. Renal dysfunction may occur in β thalassemia asymptomatic patients. Sometimes kidney injury happens before the manifestation of any other complications (60). Renal involvement in thalassemia increases with age and duration of blood transfusions, manifesting as both tubular and glomerular dysfunctions (17, 18). Kidney injury due to thalassemia is a serious condition and, once established, is not reversible. Because of this irreversibility of thalassemic renal damage, physicians should be able to detect the oncoming renal damage as early as possible. Defining early and reliable biomarkers of kidney involvement in thalassemia is very important, since they allow for early diagnosis and specific measures to be undertaken that will delay the progression of renal injury and thus reduce the incidence of renal impairment (21). The diagnosis of acute kidney injury (AKI) is based on elevation of serum creatinin, but creatinin is not a strong marker for detection of early renal dysfunction. Serum creatinin is influenced by multiple non renal parameters and also elevation of creatinin lags far behind the renal injury. Often after 48-72 hours after the initial damage to the kidney, serum creatinin rises (61). On the other hand, there are some conflicting results about the level of serum creatinin in thalassemia. Elbedewy et al., Smolkin et al., and Kacar et al. have found that there is no statistically significant difference between β thalassemia patients and healthy control group. Additionally, these authors demonstrated the same results about estimated glomerular filtration rate (e-GFR) (30, 37, 60). However Hamed et al. and Ali et al. showed that β thalassemia patients had higher serum creatinin and lower e-GFR in comparison with control group (15, 26). Moreover, Ali et al. reported that e-GFR was significantly lower in chelated group when compared with non chelated group (15).Novel renal biomarkers such as serum cystatin C, urine N-acetyl-β-D-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), liver-type fatty acid binding protein (L-FABP), and retinol binding protein (RBP) have been suggested to improve diagnosis and monitoring of renal impairments among thalassemic patients(31,34,36).

In Iran, β2 microglobin, NAG, cystatin C, NGAL, KIM-1, interleukin- 18, and calprotectin have been used in laboratories for early detection of kidney function impairment. KIM-1 is a new biomarker for diagnosis of tubulopathy. Şen et al., in a study from Turkey found no difference in urinary KIM-1 to creatinine ratio between thalassemic patients and controls (6). However Badeli et al., in a study on 40 β TM Iranian patients (21 patient on deferasirox, 19 on deferoxamine) and 20 healthy controls

revealed that urinary KIM-1/creatinine ratio was significantly higher in the deferasirox group than in the control group (62).

Cystatin C is a sensitive marker for monitoring of glomerular dysfunction (15). In many studies, the elevation of serum cystatin C was shown in thalassemia patients (14, 26, 27, 34). However, Papassotiriou et al., in a study on 150 β TM patients found that slight changes of cystatin C during deferasirox treatment might not reflect renal injury (35). Moreover, no correlation was found between cystatin C concentration and neutrophil gelatinase associated lipocalin (NGAL) in their study. Elbedewy reported significantly higher levels of serum cystatin-C in poorly chelated and inadequately transfused patients when compared with well chelated and adequately transfused patients, respectively. In addition, these authors demonstrated a positive correlation between serum cystatin-C and serum ferritin and a significantly negative correlation between serum cystatin-C on one hand and pretransfusional hemoglobin and e-GFR on the other hand among thalassemic patients with kidney involvement (60). Papassotiriou et al., found that serum cystatin-C and serum ferritin concentrations correlated positively with each other (35). Hamed and El-Melegy discovered no correlation between serum cystatin-C and age or urinary NAG level (26). Kacar et al. reported a non-significant difference between thalassemic patients and control group regarding level of serum cystatin-C and an insignificant relationship between serum cystatin-C and creatinine clearance (37). However, Hamed and El-Melegy demonstrated a strong positive correlation between serum cystatin-C and serum creatinine (26). Many authors have found significantly higher uNAG in thalassemic patients when compared with the control group (19, 21, 26, 30, 60).Tantawy et al. have shown that uNAG was positively correlated with serum ferritin. Furthermore, these authors found that uNAG was negatively correlated with creatinine clearance (19). Jalali et al., reported no significant difference in uNAG activity between thalassemic patients and healthy control group and also found that the increase in serum ferritin was correlated with the increase in NAG activity (17). Smolkin et al., found that uNAG levels were not correlated with the actual ferritin level (30). Nevertheless, Elbedewy et al., reported a negative correlations between uNAG and e-GFR (60) . Hamed and El-Melegy demonstrated no correlations between uNAG and age, serum creatinine, or serum cystatin C (26).

L-FABP can be used as a predictor for early and late stage of chronic kidney disease (57). In a study in Turkey, no correlation in urinary L-FABP to creatinine ratio between thalassemic patients and healthy control group was found (6).

Conclusion

Early identification of thalassemic patients at high risk for renal impairment is of great importance. Further studies are needed to evaluate the significance of these novel biomarkers as predictors of renal disease in thalassemic patients.

Conflicts of interest

There are no conflicts of interest.

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