

β -Thalassemia and renal complications. A narrative review of pathophysiologic mechanisms

Christos Demosthenous^{1*}, Perla Eleftheriou², Chrysa Apostolou³, Pantelis Sarafidis⁴, Vassilios Perifanis⁵ and Efthimia Vlachaki³

¹Department of Hematology and HCT Unit, General Hospital of Thessaloniki "George Papanicolaou", Thessaloniki, Greece

²Department of Haematology, University College London, London, UK

³Adults Thalassemia Unit, Second Department of Internal Medicine, Aristotle University, Hippokraton Hospital, Thessaloniki, Greece

⁴Department of Nephrology, Hippokraton Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece

⁵First Propedeutic Department of Internal Medicine, University General Hospital of Thessaloniki AHEPA, Thessaloniki, Greece

Abstract

β -thalassemia are a group of recessively autosomal inherited disorders of haemoglobin synthesis where in mutations of the β -globin gene lead to various degrees of defective β -chain production, an imbalance in α/β -globin chain synthesis, ineffective erythropoiesis, and anaemia. Improved survival in thalassaemic patients has led to the emergence of previously unrecognized complications, such as renal disease. Renal disease is considered the 4th cause of morbidity among patients with transfusion dependent thalassemia. Chronic anaemia, hypoxia and iron overload are the main mechanisms implicated in development of renal injury, whereas several studies suggested also a contributive role of iron chelators. Kidney disease may develop through progressing renal tubular and glomerular damage; thus, its early recognition and prevention is important in order to prevent and/or reverse deterioration. This review will provide an insight on the involved mechanisms implicated in kidney disease in thalassaemic patients and will discuss the updates on diagnosis and prevention of renal complications in thalassemia.

Introduction

β -thalassemia syndromes are the most common inherited monogenic disorders worldwide. They are heterogenous disorders caused by reduced or absent β -globin synthesis, a major component of adult haemoglobin A (HbA, $\alpha_2\beta_2$) leading to imbalance of the globin chains [1]. Consequently, β -thalassemia leads to reduced haemoglobin production and accumulation of α -globins which form insoluble hemichromes [2]. The former causes microcytic, hypochromic anaemia; the latter is associated with oxidative stress, ineffective erythropoiesis and haemolytic anaemia [3,4]. The clinical and haematological spectrum of β -thalassemia disease ranges from mild to clinically overt conditions including transfusion dependent (TDT) β -thalassemia major (TM) and non-transfusion dependent (NTDT) β -thalassemia intermedia (TI) or thalassemia minor (TMin) [5].

Thalassemia syndromes are prevalent in the Mediterranean, Africa and Southeast Asia [6]. Although they present a significant public health concern for poorer countries, population movements from countries where thalassemia is prevalent have also led to an increased number of patients affected in developed countries [7]. At least 20.7% of the world population carry a β -thalassemia variant, whereas annually approximately 40000 children are born with β -Thalassemia [8].

In recent years, mainly in developed countries, the prognosis of β -thalassemia has improved due to blood transfusion, iron chelation therapy and the advances in the knowledge of the disease. In addition, the numbers of new-borns with β -thalassemia have been limited in Western societies due to introduction of screening programs.

Following the improved survival noted for β -thalassemia in recent years, many patients experience dysfunctions in several systems, including cardiopulmonary disorders, endocrine organ diseases, liver

dysfunction and thromboses in different vascular beds [9]. Further, the advances in the management of patients with β -thalassemia translated into improved patient survival has allowed previously unrecognized renal complications to emerge. The effect of thalassemia on the kidney has not been extensively evaluated. This review will discuss current knowledge and future perspectives in the field.

Materials and methods

A bibliographical research was carried out in PubMed searching for articles published in the last decades, in order to provide a narrative review on the pathophysiological mechanisms of renal disease in β -thalassemia.

Epidemiology

Information on the epidemiology of renal complications in β -thalassemia is limited. Cross-sectional studies in various thalassemia groups from five thalassemia centres in North America have shown reduced creatinine clearance in 7.8%, and albuminuria in up to 59% of patients [10]. More recent studies identified renal dysfunction in 1.8% of TDT patients, whereas renal disease was classified as the 4th most common cause of morbidity (4%) after endocrine (44.7%),

*Correspondence to: Christos Demosthenous, Department of Hematology and HCT Unit, General Hospital of Thessaloniki "George Papanicolaou", Thessaloniki, Exohi, Thessaloniki 57010, Greece, E-mail: christosde@msn.com

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cardiovascular (41.3%) and hepatic (40.5%) disease in the same patient population [11,12].

However, there are currently no data from large observational cohorts on the prevalence of chronic kidney disease (CKD) following contemporary definitions in patients with β -thalassemia.

Renal manifestations of β -thalassemia

In the following lines the most common pathophysiologic and clinical manifestations of renal disease in β -thalassemia patients are discussed. The etiology, pathogenetic mechanisms involved, and possible evaluation, including emerging biomarkers are presented in Table 1.

Tubular dysfunction

Evidence of tubular dysfunction among patients with β -thalassemia, were initial described about 2 decades ago by Ong-ajyooth et al [13]. Since then, several studies confirmed and evaluated further tubular function in β -thalassemia (Table 2) Up to 60% of patients with TDT have been reported to develop signs of tubular dysfunction [14]. Common signs of tubulopathy, such as proteinuria (8.6%), hypercalciuria (12.9%), phosphaturia (9.2%), hyperuricosuria (38%), magnesiumuria (8.6%), and increased excretion of β 2-microglobulin (β 2M) (13.5%) was demonstrated in a TDT Iranian population [15].

Early recognition of renal dysfunction is of great importance both for intervening in a timely manner and for improving prognosis. Therefore, in recent years several products produced and released by proximal tubular cells as measurable proteins in a variety of renal diseases have been tested, as possible valid biomarkers of renal injury. Such proteins include N-Acetyl-beta-D-glycosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), Kidney Injury Molecule-1 (KIM-1), Liver-type Fatty Acid-Binding Protein (L-FABP) and Interleukin-8 (IL-8), all of which were studied mainly in children and younger patients with TM [16-18]. Further studies are needed to evaluate the significance of these biomarkers as predictors of renal disease in thalassemia patients.

Tubular dysfunction among patients with β -thalassemia has been related to iron overload, chronic anaemia, as well as deferoxamine (DFO) toxicity [19-22]. The mechanism of iron overload-associated renal damage has not been fully elucidated. Non-transferrin iron can lead to organelle membrane dysfunction and subsequent cell injury/death. Iron-catalyse generation of reactive oxygen species (ROS) is responsible for initiating the peroxidative reaction. The possible association of oxidative stress and its impact on nitric oxide (NO) metabolism in iron overload associated renal injury was investigated through a randomized study in rats. In this study, animals were assigned into Fe-loaded (given 500 mg elemental iron/kg body weight as iron dextran; IV), Fe-depleted

Table 1. Renal disease in β -Thalassemia

Renal manifestation	Etiology	Mechanism	Evaluation/Biomarkers
Haematuria	Nephrolithiasis	Hypercalciuria, hyperuricosuria, cystinuria, struvite stones	Dipstick urinalysis
Tubular dysfunction	Chronic anaemia/hypoxia	Oxidative stress, lipid peroxidation, endothelial damage and loss of peritubular capillaries	Serum β 2-M Urine calcium/creatinine Urine β 2-M/creatinine Urinary NAG Urinary NAGL Urinary α 1-microglobulin Urinary RBP
	Iron overload	Oxidative stress, lipid peroxidation	
	Iron chelators	Nephrotoxicity	
	Aminoglycoside, intravenous radiocontrast agents, NSAIDs	Cytotoxicity, renal vasoconstriction, acute tubular necrosis.	
	β -lactams	Mitochondrial dysfunction, lipid peroxidation, acute tubular necrosis	
	Ampicillin, ciprofloxacin, sulphonamides	Crystal precipitation within the distal tubular lumen	
Glomerular dysfunction	Chronic anaemia/hypoxia	Reduced vascular resistance, elevated RPF	Urine dipstick Serum creatinine Urine protein/creatinine Serum cystatin CrCl eGFR
		Damage and loss of peritubular capillaries, epithelial-mesenchymal trans differentiation of tubular cells to myofibroblasts, tubulointerstitial injury, glomerulosclerosis	
	Iron overload	Damage and loss of peritubular capillaries, epithelial mesenchymal trans differentiation of tubular cells to myofibroblasts, tubulointerstitial injury, glomerulosclerosis	
	Infections (e.g. HIV, HCV, HBV)	Glomerulonephritis	
	Iron chelators	Relative iron depletion, mitochondrial dysfunction in tubular cells, tubuloglomerular feedback, vasoconstriction of the afferent arteriole	
	NSAIDs, COX-2 inhibitors	Vasoconstriction of the afferent arteriole	
	ACE inhibitors, ARBs	Vasodilation of the afferent and efferent arterioles	
Nephrolithiasis	Vitamin D, calcium supplementation, deferasirox, tubular dysfunction	Hypercalciuria, calcium stones	Urine dipstick Serum electrolytes Serum creatinine 24-hour urine collection Radiographic studies
	Tubular dysfunction	Cystinuria, cystine stones	
	Splenectomy increased red cell turnover, tubular dysfunction	Hyperuricosuria, uric acid stones	
	Urinary tract infections by urease-producing bacteria (e.g. Proteus spp, Klebsiella spp, S.epidermidis, Mycoplasma spp, yeast species)	Struvite stones	

β 2M: β 2 micro globulin; NAG: N-Acetyl-beta-D-glycosaminidase; NAGL: neutrophil gelatinase-associated lipocalin; RBP: Retinol binding protein; RPF: Renal plasma flow; HIV: Human immunodeficiency virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; NSAIDs: Nonsteroidal anti-inflammatory drugs; COX2: Cyclooxygenase-2; ACE: Angiotensin-converting enzyme; ARBs: Angiotensin receptor blockers; CrCl: Creatinine clearance; eGFR: estimated glomerular filtration rate

Table 2. Studies indicated or no tubular dysfunction in patients with β -thalassemia

Authors	Study type	Number of patients	Age (years)	Chelation therapy	Biomarkers	Results-Conclusions
¹⁸ Koliakos et al, 2003	observational	91 TM with no evidence of renal disease	17.2 \pm 7.2	DFO	Urine NAG Urine IgG Urine albumin Urine β 2M Serum ferritin	- high incidence of renal proximal tubular dysfunction. -iron overload as the main cause of this dysfunction
⁸⁷ Papassotiriou et al, 2010	observational	150 TM with no evidence of renal disease	29.2 (6.4–44.2)	DFX	Plasma NGAL Cys C NT-proBNP ferritin	Cys C concentration may be influenced by hemodynamic parameters as a result of therapy with DFX. Any changes in cys C do not reflect renal impairment
¹⁶ Jalali et al, 2011	case-control study	140 TM with no evidence of renal disease	7-16	DFO	urine NAG blood sample for biochemical and ferritin tests	- Kidney dysfunction in thalassemia increases with increasing age, duration, and levels of blood transfusion and hypercalciuria. - The presence of severe renal dysfunction in thalassaemic patients should be investigated using sensitive and specific tests, mainly NAG, to prevent progress
¹⁵ Mohkam et al, 2008	cross-sectional study	103 TM with no evidence of renal disease	12.5+/- 5.53	DFO	Urine sodium (Na), potassium (K), calcium (Ca), creatinine (Cr), phosphate, uric acid (UA), NAG and amino acids	Urinary NAG excretion can be a reliable index of the tubular toxicity and a possible predictor of proteinuria, aminoaciduria and eventual renal impairment in these patients.
³⁸ Michelakakis et al, 1997	case-control study	36 TM with no evidence of renal disease	5-22	DFO	urine specimens Urine NAG a-Mannosidase ferritin	Iron overload resulted in increased urinary levels of the lysosomal enzyme NAG. Reduction of iron load, achieved by regular DFO infusion, resulted in normalization of the urinary enzyme levels.
³⁰ Smolkin et al, 2008	case-control study	37 TM and 11 TI	2.4 - 27	DFO	Urine and blood samples Urine NAG	Renal tubular function is impaired in children with TM and TI. It is not known whether these functional abnormalities would have any long-term effects on the patients.
²² Kalman et al, 2005	case-control study	32 Tmin	5.8 +/- 3.1	-	Urinary calcium excretion zinc glucosuria (mg/dL), β 2M (mg/dL), NAG, sodium, magnesium uric acid and tubular phosphorus reabsorption	Renal tubular dysfunction has not been determined in children with TMin.
⁴⁴ Tantawy et al, 2014	cross-sectional, case-control study	66 TM and 26 TI	2.5-16	DFP	Serum ferritin, bicarbonate, plasma osmolality and urinary total proteins, microalbuminuria, NAG, RBP, α -1 micro globulin, bicarbonate, osmolality, creatinine clearance (CrCl)	Asymptomatic renal dysfunctions are prevalent in young β -TM and β -TI patients that necessitate regular screening
¹²¹ Aydinok et al, 1999	case-control study	40 TM	6-24	DFO	Urinary NAG creatinine, zinc	Urinary NAG indices (U/g Cr) were significantly higher in the patients compared to controls. Urinary zinc excretion was correlated with the urinary NAG indices.
¹⁴ Ong-ajyooth et al, 1998	case-control study	95 beta-thal/Hb E	na	na	Urine NAG, β 2M Plasma and urine MDA	This is the first report of renal tubular defects found associated with beta-thal/Hb E disease. The mechanism leading to the damage is not known but it might be related to increased oxidative stress secondary to tissue deposition of iron, as indicated by the raised levels of serum and urine MDA.
²⁶ Patsoura et al, 2014	case-control study	35 TI	8-63	-	Plasma NGAL, STfR, NTBI, Cys C, β 2M, hs-CRP	The increased NGAL levels reported for the first time in TI patients in agreement with the elevated expression of NGAL observed in TI mouse models. The induction of NGAL may represent either a survival response, facilitating the survival of the less damaged thalassaemic erythroid precursors, or a consequence of the abnormal iron regulation in TI.
²⁷ Roudkenar et al, 2008	case-control study	25 adult's TM and 9 paediatric TM	24.33 \pm 7.09 and 8.28 \pm 1.49	na	Plasma NGAL with PCR and ELISA	- In all adult cases, except one sample, NGAL protein was expressed more compared to the controls - Positive correlation with ferritin - Negative correlation with sex, age - NGAL upregulation was not found in paediatric β -thalassemia patients. Iron overload and oxidative status in β -thalassemia patients induce NGAL/Lcn2 expression. Upregulation of NGAL in this disorder may play a beneficial role in decreasing ROS or chelating iron. Obviously, chelating of iron is one of the major therapeutic goals in β -thalassemia.

TM: Thalassemia major; TI: Thalassemia intermedia; TMin: Thalassemia minor; DFO: Deferoxamine; DFX: Deferasirox NAG: N-Acetyl-beta-D-glucosaminidase; β 2M: β 2 micro globulin; NAGL: Neutrophil gelatinase-associated lipocalin; IgG: Immunoglobulin G; Cys C: Cystatin C; NT-proBNP: N-terminal pro b-type natriuretic peptide; RBP: Retinol binding protein; MDA: malondialdehyde; STfR: Soluble Transferrin Receptor; NTBI: Non-transferrin-bound serum iron; hs-CRP: high-sensitivity C-reactive protein; PCR: Polymerase chain reaction; ELISA: Enzyme-linked Immunosorbent Assay; Lcn-2: Lipocalin-2; ROS: Reactive oxygen species

(given an iron-free diet for 20 weeks), and control groups. Chronic iron deposition in proximal tubules, glomeruli and interstitium was associated with significant glomerulosclerosis, tubular atrophy, and interstitial fibrosis [23]. Iron staining in the distal tubules was negligible, but iron overload resulted in iron deposition in the glomeruli and proximal tubules with various renal lesions and evidence of increased ROS activity, enhanced ROS-mediated inactivation and sequestration of NO. Iron depletion was associated with reduced malondialdehyde (MDA) and tissue nitro tyrosine abundance, increased urinary NO metabolite (NOx) excretion, normal NO synthase (NOS) expression and absence of renal injury. The iron overload-induced toxicity is supported by clinical studies demonstrating concordance between serum ferritin levels and markers of tubular injury. At the same time, chelation therapy was associated with reversal of toxicity. An initial study from Greece including 36 patients aged 5-22 years old with TM showed a significantly higher urinary excretion of NAG in patients with poor compliance to DFO and serum ferritin >3,000ng/ml as compared to regularly chelated patients with serum ferritin <3,000 ng/ml (2.86 ± 0.287 vs. 2.37 ± 0.32 nmol/mg creatinine/h, $p < 0.05$) [24]. Similar results were observed in a second Greek study with 91 TM patients, aged 5-65 years and on DFO chelation. However, urine markers did not correlate with age, indicating that DFO treatment was not related to renal dysfunction [25].

Anaemia and associated potential chronic hypoxia could also lead to activation of the oxidative stress, lipid peroxidation and lead to tubular functional abnormalities [26,27]. Moreover, hypoxia per se induces differentiation of tubular cells to myofibroblasts, while at the same time it activates fibroblasts, changes the extracellular matrix metabolism of renal cells and leads to the obliteration of peritubular capillaries [28-30]. Relationship between the degree of anaemia and tubular dysfunction markers has been demonstrated in a study performed in a paediatric population of 104 patients with β -thalassemia of various disease severity. The patients were divided into three groups: severe anaemia [haematocrit (Hct)<25%], hyper transfusion program and DFO treatment, and moderate anaemia (Hct> 25%). Patients with severe anaemia had significantly higher low-molecular weight proteinuria and malondialdehyde (MDA, an indicator of lipid peroxidation), and lower urine osmolality than those with moderate anaemia. Severity of the abnormalities were least severe in patients on hyper transfusion and DFO therapy [22].

Etiology of the precise distribution of iron deposits in the renal tubular system has not been fully clear. In parallel, kidney biopsies to delineate such early deposits are impractical because of the procedural risk and the significant patient discomfort. Nowadays, kidney magnetic resonance imaging (MRI) is essential for non-invasive evaluation of renal iron overload. Eighty-three patients suffered from TM (mean age 24.7 years) and 37 patients with TI (mean age 28.8 years) were enrolled in an interesting study performed in Tehran, Iran. The mean transfusion duration was 21.8 days, while 57.5% of patients were splenectomised. All patients received iron chelation therapy (mean therapy duration 21.8 years) with variable compliance. The results showed statistically significant, but moderate correlation between serum ferritin and kidney T2* relaxation (correlation coefficient = -0.446, $P < 0.001$). The non-significant correlation of liver or heart MRI T2* relaxation time with serum ferritin highlights the important role of kidney MRI on iron load assessment [31].

In addition to the above, splenectomy was proved to be an independent risk factor for tubular abnormalities. A cross-sectional, case-control study reported data among 40 splenectomised and 26

non-splenectomised patients with TM, aged 2.5 -13 years (mean 6 years). Increased levels of NAG (32 ± 14.3 U/g creatinine, $p < 0.01$ vs. 18.3 ± 15.2 U/g creatinine, $p < 0.01$), α -1 macroglobulin (27.5 ± 13.6 mg/g creatinine, $p < 0.01$ vs. 13 ± 8.5 mg/g creatinine, $p < 0.01$) and also marked elevation of serum ferritin (683 ± 160 ng/ml, $p < 0.01$ vs. 483 ± 227 ng/ml, $p < 0.01$) were found more frequently in splenectomised than in non-splenectomised patients [32].

Interestingly, improved renal function was illustrated in patients with TDT after curative hematopoietic stem-cell transplantation (HSCT). When comparing 29 children with TM who had undergone HSCT with 39 children of the same age and similar disease severity but who had not experienced HSCT, parameters of tubule function were better in patients that had undergone HSCT, as demonstrated by urine protein level (0.36 mg/mg creatinine vs 3.03 mg/mg creatinine, $p < 0.001$), osmolality (712 mos mol/kg vs 573 mos mol/kg, $p = 0.006$), NAG (17.7 U/g creatinine vs 42.9 U/g creatinine, $P = 0.045$), and β 2M (0.09 microg/mg creatinine vs 0.13 microg/mg creatinine, $P = 0.029$) [33].

Glomerular dysfunction

Changes in glomerular function are also evident in multi transfused patients with β -thalassemia. Chronic anaemia is thought to reduce systemic vascular resistance leading hyperdynamic circulation and subsequent increased renal plasma flow and glomerular filtration rate (GFR) [34]. Glomerular hyperfiltration appears to be deleterious on the mesangial compartment causing it to increase matrix volume and cellularity, initiating a sclerotic process [35,36]. In the long-term, such modifications may theoretically lead to a progressive decline in GFR through the typical pathway of hyperfiltration – albuminuria – progressive renal damage. Furthermore, renal tubular cells that are subject to iron overload, induce injury into the interstitium by releasing cytokines and growth factors that may cause tubulointerstitial scarring and glomerular sclerosis [37].

As noted earlier, when iron exceeds the binding capacity of transferrin as occurs in iron overload, it accumulates as a non-transferrin-bound iron which accelerates the generation of radical oxygen species and cellular damage.

Additional independent factors that contribute to renal injury may be glomerulonephritis induced by HIV, hepatitis B and hepatitis C infections as well as non-iron related liver and heart disease [38].

Calculation of GFR based on serum creatinine concentration or creatinine clearance demonstrated glomerular hyperfiltration in studies performed in children, adolescents and adults with TDT receiving iron chelation therapy [10,39,40]. Noteworthy, Milo et al demonstrated reduced GFR in 9 TDT patients through use of inulin clearance [41]. Individuals were on chelation therapy with DFX for at least 1 year. All patients had lower inulin clearance and GFR than normal healthy young adults, while 4 of 9 patients had mild to moderate chronic kidney disease (CKD). By using Cockcroft-Gault (CG) equation and serum creatinine, median creatinine clearance in the same study was 134.9 (mean 133.3) ml/min per 1.73 m², significantly higher than the GFR estimated by inulin clearance ($P < 0.008$) [41].

Economou et al, who used cystatin C (Cys C) in 42 pediatric and adult patients with TM, also described a reduction in GFR as compared to normal controls ($p \leq 0.001$). Elevated Cys C levels, but not normal, were also significantly accompanied by other indexes of glomerular dysfunction, such as proteinuria (223.28 ± 125.84 and 79.34 ± 29.1 mg/m²/day, respectively, $p < 0.0001$). All patients of the study were

chronically transfused and under regular chelation program consisted of either DFX or combination of DFP and DFO [42]. In accordance, elevated Cys C levels were also visualized in TM patients not receiving chelation therapy [43].

Quinn et al applied 24-h urine collection and reported similar levels of creatinine clearance (mean 136.1ml/min per 1.73 m²) in 106 adult patients with TM, the majority of whom were treated with DFP or DFO. When they assessed GFR using this method, they predicted hyperfiltration [10]. On the other hand, Piga et al reported normal GFR measured by plasma sampling of ⁵¹Cr EDTA in patients with TM [44].

Taken together, the estimations regarding GFR are frequently variable and inconsistent. It is most of the times obvious, that these variations derive from the different parameters applied in each study. Patient demographics, clinical characteristics, comorbidities and iron chelation therapy in addition to the study design and data analysis are important factors that might influence outcome of each study.

Milo et colleagues propose inulin clearance as a more accurate method which may lead to early detection of reduced GFR and slow the progressive deterioration to CKD. Noteworthy, as the authors state, the same study has some limitations such as the fact that involves a relatively small number of participants. Additionally, it is a cross-sectional study and all participants were adults (median age was 30, range 21 to 37 years).

Reduced estimated GFR based on serum creatinine (MDRD study simplified equation) has also been demonstrated in a cohort of TM adult patients regularly followed-up for 10 years. As in the previous study, all patients were transfusion-dependent, treated with iron chelation and adults at the time of the enrolment. Thus, it is unclear whether hyperfiltration preceded demonstrated reduced GFR or has never existed. This theory could be supported by similar studies conducted in children with TM, but to our knowledge current bibliography lacks such data.

Other factors that favor glomerular hyperfiltration are male gender and history of splenectomy according to data derived from univariate and multivariate analysis among adult, multi transfused patients with TM and TI [45].

The variability of GFR estimations might, indeed, be related to the method used for GFR calculation. Equations based on serum creatinine (e.g. CKD-EPI, MDRD) overestimate GFR due to the low serum creatinine levels observed in patients with TM, which might be related to their muscular atrophy and reduced muscle mass and therefore decreased generation. On the contrary, urinary creatinine excretion in healthy people is expected to equal creatinine generation, most of which is generated by muscle metabolism [46-48]. Other possible factors contributing to low serum creatinine in thalassemia patients are compensated increased tubular creatinine secretion which prevents its appropriate rise in serum, or extrarenal creatinine metabolism, especially when kidney function is reduced [49,50]. Lastly, variability in serum creatinine measurements among individuals may additionally contribute to overestimation of GFR [48].

Calculation of GFR based on creatinine clearance (e.g. Cockcroft-Gault Equation) might also result in overestimation of GFR, which may be encountered as hyperfiltration. In this case, increased creatinine clearance is in large part due to the proximal tubular secretion of creatinine. Urinary creatinine excretion is composed of filtered creatinine and proximal tubular secretion of creatinine. As glomerular disease progresses, the remnant tubular cells hyper secrete creatinine,

resulting in increased creatinine excretion and thus elevated creatinine clearance [49].

Measurement of the area under the plasma clearance curve (AUC) following a single intravenous injection of ⁵¹Cr-EDTA is another procedure for determining GFR. Similarly, to the methods described before, it has been proved that measurement of GFR by ⁵¹Cr-EDTA overestimates the true renal clearance by approximately 10% [51].

Cys C is a low molecular weight proteinase inhibitor that is produced in all nucleated cells and maintains a constant appearance rate in blood. Opposed to serum creatinine, serum Cys C concentration is independent of gender and muscle mass [52]. Cys C is freely filtered through renal glomeruli and then totally reabsorbed and catabolized in the proximal renal tubule and is not returned to blood. Thus, serum concentration is mainly determined by GFR [53]. Previous studies have demonstrated the superiority of serum Cys C compared to Cr in the evaluation of GFR, particularly when there is a minor reduction in GFR [54,55]. Inulin clearance is the most widely accepted method for estimating GFR [56]. However, this is a method that, due to its complexity, cannot be routinely used in clinical practice. The use of an accurate method to calculate GFR method may lead to early detection of reduced GFR and slow the progressive deterioration to chronic kidney disease.

Haematuria

An Iranian study involving a comparative evaluation in 108 children with β -thalassemia TDT or NTDT demonstrated microscopic haematuria in 19 patients. Interestingly, haematuria was more common in NTDT than TDT (90% vs. 10%) [57]. A subsequent study revealed the presence of microscopic haematuria in 10.6% from a total of 500 patients with TDT, while at the same time its frequency was significantly increased in ages over 20 years old (9.8% vs. 20%, p=.04) [58]. The presence of haematuria might be related to either hypercalciuria or hyperuricosuria and nephrolithiasis [57].

Nephrolithiasis

The prevalence of nephrolithiasis in TDT population might reach 59%, as demonstrated in a study involving 27 subjects with TM by non-iodinated contrast renal tract CT. Among affected subjects, 69% had multiple stones, whereas 56% of the affected patients had stones of variable composition. The majority of renal calculi were struvite (33%), followed by calcium oxalate (31%) and cystine (22%). Development of struvite stones may warrant an evaluation for an underlying infection [59].

Through a retrospective study of 166 participants with TDT, Wong et al showed that nephrolithiasis is associated with reduced bone mineral density of the femoral neck (odds ratio (OR)=5.59, 95 % CI 1.16–27.03) and increased risk of fracture in males (OR = 2.13) [60]. Significantly stone formers in this study had elevated creatinine and lower ferritin serum levels suggesting that DFX is involved as a causative factor [60]. The impact of hypercalciuria after vitamin D and calcium supplementation on renal stone development in the general population remains controversial [61,62]. Similarly, calcium or vitamin D supplementation was not found to be a risk factor for kidney stones in individuals with thalassemia [59]. Among other stone types, hyperuricosuria due to tubular dysfunction and increased cell turnover has been associated with uric acid urolithiasis in the general population. However, in TDT patients, uric acid stones represented only 2% of all stones which might be reflected by the increased frequency of blood transfusion which limits cell turnover [59]. Splenectomy was proved

to be an independent risk factor for developing hyperuricemia and nephrolithiasis in patients with TI by further increasing erythrocyte turnover and number. Despite xanthine oxidase inhibition, patients receiving allopurinol had both significantly higher level of serum urate and higher prevalence of urolithiasis as compared to those not receiving ($p < 0.01$) [63].

The role of iron chelating agents in renal disease

The advent of iron chelators improved patient survival in β -thalassemia, however this achievement has allowed previously unrecognized complications to come on the scene. Currently, three iron chelators are available: oral agents deferasirox (DFX), deferiprone (DFP), as well as parenteral deferoxamine mesylate (DFO). Renal manifestations attributed to chelating agents are rare. Although serum ferritin levels have been inversely correlated with GFR, iron chelation therapy has also been related to renal function deterioration [64,65]. Oral agent DFP lacks support of large randomized controlled trials. About two decades ago, Hoffbrand et al studied the efficacy and safety of DFP in 51 transfusion dependent iron overloaded patients. They reported that only 1 patient discontinued the drug after 4 months of treatment because of renal failure, however, that patient was not thalassaemic [66]. El Alfy et al from a study of 100 children under 10 years (mean 5.1 years) with TDT (91 patients with TM) and given 50-100 mg/kg DFP demonstrated a modest non-significant and non-progressive elevation in serum creatinine from a baseline mean of 29.2 ± 12 to 37.1 ± 10 micromol/L over a 6-month period. Two patients had baseline serum creatinine values of 71 and 62 micromol/L, which resolved by the end of the study, while two others had a single but transient episode of elevation of serum creatinine above the upper limit of normal, which resolved by the end of the study without a need for interruption of therapy or dose adjustment [67]. The etiology for these

fluctuations is unclear but have not seen in other currently available data. Although DFP is not considered nephrotoxic, is characterized by a narrow therapeutic window and serious adverse effects, such as agranulocytosis, neutropenia and disabling arthropathy [68]. Thus, is approved specifically for patients with TDT when treatment with other chelating agents is contraindicated or inadequate.

It also binds intracellular iron inducing ferritin degradation through proteasome mediated mechanism [69]. DFX is generally well tolerated. Due to its lipophilicity, DFX enters the tubular cells and forms a highly charged complex with iron. This triple negative charged DFX-iron complex less readily penetrates membranes, accumulates and may result in proximal tubulopathy and Fanconi syndrome (FS) [70]. FS is associated with non-anion gap metabolic acidosis, hypophosphatemia due to phosphaturia, aminoaciduria, proteinuria and glucosuria (with normal serum glucose concentrations). Despite its importance, there is limited data about the risk factors, incidence and clinical picture of FS in patients receiving DFX. A retrospective study conducted by Chuang et al indicated younger age of treatment initiation as the sole factor that was significantly associated with FS (7.8 vs. 19.2 years, $p = 0.008$) [71]. Similarly, to other reports, patients in this study did not document complete FS. FS generally affects less than 1% of patients, mainly those treated for more than 6 months [70-83]. Successful management is usual upon discontinuation of DFX and repletion of fluid and electrolyte deficits while efficacy of plasmapheresis has not been confirmed deficits [72,74,75,79,80,81,84-86]. Unfortunately, recurrence even after initiation of lower doses of drug may occur [74,79] (Table 3). Moreover, DFX at therapeutic doses in TM patients was also associated with hypercalciuria providing a biological mechanism for both increased kidney stones and accelerated bone resorption [87,88]. Dose-dependent increases in serum creatinine were observed in 38% of TDT patients treated with 20-30mg/kg DFX. However, these increases

Table 3. Deferasirox induced Fanconi syndrome

Authors	Study design	Patient population	Comorbidities/ other potentially nephrotoxic medications	Dose and duration of treatment	Clinical picture	Outcome
⁹⁹ Chuang et al, 2015	Cohort study, single centre, evaluating the incidence of DFX-related FS and its risk factors.	57 patients with TDT, aged 7.8 ± 4.4 years old	None	9299 ± 1774 mg/kg/year for 6.9 ± 1.8 years	Non-anion gap metabolic acidosis, hypophosphatemia, glucosuria	DFX was stopped. All features of proximal tubular dysfunction resolved.
¹⁰⁶ Rafat et al, 2009	Case report	78-year-old male with sideroblastic anaemia	DM, CLL, aortic valve replacement, and essential hypertension/perindopril, glibenclamide, metformin	24mg/kg/d for 1 month	Non-anion gap metabolic acidosis, hypophosphatemia, glycosuria, proteinuria, hypouricemia, increased β_2 -microglobulin urinary level. Increase in serum creatinine from 0.9 to 2mg/dl.	DFX was stopped. Resolution of tubulopathy apart from minimal proteinuria. Serum creatinine 1.2 mg/dl
¹⁰⁴ Murphy et al, 2013	Case report	21-year-old male with Ewing sarcoma	Ifosfamide	1125 mg/d for 9 months	Hypophosphatemia, hypokalaemia, glycosuria, proteinuria, kaliuresis. Increase in serum creatinine from 1.1 to 2.5mg/dl. Kidney biopsy revealed severe tubular injury with tubular epithelial cells demonstrating isometric vacuolization consistent with drug toxicity	DFX was stopped. Fluid and electrolyte supplementation. SCr fell to 1.5 mg/dL and bicarbonate normalized. Persistent hypokalemia, hypophosphatemia, proteinuria, and glycosuria
¹¹⁴ Shah et al, 2017	Case report	20-year-old male with TDT admitted for a scheduled allogenic stem cell transplant	Fludarabine	20-30 mg/kg/d for 8 years. Due to dispensing error he received a one-time dose of 90 mg/kg.	Acute onset, following high dose administration. Metabolic acidosis, glycosuria, haematuria, phosphaturia, kaliuresis and elevated β_2 -microglobulinuria	DFX was stopped. Fluid and electrolyte supplementation. Plasmapheresis. All features of proximal tubular dysfunction resolved.

112Papneja et al, 2016	Two Case reports, two centres	16-year-old male with DBA	None	30 mg/kg/d for 6 years with a transient increase to a maximum of 45 mg/kg/day during the last year.	Non-anion gap metabolic acidosis hypokalaemia, hypophosphatemia, phosphaturia, glucosuria, and proteinuria	DFX was stopped. Fluid and electrolyte supplementation. FS was resolved. DFX was restarted.
		14-year-old male with DBA	None	36 mg/kg/d for 10 months	Non-anion gap metabolic acidosis hypokalaemia, hypophosphatemia, hyper phosphaturia, glucosuria, and proteinuria. Increase in SCr from 59 μ mol/l to 204 μ mol/l.	DFX was stopped. Fluid and electrolyte supplementation. SCr was normalized. All features of proximal tubular dysfunction resolved. DFX was restarted.
100Dee et al, 2014	Cohort study, single centre, evaluating the incidence of DFX-related FS.	9 patients with TDT, aged 2.6 to 23.8 years old	One patient had influenza virus H3N2 and developed severe hypokalaemia while on DFX. Another patient had Echovirus type 6 infection 10 months before the onset of renal tubular dysfunction	Dose was not indicated. Cumulative incidence was estimated at 11% at 2 months and about 90% at 6 years of DFX therapy	Metabolic acidosis (33%), elevated urine β 2M [Odds ratio (OR) = 35.29, P = 0.0009], hypokalaemia (OR = 23.22, P = 0.015), hypophosphatemia (OR = 57.00, P = 0.001), hypocalcaemia (OR = 23.22, P = 0.015)	Normalized after reduction or withdrawal of DFX.
113Tunc et al, 2012	Cohort study, single centre, evaluating the incidence of DFX-related adverse events.	8 patients with Fanconi anaemia aged 12.8 \pm 3.6 years old	All patients were receiving oxymetholone.	10-30 mg/kg/d for 13.6 \pm 5.8 months	Proteinuria (87.5%). Increase in SCr (37.5%) in: a patient with unilateral renal agenesis (from 1.03 mg/dL to 1.44 mg/dL), a patient who undergone surgery for cellulitis (SCr 1.28 mg/dL), a patient with congenital abnormality of the right duplicated collecting system during sepsis (SCr 1.26 mg/dL)	SCr was normalized in 2/3 of patients after transient cessation of DFX. Other information is not provided.
107Rheault et al, 2011	Two case reports	7.5-year-old-male with TDT	None	30 mg/kg/d for 18 months.	Normal-anion gap metabolic acidosis, hypokalaemia, phosphaturia, glucosuria, aminoaciduria hypercalciuria.	DFX was stopped. Fluid and electrolyte supplementation. Features FS were improved. DFX was restarted at 20 mg/kg/d with evidence of mild FS (elevated urine β 2M, hypercalciuria).
		8.5-year-old-female with TDT	None	30 mg/kg/day for 12 months.	Elevated protein to creatinine ratio (0.38), urine calcium to creatinine ratio (0.46) and urine β 2M.	Stable without intervention.
108Wei et al, 2011	Case report	18.75-year-old male with TDT	Secondary hypothyroidism and hypogonadotropic hypogonadism/oral thyroxine, intramuscular testosterone cypionate	28.9-35.3 mg/kg/d for 27 months.	Proteinuria, glycosuria, hypophosphatemia, hypocalcaemia, hyponatremia, hypokalaemia, metabolic acidosis, low serum uric acid, elevated urinary β 2M. Increase in SCr from 0.5 to 1.74 mg/dL. Also developed coma, hepatic dysfunction and thrombocytopenia.	Fluid and electrolyte supplementation. DFX was stopped and patient had full recovery.
109Yacobovich et al, 2010	Four case reports	8.5-year-old female with TDT	None	30 mg/kg/d for 8 months.	Metabolic acidosis, glycosuria, hypokalaemia, hypophosphatemia.	DFX was stopped. Fluid and electrolyte supplementation. FS was resolved.
		11-year-old female with TDT	None	30 mg/kg/d for 24 months.	Metabolic acidosis, glycosuria, low serum uric acid, hypokalaemia, hypophosphatemia and elevated urine lysozyme. Also had gastroenteritis.	DFX was stopped. Fluid and electrolyte supplementation. FS was resolved.
		8-year-old male with TDT	None	30 mg/kg/d for 36 months.	Metabolic acidosis, glycosuria, low serum uric acid, hypokalaemia, hypophosphatemia and elevated urine lysozyme. Also had gastroenteritis.	DFX was stopped. Fluid and electrolyte supplementation. FS was resolved.
		32-year-old female with TDT	Calcium plus vitamin D supplement. Also, on ofloxacin and phenazopyridine HCL for a recently diagnosed urinary tract infection	38 mg/kg/d for 33 months.	Metabolic acidosis, glycosuria, low serum uric acid, hypokalaemia, hypophosphatemia, kaliurisis.	DFX was stopped. Fluid and electrolyte supplementation. FS was resolved.
103Grange et al, 2010	Case report.	77-year-old male with non- hereditary hemochromatosis.	Hypertension/ irbesartan, HCTZ, ursodesoxycholic acid	1500 mg/day for 1 month.	Glycosuria, hypokalaemia, hypophosphatemia, hypocalcaemia, kaliurisis, phosphaturia, aminoaciduria. Increase in SCr at 180 μ mol/L.	DFX was stopped. Fluid and electrolyte supplementation. FS was resolved. SCr was normalized.

102Even-Or et al, 2009	Two case reports.	18-year-old male with pure red cell aplasia.	None.	20 mg/kg/d for 6 months.	Glycosuria, uricosuria, phosphaturia, kaliuria, aminoaciduria, low serum uric acid, hypokalaemia, hypophosphatemia. Increase in SCr from 0.6 to 1.07 mg/dL.	DFX was stopped. FS and SCr were resolved. DFX was restarted and mild hypouricemia, hypophosphatemia, phosphaturia and increased urinary β 2M within a few weeks, while on 10 mg/kg/day of DFX. He continued treatment with increasing doses with no further deterioration.
		11-year-old female with	Acute graft	30 mg/kg/d	Glycosuria, uricosuria,	DFX was stopped. Electrolyte supplementation. FS was resolved.

DFX: Deferasirox; FS: Fanconi syndrome; Scr: Serum creatinine; DBA: Diamond-Blackfan anemia, β 2M: β 2 micro globulin; HCTZ: Hydrochlorothiazide; AHSCT: Allogeneic hematopoietic stem cell transplantation

were sometimes transient, never exceeded two times the upper limit of normal (ULN) and generally within the normal range [82]. Later studies in adult and paediatric patients with TDT and normal renal function, confirmed absence of progressive increases in serum creatinine over a 5-year follow up after treatment. Consecutive serum creatinine level increases > 33% above the value at the start of DFX and greater than the ULN were reported 8.8% patients, mostly in doses 25 to 35 mg/kg/day. Nonetheless, these increases were manageable and did not lead to drug interruption [83]. Furthermore, DFX proved to be safe in managing iron overload in thalassemia patients with end stage renal disease or diabetes mellitus [89,90].

DFO chelates iron located in plasma and ferritin by forming a metabolically inactive complex, that is renally excreted. DFO is poorly absorbed by the oral route. Consequently, intravenous or subcutaneous administration is required [91]. Acute renal failure necessitating dialysis following intravenous DFO overdose was described in patients who received 10-times the recommended dose due to administration pump failure or with inadequate monitoring [65,92]. Similarly, to DFX, transient and within the normal range serum creatinine increases have been documented in 14% of patients receiving DFO, whereas the same agent was also associated with increased level of NAG and tubular dysfunction in patients with TM [82,93]. Of importance, DFO poses a risk of Yersinia, Mucor mycosis (zygomycosis) and Vibrio vulnificus infection and sepsis, an additional causative factor of acute kidney injury if left untreated [94-96].

The mechanisms involved in GFR alteration concurrently with iron depletion include impaired mitochondrial function and consequent production of adenosine and adenosine triphosphate that lead to activation of the tubulo-glomerular feedback and vasoconstriction of the afferent glomerular arterioles. Interference with prostaglandin production and imbalance between vasodilating and vasoconstrictive substances is also implicated in GFR reduction [65].

The management of serum creatinine elevation should be individualized based on the magnitude of increase and the presence for additional risk factors for renal disease or comorbid conditions. Recommendations for dose modifications and monitoring in patients who experience DFX-associated adverse events are available [97].

Conclusion

In conclusion, more longitudinal data are required to fully portray any possible differences in renal disease in TDT and NTDT patients, as well the current prevalence of this comorbidity in the era of new iron chelation and blood transfusion guidelines. There is a need for close monitoring and follow-up of renal function both in NTDT and TDT patients as their life expectancy has increased, and this puts them potentially at increased risk of severe renal disease.

Disclosure of interest

The authors report no conflict of interest

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