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Renal functions in pediatric patients with beta-thalassemia major: relation to chelation therapy: original prospective study

Enas A Hamed*¹ and Nagla T ElMelegy²

Abstract

Background: In β -thalassemia, profound anemia and severe hemosiderosis cause functional and physiological abnormalities in various organ systems. In recent years, there have been few published studies mainly in adult demonstrating renal involvement in β -thalassemia. This prospective study was aimed to investigate renal involvement in pediatric patients with transfusion dependant beta-thalassemia major (TD- β TM), using both conventional and early markers of glomerular and tubular dysfunctions, and to correlate findings to oxidative stress and iron chelation therapy.

Methods: Sixty-nine TD- β TM patients (aged 1-16 years) and 15 healthy controls (aged 3-14 years) were enrolled in this study. Based on receiving chelation therapy (deferoxamine, DFO), patients were divided into two groups: group [I] with chelation (n = 34) and group [II] without chelation (n = 35). Levels of creatinine (Cr), calcium (Ca), inorganic phosphorus (PO_4), uric acid (UA) and albumin were measured by spectrophotometer. Serum (S) levels of cystatin-C (S_{CysC}) and total antioxidant capacity (S_{TAC}) and urinary (U) levels of β_2 -microglobulin (U_{β_2MG}) were measured by immunosorbent assay (ELISA). Urinary N-acetyl-beta-D-glucosaminidase (U_{NAG}) activity and malondialdehyde (U_{MDA}) were measured by chemical methods. Estimated glomerular filtration rate (eGFR) was determined from serum creatinine.

Results: In patient with and without chelation, glomerular [elevated S_{CysC} , S_{Cr} , $U_{albumin}/Cr$ and diminished eGFR]; and tubular dysfunctions [elevated S_{UA} , S_{PO_4} , U_{NAG}/Cr , U_{β_2MG}/Cr] and oxidative stress marker disturbances [diminished S_{TAC} and elevated U_{MDA}/Cr] were reported than controls. In patients with chelation, S_{CysC} was significantly higher while, S_{TAC} was significantly lower than those without chelation. In all patients, S_{CysC} showed significant positive correlation with S_{Cr} and negative correlation with eGFR; S_{TAC} showed significant positive correlation with eGFR and negative correlation with S_{CysC} , S_{Cr} , U_{NAG}/Cr ; U_{MDA}/Cr showed significant positive correlation with $U_{albumin}/Cr$, U_{β_2MG}/Cr , U_{NAG}/Cr .

Conclusions: Our data confirm high frequency of glomerular and tubular dysfunctions in TD- β TM pediatric patients which could be attributed to oxidative stress and DFO therapy.

Background

β -thalassaemia major (β TM), is a type of chronic, inherited, microcytic anemia that is characterized by impaired biosynthesis of the β -globin leading to accumulation of unpaired α -globin chain. Although the prognosis for patients with thalassaemia has greatly improved in recent decades with the use of modern newborn screening, blood transfusions and iron chelation therapy; multi-organ dysfunctions is still common. Patients with thalas-

semia are known to have severe cardiopulmonary, reticuloendothelial, and other major systems dysfunction, but renal involvement has received little attention. Increased renal plasma flow and failure of urine concentration ability had been reported in adult subjects with β -thalassemia since 1975 [1]. Also, renal tubular acidosis had also been reported in patients with thalassemia [2]. In recent years, there have been few published studies demonstrating proteinuria, aminoaciduria, low urine osmolarity, and excess secretion of the proximal tubule damage markers, such as N-acetyl-beta-D-glucosaminidase (NAG) activity in such patients [3-5]. Shortened red blood cells (RBCs)

* Correspondence: eah3a2010@yahoo.com

¹ Department of Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt

Full list of author information is available at the end of the article



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. On the other hand, deferoxamine (DFO) therapy has been proved to be nephrotoxic and induce dose-dependent proximal tubular dysfunction by an unknown mechanism [6,7]. Thus, the necessity of monitoring renal damage in thalassemic patients receiving DFO therapy has been well-recognized.

Reports investigating renal dysfunction in thalassemic have been limited in number, mainly studying adult patients. Additionally, most of them have not assessed, early markers of glomerular and tubular functions, such as serum (S) cystatin-C (S_{CysC}), urinary (U) β_2 -microglobulin (U_{β_2MG}) and U_{NAG} . Early identification of patients at high risk of developing renal damage is of great importance as it may allow specific measures to be undertaken that will delay the progression of renal injury and thus reduce the incidence of renal impairment. Therefore, the aim of the present study was to: 1) investigate the presence of glomerular and/or tubular dysfunctions in children and adolescent with transfusion dependant (TD) β TM, without any overt renal diseases, using both conventional and early markers of glomerular and tubular dysfunctions. 2) To correlate the findings to oxidative stress markers and iron chelation therapy with DFO.

Patients and Methods

Sixty-nine follow up pediatric patients with TD- β TM recruited to Hematology Clinic of Pediatric Hospital, Faculty of Medicine, Assiut University, Assiut, Egypt, were randomly selected to participate in this prospective study during the period from January 2008 to June 2009. The diagnosis of β TM was based on standard criteria [6]. The study was approved by the Ethical Committee of Faculty of Medicine, Assiut University and informed consent was obtained in every case from their legal guardians.

All patients were in a stable phase of their disease with regular erythrocyte transfusion/1-2 months (TD) since early childhood to maintain pretransfusional hemoglobin levels above 9 g/dL. Patients were divided into 2 groups, group I (n = 34) on regular iron DFO chelation therapy (20-60 mg/kg/day usually administered subcutaneously via a battery-operated portable pump over a period of 8-12 hours overnight, for 5-7 nights per week) to maintain serum ferritin level below 1000 ng/mL and group II (n = 35) without chelation therapy due to failure of treatment as a result of hypersensitivity to DFO or non-compliance. Failure of treatment was defined as an increase of serum ferritin levels >1000 ng/mL with respect to the previous values, confirmed by two determinations. Those patients who were unable to afford its prohibitive cost or unwilling to receive daily regularly rigorous subcutaneous infusions of DFO were considered as non-compliant.

Demographic and clinical conditions included age, sex, weight, height, age of puberty, disease duration, number of blood transfusions/year, history of splenectomy, post-splenectomy duration, chelation therapy were obtained by interview and chart review. Patients were splenectomized when the transfusion requirements of packed red blood cells increased to 180 mL/kg/year, presence of hypersplenism signs such as leucopenia or thrombocytopenia, or presence of large spleen. Fifteen age-, sex-, body mass index (BMI)-matching healthy volunteer subjects attending the child health promotion clinic of hospital were recruited in this study as controls. Clinical examination was implemented for all participants.

Children and adolescence with clinical or laboratory evidence of renal pathology, systemic illness (cardiac, thyroid, hepatic diseases, diabetes mellitus or sepsis, etc.) or the need of renal replacement or diuretic therapy or history of intake of trimethoprim, corticosteroids or cephalosporin in the past 7 days, were excluded from this study.

Patients were instructed to fast overnight before attending the clinic in the morning and advised to abstain from taking any medications (including chelation), vitamin or mineral supplements for the previous 24 hours. Just before blood transfusion, fasting venous blood and fresh second-morning midstream urine samples were collected from all the participants (between 7-8 a.m.) for hematological and biochemical analysis. Serum and urinary samples were recovered and immediately aliquot and frozen at -70°C till use. Glomerular functions were assessed by measuring serum and urinary creatinine (Creatinine-Jaffe enzymatic assay) and urinary albumin by colorimetrically (Egypt Company for Biotechnology, Cairo, Egypt). Patients were considered to have preclinical glomerular damage if urinary albumin/creatinine ratio was 2.5-30 mg/mmol.Cr and to have glomerular proteinuria if urinary albumin/creatinine ratio was >30 mg/mmol.Cr [8]. S_{CysC} levels were measured by quantitative human cystatin-C immunosorbent assay (ELISA) kit [Cat. No. DSC7C0; R&D systems, Inc., Minneapolis, MN, USA] [9]. Estimated glomerular filtration rate (eGFR) was calculated using Schwartz formula for children [10]: $eGFR (\text{ml}/\text{min}/1.73 \text{ m}^2) = \text{height (cm)} \times \text{constant}/\text{serum creatinine (mg/dL)}$, where height was expressed in "cm" and constants was 0.44 (for children < 2 years) and 0.55 (for children ≥ 2 years). Renal dysfunction was defined as $eGFR < 90 \text{ mL}/\text{min}/1.73 \text{ m}^2$ [11]. Tubular functions were assessed by measuring serum and urinary calcium (Ca), inorganic phosphorus (PO_4) and uric acid (UA) by colorimetric method (Egypt company for biotechnology, Cairo, Egypt); β_2 MG was measured by an ELISA kit [Cat. No. ORG5BM; Orgente Diagnostika, GmbH, Mainz, Germany] [12] and U_{NAG} by chemical method [13]. In order to minimize urine flow rate on uri-

nary enzyme levels, enzymes levels were expressed as ratio of enzyme activity to urinary creatinine level. Markers of oxidative stress were assessed by measuring serum total antioxidant capacity (S_{TAC}) (Biodiagnostic, Giza, Egypt) by specific ELISA kit [14] and urinary malondialdehyde (U_{MDA}) by chemical method [15]. The lower detection limits were 0.31 mg/dL (0.027 mmol/L) for creatinine, 0.2 ng/mL for CysC, 2.0 mg/dL for Ca, 1.0 mg/dL for PO_4 , 1.0 mg/dL (0.06 mmol/L) for UA and 0.2 μ g/mL for β_2 MG.

Data analysis

Statistical Science for Social Package (SPSS Inc, USA) software computer program version 12 was used for data analysis. Data were presented as mean \pm standard deviation (SD) or number and percentage (n, %) as appropriate. For comparison of two groups the nonparametric test for independent variables was used while, comparisons of multiple groups were done using one-way analysis of variation (ANOVA) and Kruskal Wallis tests for parametric and non-parametric variables, respectively. Spearman's and Pearson's correlation tests were used as appropriate for correlating non-parametric and parametric variables. For all tests, a probability (P) < 0.05 was considered significant.

Results

Table 1 showed the demographic characteristics of the patients and controls. There were no significant differences regarding gender, age, weight, height and BMI between patients and controls ($P > 0.05$ for all). No significant difference was found between patient's subgroups regarding disease onset, 1st blood transfusion onset, disease duration, blood transfusion duration, splenectomy duration, duration between splenectomy and 1st blood

transfusion, puberty age and family history of thalassemia ($P > 0.05$ for all) (Table 2).

In patients with and without chelation therapy, significant higher levels of serum levels of CysC ($P < 0.0001$, $P < 0.022$), creatinine ($P < 0.001$, $P < 0.008$), uric acid ($P < 0.0001$, $P < 0.002$), inorganic phosphorus ($P < 0.001$, $P < 0.001$), while significant lower levels of TAC ($P < 0.0001$, $P < 0.001$), $eGFR_{Schwartz}$ ($P < 0.001$, $P < 0.001$) were reported than controls. In patients with chelation therapy, level of S_{CysC} ($P < 0.001$) was higher while, S_{TAC} was lower ($P < 0.048$) than patients without chelation therapy. Impaired renal functions ($eGFR < 90$ mL/minute/1.73 m²) was reported in 58.82% and 45.71% of patients with and without chelation (Table 3).

In patient with and without chelation therapy, elevated urinary levels of NAG/Cr ($P < 0.000$, $P < 0.002$), β_2 MG/Cr ($P < 0.005$, $P < 0.010$), MDA/Cr ($P < 0.021$, $P < 0.045$) and albumin/Cr ($P < 0.003$, $P < 0.006$), uric acid ($P < 0.008$, $P < 0.023$), calcium ($P < 0.003$, $P < 0.011$), inorganic phosphorus ($P < 0.001$, $P < 0.006$) were reported than those of controls. Meanwhile, no significant difference was found in the levels of the urinary measured parameters between patients with and without chelation therapy. In patients with and without chelation therapy, normal urinary albumin (< 2.5 mg/mmol.Cr) was found in (5.90% and 28.60%, respectively); microalbuminuria (2.5-30 mg/mmol.Cr) in (47.10% and 25.70%, respectively) and proteinuria (>30 mg/mmol.Cr) in (47.10% and 45.70%, respectively) (Table 4).

In thalassemia patients, S_{Cr} showed significant positive correlation with S_{UA} , S_{PO_4} ($r = 0.391$, $P < 0.010$; $r = 0.339$, $P < 0.010$) and negative correlation with S_{Cr} ($r = -0.309$, $P < 0.010$). S_{UA} showed significant positive correlation with U_{β_2MG}/Cr ($r = 0.349$, $P < 0.010$). $eGFR_{Schwartz}$ showed significant positive correlation with age, height, weight and

Table 1: The demographic and anthropometric characteristics of blood transfusion-dependant β -thalassemia major patients and controls.

Parameters	Patients (n = 69)	Controls (n = 15)	P-value <
Gender (Male/Female)	45 (65.20%)/24 (34.80%)	11(73.30%)/4 (26.70%)	0.390
Age (years)	8.72 \pm 3.70 (1.00-16.00)	8.40 \pm 4.10 (3.00-14.00)	0.799
Weight (kg)	22.84 \pm 7.04 (8.00-47.00)	23.30 \pm 9.38 (11.00-40.00)	0.830
Height (meter)	1.16 \pm 0.15 (0.80-1.47)	1.15 \pm 0.18 (0.90-1.35)	0.941
Body mass index (kg/m ²)	16.59 \pm 2.13 (12.5-22.22)	16.80 \pm 3.38 (12.19-22.40)	0.759

Data presented are mean \pm SD (range) or number (%); P-value, significance versus controls.

Table 2: The clinical characteristics of blood transfusion-dependant-thalassemia major patients.

Parameters	All patients (n = 69)	Patients subgroups		P-value <
		With chelation [Group I] (n = 34)	Without chelation [Group II] (n = 35)	
Disease onset age (months)	13.74 ± 14.55 (2.00-60.00)	12.59 ± 12.77 (2.00-60.00)	14.86 ± 16.21 (2.00-60.00)	0.202
Disease duration (months)	87.42 ± 42.95 (3.00-180.00)	103.21 ± 38.95 (26.00-180.00)	72.09 ± 41.34 (3.00-174.00)	0.991
1st blood transfusion onset (months)	13.74 ± 14.55 (2.00-60.00)	12.59 ± 12.77 (2.00-60.00)	14.86 ± 16.21 (2.00-60.00)	0.202
Blood transfusions/year	9.68 ± 2.96 (6.00-15.00)	9.65 ± 2.79 (6.00-12.00)	9.71 ± 3.16 (6.00-15.00)	0.202
Splenectomy	40 (58.00%)	23 (67.60%)	17 (48.90%)	0.464
Splenectomy duration (months)	37.75 ± 22.43 (3.00-96.00)	42.87 ± 20.76 (12.00-96.00)	30.82 ± 23.35 (3.00-84.00)	0.087
Duration between splenectomy & 1st blood transfusion (months)	62.10 ± 35.28 (6.00-150.00)	69.48 ± 33.16 (6.00-137.00)	52.12 ± 36.57 (6.00-150.00)	0.520
Puberty age (years)	12.28 ± 0.46 (12.00-13.00)	12.17 ± 0.39 (12.00-13.00)	12.50 ± 0.55 (12.00-13.00)	0.055
Positive family history	13 (18.80%)	9 (26.50%)	4 (11.40%)	0.098

Data presented are mean +/- SD (range) or number (%); P-value, significance between patients with and without chelation therapy.

S_{TAC} ($r = 0.374, P < 0.002$; $r = 0.319, P < 0.008$; $r = 0.300, P < 0.012$; $r = 0.313, P < 0.009$) and negative correlation with S_{PO_4} , S_{UA} , S_{CysC} ($r = -0.298, P < 0.013$; $r = -0.349, P < 0.003$; $r = -0.517, P < 0.001$). S_{CysC} showed significant positive correlation with S_{Cr} ($r = 0.659, P < 0.001$) and negative correlation with S_{TAC} ($r = -0.295, P < 0.014$). $U_{albumin}/Cr$ showed significant positive correlation with S_{UA} , U_{NAG}/Cr , U_{MDA}/Cr , U_{β_2MG}/Cr ($r = 0.339, P < 0.010$; $r = 0.885, P < 0.001$; $r = 0.845, P < 0.001$; $r = 0.991, P < 0.001$). U_{NAG}/Cr showed significant positive correlation with U_{MDA}/Cr , U_{β_2MG}/Cr ($r = 0.845, P < 0.001$; $r = 0.900, P < 0.001$). U_{MDA}/Cr showed significant positive correlation with U_{β_2MG}/Cr and S_{UA} ($r = 0.861, P < 0.001$; $r = 0.251, P < 0.050$) (Table 5).

Discussion

Several investigations about renal involvement in adult β TM patients [4,5,7,16,17] were reported but there is a

dearth of pediatric data. This study aimed to investigate the presence of glomerular and/or tubular dysfunctions in children and adolescent with TD- β TM, without any overt renal diseases, using both conventional and early markers of glomerular and tubular dysfunctions, and to correlate the findings to oxidative stress markers and iron chelation therapy with DFO. Results of this study showed impaired in classical (Cr, albumin and eGFR) and early (CysC) glomerular function markers in β TM patients than controls. Mean serum level of Cr was significantly higher while eGFR was significantly lower in patients with and without chelation therapy than controls. Impaired eGFR (< 90 mL/minute/1.73 m²) was found in 58.82% and 45.71% of patients with and without chelation therapy. In this respect, Grundy et al. [18] reported high serum level of Cr in β TM patients. In contrast, Li Volti et al. [19] and Aldudak et al. [5] found normal S_{Cr} , and creatinine clearance (CrC) in patients who received subcutaneous DFO treatment. In consistence with others [20], we

Table 3: Biochemical parameters in serum of β -thalassemia major patients' groups and controls.

Parameters	Controls (n = 15)	Patient groups	
		With chelation [Group I] (n = 34)	Without chelation [Group II] (n = 35)
Cystatin-C (mg/L)	0.83 \pm 0.05	1.26 \pm 0.34	1.03 \pm 0.26
Range	(0.80 - 0.90)	(0.85-2.00)	(0.82-2.12)
Significance		P < 0.0001	P < 0.022, *P < 0.001
Creatinine (mg/dL)	0.55 \pm 0.08	0.75 \pm 0.18	0.70 \pm 0.21
Range	(0.50 - 0.70)	(0.50-1.10)	(0.40-1.10)
Significance		P < 0.001	P < 0.008, *P < 0.262
eGFRSchwartz (mL/min/1.73 m ²)	119.08 \pm 11.13	92.67 \pm 25.16	93.73 \pm 25.53
Range	(96.25-125.40)	(53.17-125.40)	(49.50-125.40)
Significance		P < 0.001	P < 0.001, *P < 0.852
≥ 90 mL/minute/1.73 m ²	15 (100.00%)	14 (41.18%)	19 (54.29%)
< 90 mL/minute/1.73 m ²		20 (58.82%)	16 (45.71%)
Uric acid (mg/dL)	3.81 \pm 1.19	5.69 \pm 1.69	5.30 \pm 1.49
Range	(2.50-5.50)	(2.00-8.50)	(2.50-7.50)
Significance		P < 0.0001	P < 0.002, *P < 0.292
Calcium (mg/dL)	9.35 \pm 1.11	9.10 \pm 1.41	8.59 \pm 1.86
Range	(8.01-10.95)	(6.09-11.10)	(5.35-12.37)
Significance		P < 0.121	P < 0.607, *P < 0.183
Inorganic phosphate (mg/dL)	3.32 \pm 0.72	4.69 \pm 0.87	4.50 \pm 1.14
Range	(2.38-4.40)	(3.60-7.11)	(2.31-6.92)
Significance		P < 0.001	P < 0.001, *P < 0.412
Total antioxidant capacity (mmol/L)	0.43 \pm 0.12	0.18 \pm 0.10	0.23 \pm 0.13
Range	(0.24-0.60)	(0.03-0.36)	(0.02-0.41)
Significance		P < 0.0001	P < 0.0001, *P < 0.048

Data presented are mean \pm SD (range) or number (%); eGFR, estimated glomerular filtration rate. P-value, significance versus controls; *P-value, significance versus patients with chelation therapy.

did not find any variation of serum Cr with age, meanwhile, Helin et al. [21] found a positive correlation between age and Cr. Stevens and Levey [11] found a positive correlation between eGFR and age, height, weight and 1st blood transfusion age in thalassemia patients. In accordance, microalbuminuria and proteinuria were reported in our patients with (47.10% and 47.10%) and without chelation therapy (25.70% and 45.70%). Albuminuria found in this study could be attributed mainly to

destruction of glomerular filtration membrane which proven by decreased in eGFR as well as tubulopathy. In accordance, Mohkam et al. [22] found proteinuria in 89.3% of β TM patients. Katopodis et al. [23] reported proteinuria and microalbuminuria in patients with sickle cell beta-thalassemia and related these finding to prolonged glomerular hyperfiltration, glomerulosclerosis, prostaglandin secretion and chronic anemia.

Table 4: Biochemical parameters in urine of β -thalassemia major patients' groups and controls.

Parameters	Controls (n = 15)	Patients groups	
		With chelation [Group I] (n = 34)	Without chelation [Group II] (n = 35)
Albumin/Cr (mg/mmol)	1.56 \pm 0.51	68.19 \pm 79.85	63.35 \pm 79.89
Range	(0.50-2.00)	(1.20-216.00)	(0.50-300.00)
Significance		P < 0.003	P < 0.006 , *P < 0.779
Normal albumin, < 2.5 mg/mmol.creatinine	15 (100.00%)	2 (5.90%)	10 (28.60%)
Microalbuminurea, 2.5-30 mg/mmol.creatinine	-	16 (47.10%)	9 (25.70%)
Proteinuria, > 30 mg/mmol.creatinine	-	16 (47.10%)	16 (45.70%)
NAG/Cr (U/mg)	7.47 \pm 2.28	15.00 \pm 6.17	13.84 \pm 7.60
Range	(3.00-9.50)	(6.00-25.00)	(3.00-30.00)
Significance		P < 0.000	P < 0.002 , *P < 0.453
β2MG/Cr (ug/mg)	11.40 \pm 6.70	70.90 \pm 71.40	65.40 \pm 74.00
Range	(1.80-22.00)	(3.00-220.00)	(0.90-280.00)
Significance		P < 0.005	P < 0.010 , *P < 0.730
Uric acid (mg/dL)	25.20 \pm 5.62	38.97 \pm 18.53	36.86 \pm 17.05
Range	(16.00-32.00)	(15.00-70.00)	(16.00-70.00)
Significance		P < 0.008	P < 0.023 , *P < 0.593
Calcium (mg/dL)	9.01 \pm 1.20	13.24 \pm 4.53	12.63 \pm 5.26
Range	(7.50-23.00)	(7.50-23.00)	(7.00-25.00)
Significance		P < 0.003	P < 0.011 , *P < 0.569
Inorganic phosphorus (mg/dL)	11.83 \pm 7.42	48.32 \pm 38.83	42.53 \pm 38.04
Range	(6.00-25.00)	(6.00-110.00)	(5.00-120.00)
Significance		P < 0.001	P < 0.006 , *P < 0.495
MDA/Cr (nmol/mg)	13.57 \pm 3.69	21.29 \pm 9.77	20.20 \pm 12.93
Range	(2.50-18.00)	(10.00-41.00)	(4.00-50.00)
Significance		P < 0.021	P < 0.045 , *P < 0.671

Data presented are mean \pm SD (range) or number (%); Cr, creatinine; NAG, N-acetyl-beta-D-glucosaminidase; β_2 MG, β_2 -microglobulin; MDA, malondialdehyde; P-value, significance versus controls; *P-value, significance versus patients with chelation therapy.

Cystatin-C is a 122-amino acid non-glycosylated low molecular weight (13 kDa) protein that inhibits cysteine proteases. It is considered to be a housekeeping gene since it is transcribed at a relatively constant level and is expressed in all nucleated cells. CysC is filtered by glomeruli and is followed by tubular reabsorption and degra-

tion resulting in excretion of a minute amount in the urine. It is not secreted by the renal tubules and not reabsorbed back into the serum. Therefore, its serum levels serve as an endogenous parameter of GFR [24-26]. In this study, S_{CysC} was significantly higher in patients with and without chelation therapy than controls. Also, S_{CysC}

Table 5: Correlation coefficient (r) among various parameters in β -thalassaemia major (β TM) pediatric patients.

Parameters	Duration of disease	Age	BMI	SCysC	SCr	STAC	SUA	SCa	SPO4	eGFR	UNAG/Cr	UB2MG/Cr	UMDA/Cr
Age	0.941***												
BMI	0.387**	0.356**											
SCysC	0.168	0.101	0.046										
SCr	0.051	-0.078	-0.027	0.659***									
STAC	0.161	0.042	0.193	-0.293*	-0.309**								
SUA	0.073	0.002	-0.091	0.142	0.319**	-0.077							
SCa	-0.101	-0.077	-0.047	0.091	-0.169	0.105	0.078						
SPO4	0.054	0.023	0.055	0.150	0.339**	0.212	0.055	-0.325**					
eGFR	0.229	0.374**	0.097	-0.517**	-0.892***	0.312**	-0.349**	0.144	-0.298*				
UNAG/Cr	0.070	0.144	-0.021	0.141	0.159	0.014	0.220	0.020	0.020	-0.083			
UB2MG/Cr	0.036	0.117	-0.081	0.094	0.0161	-0.018	0.349**	-0.076	0.076	-0.096	0.900***		
UMDA/Cr	0.010	0.068	-0.072	0.142	\pm 0.188	0.041	0.251*	0.023	0.108	-0.091	0.853***	0.861***	
Ualbumin/Cr	0.089	0.088	-0.105	0.071	0.146	0.024	0.339**	-0.084	0.085	-0.088	0.885***	0.991***	0.845***

Significance: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ BMI, body mass index; S_{CysC} , serum cystatin-C; S_{Cr} , serum creatinine, S_{TAC} , serum total antioxidant capacity; S_{UA} , serum uric acid; S_{Ca} , serum calcium; S_{PO4} , serum inorganic phosphorus; eGFR, estimated glomerular filtration rate; $U_{NAG/Cr}$, urinary N-acetyl-beta-D-glucosaminidase to creatinine; $U_{B2MG/Cr}$, urinary β_2 -microglobulin to creatinine; $U_{MDA/Cr}$, urinary malondialdehyde to creatinine; $U_{albumin/Cr}$, urinary albumin to creatinine.

showed significant positive correlation with S_{Cr} but negative correlation with eGFR. In consistence, Voskaridou et al. [27] reported high S_{CysC} levels in 32.1% of patients with sickle cell/beta-thalassemia. Economou et al. [28] reported 36% of their β TM patients had elevated S_{CysC} . The data compared correlation coefficients between GFR and reciprocals of serum creatinine and CysC in 3,703 subjects, finding significantly better correlations for CysC and suggesting that CysC is superior to serum Cr for detection of impaired GFR in cross-sectional studies [24]. In consistence with others [20], we did not find any correlation of CysC with age, weight, height or gender. In contrast, MacDonald et al. [29] reported that S_{CysC} is not independent of body composition, as previously thought. These authors showed that past studies were based on the fact that CysC is produced at a constant rate by all nucleated cells, but 60% of cellular mass comes from muscles, which varies with anthropometric measures, diet, genetics and physical activity.

The results of the present study also showed signs of renal tubulopathy, such as increase in serum levels uric acid and inorganic phosphorus and urinary excretion of albumin, uric acid, calcium and inorganic phosphorus in thalassaemic patients with and without chelation therapy compared to healthy controls. The increased serum and excretory levels of UA and PO_4 can be explained by rapid erythrocyte turnover in combination with decreased reabsorption of filtered UA and PO_4 from damaged renal tubules. Similarly, Lapatsanis et al. [30] reported phosphaturia and increase in serum phosphorus levels in β TM patients. In Turkey, Aldudak et al. [5], reported elevated urinary levels of protein/Cr and serum levels of UA and PO_4 in β TM patients. Mohkam et al. [22] and Economou et al. [28] reported hypercalciuria in a large number of thalassemia patients. Smolkin et al. [31] found hyperuricosuria without hyperuricemia and hypercalciuria in β TM patients without relation to DFO treatment. The authors attributed hypercalciuria to hypoparathyroidism and related treatment with calcium and vitamin D [31]. Grundy et al. [18] reported lower serum levels of calcium and phosphate in children with β TM and contributed their findings to either liver or parathyroid glands involvement. In our studied patients, these findings reflect proximal tubular damage, as none of our patients suffered from liver diseases or hypoparathyroidism or received relative supplements.

N-acetyl-beta-D-glucosaminidase (EC 3.2.1.30) is a high molecular weight (140,000 Dalton) lysosomal enzyme. It is not specific to the kidney and found in other tissues as well. It cannot pass into glomerular ultrafiltrate due to its high molecular weight. This enzyme shows high activity in renal proximal tubular cells. As, urinary NAG is not of plasmatic origin and is not filtered through

the glomeruli, so enhanced U_{NAG} is due to nonspecific release of tubular proteins as results of tubular dysfunction and tubular proteinuria and not secondary to loss of glomerular selectivity [27,32]. β_2 MG is a low molecular weight protein (11.8 kDa) which, under normal circumstances, is freely filtered at the glomerulus but almost totally reabsorbed and degraded by renal tubules [33]. Elevation of urinary β_2 MG is a sensitive and reliable early marker of tubular dysfunction [34,35]. In our study, the significant elevated excretion of NAG/Cr and β_2 MG/Cr in β TM patients with and without chelation therapy than those of controls indicate proximal tubular renal damage. In a similar way, others reported high U_{NAG} activities [3,4,16,22,27,31] and β_2 MG excretion [27,28,36] in thalassaemic patients. In this study, a significant positive correlation was found between U_{NAG}/Cr and U_{β_2MG}/Cr ratios and between them and $U_{albumin}/Cr$. In consistence, Mohkam et al. [22] found that proteinuria was positively correlated with U_{NAG}/Cr ratio. As such, U_{NAG} can be considered a sensitive and reliable index of proximal tubular dysfunctions in β TM patients.

The underlying mechanism for renal dysfunctions in patients with β TM is not clear. They seem to be multifactorial, attributed mainly to include long-standing anemia, chronic hypoxia, iron overload and deferoxamine toxicity [5,16,37]. In β TM patients with and without chelation, we found significant decreased serum levels of TAC (irrespective of high serum uric acid in our patients) and increase urinary excretion of MDA. Also, we found in thalassemia patients, S_{TAC} was significantly positively correlated with eGFR, negatively with S_{CysC} , S_{Cr} and U_{MDA}/Cr was positive significant correlation with each of U_{NAG}/Cr , U_{β_2MG}/Cr and $U_{albumin}/Cr$. This emphasis the role of oxidative stress in inducing renal tubular damage [5,38,39]. Although severe anemia and chronic hypoxia are believed to play a role in renal involvement in β TM, lipid peroxidation is currently the most favored hypothesis: according to this hypothesis, the imbalance in synthesis of hemoglobin (Hb) leads to excess unpaired globin chain and high intracellular content of non-Hb iron. The unstable Hb subunits are known to generate free oxygen radical species that starting a chain of oxidative events which leading to disintegration of denatured globin chains, heme, and iron, which bind to different membrane proteins and altering their normal structure and functions. In addition, the excess free iron is known to be a catalyst of lipid peroxidation via the fenton reaction [39].

Although transfusion therapy prolongs survival in β TM, the absence of a physiological iron excretion mechanism leads to uneven accumulation of this metal in various body organs results in death, usually during the second decade of life [40]. Therefore, lifelong chelation

therapy, the most common of which is deferoxamine, which produces iron excretion in urine and stool, is an essential facet in the management of β -thalassemia patients [41]. The data obtained from this study showed decreased in both glomerular and tubular functions in β TM pediatric patients on a hypertransfusion protocol with regular DFO chelation therapy program. In addition, S_{CysC} (indicator of glomerular dysfunction) was significantly higher while S_{TAC} (indicator of total antioxidant capacity) was significantly lower in patients on DFO therapy than those without chelation therapy. Meanwhile, large number of patients on DFO therapy showed impaired eGFR (58.82%), microalbuminuria (47.10%) and proteinuria (47.10%) suggesting nephrotoxic effect of DFO. Although our patients received DFO subcutaneously in doses that are considered non-nephrotoxic according to a generally accepted protocol [42], the possibility that chronic administration of this agent caused duration dependent kidney damage cannot be excluded. In consistency, Koren et al. [6] reported that subcutaneous administration of DFO was associated with a clinically significant decrease in GFR in 40% of β TM patients and with a mild decrease in another 40%. Mohkam et al. [22] found a significant relationship between U_{NAG} and duration of DFO therapy. On contrary, others [4,5,16-18,31] reported no detrimental effect of DFO on renal functions. On contrary, others [4] reported lower level of U_{MDA} in groups treated with DFO than those without treatment which supports the hypothesis of direct suppressive effect of DFO on peroxidation.

Conclusions

The presented data confirm that glomerular and tubular dysfunctions exist in children and adolescent with TD- β TM. These abnormalities are mainly sub-clinical, but, with prolonged repeated tubule damage, tubulointerstitial fibrosis may occur. As renal dysfunction may not be detected by routine tests, use of early markers is recommended. CysC is a promising marker of monitoring of glomerular dysfunction. Urinary NAG excretion can be considered a reliable index of the tubular toxicity and a possible predictor of proteinuria. In β TM, the renal dysfunction may be partially explained by excessive oxidative stress as well as by deferoxamine toxicity. Based on the results of our study, the usefulness of alternative chelation drugs and their effects on glomerular and tubular functions should be studied. The administration of selective antioxidants, along with an appropriate, nutritionally balanced diet would represent a promising approach towards counteracting oxidative damage and its deleterious effects on thalassemia.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EAH designed the study, drafted the manuscript, carried out collection of serum and urine samples from patients, laboratory analysis of serum and urinary electrolytes and statistical analysis. NTM participated in the design of the study, drafted the manuscript and carried out the other laboratory analysis. Both authors read and approved the final manuscript.

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Author Details

¹Department of Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt and ²Department of Biochemistry, Faculty of Medicine, Assiut University, Assiut, Egypt

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