Renal Functions in Pediatric Patients with β-Thalassemia Major: Relation to Iron Chelation Therapy

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ABSTRACT

Renal failure is one of the main complications in β-thalassaemia as a result of long-standing anemia, therefore the objectives of the study were: (1) To investigate glomerular and tubular functions in transfusion dependant (TD) β-thalassaemia major (βTM) pediatric patients without any occult renal diseases. 2) To correlate the findings with clinical parameters, oxidative stress status [by measuring serum total antioxidant capacity (TAC) and urinary malondialdehyde (MDA)] and desferrioxamine (DFO) chelation therapy. ***Patients and methods:*** *The study included sixty-nine TD-βTM patients (45 males and 24 females). They were subdivided into those with (34 patients) and without chelation therapy (35 patients). In addition to fifteen age-, sex-, body mass index (BMI)-matched healthy subjects as a control group. From each participant blood sample was taken for determination of the serum (S) levels of creatinine (Cr), albumin, calcium (Ca), inorganic phosphorus (PO_4), uric acid (UA), cystatin-C (CysC) and TAC. Also a urine sample was taken for determination of urinary (U) levels of creatinine, albumin, N-acetyl-beta-D-glucosaminidase (NAG) activity, β_2-microglobulin (β_2MG) and MDA.* ***Results:*** *The results revealed that in βTM patients the serum levels of Cr, albumin, PO_4, UA, CysC, and urinary levels of NAG/Cr, β_2MG/Cr, MDA/Cr, albumin/Cr were significantly higher; while serum TAC, estimated glomerular filtration rate (eGFR) were significantly lower than those of the controls. In patients with chelation therapy, serum levels of CysC, and albumin were significantly higher while, TAC was significantly lower than those without chelation therapy. Significant positive correlations were observed in TD-βTM patients between SCysC and each of Salbumin and SCr; STAC and eGFR and UNAG/Cr with each of Uβ_2MG/Cr, UMDA/Cr and Ualbumin/Cr. Also, significant negative correlations were found between SCysC and eGFR; STAC and each of SCys, SCr, and Salbumin.* ***Conclusion:*** *The results of the present study confirm that renal tubular dysfunction exists in children with βTM which could be attributed to iron overload, oxidative stress and DFO therapy.*

Key words: *β-thalassaemia major; β_2-microglobulin; Cystatin-C; Desferioxamine; N-acetyl- beta-D-glucosaminidase activity; Oxidative stress.*

INTRODUCTION

β-thalassaemia major (βTM), 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. The cause of multiple organ dysfunctions in βTM is not known, but anemia, iron overload coming from multiple transfusions, enhanced iron absorption results in secondary haemosiderosis [(1)], auto-oxidation of RBCs membrane protein as result of excess α-globin chains [(2)] and desferrioxamine (DFO) therapy toxicity [(3)] might be important factors.

Renal involvement in βTM has received little attention. Various glomerular pathologies have been sporadically reported, and it is still unknown whether those abnormalities are genuinely associated with thalassemic syndromes [(4)].

Detection of the progressive renal damage using conventional parameters, such as serum creatinine levels (Cr) or creatinine clearance (CrC) is often misleading. As Cr could be affected by factors other than renal function (e.g., age, sex, muscle mass, protein intake, inflammatory illness and hepatic diseases) and it is secreted by the renal tubules. The early development of glomerular hypertrophy enhances creatinine excretion and gives false normal results of both Cr and CrC. Therefore, the renal dysfunction becomes evident rather late. For that reason, the identification of markers that indicate early renal dysfunction as well as further progression to end stage renal disease is highly desirable [(5)].

Cystatin-C (CysC) 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 degradation resulting in excretion of a minute amount in the urine. It is not secreted in the tubules and also, not reabsorbed back into the serum. Therefore, its serum levels serve as an endogenous parameter of glomerular filtration rate (GFR)[(6,7)]. CysC level was shown to be independent of age, sex, inflammatory state, or nutritional conditions [(8, 9)].

N-acetyl-β-glucosaminidase (NAG) (EC 3.2.1.30) is a widely distributed lysosomal enzyme, located predominantly in the epithelial cell of the renal proximal tubules. NAG has a molecular weight of about 140.000 Daltons. This enzyme shows high activity in renal proximal tubular cells, and leaks into the tubular fluid as the ultrafilterate through proximal convoluted tubules. When proximal tubular cell are injured due to disease or nephrotoxic agent, urinary NAG level increases. Thus, increased urinary NAG reflects proximal tubular cell damage [(10)].

β_2-microglobulin (β_2MG), a low molecular weight protein (11.8 kDa), is freely filtered in the glomerulus wherefrom it is totally reabsorbed and degraded in the renal tubules. Thus, it is a sensitive marker of the glomerular filtration capacity of the kidney. In healthy individuals, β_2MG serum levels remain low and their urine contains almost no β_2MG, while in tubular kidney disease, the tubules can't reabsorb β2MG back into the blood, so urine levels rise. β_2MG is

not influenced by age, gender, or muscle mass but its production increases in inflammatory processes, proliferative syndromes, hepatic and autoimmune illnesses [(11)].

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 [(12)]. Therefore, the aim of the present study was to: 1) investigate glomerular and tubular functions in transfusion dependant (TD)-βTM pediatric patients without any occult renal diseases by measuring serum levels of CysC, urinary levels of NAG activity and β_2MG. 2) correlate the findings with clinical parameters, oxidative stress status (by measuring serum total antioxidant capacity and urinary malondialdehyde) and DFO chelation therapy.

PATIENTS & METHODS

Sixty-nine follow up pediatric patients aged (mean ± SD) 8.72 ± 3.70 years with TD-βTM at Hematology Clinic of Pediatric Hospital, Faculty of Medicine, Assiut University, Assiut, Egypt, were randomly selected to participate in this prospective study during the period January 2008 – June 2009. The diagnosis of βTM was based on standard criteria [(12)]. The study approved by the Ethical Committee of Faculty of Medicine, Assiut University and informed consent was obtained in every case from their legal guardians.

The patients were in a stable phase of their disease with regular erythrocyte transfusion 1-2 times per months since early childhood to maintain pretransfusional hemoglobin levels above 9 g/dl. Patients were divided into 2 groups, group I: thirty four patients on regular iron DFO chelation therapy (20-50 mg/kg/day for 5 times per week as slow overnight 8-12 hours minipump subcutaneous infusion) and group II : thirty five patients without the chelation therapy due to failure of treatment as result of hypersensitivity to DFO or failure of 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. 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. These data are presented in tables 1and 2. A control group of fifteen age-, sex-, body mass index (BMI)-matched healthy subjects attending the child health promotion clinic of hospital was recruited. 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 on diuretic therapy or with history of intake of trimethoprim, corticosteroids or cephalosporin in the past 7 days, were

excluded from the study. Patients were instructed to fast overnight before attending the clinic in the morning and advised to abstain from taking any medications (including chelation)/ vitamins or mineral supplements for the previous 24 hour. Just before blood transfusion, fasting venous blood and fresh second-morning midstream urine samples were collected from all the participants (between 7 and 8 a.m.) for hematological and biochemical tests. The blood samples were allowed to clot at room temperature, centrifuged at 4000 *g* for 15 minutes. The sera were divided in aliquots, and stored at -20˚C till the time of assay of serum (S) calcium (Ca), inorganic phosphorus (PO_4), uric acid (UA), albumin, creatinine, cystatin-C and TAC. Aliquots of urine samples were centrifuged for 10 min at 3000 rpm. After centrifugation of urine sample, microscopic examination of urinary sediments was carried out for detection of urinary tract infection and contaminated urine specimens were excluded. The clear supernatant of each urine specimen was stored frozen in aliquots at -20°C without preservatives till the time of assay of urinary (U) albumin, creatinine, NAG activity, β_2-MG and MDA. Serum calcium, inorganic phosphorus, uric acid, serum and urinary albumin were measured colorimetrically by commercial assay kits (Egypt Company for Biotechnology, Cairo, Egypt). Serum and urinary creatinine levels were determined by quantitative colorimeteric Stanbio creatinine Kit, Cat. No. 0400, manufactured by Stanbio laboratory, Inc, San Antonio, Texas, U.S.A., according to Jaffé [(13)]. Serum CysC levels were measured by Quantikine_ human cystatin C immunoassay kit Cat. No.DSCTC0, R&D Systems, Inc., Minneapolis, MN, USA [(14)]. Serum TAC was determined by an ELISA kit (Biodiagnostic, Giza, Egypt) according to the method described by **Miller et al.** [(15)]. Urinary NAG activity was measured spectrophotometerically according to a method described by **Maruhun,** [(16)]. $Beta_2$-MG levels in urine were measured by an ELISA kit, Cat. No. ORG5BM, manufactured by: ORGENTE, Diagnostika GmbH, Germany. The measurement was performed according to the method described by **Hemmingsen and Skaarup** [(17)]. Urinary MDA was estimated according to the method described by **Buege and Aust** [(18)] in which MDA reacts with thiobarbituric acid (TBA) with the production of a pink pigment.

Patients with microalbuminuria (urinary albumin/creatinine ratio of 2.5–30 mg/mmol.Cr) were considered to have preclinical glomerular damage and to have glomerular proteinuria if the urinary albumin/creatinine ratio was >30 mg/mmol.Cr [(19)]. eGFR was estimated through Schwartz formula [(20)]: GFR (ml/min/1.73 m2) = height (cm) X constant / serum creatinine (mg/dL). Height was expressed in "cm" and the constants of 0.55 (for children ≥2 years) and 0.44 (for children <2 years) were used. The cut-off for eGFR was <90 ml/min/1.73m^2 for low eGFR and ≥90 ml/min/1.73m^2 for normal eGFR, based on renal disease stages adapted from recommendations of the National Kidney Foundation [(21)].

Statistical analysis: Statistical Science for Social Package (SPSS Inc,

USA) software Computer program version 12 was used for data analysis. Data were presented as mean±SD or number and percentage 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 Kruskall Wallis tests for parametric and nonparametric variables, respectively. Chi-square test was used to compare frequency of qualitative variables among different groups. 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) shows the demographic characteristics of the patients and controls. There were no significant differences regarding gender, age, weight, height, BMI between patients and controls. No significant difference was found between patient's subgroups regarding onset of the disease, onset of 1st blood transfusion, disease duration, blood transfusion duration, splenectomy duration, duration between splenectomy and 1st blood transfusion, puberty age and family history of thalassemia (Table 2).

Patient with βTM showed significantly higher serum levels of CysC, creatinine, albumin, uric acid, inorganic phosphorus and significantly lower levels of serum TAC and eGFR than those of the controls. Significantly higher serum levels of CysC (P <0.001), albumin (p<0.05) and significantly lower levels of serum TAC (P < 0.05) were observed in patients with chelation therapy than those without chelation therapy (Table 3).

In patient with and without chelation therapy, urinary levels of NAG/Cr, β_2MG/Cr, MDA/Cr and albumin/Cr were significantly higher than those of the controls. Meanwhile, no significant difference was found in the levels of the urinary measured parameters between patients with and without chelation therapy (Table 4).

Correlation coefficient (r) among various bioindices in βTM patients is presented in table (5). In thalassemic patients, serum cystatin-C was significantly positively correlated with each of serum creatinine, serum albumin and significantly negatively correlated with each of serum TAC and eGFR. Serum TAC levels were significantly negatively correlated with each of serum levels of creatinine, albumin, cyst-C and significantly positively correlated with eGFR. Each of serum uric acid and inorganic phosphorus was significantly positively correlated with each of serum creatinine, serum albumin and significantly negatively correlated with eGFR. Urinary NAG/Cr was significantly positively correlated with levels of urinary albumin/Cr. Also urinary β_2MG/Cr was significantly positively correlated with each of serum uric acid and urinary NAG/Cr. Moreover, levels of MDA/Cr were significantly positively correlated with each of NAG/Cr, β_2MG/Cr and serum uric acid levels.

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

Parameter	Patients (n = 69)	Controls (n = 15)	*P*-value
Gender: Male/Female (n, %)	45 (65.2%)/24 (34.8%)	11(73.3%)/4 (26.7%)	NS
Age (years **)**			
Mean±SD	8.72 ± 3.70	8.40 ± 4.10	NS
Range	1.00-16.00	3.00-14.00	
Weight (kg**)**			
Mean±SD	22.84 ± 7.04	23.30 ± 9.38	NS
Range	8.00-47.00	11.0-40.0	
Height (meter)			
Mean±SD	1.16 ± 0.15	1.15 ± 0.18	NS
Range	0.80-1.47	0.90-1.35	
Body mass index(kg/m^2 **)**			
Mean±SD	16.59 ± 2.13	16.80 ± 3.38	NS
Range	12.5-22.22	12.19-22.4	

NS: not significant

Table (2): The clinical characteristics of patients with blood transfusion-dependant β-thalassemia major

Parameters	All patients (n = 69)	Patients subgroups With chelation (n = 34)	Patients subgroups Without chelation (n = 35)	P-value
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	NS
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	NS
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	NS
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	NS
Splenectomy (n, %)	40 (58.00%)	23 (67.60%)	17 (48.90%)	NS
Post-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	NS
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	NS
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)	NS
Family history (n, %)	13 (18.80%)	9 (26.50%)	4 (11.40%)	NS

Data presented are mean±SD and range or (n, %).
P-value: patients with versus without chelation therapy.
NS: not significant

Table (3): Biochemical parameters in serum of β-thalassemia major patient subgroups and controls

Parameters	Controls	Patients subgroups	
		With chelation	Without chelation
	(n=15)	(n=34)	(n=35)
Cystatin-C (mg/l)			
Mean±SD	0.83±0.05	1.26±0.34	1.03±0.26
Range	0.80 -.0.90	0.85 -2.00	0.82 -2.12
Significance	**˙P <0.05**	**˙P <0.001**	***P <0.001**
Creatinine (mg/dl)			
Mean±SD	0.55±0.08	0.75±0.18	0.70±0.21
Range	0.50 -.0.70	0.50 -1.10	0.40 -1.10
Significance	**˙P <0.001**	**˙P <0.001**	***NS**
Total antioxidant capacity (mmol/l)			
Mean±SD	0.43±0.12	0.18±0.10	0.23±0.13
Range	0.24-0.60	0.03-0.36	0.02-0.41
Significance	**˙P <0.001**	**˙P <0.001**	***P <0.05**
Albumin (mg/l)			
Mean±SD	3.52±0.40	7.09±2.33	5.98±2.01
Range	3.00-4.00	3.50-11.00	3.00-10.00
Significance	**˙P <0.001**	**˙P <0.001**	***P <0.05**
Uric acid (mg/dl)			
Mean±SD	3.81 ±1.19	5.69±1.69	5.30±1.49
Range	2.50-5.50	2.00-8.50	2.50-7.50
Significance	**˙P <0.001**	**˙P <0.001**	***NS**
Calcium (mg/dl)			
Mean±SD	9.35±1.11	9.10±1.41	8.59 ±1.86
Range	8.01-10.95	6.09-11.10	5.35-12.37
Significance	**˙ NS**	**˙ NS**	***NS**
Inorganic phosphorus (mg/dl) Mean±SD	3.32±0.72	4.69±0.87	4.50±1.14
Range	2.38-4.40	3.60-7.11	2.31-6.92
Significance	**˙P <0.001**	**˙P <0.001**	***NS**
eGFR (ml/min/1.73 m^2)			
Mean±SD	119.08±11.13	92.67±25.16	93.73±25.53
Range	96.25-125.40	53.17-125.40	49.50-125.40
Significance	**˙P <0.001**	**˙P <0.001**	***NS**
≥90 ml/minute/1.73 m^2		14 (41.18 %)	19 (54.29%)
<90 ml/minute/1.73 m^2		20 (58.82%)	16 (45.71%)

eGFR: estimated glomerular filtration rate.
˙P: significance versus controls
**P: significance versus patients with chelation therapy* *NS: not significant*

Table (4): Biochemical parameters in urine of β-thalassemia major patient subgroups and controls.

Parameters	Controls (n=15)	Patients subgroups	
		With chelation (n=34)	Without chelation (n=35)
NAG/Cr, U/mg			
Mean±SD	7.47±2.28	15.00±6.17	13.84±7.60
Range	3.00-.9.50	6.00-25.00	3.00-30.00
Significance	**˙P <0.001**	**˙P <0.001**	***NS**
β_2MG/Cr, µg/mg			
Mean±SD	11.40±6.70	70.90±71.40	65.40±74.00
Range	1.80-22.00	3.00-220.00	0.90-280.00
Significance	**˙P <0.01**	**˙P <0.001**	***NS**
MDA/Cr, nmol/mg			
Mean±SD	13.57±3.69	21.29±9.77	20.20±12.93
Range	2.50-18.00	10.00-41.00	4.00-50.00
Significance	**˙P <0.05**	**˙P <0.05**	***NS**
Albumin/Cr, mg/mmol			
Mean±SD	1.56±0.51	68.19±79.85	63.35±79.89
Range	0.50-2.00	1.20-216.00	0.50-300.00
Significance	**˙P <0.001**	**˙P <0.001**	***NS**
Normal albumin, <2.5 mg/mmol creatinine (n,%)	15 (100%)	2 (5.90%)	10 (28.60%)
Microalbuminurea,2.5-30mg/mmol creatinine (n,%)		16 (47.10%)	9 (25.70%)
Proteinuria, >30mg/mmol creatinine (n,%)		16 (47.10%)	16 (45.70%)

NAG: N-acetyl-beta-D-glucosaminidase *β_2MG: β_2-microglobulin*
MDA: malondialdehyde ***NS**: not significant*
˙P: significance versus controls
**P: significance versus patients with chelation therapy*

Table (5): Correlation coefficient (r) among various bioindices in β-thalassaemia major (βTM) pediatric patients

	Duration of disease	Age	BMI	S Cyst-c	s Cr	TAC	S Albumin	♀♀S UA	S Ca	S PO_4	eGFR	NAG/Cr	B_2MG/Cr	MDA/Cr
Age	0.941***													
BMI	0.387**	0.356**												
Cyst-c	0.168	0.101	0.046											
sCr	0.051	-0.078	-0.027	0.659***										
TAC	0.161	0.042	0.193	-0.293*	-0.309**									
S Albumin	0.143	-0.020	0.021	0.788***	0.780***	-0.351**								
S UA	0.073	0.002	-0.091	0.142	0.319**	-0.077	0.262*							
S calcium	-0.101	-0.077	-0.047	0.091	-0.169	0.105	-0.022	0.078						
PO_4	0.054	0.023	0.055	0.150	0.339**	0.212	0.300*	0.055	-0.325**					
eGFR	0.229	0.374**	0.097	-0.517**	-0.892***	0.312**	-0.680***	-0.349**	0.144	-0.298*				
NAG/Cr	0.070	0.144	-0.021	0.141	0.159	0.014	0.033	0.220	0.020	0.020	-0.083			
B_2MG/Cr	0.036	0.117	-0.081	0.094	0.0161	-0.018	0.023	0.349**	-0.076	0.076	-0.096	0.900***		
MDA/Cr	0.010	0.068	-0.072	0.142	0.188	0.041	0.097	0.251*	0.023	0.108	-0.091	0.853***	0.861***	
Albumin/Cr	0.089	0.088	-0.105	0.071	0.146	0.024	0.099	0.339**	-0.084	0.085	-0.088	0.885***	0.991***	0.845***

**: P< 0.05 **: P< 0.01 ***: P<0.001*

DISCUSSION

In thalassemic patients, kidney involvement is related to the patient's age, severity of anemia, type of thalassemia, frequency of blood transfusion and chelation therapy. Deferoxamine treatment has been reported to cause changes in renal function in these patients. There have been several investigations in which renal involvement occur in adult β-thalassemia major patients, but there is a dearth of pediatric data [(22)].

In the present study, patients with βTM, showed significantly higher levels of serum creatinine, urinary albumin/Cr levels and significantly lower eGFR. No significant difference was observed in the levels of these parameters between patients with and without chelation therapy (tables 3, 4). These findings agree with those of previous studies [(5, 23, 24, 25)] who reported that the increased serum creatinine, decreased Ccr and proteinuria are considered routine findings of renal failure in βT. However, **Aldudak et al.** [(22)] reported no significant difference between serum creatinine and Ccr of the studied thalassemic patients and controls. Patients with βTM of the present study showed proteinuria in 46.37% of them and microalbumiuria in 36.23 %of them. This finding agrees with those of **Voskaridou et al.** [(5)] and **Katopodis et al.** [(25)]. They attributed proteinuria and microalbumiuria to prolonged glomerular hyperfilteration and glomerulosclerosis. These phenomena are prostaglandin mediated and have been attributed to chronic anemia [(25)]. Serum creatinine is the metabolite most commonly used for this purpose. The widely-held belief that serum creatinine is insensitive to early changes in renal function is typically based on identification of several drawbacks: First, tubular secretion plays an important role in creatinine elimination with declining GFR. Therefore, serum creatinine concentrations could be within the normal range even with a GFR around 60 mL/min/1.73m2 resulting in a "creatinine-blind range". Second, creatinine production varies considerably intra- and inter-individually [(26)].

Progressive renal failure is one of the main complications in beta-thalassemia. Early identification of patients at high risk of developing renal failure is of great importance as it may allow specific measures to delay the progression of renal damage and thus reduce the incidence of end-stage renal failure and mortality. Early predictors of renal impairment in beta-thalassemia remain to explore [(5)].

One of these potential markers of renal dysfunction is cystatin-C. The present study revealed significantly higher levels of serum cyst-C in βTM patients in comparison to those of the controls, with significantly higher levels in those patients with chelation therapy than those without (table 3). Also, levels of serum cyst-C was significantly positively correlated with each of serum creatinine, serum albumin and significantly negatively correlated with eGFR (table 5). Cyst-C is considered as a perfect endogenous marker of GFR. This observation has already been reported for various types of kidney disease [(6)]. The findings of the present study

confirmed those of **Voskaridou et al.**[5] who reported that abnormal levels of serum Cys-C were observed in 32% of βT patients in contrast with only 6.8% of them who had increased serum Cr values. Furthermore, increased Cys-C levels were strongly associated with reduced Ccr values. These findings are consistent with those of the present study. Moreover, no significant correlation was found between serum cyst-C and each of age and BMI which is consistent with those of **Cordeiro et al.**[27]. However, this finding disagrees with those of **Macdonald et al.**[28] who reported that serum cyst-C is dependent upon body composition. There are several possible advantages to cystatin C as an endogenous marker, including reported constant rate of production, lack of effect of age, sex or muscle mass on cystatin C generation, free filtration at the glomerulus because of its small size and basic pH, complete reabsorption and catabolism by the proximal tubule cells, lack of renal tubular secretion or reabsorption and absence of known problems with analytical interference[9,29]. Furthermore, **Filler et al.** [6] reported that Cys-C is a fascinating novel marker of GFR that bears certain advantages over the most widely used surrogate marker of GFR: serum creatinine. There is strong evidence that the diagnostic sensitivity of Cys-C for the detection of mildly impaired GFR is superior [5, 6, 30]. Moreover, the study of **Narvaez-Sanchez et al.** [31] added that cyst-C is a very interesting option and could be a replacement to serum creatinine for diagnosing and possibly monitoring kidney function in children.

Under normal conditions, the proximal tubule performs major functions, such as the reabsorption of more than one-half of the filtered sodium and of almost all of the filtered amino and uric acids. Changes in the reabsorption of these factors may be an indication of renal tubular dysfunction[12]. The present study revealed significant higher levels of serum uric acid and inorganic phosphorus in thalassaemic patients in comparison to those of the controls with no significant difference between those with and without therapy. Each of serum uric acid and inorganic phosphorus was significantly positively correlated with each of serum creatinine, serum albumin and significantly negatively correlated with eGFR (table 5). Moreover, serum calcium levels showed no significant difference between patients and controls, however, some of the patients showed low calcium levels (table 3). These findings agree with those reported by **Aldulak et al.** [22] **and Lapatsanis et al.** [32]. The old study of **Lapatsanis et al.** [32] reported a negative phosphorus balance in thalassaemic patients. The increased serum phosphorus levels may be due to rapid erythrocyte turnover rather than tubular dysfunction. The proximal tubular damage may lead to hyperuricemia in these patients because the filtered uric acid can be reabsorbed from the proximal tubules [22]. However, **Smolkin et al.** [4] found hyperuricosuria without hyperuricemia in βTM patients and attributed this to the same explanation. Also, serum calcium levels were significantly negatively correlated with serum phosphorus (table 5).

These observations about serum calcium could be attributed to hypoparathyroidism which is a well-known complication of iron overload in patients with thalassaemia[4]. So, these patients must be observed for the early manifestation of hypocalcaemia. Oral calcium and vitamin D should be started as soon as possible to alleviate acute symptoms and prevent complications of chronic hypocalcaemia [4, 33].

It is well known that, enzyme-protein substances such as NAG and β_2-microglobulin (β_2MG) could be reliable indicators of proximal tubular damage. NAG is a widely distributed lysosomal enzyme contained in the tubular epithelial cells and released in the urine as a result of the tubulo-toxicity of proteinuria in the early stages of idiopathic membranous nephropathy, glomerular hypertrophy and minimal changes disease [10]. In the present study, urinary NAG/Cr levels were significantly elevated in βTM patients in comparison to those of the controls, with no significant difference between patients with and without therapy (table 4). These findings are consistent with those of previous studies[2,4,5,12,22,34,35,36,37]. Since NAG has not been of plasmatic origin and has not been filtered through the glomeruli, the enhanced NAG urinary output may be due to nonspecific release of tubular proteins and not secondary to the loss of glomerular selectivity. Additionally, the quantity of daily protein excretion in the urine displayed a significant correlation with the levels of NAG [5, 12]. This finding is consistent with that of the present study that urinary NAG/Cr levels were significantly positively correlated with levels of urinary albumin/Cr (table 5). Therefore, urinary NAG excretion might be considered as a reliable index of the tubular cell status in thalassaemia and its assessment might be useful for the early recognition of the malfunction of the proximal tubular epithelial cells in thalassaemia patients [5, 12].

Another low molecular weight protein, β_2MG is freely filtered in the kidney wherefrom it is reabsorbed and degraded in the renal tubules [12]. Urinary levels of β_2MG/Cr of the βTM patients of the present study were significantly higher than those of the controls, with no significant difference between those with and without therapy (table 4). Also, urinary β_2MG/Cr levels were significantly positively correlated with each of serum uric acid and urinary NAG/Cr levels (table 5). These findings are consistent with those of **Aldudak et al.[22]; Koliakos et al.[36]; Sumboonnanonda et al[37]** ; **Tomlinson[38]; Lena et al.[39]**. Moreover, **Voskaridou et al.[5]** reported that the levels of this microprotein may serve as an endogenous marker of GFR and it is a sensitive and reliable marker of tubular dysfunction in βTM patients.

The cause of renal tubular dysfunction in βTM patients is not known but anemia and iron deposition may be the key factors. In thalassemia, the imbalance in synthesis of hemoglobin (Hb) leads to excess unpaired globin chain and a high intracellular content of non-Hb iron. The unstable Hb subunits are known to generate free oxygen radical species, starting a chain of oxidative

events, leading to disintegration to denatured globin chains, heme, and iron, which bind to different membrane proteins, altering the normal structure and function. In addition, the excess free iron is known to be a catalyst of lipid peroxidation via the Fenton reaction [37]. The current study supports this hypothesis as the levels of urinary MDA/Cr, a lipid peroxidation metabolite, were significantly higher in βTM patients in comparison to those of the controls, with no significant difference between those patients with therapy and those without (table 4). This finding agrees with those of previous studies[2,22,35,36,37,39,40,41]. The non significant difference in the levels of urinary MDA/Cr between patients with and without therapy, which disagreed with those of **Sumboonnanonda et al.[2] and Walter et al.[41]**, who reported reduction of MDA levels in treated group and attributed this to direct suppressive effect of DFO on peroxidation. Also, the levels of MDA/Cr were significantly positively correlated with each of NAG/Cr, β_2MG/Cr and serum uric acid levels (table 5). These correlations support the contribution of the oxidative stress to the observed renal tubular dysfunction. Moreover, the present study revealed significant lower levels of serum TAC in βTM patients in comparison to those of the controls particularly in those patients treated with chelation therapy in comparison to those without (table 3). The primary defense against oxidative stress in extracellular fluids results from a number of low molecular weight antioxidant molecules being either water- (e.g. ascorbic acid) or lipid-soluble (e.g. vitamin E). These antioxidants are either generated during normal metabolism (e.g. uric acid, bilirubin, albumin, thiols) or introduced in the body by the consumption of dietary products rich in antioxidants (olive oil, fruits, vegetables, tea, etc)[44]. The sum of endogenous plus exogenous (food-derived) antioxidants represents the total antioxidant capacity of extracellular fluids. The findings of the present study agree with those of **Cighetti et al.[40]; Walter et al.[41]; Tesoriere et al.[43]; Walter et al.[44]**. The study of **Walter et al.[41]** suggested that the increased oxidative stress in thalassemia might be explained by three mechanisms: (i) the excess α-chains in β-thalassemic erythrocytes and erythroblasts being unstable and prone to denaturation and oxidation; (ii) peroxidation of tissues that leak malondialdehyde into the blood; and (iii) depleted antioxidant capacity that in turn lowering the defense to oxidants. Moreover, the significantly higher levels of serum albumin (particularly in patients with chelation therapy) were observed in βTM patients of the present study. This increase together with the increased serum uric acid levels which showed significant positive correlation with levels of MDA/Cr could be explained as a counterbalance to the increased oxidative stress to maintain the serum antioxidant capacity at normal range [41]. However, this is not the case of the present study as the high levels of these antioxidants could not normalize the decreased levels of serum TAC observed in βTM patients. Moreover,

serum TAC levels were significantly negatively correlated with each of serum levels of creatinine, albumin, cyst-C and significantly positively correlated with eGFR (table 5). These correlations further support the contribution of oxidative stress to renal tubular dysfunction in βTM patients.

In the current study βTM patients treated with DFO showed more severe renal dysfunction as manifested by, significantly higher serum levels of cyst-C, albumin and significantly lower levels of serum TAC in comparison to those of patients without DFO therapy. Moreover, normal urinary albumin levels were present in 5.9 % of them in comparison to 28.6 % of patients without DFO therapy (tables 3, 4). These findings agree with those of previous studies [3, 12, 45, 46, 47, 48]. **Koren et al.** [46] reported that the subcutaneous administration of DFO was associated with a clinically significant decrease in GFR in 40% of the patients and in a mild decrease in another 40%. In all cases of severe decreases in GFR, it tended to return to baseline values upon discontinuation of DFO. Moreover, **Cappellini et al.**[49] **and Raphael et al.,**[50] reported that satisfaction and convenience were significantly higher in patients who are taking the once-daily, oral deferasirox than for DFO infusions. However, these findings disagreed with those **of Sumboonnanonda et al.**[2]**; Smolkin et al.**[4]**; Aldudak et al.,** [22]**; Michelakakis et al.,** [34]**; Koliakos et al.** [36]**; Li Volti et al.** [51]. They found no detrimental effect of DFO on renal function and suggested that renal dysfunction in these patients could be a result of chronic anemia and iron overload. The present study findings support the nephrotoxic effect of DFO inducing a dose-dependent proximal tubular dysfunction by an unknown mechanism [3, 47, 48]. Although our patients received DFO subcutaneously in doses that are considered non-nephrotoxic according to a generally accepted protocol, the possibility that chronic administration of this agent caused a duration of therapy-dependent kidney damage cannot be excluded.

Study limitations:

The present study has the following limitations: the small number of the studied subjects. Also, the present study is a short-term follow up of βTM patients. A long-term follow-up in thalassemic patients before and after efficient iron chelation and hypertransfusion, is needed to clarify our observations.

Conclusion:

The results of the present study confirm that renal tubular dysfunction exists in children with βTM even in absence of clinical findings. The cause of such dysfunction could be due to iron overload, oxidative stress and DFO therapy. It is necessary and cost effective to diagnose renal dysfunction in thalassemic patients in its early stages as an approach to prevent the ongoing renal injury. Serum cystatin-C is a promising marker for diagnosing and monitoring renal function particularly in children. Urinary NAG excretion could be considered to be a reliable index of the tubular toxicity and a possible predictor of proteinuria. Moreover, the usefulness of new-generation oral

chelators instead of DFO should be studied further. Together with supplementation of antioxidants along with balanced diet would represent a promising approach to counteract the oxidative damage in patients with βTM.

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وظائف الكلى فى الأطفال المرضى بالبيتا ثلاسميا الرئيسية: العلاقة مع العلاج المزيل للحديد

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يعد مرض الفشل الكلوى من المضاعفات الأساسية لمرض البيتا ثلاسميا نتيجة فقر الدم الطويل الأمد. لهذا كان الغرض من هذة الدراسة : ١) دراسة وظائف الكبيبات و الأنيبيبات الكلوية فى الأطفال المرضى بالبيتا ثلاسميا الرئيسية المعتمد على نقل الدم بدون وجود مرض كلوى ظاهر. ٢) عمل ارتباطات بين النتائج و حالة أجهاد الأكسدة (عن طريق قياس القدرة الكلية لمضادات الأكسدة فى مصل الدم و ثنائى الدهيد المالون فى البول) و العلاج المزيل للحديد : ديسفرواوكذامين. قد اشتملت الدراسة على تسعة و ستون مريضا (٤٥ ولد و ٢٤ بنت) تم تقسيمهم الى ٣٤ مريض معالج و ٣٥ مريض غير معالج بالعلاج المزيل للحديد. هذا بالأضافه الى ١٥ طفل صحيح من نفس السن و النوع و مؤشر كتلة الجسم تم اختيارهم كمجموعة ضابطة. تم أخذ عينة دم من كل مشارك لتقدير مستويات الكرياتنين و الألبيومين و الكالسيوم و الفوسفور و حمض اليوريك و السيستاتين-س و القدرة الكلية لمضادات الأكسدة فى المصل. و عينة بول لقياس مستويات الكرياتنين و الألبيومين و نشاط إن-أسيتيل-بيتا-جلوكوزامينيدز و البيتا-٢-ميكروجلوبيولين و ثنائى الدهيد المالون. تم تقدير المعدل التقديرى للترشيح الكبيبى بأستخدام كرياتنين المصل. . أظهرت النتائج ان حالات المرضى بالبيتا ثلاسميا الرئيسية لديهم زيادة ذات دلالة إحصائية فى مستويات الكرياتنين و الألبيومين و الفوسفور و حمض اليوريك و السيستاتين-س فى المصل و مستويات نشاط إن-أسيتيل-بيتا-جلوكوزامينيدز/ كرياتنين و البيتا-٢-ميكروجلوبيولين/ كرياتنين و المالونداليدهيد / كرياتنين و الألبيومين/كرياتنين فى البول. ايضا لديهم نقص ذو مدلول إحصائى فى مستويات القدرة الكلية لمضادات الأكسدة فى المصل و المعدل التقديرى للترشيح الكبيبى بالمقارنة بالمجموعة الضابطة. لوحظ ايضا ان المرضى بالبيتا ثلاسميا الرئيسية المعالجين بالعلاج المزيل للحديد لديهم زيادة ذات دلالة إحصائية فى مستويات السيستاتين-س و الألبيومين فى المصل و نقص ذو مدلول إحصائى فى مستويات القدرة الكلية لمضادات الأكسدة فى المصل بالمقارنة بالمرضى الغير المعالجين بالعلاج المزيل للحديد. . أيضا أظهرت الدراسة علاقات إرتباط إيجابية ذات دلالة إحصائية بين السيستاتين-س فى المصل وكلا من كرياتنين و ألبيومين المصل. و بين القدرة الكلية لمضادات الأكسدة فى المصل و المعدل التقديرى للترشيح الكبيبى. و بين نشاط إن-أسيتيل-بيتا-جلوكوزامينيدز/ كرياتنين وكلا من البيتا-٢-ميكروجلوبيولين/ كرياتنين و ثنائى الدهيد المالون / كرياتنين و الألبيومين/كرياتنين فى البول. أيضا وجدت علاقات إرتباط سلبية ذات دلالة إحصائية بين السيستاتين-س فى المصل و المعدل التقديرى للترشيح الكبيبى. و بين القدرة الكلية لمضادات الأكسدة فى المصل و كلا من كرياتنين و الألبيومين و السيستاتين-س المصل. ومن هذه الدراسة يمكن إستخلاص ان هذة النتائج تؤكد وجود إعتلال الأنيبيبات الكلوية فى الأطفال المرضى بالبيتا ثلاسميا الرئيسية و يعذى هذا الأعتلال الى تكدس الحديد و اجهاد الأكسدة و العلاج المزيل للحديد : ديسفرواوكذامين