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RENAL FUNCTIONS AND ERYTHROPOIETIN STATUS OF BETA THALASSEMIA PATIENTS

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Abstract

Background

Thalassemias are heterogeneous group of hereditary disorders resulting from mutations that impact the synthesis of globin chains of hemoglobin. Evidence of both glomerular and proximal tubular damage is observed in β-TM patients. Erythropoietin (EPO) is one of the key regulators of erythropoiesis, promoting the proliferation of erythroid progenitor cells and increasing the production of red blood cells with recently discovered receptors in multiple organs. Inspired by studies proposing EPO as a part of the treatment in β-hemoglobinopathies and more studies with significant effects of EPO to reduce tubular dysfunction and glomerular injury, our study aimed to assess the tubular and glomerular renal functions of β-TM patients along with their EPO status.

Materials and methods

This prospective case-control study included a total of 71 TDT beta thalassemia patients and 22 healthy, age- and sex-matched controls. All patients were examined for their current weight and height, body mass index (BMI), z score, blood pressure, renal glomerular and tubular status and erythropoietin levels.

Results

There were 16 malnourished patients with VKI <18 and -2 SDS. Forty-eight patients (67,6% of patient group) had proteinuria and 16 (22,5%) had albuminuria. Twenty nine of β-TM (40,8%) patients had high fractional excretion of uric acid (FEUA). TmP/GFR was <2,8 in 22,5% (n=16) of patients demonstrating higher phosphate wasting. Thirty eight percent (n=27) of the patients showed hypercalciuria. Serum beta-2-microglobulin and urine β-2-MG were elevated in 80,3% (n=57) and 49,3% (n=35) of the patients respectively. Sixty four (90,1%) patients had elevated serum EPO.

Conclusion

Compared to healthy group, patient group had significantly higher levels of serum urea, proteinuria, albuminuria, hyperuricosuria, phosphaturia and significantly elevated eGFR, EPO, urine and serum β-2-MG. Both renal tubular and glomerular functions of a β-TM patient deteriorate beginning from

childhood years. Routine follow-up of serum creatinine, eGFR, serum cystatin C, spot urine protein/creatinine, spot urine Ca/Cr, FENa and FEUA could fail to detect early renal damage but still essential to analyze as they may be discovered impaired.

Introduction

Thalassemias are heterogeneous group of hereditary disorders resulting from mutations that impact the synthesis of globin chains of hemoglobin. Imbalanced globin chain synthesis is the hallmark of all forms of thalassemia. Beta thalassemia is due to defective production of beta globin chains, leading to excessive accumulation of alpha globin chains. Beta thalassemia comprises transfusion dependent (TDT) beta thalassemia major (β-TM), non-transfusion dependent (NTDT) beta thalassemia intermedia (TI) or thalassemia minor. Microcytic, hypochromic anemia, oxidative stress, ineffective erythropoiesis and hemolytic anemia necessitates regular blood transfusions and iron chelation therapy (ICT) for TM patients (1-5).

Due to the shorter lifespan of red blood cells and the presence of excess iron, β-TM patients experience functional and physiological problems in multiple organ systems, especially heart, liver, endocrine glands and contemporarily also kidney (4,6).

The progression of iron chelation therapy and patient care has enabled extended survival rates leading previously underestimated complications such as renal dysfunction to emerge. Evidence of both glomerular and proximal tubular damage is observed in β-TM patients (1-5).

Erythropoietin (EPO) is one of the key regulators of erythropoiesis, promoting the proliferation of erythroid progenitor cells and increasing the production of red blood cells. The production of approximately 80% of erythropoietin (EPO) occurs within the kidney as a response to diminished oxygen delivery (7).

Inspired by studies proposing EPO as a part of the treatment in β-hemoglobinopathies and more studies with significant effects of EPO to reduce tubular dysfunction and glomerular injury (8,9,10,11), our study aimed to assess the tubular and glomerular renal functions of β-TM patients along with their EPO status and comparing with healthy controls.

Methods

Patients

This prospective case-control study included a total of 71 TDT beta thalassemia patients being followed by Pediatric Hematology Units of Bursa Health Sciences University Bursa City Hospital and Yuksek Ihtisas Training and Research Hospital, in Bursa, Turkiye and 22 healthy, age- and sex-matched controls. The study was conducted from January 2021 to May 2023. Children who were diagnosed as β-TM using established criteria, such as Hb electrophoresis, were frequently transfused with packed red blood cells, and received chelation therapy on a regular basis, were evaluated for eligibility. Patients who do not comply to regular transfusions, patients with chronic disease other than β-TM and patients receiving medication affecting renal functions were excluded. All patients were examined for their current weight and height, had their BMI checked, z score calculated, blood pressure monitored and had a physical examination. Blood pressure was measured with an automated oscillometric device (Welch Allyn ProBP 2400 Inc, Skaneateles Falls, NY, USA) with an appropriately sized cuff 3 times with 15 minutes intervals and the mean systolic and diastolic blood pressure values were calculated. Hypertension was categorized and staged according to the clinical practice guidelines of the American Academy of Pediatrics.

Sample Collection

Blood samples and fresh morning urine samples were collected during routine follow-up visits prior to administering the scheduled blood transfusion. Both blood samples and fresh morning urine samples were directly tested for complete blood count, serum creatinine (Cr), urea , uric acid, fasting blood sugar, serum electrolytes, serum ferritin, serum osmolality, serum cystatin C, serum beta-2-microglobulin (β-2-MG), serum erythropoietin (EPO), dipstick urinalysis, spot urine Na, spot urine K, spot urine Ca, spot urine P, spot urine uric acid, spot urine β-2-MG , spot urine protein, spot urine

albumin, spot urine creatinine and in order to calculate urine osmolality, spot urine glucose and spot urine blood urea nitrogen (BUN).

Renal Function Tests

Renal tubular functions were evaluated analyzing urine osmolality, fractional excretion of sodium (FENa), fractional excretion of potassium (FEK), spot urine Ca/Cr ratio, fractional excretion of uric acid (FEUA), tubular phosphorus reabsorption (TRP) , the ratio of the renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR) and serum and urine β-2-MG using standard formulas and reference values (12-17).

Renal glomerular functions were assessed by serum creatinine (Cr), urea, serum cystatin C, spot urine protein/creatinine ratio and spot urine albumin/ratio. Estimated GFR (eGFR) was calculated according to the following Schwartz formula: (height x k)/Cr, where the height is calculated in centimeters, k=0,45 for children under 2 years, 0,55 for children over 2 years and adolescent girls, 0,7 for adolescent boys up to age 18 years, and Cr is serum creatinine (18,19).

Statistical Analyses

Statistical analyses were performed using the SPSS for windows (version 21, SPSS Inc.,Chicago,IL) statistical package. In the analysis of the data, "descriptive statistics, mean, compliance of the data with the normal distribution were determined by Kurtosis and skewness coefficients, Shapiro-Wilk and Kolmogorov-Smirnov tests. Continuous variables with normal distribution were expressed by the means ± standard deviations (range) and categorical variables were stated as frequencies (numbers with percentages) in descriptive analysis. The chi-square test was used to compare the nominal data. "T test was used for and ANOVA test for independent groups" were used for normal distributions The 'Student's t-test' was used to compare two normally distributed independent groups, and the 'One Way ANOVA' test was used to compare more than two normally independent distributed groups. "Kruskal-Wallis and Mann-Whitney U" tests were used for non-normal distributions. The Mann Whitney U test was used to compare two independent groups that did not show normal distribution, and the test of the "Kruskal Wallis" was used to compare more than two groups that did not show normal distribution. Significance level was accepted as $p<0.05$.

Results

A total of 71 patients and 22 age- and sex-matched healthy children were enrolled in this study. The age of the study group ranged from 1 to 28 years old (mean range 140,49±,14 months), and 60,2% of total group were males. Systolic and diastolic blood pressures of all patients were <90th percentile on the basis of age, sex, and height percentiles. There were 6 β-TM patients with VKI >25 whereas there were 16 malnourished patients with VKI <18 and -2 SDS. Forty-eight patients (67,6% of patient group) had proteinuria and 16 (22,5%) had albuminuria. Twenty nine of β-TM (40,8%) patients had high fractional excretion of uric acid (FEUA) whereas only 4 (5,6%) patients had high FENa and 2 (2,8%) had high FEK. Tubular phosphorus reabsorption was <85% in 4,2% of patients while TmP/GFR was <2,8 in 22,5% (n=16) of patients demonstrating higher phosphate wasting. Thirty eight percent (n=27) of the patients showed hypercalciuria. Serum beta-2-microglobulin and urine β-2-MG were elevated in 80,3% (n=57) and 49,3% (n=35) of the patients respectively. Six (8,5%) patients had increased urine osmolality. Sixty four (90,1%) patients had elevated serum EPO. There was only 1 patient with raised serum cystatin C. Compared to healthy group, patient group had significantly higher levels of serum urea, proteinuria, albuminuria, hyperuricosuria, phosphaturia and significantly elevated eGFR, EPO, urine and serum β-2-MG (Table 1).

Fourteen patients with normal serum beta-2-microglobulin levels were detected to have a mean EPO level of 64,07±51,58 mIU/ml while 57 patients with increased serum β-2-MG had a mean EPO level of 368,51±975,46 mIU/ml (p=0,25). Thirty five patients with increased urine β-2-MG levels also had a concurrent elevation in EPO levels, 246,86±593,51 mIU/ml (p=0,35). Sixteen patients with albuminuria and 48 patients with proteinuria had low EPO levels, 260,93±306,6 mIU/ml (p=0,8) and 242±515,3 mIU/ml (p=0,36) respectively. Mean FENa of 64 EPO increased patients was 0,59±0,52%

and FENa of 1 patient with low EPO level (<9 mIU/ml) was 8,3% which was statistically significant (p<0,01). That one patient also exhibited increased FEUA and lower serum osmolality. There was no significant correlation between EPO and FEK, FEUA, TRP, TmP/GFR, e-GFR and serum cystatin C. Fourteen patients with normal serum β-2-MG levels revealed higher urine osmolality (Table 2). There was no significant correlation between serum cystatin C and serum β-2-MG , between serum cystatin C, serum β-2-M, uine β-2-M, serum EPO and ferritin levels.
Six patients with increased body mass index (VKI >25) presented hypercalciuria by a mean value of 0,58 mg/mg creatinine and lower serum cystatin C levels which were statistically significant (p<0,05). There were 4 patients who did not require an iron chelator, three who used deferiprone (DFP), four who used both deferasirox (DFX) and DFP, and the rest were on DFX. The eGFR was in the normal range for all patients. Estimated GFR of 60 patients using DFX was significantly low (Table 3).
Urine dipstick results were shown in Table 4.

Discussion

Knowledge on the epidemiology of renal complications in beta-thalassemia is limited. Improved survivaI of β-TM patients enhanced studies on renal status of those patients, declaring renal dysfunction in 1,8% of TDT patients and classifying renal problems as the fourth most common cause of morbidity (1, 20, 21). In beta thalassemia syndromes, iron overload due to regular blood transfusion leads to iron deposition in renal proximal tubules, glomeruli, and interstitium, leading to tubular atrophy, glomerulosclerosis, and interstitial fibrosis.(3, 22). Moreover, hypoxia and chronic anemia lead to oxidative stress and lipid peroxidation, resulting in tubular cell dysfunction (3, 23). In addition, iron chelator toxicity can result in glomerular dysfunction (3, 24).
Our study focused on the tubular and glomerular functions of β-TM patients and also comparing those functions with healthy children. Our patient group had significantly higher levels of urea and eGFR than the control group compatible with the study of Mahmoud et al also declaring elevated serum cystatin C (3) and Bilir et al proclaiming elevated eGFR and serum cystatin C in their patient group (2). Increased eGFR could be explained by 'glomerular hyperfiltration' secondary to the decrease in systemic vascular resistance in the anemic state leading to increased renal plasma flow. Unfortunately, these changes can eventually progress to glomerular dysfunction (23-25). Thirthy eight percent of our β-TM patients had hypercalciuria in harmony with the studies of Bilir et al., Mahmoud et al., Tanous et al., Mohkam et al. and Smolkin et al. (2,3, 5, 26, 27). Aldudak et al. found lower TPR values in the patient group than in the control group, 16 patients in our study showed phosphate loss presenting with TmP/GFR values <2,8 mg/dl (28). Incidences of FEUA, proteinuria of our β-TM patients were similar to literature and significantly higher in the patient group (2, 3, 5, 6, 26-28). We detected no significant differences in FENa and FEK between patients and controls as Smolkin et al. and Aldudak et al. did (27, 28). Tanous et al. and Mohkam et al. mentioned FEK in 33% and 7,8% of the patients, respectively (5, 26). FEK was increased in just 2 (2,8%) of our patients. In contrast to the findings of Smolkin and Aldudak, urine osmolality of the patient group was higher than the control group's (27, 28). In concordance with the study of Uzun et al, urine β-2-MG levels were significantly increased in β-TM patients (6). Bilir et al. found an insignificant increase in urine β-2-MG levels of the patient group (2).
We also studied EPO and its relation with renal dysfunction as there studies analyzing renoprotective effects of EPO or EPO as a supportive treatment in TM as well as EPO's contribution to iron overload in TM patients by stimulating dietary iron absorption (9-11, 29-32). EPO is a glycoprotein, the cardinal hematopoietic growth factor regulating red blood cell production by promoting the survival, proliferation and differentiation of erythroid progenitor cells in the bone marrow. In recent years, the discovery of EPO receptors in multiple tissues like vasculature, brain, heart, uterus and skeletal muscle has changed this sole physiological role of EPO and enabled studies to discover EPO's beneficial effects on kidney (9, 33).
EPO attenuates the dysfunction and histological changes associated with ischaemia-reperfusion injury, with a reduction in apoptotic cell death. In vitro studies have shown that EPO has direct effects

on proliferation and cell death in proximal tubular epithelial cells and reduces glomerular injury (9, 34).

In our study, 64 patients (90,1%) had elevated serum EPO as expected (35-38).

Fourteen patients with normal serum beta-2-microglobulin levels were detected to have a mean EPO level of 64,07±51,58 mIU/ml while 57 patients with increased serum β-2-MG had a mean EPO level of 368,51±975,46 mIU/ml. Although it wasn't statistically significant, as serum β-2-MG levels increase, serum EPO levels may be rising as a response of kidney to protect itself (9,10, 39-42). In accordance with the aforementioned data, 35 patients with increased urine β-2-MG levels also had a concurrent elevation in EPO levels, 246,86±593,51 mIU/ml. In contrast to the aforementioned data, 16 patients with albuminuria and 48 patients with proteinuria had low EPO levels, 260,93±306,6 mIU/ml and 242±515,3 mIU/ml respectively. This condition could potentially be elucidated by examining the distinct response of erythropoietin (EPO) to glomerular and tubular proteinuria. However, the current body of knowledge lacks comprehensive information regarding this matter (43-45). We found no relevant correlation between EPO and FEK, FEUA, TRP, TmP/GFR, e-GFR, serum cystatin C and spot urine Ca/Cr ratio.

Estimated GFR of 60 patients utilizing DFX was significantly low, compatible with the findings of Tanous et al. (5). The clinical impact of chelation toxicity is believed to be moderate, transient and non-progressive. Deferasirox associated reversible Fanconi syndrome and significant hypercalciuria were reported (46-50).

The major limitation of this study is relatively small sample size. However this is the first study searching for tubular and glomerular dysfunction along with EPO levels and we believe that our study will contribute for an awareness to recognize renal impairment of beta thalassemia patients.

Conclusion

Both renal tubular and glomerular functions of a β-TM patient deteriorate beginning from childhood years. Routine follow-up of serum creatinine, eGFR, serum cystatin C, spot urine protein/creatinine, spot urine Ca/Cr, FENa and FEUA could fail to detect early renal damage but still essential to analyze as they may be discovered impaired. Serum and urine β-2-MG levels are accurate indicators of tubular dysfunction in β-TM patients. Studies with larger sample sizes are needed to analyze the EPO status of β-TM patients and its effects on renal function.

Table 1

	Patients (n=71)	**Healthy group (n=22)**	***p***
Serum ferritin (ng/ml)	1923.39±1335.4	53.82±51.23	***<0.0001***
Serum urea (mg/dl)	23.04±9.64	18.91±7.33	***0.03***
Serum creatinin (mg/dl)	0.42±0.13	0.5±0.17	0.06
GFR (mg/dl)	189.15±56.98	162±24.95	***0.002***
UPCR (mg/mg)	0.30±0.24	0.19±0.16	***0.018***
UACR (mg/g)	33.4±58.03	14.75±7.85	***0.01***
UCaCr	0.25±0.35	0.16±0.18	0.126
FEUA (%)	12.7±6.92	9.88±4.66	***0.034***
FENa (%)	0.69±1.04	0.7±0.38	0.947
FEK (%)	8.74±8.04	8.28±5.46	0.759
FEP (%)	4.17±5.22	8.57±5.51	***0.002***
TRP (%)	95.84±5.23	91.42±5.51	***0.002***
TmP/GFR (mg/dl)	4±1.5	4.5±0.62	***0.026***
Urine osmolality (mOsm/kg)	551.80±192.88	453.5±103.94	***0.003***
Serum osmolality (mOsm/kg)	283.12±9.66	285.54±5.9	0.160
Urine B2M (mg/L)	1.7±3.16	0.61±1.22	***0.019***
Serum B2M (mcg/ml)	2.3±0.51	1.77±0.37	***<0.0001***
Cystatin C (mg/L)	0.82±0.16	0.75±0.16	0.09
EPO (mU/ml)	308.48±881.24	11.79±5.88	***0.006***

B2M; Beta-2 microglobulin, EPO; erythropoietin, FENa; fractional excretion of sodium, FEK; fractional excretion of potassium, FEP; fractional excretion of phosphate, FEUA; fractional urinary excretion of uric acid, GFR; glomeruler filtration rate, TRP; tubular reabsorption of phosphate, UACR; urine albumin/urine creatinin, UCaCr; urine calcium/creatinine, UPCR; urine protein/urine creatinin

Table 2

	Serum Erythropoietin			Serum Beta-2 microglobulin	
	Normal (n=6)	Increased (n=64)	Decreased (n=1)	Normal (n=14)	Increased (n=57)
Ferritin (mg/dl)	2238.3±2558.8	1855.17±1162	4400	1820.71±1706.85	1948.61±1244.7
GFR	176.83±53.3	190.62±57.96	169	182.35±42.42	190.82±60.22
UPCR (mg/mg)	0.21±0.1	0.31±0.25	0.15	0.26±0.15	0.31±0.26
UACR (mg/g)	21.92±22.09	34.83±60.66	10.85	15.58±12.77	37.77±63.82
UCaCr	0.19±0.13	0.26±0.36	0.05	0.19±0.17	0.27±0.38
FEUA (%)	11.65±3.56	12.75±7.2	16**	15.56±9.28	12±6.11
FENa (%)	0.55±0.38	0.59±0.52	8.3**	0.38±0.32	0.77±1.14
FEK (%)	10.33±5.2	8.6±8.33	8	10.33±8.08	8.35±8.05
FEP (%)	3.26±5.64	4.21±5.25	6.9	3.47±4.41	4.34±5.43
TRP (%)	96.73±5.64	95.8±5.26	93.1	96.52±4.41	95.67±5.43
TmP/GFR (mg/dl)	4.21±0.54	4.02±1.54	1.39	4.16±1.47	3.96±1.53
Urine osmolality (mOsm/kg)	593.55±163.05	543.67±194.5	821.9	657.87±185.76	525.75±187.05*
Serum osmolality (mOsm/kg)	289.3±2.13	283.03±9.16	251.5**	285.23±3.93	282.6±10.57
CystatinC (mg/L)	0.8±0.09	0.82±0.16	0.91	0.78±0.2	0.84±0.14

*p<0.05; **p<0.001

FENa; fractional excretion of sodium, FEK; fractional excretion of potassium, FEP; fractional excretion of phosphate, FEUA; fractional urinary excretion of uric acid, GFR; glomeruler filtration rate, TRP; tubular reabsorption of phosphate, UACR; urine albumin/urine creatinin, UCaCr; urine calcium/creatinine, UPCR; urine protein/urine creatinin

Table 3

	Chelation treatment			
	DFX (n=60)	DFP (n=3)	DFX+DFP (n=4)	None (n=4)
Ferritin (mg/dl)	1832.07±1118.13	1278.33±307.43	4823.75±1904.86*	876.75±267.41
GFR	182.58±51.05*	270.33±112.6	197.25±60.78	218.75±60.46
UPCR (mg/mg)	0.29±0.21	0.24±0.02	0.58±0.63	0.2±0.05
UACR (mg/g)	29.89±44.22	18.6±17.93	40.07±54.71	90.5±179
UCaCr	0.26±0.37	0.15±0.03	0.27±0.11	0.26±0.39
FEUA (%)	13.03±6.77	16.66±12.66	10.75±5.37	6.75±3.09
FENa (%)	0.73±1.12	0.4±0.26	0.52±0.41	0.55±0.26
FEK (%)	8.64±7.86	14±17.43	8.75±5.9	6.32±4.19
FEP (%)	4.3±5.52	3.06±2.91	5.4±3.85	1.85±2.85
TRP (%)	95.69±5.52	96.93±2.91	94.95±4.19	98.12±2.54
TmP/GFR (mg/dl)	4.04±1.47	2.46±2.11	4.49±0.94	4.04±1.83
Urine osmolality (mOsm/kg)	560.28±194.88	540.26±234.17	497.75±180.34	487.4±202.7
Serum osmolality (mOsm/kg)	282.88±10.26	285.53±1.51	283.4±9.17	284.65±3.78
Cystatin C (mg/L)	0.83±0.16	0.73±0.1	0.74±0.13	0.85±0.13
EPO (mU/ml)	332.93±954.24	371±325.08	131.9±96.9	71.45±21
Urine B2M (mg/L)	1.98±3.36	0.05±0.02	0.32±0.32	0.14±0.06
Serum B2M (mcg/ml)	2.32±0.53	1.98±0.18	2.1±0.26	2.38±0.57

*p<0.001

B2M; Beta-2 microglobulin, DFP; deferiprone, DFX; deferoxamine, EPO; erythropoietin, FENa; fractional excretion of sodium, FEK; fractional excretion of potassium, FEP; fractional excretion of phosphate, FEUA; fractional urinary excretion of uric acid, GFR; glomeruler filtration rate, TRP; tubular reabsorption of phosphate, UACR; urine albumin/urine creatinin, UCaCr; urine calcium/creatinine, UPCR; urine protein/urine creatinin

Table 4

Urine dipstick test	Patients (n=71)	Healthy group (n=22)
Normal	34	20
+1 proteinuria	1	None
Microscopic hematuria	12	1
Hematuria+proteinuria	6	None
Hemoglobinuria	5	None
Hemoglobinuria+proteinuria	5	None
Pyuria	2	1
Pyuria+hematuria	4	None
Trace proteinuria	2	None

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