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CLINICAL STUDY

Glomerular and tubular functions in children with different forms of beta thalassemia

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Abstract

Background: Although there are many available data about renal involvement in patients with beta thalassemia major (TM), the changes in renal functions of other types, such as thalassemia intermedia (TI) and thalassemia minor (TMin), were reported less. Therefore, we aimed to evaluate renal tubular and glomerular functions in patients with three types of beta thalassemia. *Methods*: This prospective case–control study was conducted on 118 beta-thalassemia patients (49 in TM, 18 in TI and 51 TMin) and 51 healthy controls. Glomerular functions [estimated glomerular filtration rate (GFR), serum cystatin C and urinary protein creatinine ratio] and tubular functions [fractioned sodium excretion (FENa), tubular reabsorption of phosphorus, urinary excretion of uric acid, levels of retinol-binding protein, alpha-1 macroglobulin (alpha-1M), and beta-2 microglobulin, calcium creatinine ratio] were assessed in all patients and controls. *Results*: The mean ages of the groups and controls at presentation were similar. Although GFR was similar in all patients and control groups, serum levels of cystatin C in patients with TM and TI were significantly higher compared to TMin and controls. Alpha-1M, FENa, urinary excretion of uric acid, and urine protein/creatinine ratio in TM and TI groups were significantly higher than the others. Mean cystatin C level was also higher in patients with TMin compared the controls. However, there were no significant differences according to all tubular and other glomerular functions between TMin and control groups. *Conclusions*: Although all types of beta thalassemia patients should be closely monitored to prevent further decrease in renal functions, the patients with TI should be considered to have a higher risk of glomerular and tubular deterioration as well as TM.

Keywords

Alpha-1 macroglobulin, cystatin C, hemoglobinopathies, renal function, retinol-binding protein

History

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Introduction

Beta thalassemia is an inherited disorder which is characterized with various degrees of defective hemoglobin beta-chain production and ineffective erythropoiesis. Patients with thalassemia major (TM) present with profound anemia in the first year of life and require regular blood transfusions for survival, whereas those who had thalassemia intermedia (TI) and thalassemia minor (TMin) types are transfusion independent. Because of regular blood transfusions, an increase of iron intestinal absorption and subsequent iron load has been well established as the potential cause of kidney disease in patients with beta TM. Shortened red cell lifespan and excess iron cause functional and physiological abnormalities in various organ systems in thalassemia patients. Meanwhile, they have a high prevalence of renal tubular abnormalities. The severity correlated with the degree of anemia is least severe in patients on hyper transfusion and iron chelation therapy, suggesting that the damage might be caused by the anemia and increased oxidation induced by excess iron deposits.[1–9]

Although it was reported that urinary excretion of proximal renal tubular damage parameters increased in patients with TM and TI, renal functional parameters were not separately studied as glomerular and tubular markers in all types of the disease. The aims of the present study were to assess the glomerular and tubular renal functions in three different types of beta thalassemia, and to identify the similarities and differences between groups and to compare with healthy controls.

Methods

Patients

This prospective case–control study included a total of 118 beta thalassemia patients and 51 healthy, age- and sex-matched controls. The study patients were divided into three groups; beta TM (Group 1), TI (Group 2), and TMin (Group 3), and thus, analyses were performed on four

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Table 1. According to the groups, demographic and basic laboratory data.

	Group 1 (TM) $N=49$	Group 2 (TI) $N=18$	Group 3 (TMin) $N=51$	Group 4 (controls) $N=51$
Age (year)	10.9 ± 4.2	9.5 ± 4.0	9.6 ± 4.0	9.0 ± 4.0
Gender (female/male)	26/23	7/11	23/28	22/29
Hemoglobin (g/dL)	9.0 ± 0.9	8.4 ± 1.5	11.0 ± 0.7	13.2 ± 0.8
Ferritin (ng/mL)	1925.1 ± 1100.2	773.8 ± 340.3	52.1 ± 35.7	47.5 ± 34.3
Creatinine (mg/dL)	0.43 ± 0.07	0.37 ± 0.11	0.44 ± 0.11	0.42 ± 0.10

Notes: TM, thalassemia major; TI, thalassemia intermedia; TMin: thalassemia minor.

groups together with controls (Group 4). The study was approved by the institutional ethics board of Pamukkale University Ethics Committee (23.11.2010/08). Informed consents and parental informed consents were obtained for each patient on the day of admission to hospital. Data regarding the patient demographics and clinical information were retrospectively collected. Patients who had other systemic diseases, formerly poor renal functions, urinary tract infections and those who were on medications that affect renal functions were excluded. The control group consisted of healthy children. In this study, all transfusion dependent patients that need chelators were receiving Deferasirox (10–30 mg/kg/day).

There were 49 TM patients (23 males) in Group 1, 18 TI patients (11 males) in Group 2, 51 TMin patients (28 males) in Group 3, and 51 healthy controls (29 males) in Group 4.

Samples and measurements

In TM and TI groups, blood samples were taken before transfusion of erythrocytes; first-morning urine was taken at the same time. After the samples were centrifuged, serum and urine levels of calcium, phosphorus, creatinine, uric acid, protein were detected using the auto analyzer (Roche Cobas 6000, Roche-Hitachi Diagnostics, Mannheim, Germany). After routine laboratory detections were made, remaining samples were stored at $-80\,^{\circ}C$ for future detection of cystatin-C in serum and retinol-binding protein (RBP), alpha-1 macroglobulin (alpha-1M) and beta-2 microglobulin (beta-2 m) in urine. Total blood count was made in samples collected in EDTA containing tubes (Siemens ADVIA® 2120i System, Siemens Healthcare Diagnostics, Ireland, UK). Serum ferritin was detected using electro chemiluminescence technology. Tubular reabsorption and excretion of urine electrolytes were calculated using appropriate formulas. Glomerular filtration rate was calculated using Schwartz formula. The stored blood and urine samples were defrosted at room temperature. AssayMax Retinol-Binding Protein ELISA kit was used for urine RBP detection, AssayMax Human alpha 1-Microglobulin ELISA kit was used for urine alpha-1M and DRG microglobulin, (beta-2) Enzyme Immunassay-1789 kit was used for urine beta-2 m. Human Cystatin C ELİSA kit was used for serum cystatin C detection.

Statistical analysis

All statistical analyses were performed using SPSS (SPSS version 10.0 Inc., Chicago, IL) packaged software. Shapiro–Wilk's test was used for determination of normal distribution. Continuous variables were defined by the mean ± standard deviations. Kruskal–Wallis variance analysis, Bonferroni corrections, Mann–Whitney *U* test, one-way ANOVA test was used for comparison of continuous data. Intergroup comparisons of categorical parameters were made using chi-square test. Pearson's linear regression analysis was performed. A *p*-value of less than 0.05 was considered to be statistically significant.

Results

There were no significant differences in the mean age of the groups (10.9 ± 4.2 years in group of TM, 9.5 ± 4.8 in TI, 9.6 ± 4.1 in TMin, and 9 ± 4 in controls). Patients with TM and TI had significantly lower levels of hemoglobin than patients with TMin and controls ($p < 0.001$, Table 1). At the same time, serum ferritin levels in the groups of TM and TI were significantly higher than in the groups of TMin and the healthy subjects ($p < 0.001$, Table 1). According to tubular function parameters levels of fractioned sodium excretion (FENa), urinary excretion of uric acid and alpha-1M were significantly higher in TM and TI groups than in TMin and control groups, whereas the difference in these three parameters was not significant between TM and TI groups and between TMin and control groups. However, in terms of all tubular function tests, there was no significant difference between patients with TM and TI, as well as between patients with TMin and healthy subjects. RBP and urine calcium/creatinine ratios were only higher in TM group when compared to controls. The other groups were similar in regard to excretions of these two tubular parameters. Urine beta-2 m was significantly higher in TM group than in TMin and control groups. Tubular reabsorption of phosphorus (TPR) was similar among groups. Tubular function parameters are given in Table 2.

In terms of glomerular functions, serum cystatin-c levels were significantly higher in TM and TI groups than in both TMin group and the control group. There was no significant difference between TM and TI group, whereas serum cystatin-c levels were significantly higher in TMin group than in control group. Mean spot urine protein/creatinine ratio was significantly higher in TM and TI groups than in both TMin and control groups. However, there was no significant difference between TM and TI groups in regard to protein/creatinine ratio. TMin and control groups were also similar in regard to this parameter. Creatinine clearance values were similar among all groups (Table 3).

There was a significant positive correlation between ferritin and FENa, RBP, alpha-1M beta-2 m, protein/creatinine ratio and cystatin-c (Table 4).

Table 2. Comparison of renal tubular functions among groups.

	Group 1—TM ($N=49$)	Group 2—TI ($N=18$)	Group 3—TMin ($N=51$)	Group 4—Controls ($N=51$)	*p*-Values					
					Group 1–Group 4	Group 2–Group 4	Group 3–Group 4	Group 1–Group 2	Group 1–Group 3	Group 2–Group 3
FENa (%)	0.50 ± 0.30	0.70 ± 0.40	0.40 ± 0.40	0.30 ± 0.30	0.009	0.0001	ND	ND	0.0001	0.0001
Urinary excretion of uric acid (mg/dL GFR)	0.50 ± 0.20	0.50 ± 0.30	0.30 ± 0.10	0.30 ± 0.10	0.0001	0.0001	ND	ND	0.0001	0.0001
alpha-1M (mcg/mL)	25.00 ± 7.70	25.00 ± 9.50	12.20 ± 9.10	11.10 ± 8.90	0.0001	0.0001	ND	ND	0.0001	0.0001
RBP (mcg/mL)	0.06 ± 0.04	0.06 ± 0.04	0.05 ± 0.03	0.04 ± 0.02	0.022	ND	ND	ND	ND	ND
beta-2 m (mcg/mL)	21.50 ± 37.90	16.50 ± 34.20	7.70 ± 5.10	5.90 ± 4.60	0.013	ND	ND	ND	0.023	ND
Urinary calcium/ creatinine (mg/mg)	0.10 ± 0.10	0.20 ± 0.10	0.10 ± 0.10	0.10 ± 0.10	0.007	ND	ND	ND	ND	ND
TPR (%)	95.20 ± 2.40	94.00 ± 4.70	94.50 ± 3.20	94.60 ± 3.20	ND	ND	ND	ND	ND	ND

Notes: TM, thalassemia major; TI, thalassemia intermedia; TMin, thalassemia minor; FENa (%), fractioned sodium excretion; alpha-1M, alpha-1 macroglobulin; RBP, retinol-binding protein; beta-2 m, beta-2 microglobulin; TPR, tubular reabsorption of phosphorus; ND, no difference.

Table 3. Comparison of renal glomerular functions among groups.

	Group 1—TM ($N=49$)	Group 2—TI ($N=18$)	Group 3—TMin ($N=51$)	Group 4—Controls ($N=51$)	*p*-Values					
					Group 1–Group 4	Group 2–Group 4	Group 3–Group 4	Group 1–Group 2	Group 1–Group 3	Group 2–Group 3
Cystatin C (ng/mL)	3.70 ± 0.90	4.10 ± 0.90	3.20 ± 0.60	2.60 ± 0.90	0.0001	0.0001	0.003	ND	0.016	0.001
Urinary protein/creatinine (mg/mg)	0.20 ± 0.10	0.20 ± 0.10	0.10 ± 0.10	0.10 ± 0.00	0.01	0.0001	ND	ND	0.012	0.0001
e-GFR (mL/min/1.73m2)	121.40 ± 10.70	123.20 ± 15.30	117.70 ± 8.20	120.30 ± 11.40	ND	ND	ND	ND	ND	ND

Notes: TM, thalassemia major; TI, thalassemia intermedia; TMin, thalassemia minor; e-GFR, estimated glomerular filtration rate; ND, no difference.

Table 4. Correlation between ferritin and glomerular and tubular functional parameters.

Variables	*R* (neg)	*p*-Value
FENa (%)	0.229	0.003
RBP (mcg/mL)	0.185	0.036
Alpha-1M (mcg/mL)	0.470	0.0001
Beta-2 m (mcg/mL)	0.251	0.001
Cystatin-C (ng/mL)	0.293	0.0001
Protein/creatinine	0.187	0.02

Notes: *R*, correlation coefficient, FENa (%), fractioned sodium excretion; RBP, retinol-binding protein; alpha-1M, alpha-1 macroglobulin; beta-2 m, beta-2 microglobulin.

Discussion

We found that tubular and glomerular functional parameters showed similar deterioration between patients with TM and TI and the deterioration in these forms of the disease was more profound than that in patients with TMin and healthy subjects. To the best of our knowledge, our study was the first to compare renal functional parameters among three types of beta thalassemia and healthy controls. Our results may suggest that in patients with beta thalassemia, tubular and glomerular dysfunction may be depend on the severity of anemia, frequency of blood transfusion, and iron load.

We found that FENa levels in beta thalassemia patients tended to increase with disease severity. As a predictive marker of poor tubular function, increases in FENa levels were similar between TM and TI groups. Similarly, increases in urinary excretion of uric acid and alpha-1M were also significant in these groups. There have been studies showing FENa levels increased as an indicator of poor renal function in beta thalassemia patients,[5,10–12] whereas there has also been studies suggesting that this parameter showed no difference.[2,4,6,13] Data regarding the significance of alpha-1M excretion in thalassemia are limited. Our study showed that alpha-1M excretion increased in patients with TM and TI. Thus, we suggest that alpha-1M may be taken as a reliable marker of tubular dysfunction with TM patients. Similarly, our results also showed that uric acid excretion increased in patients with TM and TI. In the past, some studies reported that what was found high in patients with thalassemia.[2,5,11,13] However, these three tubular parameters (FENa, alpha-1M, and uric acid excretion) were similar between TMin and healthy subjects.

RBP is a low molecular weighted protein and it is found in trace amounts in urine even in the presence of massive proteinuria. Increased RBP excretion is specific to tubular dysfunction and it is well stabilized in acidic urine.[14,15] However, data are limited regarding the urinary RBP levels in patients with different forms of thalassemia. In our study, urinary RBP excretion in TM group was highest among all groups and the difference between the TM group and the control group was found significant. The differences in regard to RBP values were not significant among other groups. We also found a positive correlation between RBP and ferritin levels. Unlike urine FENa, urinary excretion of uric acid and alpha-1M, we found that RBP was higher only in TM group. Therefore, it may be justified that RBP is a reliable marker of tubular dysfunction in patients with TM.

Beta-2 m is a protein which is normally freely filtered through glomerulus and is almost completely reabsorbed by the tubular cells. Therefore, it is found in negligible amounts in urine.[3,6,16] In our study, urinary excretion of beta-2 m was found significantly different in TM group than that in TMin and control groups. In some studies, urinary beta-2 m levels were found significantly higher in children with TM.[3,10,12,16] One study showed that urinary excretion of beta-2 m was not different between children with TMin and healthy controls.[4] Thus, we suggest that beta-2 m may be taken as a reliable marker of tubular dysfunction in patients with thalassemia major (TM).

In our study, calcium/creatinine ratio in spot urine measurement was found significantly higher in TM group than that in healthy controls. Similarly, there have been studies suggesting that urinary calcium excretion was found higher in patients with TMin.[2,3,5,13,17] Two other studies reported that urinary calcium excretion was high in patients with TMin and it was emphasized that these patients had an increased osteoporosis.[4,11]

Urinary excretion of phosphorus may increase in patients with beta thalassemia, due to rapid erythrocyte turnover, hemolysis and proximal tubular damage.[6] Phosphaturia can be shown by decreased TPR.[14] In our study, we found no difference among groups in regard to TPR values. There have also been other studies that showed no significant difference between thalassemia patients and healthy controls in regard to TPR values.[2,3,12] In one study where TPR values were compared between patients with TMin and healthy subjects, no difference was found.[4] However, there have also been studies on the contrary to our results. Aldudak et al.[6] and Hamed and Elmelegy[16] reported that TPR levels were significantly lower in patients with TM than in healthy controls. Since the results in the literature are highly variable, we think that TPR is not a reliable marker of tubular functions in patients with beta thalassemia.

Since serum cystatin C is not reabsorbed following its filtration through glomerulus and also not secreted by tubular cells, it is a reliable marker for glomerular dysfunction.[16,18] Hamed and Elmelegy[16] found in their study that serum cystatin C values were significantly higher in patients with TM than that in healthy subjects. However, there has been no study that sought to determine serum cystatin values in patients with TI. In our study, serum cystatin C level was found higher in TM and TI groups, whereas there was no significant difference between these groups. Also, we found that serum cystatin C levels were also higher in TMin group than that in the control group. Since we found that an increase was found even in the mildest form of the disease, we suggest that serum cystatin C values may be considered as an early precursor of glomerular dysfunction in patients with beta thalassemia.

Three of several studies that sought urine protein/creatinine ratios in patients with beta-thalassemia found that this parameter was significantly higher in patients with TM than in other forms of the disease.[3,5,6] Similar to our results, Kalman et al.[4] found that urinary protein excretion was not different between children with TMin and normal healthy subjects. It was reported that urinary protein excretion was not significantly different between children with thalassemia (intermedia and major) and healthy controls.[2] In our study, we found that the urinary protein/creatinine ratio was significantly higher in TM and TI groups, whereas there was no significant difference between TMin and control

groups. Therefore, we think that the protein/creatinine ratio may be an important marker of glomerular dysfunction in patients with TM and TI.

We found no differences among the four groups in regard to glomerular filtration rate (GFR), which was calculated using the Schwartz formula. There have been several studies reporting similar results to ours.[2,5,6,13] Although there have also been some studies that reported dissimilar results to ours,[17,18] our results showed that serum cystatin C levels, as an early precursor of glomerular dysfunction, changed well before the decrease in GFR occurred. Thus, we suggest that increased serum cystatin C level is an earlier precursor of glomerular dysfunction than GFR calculation in thalassemia patients.

There were two limitations in this study. First, we did not evaluate the levels of urine microalbuminuria as a glomerular function test. Second, we did not also calculate urine osmolality as a tubular function. However, we suggested that other tests which were included to this study were convenient to assess glomerular and tubular function in those patients.

In our study, serum ferritin levels were put into correlation analysis with renal functional parameters. As a result, we found a significant positive correlation between serum ferritin and FENa, RBP, alpha-1M, beta-2 m, cystatin C levels and protein/creatinine. These findings may suggest that increased iron accumulation in the body may be associated with an increased risk for glomerular and tubular dysfunction.

Conclusions

Tubular and glomerular dysfunctions were found at different levels in patients with three types of beta thalassemia. Functional impairment is subclinical in general and associated with tubular and glomerular damage. Routine laboratory tests fail in early detection of renal damage. We think that glomerular and tubular functional impairment may be revealed by measurement of serum cystatin C, urinary RBP and alpha-1M levels. More importantly, since we found that the decrease in tubular and glomerular functions were similar between patients with TM and TI, and the deterioration was more profound in patients with TM and TI, we suggest that TM and TI patients should be more closely monitored to prevent further decrease in renal functions.

Declaration of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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