Renal Tubular Dysfunction in β-Thalassemia Minor

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• ***Background:*** **Persons with β-thalassemia minor usually are symptomless. However, we previously reported renal tubular dysfunction in a patient with β-thalassemia minor. The aim of this study is to investigate renal function in patients with β-thalassemia minor.** ***Methods:*** **Forty-one subjects with β-thalassemia minor and 20 sex- and age-matched healthy subjects were enrolled in the study. For analysis, patients were divided into 2 groups: group A, all patients with anemia (n = 19), and group B, patients without anemia (n = 22). Blood and 24-hour urine samples were obtained for hematologic and biochemical analysis.** ***Results:*** **Anemic patients had increased urinary zinc excretion (U_{zinc}) and fractional excretion of sodium (FE_{Na}) and uric acid (FE_{UA}) compared with both controls and patients without anemia. Hemoglobin levels correlated significantly in a negative manner with U_{zinc}, FE_{Na}, and FE_{UA} in patients with β-thalassemia minor. However, serum lactate dehydrogenase levels correlated significantly in a positive manner with the same parameters. In addition, 6 of 41 patients (14.6%) with β-thalassemia minor showed significant signs of renal tubulopathy, such as hypercalciuria, decreased tubular reabsorption of phosphorus with hypophosphatemia, hypomagnesemia with renal magnesium wasting, hypouricemia with renal uric acid wasting, and tubular proteinuria.** ***Conclusion:*** **Proximal renal tubular dysfunction is not rare in patients with β-thalassemia minor.** ***Am J Kidney Dis*** **42:1164-1168.**

INDEX WORDS: Renal tubular dysfunction; β-thalassemia minor.

β-THALASSEMIA minor (β-thalassemia trait or heterozygous β-thalassemia), which has been known as a symptomless carrier state, is caused by the presence of a single β-thalassemia mutation and a normal β-globin gene on the other chromosome.[1] It is characterized by profound microcytosis with hypochromia, but mild or minimal anemia. No treatment is necessary for β-thalassemia minor. In particular, iron therapy is not necessary or advised. Many functional abnormalities of the kidney have been reported in patients with β-thalassemia major.[2-4] However, only 1 case report exists in the literature concerning renal tubular dysfunction in β-thalassemia minor.[5] Furthermore, Kalef-Ezra et al[6] reported no significant differences between patients with β-thalassemia minor and controls for such biochemical markers of bone metabolism as serum calcium, phosphorus, alkaline phosphatase, osteocalcin, and parathyroid hormone and urine calcium-creatinine ($U_{Ca\text{-}Cr}$) ratio. The aim of this study therefore is to investigate renal function in subjects with β-thalassemia minor.

METHODS

Forty-one subjects with β-thalassemia minor (29 men, 12 women; age, 19 to 40 years) and 20 age- and sex-matched healthy volunteers were recruited into the study. Subjects with renal disorders, urinary infection, or other causes of anemia and those administered medication that might alter renal function were excluded from the study. A previously reported case[5] was not included in the present study. Physical examination and blood pressure were normal in all patients. For analysis, patients with β-thalassemia minor were divided into 2 groups: group A, all patients with anemia (hemoglobin [Hb] < 13 g/dL [130 g/L] for men, <12 g/dL [120 g/L] for women; n = 19), and group B, patients without anemia (n = 22).

Fasting venous blood samples were obtained for hematologic tests and measurement of serum urea, creatinine, sodium, potassium, chloride, magnesium, calcium, phosphorus, uric acid, albumin, lactate dehydrogenase (LDH), vitamin B_{12}, folic acid, and ferritin. A 24-hour urine specimen was collected for determination of zinc, creatinine, sodium, potassium, chloride, magnesium, calcium, phosphorus, uric acid, protein, glucose, and amino acid levels. Fractional urinary excretion (FE) of filtered electrolytes (sodium [FE_{Na}], potassium [FE_K], chloride [FE_{Cl}], uric acid [FE_{UA}], and magnesium [FE_{Mg}]) and tubular reabsorption of phosphorus (TRP) were calculated using standard equations. Urine protein-creatinine and $U_{Ca\text{-}Cr}$ ratios also were calculated. Glomerular filtration rate was determined using Modification of Diet in Renal Disease Study equations.[7]

Five milliliters of blood were drawn into tubes (Vacu-

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Table 1. Mean Levels and Comparisons of Parameters Among the Patient and Control Groups

Parameters	Group A (n = 19)	Group B (n = 22)	Controls (n = 20)	Group A *v* Group B	Group A *v* Controls	Group B *v* Controls
Hb (g/dL)	11.10 ± 0.85	13.69 ± 0.62	13.77 ± 0.72	<0.001	<0.001	0.686
HbA (%)	93.98 ± 0.41	93.97 ± 0.78	97.24 ± 0.33	0.712	<0.001	<0.001
HbF (%)	1.13 ± 0.30	2.37 ± 0.44	0.42 ± 0.08	<0.001	<0.001	<0.001
HbA_2 (%)	4.88 ± 0.42	3.66 ± 0.92	2.34 ± 0.32	<0.001	<0.001	<0.001
Hematocrit (%)	34.71 ± 2.13	43.75 ± 2.74	44.08 ± 1.99	<0.001	<0.001	0.970
Red blood cell count ($\times 10^6/\mu$L)	6.01 ± 0.11	5.99 ± 0.11	4.61 ± 0.40	0.523	<0.001	<0.001
LDH (U/L)	354.95 ± 172.41	138.82 ± 33.92	116.65 ± 20.36	<0.001	<0.001	0.027
FE_{Na} (%)	1.14 ± 0.35	0.69 ± 0.25	0.64 ± 0.14	<0.001	<0.001	0.476
FE_{UA} (%)	7.76 ± 2.66	3.83 ± 1.54	3.22 ± 1.26	<0.001	<0.001	0.158
U_{zinc} (μg/dL)	988.89 ± 262.70	734.68 ± 198.85	314.40 ± 136.87	0.002	<0.001	<0.001

NOTE. To convert hemoglobin in g/dL to g/L, multiply by 10; hematocrit in percent to proportion of 1.0, multiply by 0.01; red blood cell count in $\times 10^6/\mu$L to $\times 10^{12}$/L, multiply by 1; U_{zinc} in μg/dL to μmol/L, multiply by 0.153.

Abbreviations: U, urine; RBC, red blood cell.

tainer system; Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 4,500 revolutions per minute for 10 minutes. Sera were extracted from samples and levels of urea, creatinine, uric acid, albumin, LDH, calcium, phosphorus, magnesium, sodium, potassium, and chloride were measured. Urine creatinine, uric acid, glucose, calcium, magnesium, phosphorus, sodium, potassium, and chloride were measured from 24-hour urine samples. Blood and urine tests were performed on an Olympus AU-600 (Mishima, Japan) autoanalyzer using its own commercial kits (Olympus Diagnostika GmbH, Hamburg, Germany) with an ion-selective and enzymatic colorimetric method. Complete blood count was determined by using a hematology analyzer, Advia 120 (Advia 120; Bayer Corp, Tarrytown, NY). Serum ferritin levels were determined by chemiluminescence method using an automated hormone analyzer, Advia Centaur (Bayer Corp). Vitamin B_{12} and folic acid levels were measured by means of radioimmunoassay with reagents from Diagnostic Product Corp (Los Angeles, CA). Quantitative analysis of total protein in urine was measured by a turbidimetric method, trichloroacetic acid. Urine amino acid screening was performed by means of a thin-layer chromatography method. U_{zinc} concentrations were measured by means of the atomic absorption method using an automated analyzer, AA 400 (Varian Inc, Melbourne, Australia).

Plasma renin, aldosterone, and parathyroid hormone levels were measured only in patients with tubulopathy. Plasma renin levels were determined by immunoradiometric assay method using a Diagnostic Systems Laboratories (DSL) kit (DSL-25100; Webster, TX). Plasma aldosterone levels were measured by radioimmunoassay method using DSL-8600. Plasma intact parathyroid hormone levels were determined by chemiluminescence method (Immulite 1000; Diagnostic Product Corp, Los Angeles, CA). β_2-Microglobulin levels in 24-hour urine samples were studied by means of an indirect solid-phase enzyme immunometric assay (Orgentec Diagnostika GmbH, Mainz, Germany). Urine microalbumin concentrations were determined by nephelometric method using an automated analyzer, Array 360 (Beckman Coulter, Brea, CA). Urine microalbumin–β_2-microglobulin ratio was calculated only in patients with tubulopathy. Bone mineral density (BMD) also was determined in these patients at the lumbar spine (L1 to L4) by dual-energy X-ray absorptiometry (Hologic QDR 4500; Hologic Inc, Bedford, MA). BMD is expressed in grams per centimeter squared and T score of young adults from the manufacturer's controls. In accordance with World Health Organization criteria,[8] osteopenia is defined as a T score less than −1, whereas osteoporosis is defined as a T score less than −2.5.

All subjects gave informed consent to participate in the study protocol. The study was approved by the local ethics committee of Gülhane Military Medical Academy (Ankara, Turkey).

Statistical Analysis

All statistical analyses were performed using StatsDirect Statistical Software (StatsDirect Limited, version 2,2,0, Cheshire, UK). Descriptive statistics are shown as the arithmetic mean ± SD. Logarithmic transformations were performed for variables not normally distributed. For comparisons of more than 2 groups, we used 1-way analysis of variance or Kruskal-Wallis test. To investigate differences between 2 groups, we used the independent-samples *t*-test or Mann-Whitney *U* test (with Bonferroni correction), when appropriate. Pearson's correlation coefficient was calculated. *P* of 0.05 or less is considered statistically significant.[9]

RESULTS

The diagnosis of β-thalassemia minor was based on Hb electrophoresis. No statistically significant difference was found with respect to age (data not shown). Mean levels and comparisons of parameters in patients with β-thalassemia minor and controls are listed in Table 1. As expected, mean red blood cell volume was lower

in subjects with β-thalassemia minor (63.37 $\pm$ 2.75 fl in group A, 63.22 $\pm$ 2.95 fl in group B) than the control group (87.95 $\pm$ 3.17 fl; chi-square, 39.762; $P < 0.001$). Mean values for Hb, HbA, HbF, HbA_2, hematocrit, red blood cell count, LDH, FE_{Na}, FE_{UA}, and U_{zinc} were significantly different among groups ($F = 58.734$; $P < 0.001$; chi-square, 39.995; $P < 0.001$; chi-square, 53.658; $P < 0.001$; chi-square, 43.583; $P < 0.001$; chi-square, 36.951; $P < 0.001$; chi-square, 40.802; $P < 0.001$; chi-square, 33.112; $P < 0.001$; chi-square, 26.971; $P < 0.001$; chi-square, 33.278; $P < 0.001$; and chi-square, 41.244; $P < 0.001$, respectively). However, mean ferritin levels did not differ statistically significantly among groups (chi-square, 1.267; $P = 0.531$).

Anemic patients had increased levels of serum LDH, U_{zinc}, FE_{Na}, and FE_{UA} and percentages of HbA_2 compared with both controls and patients without anemia. Anemic patients had lower percentages of HbF, whereas they had higher percentages of HbA_2 than those without anemia. Interestingly, no significant differences were found with regard to mean levels of LDH, FE_{Na}, and FE_{UA}, whereas percentages of HbA, HbF, and HbA_2, red blood cell counts, and mean levels of U_{zinc} were significantly different between controls and patients without anemia. Mean levels of serum vitamin B_{12}, folic acid, urea, creatinine, albumin, sodium, chloride, potassium, calcium, magnesium, phosphorus, and uric acid were not significantly different among groups (data not shown). No statistically significant difference was found with regard to mean glomerular filtration rate, TRP, FE_K, FE_{Cl}, FE_{Mg}, $U_{Ca\text{-}Cr}$ ratio, and urinary protein-creatinine ratio among groups (data not shown). In addition, glucosuria and aminoaciduria were not observed in any patient.

Hb levels correlated significantly in a negative manner with FE_{Na}, FE_{UA}, and U_{zinc} in patients with β-thalassemia minor ($r = -0.842$; $P < 0.001$; $r = -0.854$; $P < 0.001$; $r = -0.793$; $P < 0.001$, respectively). However, serum LDH levels correlated significantly in a positive manner with FE_{Na}, FE_{UA}, and U_{zinc} in patients with β-thalassemia minor ($r = 0.508$; $P < 0.001$; $r = 0.492$; $P < 0.001$; $r = 0.637$; $P < 0.001$, respectively).

In the current study, 6 of 41 patients (14.6%) with β-thalassemia minor showed significant signs of renal tubulopathy based on the parameters investigated (Table 2). Increased calcium excretion was found in 4 of 6 patients (66.7%) with renal tubulopathy. Decreased phosphorus reabsorption was found in 2 of 6 patients (33.3%), whereas 3 of 6 patients (50%) with tubulopathy showed elevated magnesium excretion. In 3 patients with tubulopathy (50%), urine analysis for β_2-microglobulin showed the majority of protein excreted was renal tubular in origin. Hypophosphatemia and hypouricemia caused by renal tubular phosphorus and uric acid wasting occurred in 1 patient (16.6%), whereas hypomagnesemia with renal magnesium wasting occurred in 2 patients (33.3%). Serum total calcium, chloride, potassium, plasma renin, aldosterone, and parathyroid hormone levels were normal in patients with tubulopathy (data not shown). FE_{Na}, FE_K, FE_{Cl}, and blood and urine pH also were normal (data not shown).

At lumbar levels, 4 of 6 patients (66.7%) with renal tubulopathy showed reduced BMD values, indicating the presence of bone loss. In particular, 1 patient had a T score lower than -2.5 (osteoporosis), and 3 patients had a T score between -1 and -2.5 (osteopenia).

DISCUSSION

The most interesting finding in the current study is that renal tubular dysfunction is not uncommon in patients with β-thalassemia minor. Several abnormalities in renal tubular function, including increased urinary secretion of uric acid and sodium, were observed in our patients. Because sodium is reabsorbed mainly in the proximal tubule, increased mean levels of FE_{Na} indicate proximal tubular dysfunction. High mean levels of FE_{UA} also may result from supranormal proximal tubular function. Furthermore, 6 of 41 patients (14.6%) with β-thalassemia minor showed such significant signs of renal tubulopathy as hypercalciuria, decreased TRP with hypophosphatemia, hypomagnesemia with renal magnesium wasting, hypouricemia with renal uric acid wasting, and tubular proteinuria. Conversely, significantly greater urinary excretion of sodium, uric acid, and zinc were observed in anemic patients compared with both patients without anemia and control subjects.

In addition, the amount of urinary excretion of sodium and uric acid correlated negatively with

Table 2. Clinical and Laboratory Characteristics of Patients With Renal Tubular Dysfunction

Parameters	Patient No.						Reference Values
	8	16	23	27	29	35	
Age (y)	34	31	24	26	20	20	
Sex	Male	Female	Male	Male	Male	Male	
Hb (g/dL)	9.70	10.70	11.90	14	10.90	10	Males, >13; females, >12
Hematocrit (%)	31.60	34.80	37	44.70	35.10	32	Males, 40-52; females, 36-48
LDH (U/L)	750	588	350	214	405	640	91-232
Serum magnesium (mg/dL)	N	1.80	1.80	N	N	N	1.9-2.5
Serum phosphorus (mg/dL)	N	N	2.4	N	N	N	2.6-5.9
Serum uric acid (mg/dL)	N	1.4	N	N	N	N	2.3-8.2
Urine microalbumin–β_2-microglobulin ratio	N	N	N	19	14	24	50-200
$U_{Ca\text{-}Cr}$	0.27	N	0.41	N	0.32	0.39	<0.2
FE_{Mg} (%)	7.50	4.10	4.40	N	N	N	<4
FE_{UA} (%)	N	7.20	N	N	N	N	<7
TRP (%)	74	N	70	N	N	N	>85
U_{zinc} (μg/dL)	1155	1228	1786	1004	1200	980	137-720
BMD L_1-L_4 (g/cm^2)	1.133	0.841	0.908	1.117	0.763	0.973	>1.1
BMD T score*	+0.39	−1.87	−1.66	+0.24	−2.98	−1.07	>−1
Presentations	—	—	—	—	Renal stone	Renal stone	

NOTE. To convert Hb in g/dL to g/L, multiply by 10; hematocrit in % to proportion of 1.0, multiply by 0.01; serum magnesium in mg/dL to mmol/L, multiply by 0.411; serum phosphorus in mg/dL to mmol/L, multiply by 0.323; serum uric acid in mg/dL to μmol/L, multiply by 59.48; U_{zinc} in μg/dL to μmol/L, multiply by 0.153.

Abbreviation: N, within normal limits.

*−1 and −2.5, osteopenia; ≤−2.5, osteoporosis.

Hb levels, suggesting renal tubular dysfunction might have developed secondary to anemia. In this context, altered cell function caused by reduced oxygen delivery to renal tubular cells may be a key factor. Experimental evidence has established that anemia, even under normal blood flow conditions, could lead to kidney hypoxia.[10,11] Furthermore, it has been suggested that the most striking anemia-related morphological change in tissues from anemic rats is damage to the proximal tubule caused by focal proximal tubular necrosis in the renal cortex.[12] Similarly, in a more recent study, proximal tubular dysfunction was shown in patients with iron-deficiency anemia.[13] In addition, increased iron turnover from low-grade hemolysis of microcytic erythrocytes evident with increased LDH levels may be another factor that contributes to proximal renal tubular dysfunction in patients with β-thalassemia minor. Also, urinary excretion of zinc, sodium, and uric acid correlated positively with serum LDH levels. Thus, tubular iron load or other red blood cell–derived toxins caused by hemolysis may disturb proximal renal tubular function.

Several studies of renal involvement in patients with β-thalassemia major reported a high frequency of proximal tubular dysfunction.[2-4] Likewise, renal proximal tubular dysfunction recently was reported in patients with α-thalassemia major.[14] In various forms of thalassemia, shortened red blood cell life span, rapid iron turnover, multiple blood transfusions, desferrioxamine toxicity, iron overload, and tissue deposition of excess iron are contributing factors to functional and physiological abnormalities.[2-4,15] However, our patients did not have severe anemia, as seen in major thalassemias, or such independent influencing variables as iron overload, multiple blood transfusions, and desferrioxamine toxicity.

Renal tubular dysfunction has some long-term effects, such as renal stone and reduction in bone mass in our patients. It was suggested that β-thalassemia minor is not a risk factor for osteoporosis.[6] Therefore, it is highly probable

that reduction in bone mass is related to urinary loss of such bone minerals as calcium, magnesium, and phosphorus. Furthermore, it is well known that chronic hypercalciuria is associated with renal stone formation.

This study has some limitations. First, it lacks a more specific test for tubular dysfunction, such as *N*-acetyl-β-glucosaminidase measurement or urine electrophoresis. Another limitation of this study is that no specific parameter of oxidative stress is available, although increased U_{zinc} levels serve as an indirect evidence of oxidative stress. Finally, urinary excretion of β_2-microglobulin as evidence of renal tubular proteinuria was studied only in patients with tubulopathy. Future studies addressing this issue should take these points into consideration.

In conclusion, proximal renal tubular dysfunction is not rare in patients with β-thalassemia minor.

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