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ORIGINAL ARTICLE

Early markers of renal dysfunction in patients with beta-thalassemia major

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Abstract Studies of renal involvement in thalassemia syndromes have been varied and few. The most important cause of mortality and morbidity in these patients is organ failure due to iron deposition. We report here a cross-sectional study carried out between February 2005 and February 2006 on all beta-thalassemia major patients being treated in Mofid Children's hospital, Tehran. The aim of the study was to detect renal dysfunction in these patients. The patient cohort consisted of 103 patients with various disease severities. Fresh first morning urine samples were collected and analyzed for sodium (Na), potassium (K), calcium (Ca), creatinine (Cr), phosphate, uric acid (UA), *N*-acetyl beta-D-glucosaminidase (NAG) and amino acids. We also carried out a complete blood count evaluation and assayed fasting blood sugar and serum ferritin, sodium, potassium, creatinine, uric acid and amino acids in all patients. The mean age of our patient cohort was 12.5±5.53 years and 53.4% were female. Abnormal levels of urinary NAG were detected in 35.9% of patients (confidence interval 26–45%). Abnormal levels of fractional excretion (FE)-Na, FE-K and FE-UA and abnormal urine protein Pr/Cr and urine Ca/Cr ratios were present in 29.1, 7.8, 52.4, 0.3 and 22.3% of the patients, respectively. There was a significant relationship between urinary NAG and the age of the patient (R=0.35), duration of deferoxamine therapy (R=0.31), duration of receiving blood transfusions (R=0.34) and level of fasting blood sugar (R=0.2). We concluded that renal disorders are not rare in patients with beta-thalassemia major and that they may increase in terms of frequency with age, increased duration of transfusion and deferoxamine usage and high levels of blood sugar.

Keywords Beta-thalassemia major · Iron overload · NAG (*N*-acetyl beta-D-glucosaminidase) · Tubulopathy

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Introduction

There is little information available on renal involvement in patients with beta-thalassemia major [1–3], and although the main underlying cause of tubular dysfunction in these patients remains unknown [3], renal damage can be attributed to chronic anemia, iron overload and deferoxamine therapy. Excess free iron is known to be a catalyst of lipid peroxidation, which damages cells [4]. *N*-acetyl beta-D-glucosaminidase (NAG) is a proximal renal tubular protein that is excreted in the urine during tubular damage. Glomerular damage can also occur in these patients due to recurrent infections and the repeated use of deferoxamine (desferal), resulting in a decreased ability of the kidneys to clear immune complexes [5]. Although open to discussion, there appears to be a direct relationship between the duration

of the disease and increased renal complications [1, 4, 5]. The presence of severe anemia along with tissue deposition of iron due to multiple transfusions is the main cause of multiple organ dysfunction, especially in the cardiovascular, endocrine and hepatic systems.

There are about 20,000 beta-thalassemia patients in Iran [6]. The aim of this study was to evaluate the prevalence and nature of renal involvement in beta-thalassemia patients and to correlate the findings with clinical parameters, iron status and the duration of deferoxamine therapy.

Material and methods

This cross-sectional study was carried out on 103 confirmed patients with of beta-thalassemia major who had been referred to the Clinic of Hematology/Oncology of Mofid Children's Hospital during a 1-year period (February 2005 to February 2006) and 64 age- and sex-matched healthy volunteers. The diagnosis of beta-thalassemia major was based on standard criteria. The glomerular filtration rate (GFR) and results of a kidney ultrasonography were normal in all of the patients. Demographic data, including age, sex, duration of transfusion therapy and deferoxamine therapy, were gathered on all patients. Fresh first morning urine samples were collected and analyzed for sodium, potassium, calcium (Ca), creatinine (Cr), phosphate, uric acid, NAG and amino acids. A blood sample from each patient was collected for a complete blood count evaluation and assays of fasting blood sugar (FBS), serum ferritin, sodium, potassium, creatinine, uric acid and amino acid levels. A colormetric method was used to measure NAG (Diazyme, General Atomics, La Jolla, CA). The urinary ratios of NAG/Cr, Ca/Cr, protein (Pr)/Cr and fractional excretion of sodium, potassium and uric acid were also estimated. Applied laboratory methods were:

- urine and serum sodium, potassium, urea and FBS by the flame photometry method (Coning480)
- urine and serum creatinine by Jaffee method (RA 1000)
- urine and serum uric acid by the uricase method (RA 1000)
- urine and serum calcium by Timol Blue method (Hitachi-717)
- venous blood gas by the ion-specific electrode (ISE) method (AVL)
- urine and serum amino acid by the thin-lay chromatography (TLC) method
- ferritin by radioimmunoassay (RIA) method (Kavosh yar kit)
- urine protein by turbidometry method (spectrophotometer).

Data were analyzed using the SPSS statistic package. The chi^2 test, Spearman's correlation regression analysis and the *t* test were performed and the confidence interval (CI) was calculated. A *P* value of less than 0.05 was considered to be significant.

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Results

A total of 103 patients and 64 age- and sex-matched healthy volunteers were enrolled in this study. The age of the patients ranged from 1 to 24 years old (mean range 12.5± 5.53 years), and 53.4% were females. The mean GFR (evaluated by Schwartz formula) in our study group was 107.5±12.7 ml/min per 1.73 m^2. The mean systolic and diastolic blood pressure in the study group were 97±10.96 and 66.9±8.89 mmHg, respectively, and in all participants of the study, blood pressure was under the 95th percentile of height.

Urinary NAG was in the range of 0.5–86.6 IU/l and urinary NAG/Cr was 0.33±0.63 in patients and 0.06±0.12 in controls ($P<0.001$).

Other results are listed in Table 1. Anemia was detected in 95% of our patients, and 97.1% had high serum ferritin levels based on reference normal ranges of hemoglobin and ferritin [7]. Of the 167 participants, 13.16% had hyperglycemia, 2.9% showed hypoglycemia and the remainder were normoglycemic based on a reference normal range of FBS [8].

Abnormal levels of urinary NAG were detected in 35.9% of patients (CI 26–45%). Abnormal levels of fractional excretion of sodium (FE-Na), fractional excretion of potassium (FE-K) and fractional excretion of uric acid (FE-UA) were found and abnormal urine Pr/Cr and urine Ca/Cr ratios were present in 29.1, 7.8, 52.4, 89.3 and 22.3% of patients, respectively. An abnormal pattern in the urinary excretion of amino acids was also found in 12.6% of the patients. There was a significant difference in terms of mean age between patients with abnormal levels of urinary NAG and those with normal levels of this protein: 15.2 versus 10.9 years, respectively ($P<0.001$). There was a statistically significant relationship between urinary NAG and patients' age ($P<0.001$, $R=0.35$), duration of deferoxamine therapy ($P<0.001$, $R=0.31$), duration of receiving blood transfusions ($P<0.001$, $R=0.34$) and level of fasting blood sugar ($P<0.043$, $R=0.2$) (Figs. 1, 2, 3, 4). However, we found no difference between the urinary NAG/Cr ratio in hyperglycemic and normoglycemic patients. We also found a significant correlation between

Table 1 Patients characteristics (n=103)

Demographic and clinical characteristics	Mean ± standard deviation
Age (years; range 1–24 years)	12.5±5.53
Duration of deferoxamine therapy (years)	9.5±5.06
Transfusion duration (years)	10.7±5.38
Systolic blood pressure (mgHg)	97±10.96
Diastolic blood pressure (mgHg)	66.9±8.89
Hemoglobin level (g/dl)	9.23±0.95
Hematocrit (%)	27.7±1.9
Serum ferritin (mg/dl)	1976±1389
Serum urea (mg/dl)	19.8±8.93
Serum creatinine (mg/dl)	0.55±0.13
Serum sodium (mEq/l)	139.3±3.58
Serum potassium (mEq/l)	4.36±0.37
Serum calcium (mg/dl)	9.54±0.51
Serum phosphate (mg/dl)	4.87±0.83
Serum uric acid (mg/dl)	4.52±1.29
Fasting blood sugar (mg/dl)	105.4±33.78
Serum bicarbonate (mg/dl)	24.7±2.75
Urine sodium (mEq/l)	146.6±68.24
Urine potassium (mEq/l)	59.7±37.24
Urine calcium (mg/dl)	10.7±6.8
Urine phosphate (mg/dl)	85.7±54.9
Urine uric acid (mg/dl)	73.7±27.1
Urine protein (mg/dl)	46.2±35.2
Urine NAG (IU/l)	13.69±13.5
Urine NAG/creatinine	0.33±0.63

NAG, *N*-acetyl beta-D-glucosaminidase

the urinary Pr/Cr and urinary NAG/Cr ratios by Spearman's correlation analysis and the ANOVA test (R=0.624, R^2 = 0.390, P<0.00) (Fig. 5). However, there was no relationship between patients' urinary NAG and sex, level of hemoglobin, ferritin and other variables.

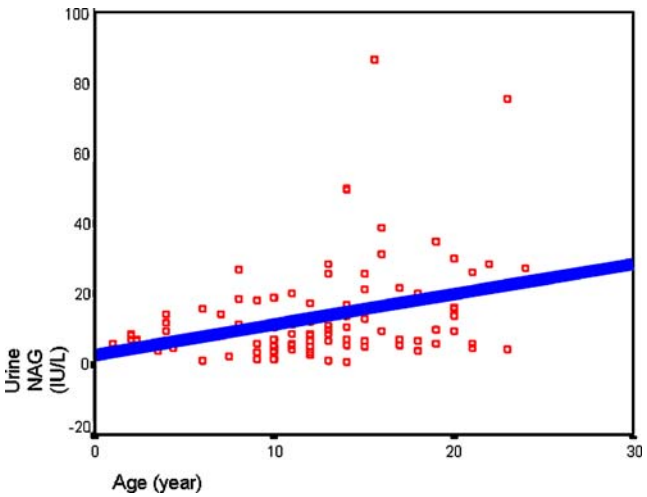

Fig. 1 Relationship between urinary *N*-acetyl beta-D-glucosaminidase (NAG) and age (P<0.001, R=0.35)

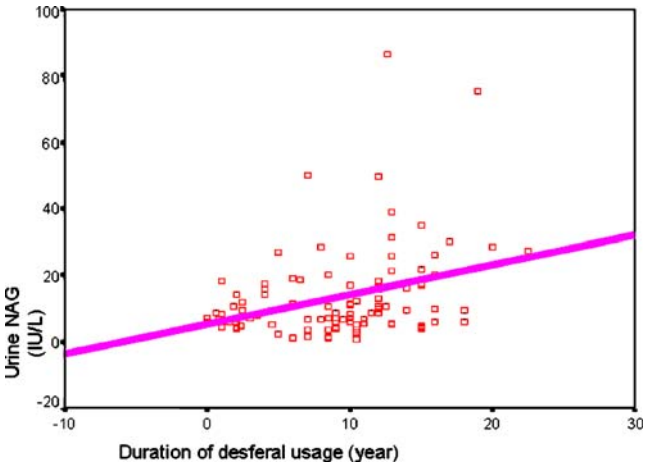

Fig. 2 Relationship between urinary NAG and duration of deferoxamine (desferal) therapy (P<0.001, R=0.31)

Discussion

The significant results of our study are as follows.

1. Urinary excretion of NAG was found to be abnormal in more than one third of the patients. Since urinary excretion of NAG is a sensitive marker of proximal tubular damage, we can conclude that more than one third of our patients are afflicted with renal tubular damage.
2. Proteinuria was found in 89.3% of our patients; this condition was significantly correlated with the urinary NAG/Cr ratio.
3. Uricosuria was found in 52.4% of our patients and natriuresis in 29.1%; both of these are other markers of proximal tubular dysfunction.

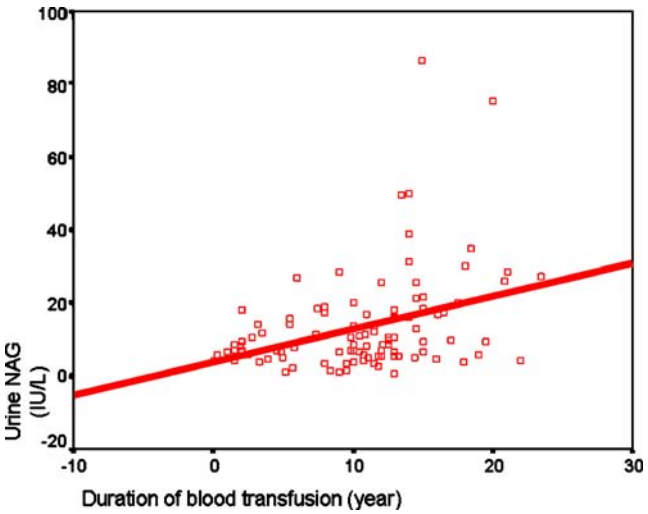

Fig. 3 Relationship between urinary NAG and duration of receiving blood transfusion (P<0.001, R=0.34)

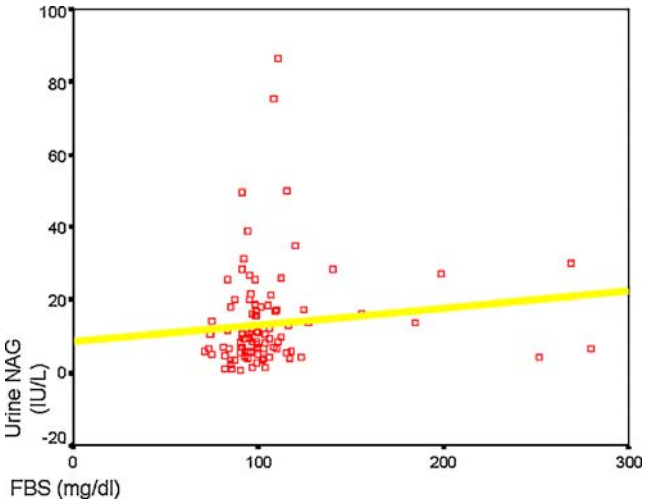

Fig. 4 Relationship between urinary NAG and level of fasting blood sugar (*FBS*) ($P<0.043$, $R=0.2$)

4. The older patients and those who had received deferoxamine and blood transfusions for relatively longer periods and those with higher levels of FBS were more prone to develop renal dysfunction.
5. Renal dysfunction may occur in beta-thalassemia major patients showing no clinical symptoms and before the manifestation of any other complications.

Our study indicated that the urinary NAG/Cr ratio is significantly correlated with the urinary Pr/Cr ratio; as such, urinary NAG can be considered to be a sensitive and reliable index of proximal tubular toxicity and a possible predictor of proteinuria. Our findings are in accordance with those of previous studies in thalassemic patients. In 1998, Lena evaluated the rate of proteinuria, aminoaciduria, urine osmolality and electrophoresis of urinary proteins in 95 thalassemia patients in Thailand and found that excretion of urinary NAG and B_2 microglobulin was markedly higher in these patients than in the control group [1]. Cianciulli and colleagues evaluated renal function in 19 beta-thalassemia patients in Italy and found evidence of renal tubular damage in 13 patients [9]. Sumboonnanonda et al. in a similar study demonstrated the renal function of 34 alpha-thalassemia patients and found that levels of urinary NAG, B_2 microglobulin and malondialdehyde (MDA) were elevated in this group relative to the controls. These researchers showed that the ratio of Pr/Cr was higher than normal in 60% of patients and that two patients had generalized aminoaciduria [3]. Katopodis et al. examined renal abnormality in patients with sickle cell beta-thalassemia and concluded that proteinuria and microalbuminuria in their study group may be related to prolonged glomerular hyperfiltration and glomerulosclerosis. These phenomena are prostaglandin mediated and have been attributed to chronic anemia [10]. *N*-acetyl beta-D-glucosaminidase is a widely distributed lysosomal enzyme contained in the tubular epithelial cells and released in the urine as a result of tubulo-toxicity of proteinuria in the early stages of idiopathic membranous nephropathy, glomerular hypertrophy, focal segmental glomerulosclerosis and minimal change disease. Since NAG is not of plasmatic origin and is not filtered through the glomeruli, the enhanced urinary NAG is due to tubular dysfunction and tubular proteinuria [11]. The underlying mechanisms for tubulopathy in patients with major beta-thalassemia include long-standing anemia, chronic hypoxia, iron overload and deferoxamine toxicity. Previous studies of renal involvement in these patients reported a high frequency of proximal tubular dysfunction and increased prostaglandin secretion; they also provide evidence suggesting that these two conditions are induced by oxidative stress and lead to tubular ischemia, medullary fibrosis, glomerular hypertrophy, increased glomerular permeability and proteinuria [5, 10, 12].

We also showed that 52.4% of our patients had uricosuria and 29.1% had natriuresis. In the study carried out by Aldulak et al. in Turkey, there was a significant difference in the serum levels of potassium, phosphate and uric acid and in the urinary ratio of Pr/Cr and NAG/Cr between beta-thalassemic patients and the control group [4]. In 2003, Qzcay evaluated patients with iron-deficiency anemia and showed that mean fractional excretion of sodium and mean urinary NAG were significantly higher in the patient group than in controls [13]. 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 acids and acid uric [14]. Changes in the reabsorption of these factors may be an indication of renal tubular dysfunction.

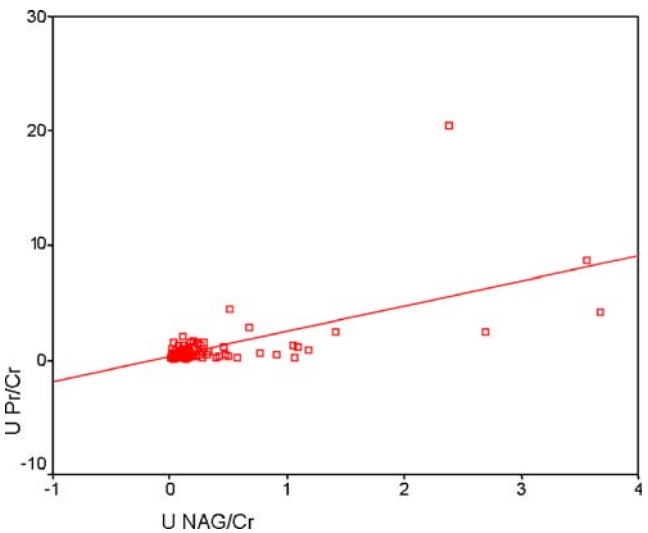

Fig. 5 Relationship between the urinary protein/creatinine (*Pr/Cr*) and urinary NAG/Cr ratios ($P<0.001$, $R=0.624$)

We did not adjust the level of urinary solutes with dietary intake so the differences observed in fractional excretion of salts may simply be due to variances in dietary intake.

We also reported a statistically significant relationship between urinary NAG and patients' age, the duration of deferoxamine therapy and the duration of receiving blood transfusions. These findings suggest that both iron overload and deferoxamine can induce tubular dysfunction. Advinok evaluated the urinary excretion of zinc and NAG and showed that urinary zinc excretion was significantly higher in patients receiving deferoxamine than in the control group, whereas urinary zinc excretion in the patients not given deferoxamine was not different from the controls. Urinary NAG indices were significantly higher in the patients than in the controls and urinary zinc excretion was correlated with the urinary NAG indices [15]. Koren showed a clinically significant decrease in GFR in thalassemic patients receiving deferoxamine administered subcutaneously [16]; in contrast, Koliakos and Sumboonnanonda found no detrimental effect of deferoxamine on renal function [11, 17] and suggested that renal dysfunction in these patients could be a result of chronic anemia and iron overload.

Surprisingly we found that there was a statistically significant relationship between urinary NAG levels and the level of FBS, although only 13.16% of our patients had hyperglycemia and there was no significant difference in the urinary NAG levels between hyperglycemic and normoglycemic patients. We conclude that there is no need for a hyperglycemia state in terms of inducing tubular dysfunction and that the upper limit of glucose level may also cause tubular dysfunction.

The results of these studies suggest that the cause of renal dysfunction in patients with homozygous beta-thalassemia is iron overload and deferoxamine therapy. 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. The mechanism leading to tissue damage is not known, but it may be related to increased oxidative stress secondary to iron deposition in tissues, as indicated by the raised levels of serum and urine MDA [1]. Consequently, kidney lysosomes appear to be a target and, possibly, a mediator of iron toxicity in the kidney [18]. We conclude that urinary NAG excretion can be considered to be a reliable index of the tubular toxicity and a possible predictor of proteinuria, aminoaciduria and eventual renal impairment in these patients.

Since about 20,000 patients with beta-thalassemia major are covered by the Iranian Association of Thalassemia patients and the cost of treatment is about $ 5000 per patient annually, it would see to be both necessary and cost effective to diagnose renal dysfunction in its early stages as an approach to prevent ongoing renal injury. Based on the results of our study and those of others, the usefulness of alternative drugs, such as deferiprone (L_1) instead of deferoxamine, should be studied further.

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