



Research Article

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Early markers of renal dysfunction in Syrian beta thalassemia major patients

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ABSTRACT

β-thalassemia syndromes are the most common inherited anemia disorders, caused by impaired β-globin chain synthesis. The patients with thalassemia major have severe anemia along with tissue deposition of iron, due to multiple transfusions, which is the main cause of multiple organ dysfunctions, especially in the cardiovascular, endocrine and hepatic systems. The aim of this study was to evaluate renal function in beta thalassemia patients using conventional tests (as creatinine, urea, microalbuminuria) and early markers (as N-acetyl-beta-D-glucosaminidase) of renal dysfunctions, and to correlate results with clinical parameters. 170 β-thalassemia patients (aged 4-28 years) and 30 healthy controls (aged 4-28 years) were enrolled in this study. Blood samples were collected and serum was separated for measurement of creatinine, urea, and ferritin. Urine samples were also collected for measurement of albumin and N-acetyl-beta-D-glucosaminidase (NAG). There was a significant increase in serum creatinine, and Albumin: Creatinine ratio (microalbuminuria) in beta thalassemia patients when compared with their control ($p=0.017$ and $p<0.0001$, respectively). NAG activity levels were elevated in beta thalassemia patients compared with control group (mean \pm SD: 13.69 ± 10.85 U/L and 3.77 ± 1.86 U/L respectively) and were correlated to ferritin levels ($r=0.579$, $p<0.0001$). On the other hand, there were no significant changes in serum urea in beta thalassemia patients when compared with their controls ($p=0.480$). In thalassemia patients, Albumin: creatinine (A:C) and NAG showed significant positive correlation with blood transfusion ($r=0.578$, $p<0.0001$ and $r=0.734$, $p<0.0001$, respectively) and age ($r=0.586$, $p<0.0001$ and $r=0.735$, $p<0.0001$, respectively). As a Conclusion Renal tubular dysfunction is a common complication in beta thalassemia patients, without clinical manifestation of renal dysfunction. Therefore it is recommended to investigate presence of severe renal dysfunction in thalassemic patients, using sensitive and specific tests, mainly NAG, to prevent progress towards the complications.

Keywords: Urinary NAG, Beta-thalassemia major, Renal tubular dysfunction.

INTRODUCTION

Beta-thalassemia syndromes are a group of hereditary blood disorders, characterized by reduced or absent beta globin chain synthesis and accumulation of unpaired alpha-globin chain, resulting in reduced Hemoglobin (Hb) in red blood cells, decreased red blood cells production and anemia. The presence of severe anemia along with tissue deposition of iron, due to multiple transfusion, is the main cause of multiple organ dysfunction, especially in the heart, liver, and endocrine glands(1,2).

Little attention has been paid to the possible involvement of the kidney in patients with beta thalassemia major. However recent studies outlined the presence of different tubular abnormalities in thalassemia major before clinical signs develop(3). Renal dysfunction in these patients is not fully understood and seems to be multifactorial; attributed mainly to long-standing anemia, chronic hypoxia, iron overload, and toxicity of iron chelators. Moreover, several studies reported increased urinary excretion of early markers of proximal tubular damage in a considerable number of

patients with beta thalassemia major including N-acetyl-b-D-glucosaminidase (3-5).

NAG is a lysosomal enzyme which plays a role in the breakdown of glycoproteins in proximal renal tubular cells, although this enzyme is of high molecular weight (140 KD), it is considered mainly as a marker of renal tubular function. NAG is secreted by tubular epithelium, its measurement has been undertaken in a variety of diseases associated with renal injury, such as thalassemia major patients(6-9), glomerulonephritis(10), lupus(11), diabetes(12), and rheumatoid arthritis(13). The urinary NAG levels are increased before the increase in serum creatinine and conventional marker in beta thalassemia patients (as urea, microalbuminuria)(8,9).

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(3-5).

The aim of this study was to evaluate renal function in beta thalassemia major patients, using conventional and early markers (NAG) of renal dysfunctions, and to correlate results with clinical parameters.

EXPERIMENTAL SECTION

A total of 170 patients of age ranged 4-28 years old, all are transfusion dependent beta thalassemia patients attending the thalassemia center in Ministry of health in Damascus, Syria.

The study also included 30 healthy subjects, age and sex matched to patients group (4 to 28 years), as control group.

Fresh first morning urine sample from each patient were collected and divided into two tubes, then analyzed for albumin, creatinine and NAG activity. Blood sample from each patient was collected to measure urea, creatinine and serum ferritin. The same were carried out with controls

Applied laboratory methods were:

- ✓ N-acetyl-BD-glucosaminidase (NAG) was measured by a colorimetric assay.
- ✓ Urine and serum creatinine by Jaffee method.
- ✓ Serum urea by Urease method.
- ✓ Microalbuminuria by Immunoturbidimetric assay (Albumin:Creatinine ratio)
- ✓ Serum ferritin by Immunoenzymatic colorimetric assay.

Statistical analysis was done by SPSS13 program.

The mean, standard deviation and range were calculated.

Student t test was used for comparative analysis between groups, and Pearson's correlation coefficient (r) was applied for the correlation study.

Frequency of renal abnormalities among patients was calculated at cut-off levels, corresponding to mean \pm 2SD of healthy controls, and Chi-Square test was applied to compare different frequencies. For all tests, a probability (P) < 0.05 was considered significant.

RESULTS

The study was conducted on 170 patients with beta thalassemia major and 30 healthy controls. The cases and controls were age and sex matched (mean \pm SD: 14 ± 5.4 years and 13.9 ± 6 years, respectively). Patients with beta thalassemia major consisted of 100 males and 70 females. In the control group, there were 15 males and 15 females (Controls).

The biochemical data of patients and control group are summarized in Table (1). As demonstrated in this table, mean serum creatinine levels were increased in beta thalassemia major patients, when compared to controls, but were within normal limits.

There was no statistical significant difference in the mean values of serum urea levels between studied groups.

Mean values of Albumin: Creatinine were significantly increased in beta thalassemia major patients when compared to controls ($P < 0.0001$).

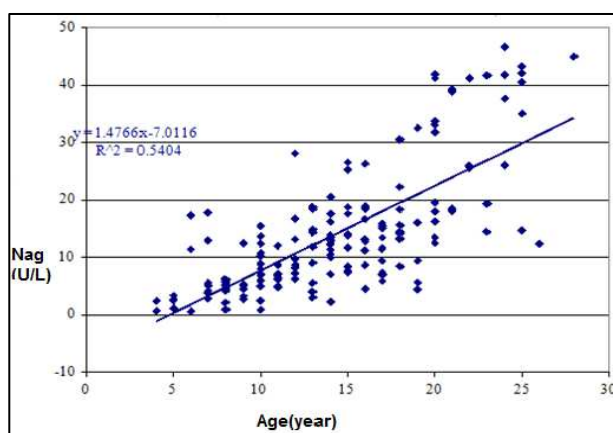
The mean values of NAG activity between thalassemic patients and controls (13.69 U/L versus 3.77 U/L) were compared and the significant difference was found ($P < 0.0001$).

Table 1: Biochemical parameters of β -thalassemia major patients and controls

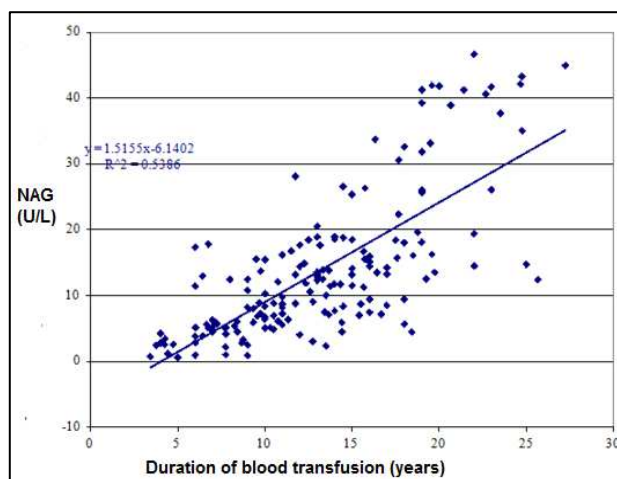
parameters	Patients with thalassemia major n=170	Controls n=30	P-value
Serum Creatinine (mg/dl)	0.521 \pm 0.09	0.481 \pm 0.06	0.017
Serum urea (mg/dl)	26.21 \pm 7.14	25.27 \pm 3.22	0.480
Albumin: Creatinine (mg/g)	13.44 \pm 14.03	2.67 \pm 3.59	<0.0001
Urine NAG (U/L)	13.69 \pm 10.85	3.77 \pm 1.86	<0.0001
Serum ferritin (ng/ml)	3060.59 \pm 1005.03		

We found a positive correlation between Microalbuminuria and each of age ($r = 0.586$; $P < 0.0001$) and duration of blood transfusions ($r = 0.578$; $P < 0.0001$).

There was a significant positive correlation between NAG and each of age ($r = 0.735$; $P < 0.0001$), duration of blood transfusions ($r = 0.734$; $P < 0.0001$) (Figs.1,2). However, there was no significant correlation between age, duration of blood transfusions and each of serum urea, creatinine.



Figure(1): Correlation between urinary NAG and age in patients of Beta thalassemia major



Figure(2): Correlation between urinary NAG and duration of blood transfusion in patients of Beta thalassemia major

The results show that increase in serum ferritin is significantly correlated with the increase in NAG activity ($r = 0.579$; $P < 0.0001$), but we found no significant correlation between ferritin and each of Albumin: Creatinine ratio, serum urea and creatinine ($P = 0.231$, $P = 0.387$ and $P = 0.203$, respectively)

The frequencies of renal abnormalities were calculated in the studied patients at cut-off levels, corresponding to mean \pm 2SD of healthy controls. The most-prevalent renal abnormalities were in NAG (47.6%), and microalbuminuria (30%) (Table 3).

Comparative statistics of the calculated frequencies of renal abnormalities (Chi-Square test) revealed the presence of a significant difference between thalassemic patients and controls ($\chi^2= 12.081$; $P<0.001$)

Table 3. Frequency of renal abnormalities among thalassemic patients

parameters	Number of affected patients /(total)	%
Serum Creatinine (Mean 0.7-1.2 mg/dl) (Woman 0.5-0.9 mg/dl)	0/170	0
Serum urea (10-50 mg/dl)	1/170	0.6%
Microalbuminuria (<30 mg /g of creatinine)	51/170	30%
Urine NAG (<12 U/L)	81/170	47.6%

DISCUSSION

Serum levels of Cr was significantly higher in patients with Beta thalassemia major than controls ($P=0.017$), but were within normal limits. we observed that we cannot rely on serum creatinine levels to assess renal dysfunctions in beta thalassemia major patients. In this respect, Mensi K et al.(2013)(14) and Ismail N et al.(2012)(15) reported high serum level of Cr in beta thalassemia patients. In contrast, Doddamani P et al.(2012)(16), have found normal serum creatinine in beta thalassemia patients.

These discorded results could be explained by the difference in age and iron chelation therapy in studied groups. In consistence with Hamed E et al.(2010)(8),we didn't find any relation of serum creatinine with age, meanwhile, Fathi F et al.(2013)(17),found a positive correlation between age and Cr. These discorded results could be explained by the difference in age in studied groups.

Serum urea was within normal range in patients with Beta thalassemia major, no significant difference was found between patients and controls ($P=0.480$). It seems that no apparent deterioration in glomerular function regarding the filtration of urea, was detected in beta thalassemia patients. Similarly, Fathi F et al.(2013)(17) and Yonus Z et al.(2012)(18) ,found normal serum urea in beta thalassemia patients. In contrast, Mensi K et al.(2013)(14) and Ismail N et al.(2012)(15) reported high serum level of urea in beta thalassemia patients. These discorded results could be explained by the difference in age and iron chelation therapy in studied groups.

Microalbuminurea was present in 30% of our patients. concordance, Hamed et al.(2010)(8) and Doddamani P et al.(2012)(16), found microalbuminuria in β TM patients. Tantawy A et al.(19) reported microalbuminuria in 29% of patients with beta-thalassemia. In contrast, Yonus Z et al.(2012)(18)found no significant change in the level of microalbuminurea between beta thalassemia patients and controls. These discorded results could be explained by the difference in age and serum Ferritin in studied groups.

In consistence with Doddamani P et al.(2012)(16), we found a positive correlation between age, duration of blood transfusions and microalbuminurea, meanwhile, Hamed et al.(2010)(8) found no significant correlation of microalbuminurea with age. These discorded results could be explained by the difference in age in studied groups.

The significant elevated excretion of NAG activity in beta thalassemia patients than those of controls ($P<0.0001$). urinary excretion of NAG was found to be abnormal in 47.6% of the patients. Since urinary excretion of NAG is a sensitive and reliable marker of proximal tubular damage. We can conclude that 47.6% of our patients are afflicted with renal tubular damage. In a similar way, Mohkan et al.(2008)(7)found elevated of urinary NAG in 35.9% of thalassemia patients. Jalali A et al.(2011)(9) reported high urinary NAG in 58.6% of patients with thalassemia. Smolkin V et al.(2008)(6) and Hamed et al.(2010)(8) found abnormal levels of urinary NAG in beta-thalassemia patients.

In consistence with Mohkan et al.(2008)(7) and Jalali A et al.(2011)(9) We found a positive correlation between age, duration of blood transfusions and NAG, meanwhile, Hamed et al.(2010)(8) found no significant correlation of NAG with age. These discorded results could be explained by the difference in age in studied groups.

There was no correlation of serum ferritin with serum creatinine, serum urea and microalbuminurea. Fathi F et al.(2013)(17)and Yonus Z et al.(2012)(18),did not find any correlation of these markers with serum ferritin.

In our results there was a positive correlation of serum ferritin with NAG. This suggest that iron overload can induce tubular dysfunction, excess free iron is known to be a catalyst of lipid peroxidation, which damage cells. Jalali A et al.(2011)(9) have reported that serum ferritin was positively correlation with NAG, meanwhile, Mohkan et

al.(2008)(7) and Smolkin V et al.(2008)(6) found no significant correlation of NAG with serum ferritin. These discorded results could be explained by the difference in serum ferritin in studied groups.

CONCLUSION

Renal dysfunction may occur in beta thalassemia major patients without clinical symptoms and before the manifestation of any other complications, these renal dysfunction may not be detected by routine tests. The older patients and those who are blood transfusions for relatively longer periods and those with higher levels of serum ferritin were more prone to develop renal dysfunction.

It is therefore recommended to detect the presence of severe renal dysfunction in thalassemic patients using sensitive and specific tests, mainly NAG, to prevent progress towards the complications. Urinary NAG excretion can be considered a reliable index of the tubular toxicity and a possible predictor of proteinuria.

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