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# Renal tubular function in β-thalassemia

**Achra Sumboonnanonda[1], Prida Malasit[2], Voravarn S. Tanphaichitr[1], Sompong Ong-ajyooth[3], Sunthorn Sunthornchart[1], Sa-nga Pattanakitsakul[4], Siripan Petrarat[1], Amara Assateerawatt[1], and Arun Vongjirad[1]**

[1] Department of Pediatrics, Siriraj Hospital, Mahidol University, Prannok Road, Bangkok 10700, Thailand
[2] Department of Medicine, Siriraj Hospital, Mahidol University, Prannok Road, Bangkok 10700, Thailand
[3] Department of Biochemistry, Siriraj Hospital, Mahidol University, Prannok Road, Bangkok 10700, Thailand
[4] Office for Research and Development, Siriraj Hospital, Mahidol University, Prannok Road, Bangkok 10700, Thailand

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**Abstract.** Studies of the renal involvement in thalassemic syndromes have been varied and few. This study was designed to define the renal abnormalities associated with β-thalassemia and to correlate the renal findings with clinical parameters. One hundred and four β-thalassemic children with various disease severity were studied. The patients were divided into three groups: 48 with severe anemia [hematocrit (Hct) $<25\%$], 31 on a hypertransfusion program and desferrioxamine treatment, and 25 with moderate anemia (Hct $>25\%$). The results were compared with 15 normal children. Significantly higher levels of proteinuria and low molecular weight proteinuria were found in all patients compared with normal children. Aminoaciduria was detected in one-third of patients. Thalassemic patients had significantly lower morning urine osmolarity, higher urine *N*-acetyl-β-D-glucoseminidase and malondialdehyde (MDA, an indicator of lipid peroxidation). Patients with severe anemia had significantly higher low-molecular weight proteinuria and MDA, and lower urine osmolarity than those with moderate anemia. Our data confirmed the high frequency of renal abnormalities in β-thalassemia patients and indicated some degree of proximal tubular dysfunction. Severity of the abnormalities correlated with the degree of anemia and were least severe in patients on hypertransfusion and desferrioxamine therapy. This suggested that the damage might be caused by *anemia and* increased oxidation induced by excess iron deposits.

**Key words:** β-Thalassemia – Hemoglobin E – Renal tubular function – Low molecular weight proteinuria – Malondialdehyde – Urine osmolarity

*Correspondence to:* A. Sumboonnanonda

## Introduction

Thalassemia hemoglobinopathies are prevalent in Thailand. The frequencies of β-thalassemia (thal) and hemoglobin (Hb) E, β-globin chain variant, are 3%–9% and 13%–60%, respectively [1]. The disease forms of β-thal are homozygous β-thal and β-thal/HbE. Half of the homozygous β-thal patients die before the age of 12 years due to severe infection, anemia, and multiple organ failure [2–4]. The abnormal synthesis of the globin chains or the production of abnormal Hb have wide impacts on the physiological functions of virtually every major organ. Patients with thal are known to have dysfunctions of the cardiopulmonary, reticuloendothelial, and other major systems [2–7]. Abnormalities of renal function have also been reported, but there is no systematic study of the prevalence and type of renal involvement. Various glomerular pathologies have been sporadically reported, and it is still unknown whether those abnormalities are genuinely associated with the thalassemic syndromes [3, 6, 8, 9]. One study of ten patients with Cooley's anemia suggested some abnormalities associated with renal medulla [10]. Renal tubular acidosis has also been reported in patients with thal [10, 11]. The purpose of this study was to systemically investigate the prevalence and nature of renal abnormalities in 104 patients with β-thal.

## Patients and methods

Patients aged between 3 and 15 years with homozygous β-thal or β-thal/HbE diseases attending the Department of Pediatrics, Siriraj Hospital from November 1993 through October 1994 were recruited into the study with informed consent from their parents. Patients with acute febrile illness were excluded. The diagnosis of β-thal was made by standard methods [12]. Height and weight standard deviation scores (SDS) were calculated according to Tanner et al. [13].

Patients were instructed to fast overnight before attending the clinic in the morning. Blood samples were collected from each patient for Hb and hematocrit (Hct), urea nitrogen, creatinine, and electrolytes. Fresh second-morning urine samples were collected; all samples were immediately aliquoted and frozen until further analysis. The remaining

**Table 1.** Summary of demographic and biochemical data of three groups of patients and controls[a]

| | Group A ($n$ = 48) | Group B ($n$ = 31) | Group C ($n$ = 25) | Controls ($n$ = 15) |
|---|---|---|---|---|
| Female/male | 21/27 | 13/18 | 15/10 | 5/10 |
| Age (years) | 9.9 ± 0.4 | 9.0 ± 0.5 | 10.2 ± 0.6 | 9.8 ± 0.9 |
| Height (SDS) | –2.5 ± 0.2 | –1.0 ± 0.3 | –2.0 ± 0.3 | |
| Weight (SDS) | –2.2 ± 0.1 | –0.9 ± 0.3 | –1.5 ± 0.2 | |
| Ratio of patients with intact spleen/splenectomized patients | 31/17 | 23/8 | 20/5 | 15/0 |
| Serum ferritin (ng/ml) | 1,078.9 ± 178.8 | 3,293.6 ± 343.8 | 1,869.8 ± 385.3 | |
| Estimated glomerular filtration rate[b] (ml/min per 1.73 $m^2$) | 137.8 ± 4.5 | 114.2 ± 3.5 | 126.3 ± 4.5 | |
| Serum creatinine (mg/dl) | 0.5 ± 0.02 | 0.6 ± 0.02 | 0.6 ± 0.02 | |
| Serum $HCO_3$ (mEq/l) | 23.0 ± 0.3 | 24.4 ± 0.4 | 24.2 ± 0.5 | |
| Urine urea N (mg/dl) | 548.8 ± 41.5** | 611.3 ± 53.9** | 585.2 ± 60.4** | 1033.3 ± 101.7 |
| Urine osmolarity (mosmol/kg) | 630.3 ± 25.6** | 739.1 ± 28.5 | 682.3 ± 40.9* | 836.6 ± 70.7 |
| Urine protein (mg)/mg Cr | 0.27 ± 0.03* | 0.20 ± 0.05 | 0.22 ± 0.04* | 0.07 ± 0.01 |
| Urine LMW protein (mg)/mg Cr | 0.15 ± 0.02** | 0.10 ± 0.02* | 0.12 ± 0.02* | 0.03 ± 0.00 |
| Urine NAG (unit)/g Cr | 42.3 ± 6.2* | 32.3 ± 5.4* | 26.1 ± 5.2* | 3.5 ± 0.4 |
| Urine MDA (nmol)/mg Cr | 0.056 ± 0.008** | 0.025 ± 0.003 | 0.050 ± 0.008** | 0.013 ± 0.001 |

SDS, Standard deviation score; $HCO_3$, bicarbonate; *N*, nitrogen; Cr, creatinine; LMW, low molecular weight; NAG, *N*-acetyl-β-D-glucosaminidase; MDA, malondialdehydr

*$P$ < 0.05; **$P$ < 0.001

[a] Values are mean ± standard error

[b] As calculated by Schwartz's formula: 0.55 × height (cm)/plasma Cr (mg/dl)

[c] Group A consists of patients with severe anemia; group B are on hypertransfusion with desferrioxamine treat; group C are those with mild anemia

urine was tested for osmolarity, protein, sugar (by Labstix, Bayer Diagnostics) and examined microscopically. *N*-Acetyl-β-D-glucosaminidase (NAG) (by a spectrophotometric method [14], creatinine, (Jaffe reaction, autoanalyzer), and amino acids (by paper chromatography [15] were measured. Urine malondialdehyde (MDA) was measured by a spectrophotometric technique described by Knight et al. [16]. Urinary protein was assayed by a modified Bradford method [17] (Bio-Rad Laboratories, Richmond Calif., USA). The urine was electrophoresed in 8%–15% polyacrylamide gels and silver stained [18]. The stained gels were scanned in a flat-bed scanner, and the amount of low molecular weight (LMW) proteins was calculated from the proportion of the protein bands smaller than 45 kilodaltons [19]. Fresh morning urine from 15 healthy children of the same age group were used as controls.

*Statistical methods.* All calculations were carried out using Statview statistical package (Abacus Concepts, USA). Comparison between groups was performed using unpaired Student's *t*-test and in categorical data using the chi-squared test. A $P$ value of less than 0.05 was regarded as significant.

## Results

One hundred and four patients comprising 27 homozygous β-thal and 77 β-thal/HbE patients were included in this study. Mean age was 9.7 years with a range of 3–15 years. Thirty patients were splenectomized (mean age 10.7 years vs. 9.3 years of those with intact spleens). The patients were divided into three groups. Group A included 48 patients with severe anemia (Hct less than 25%), group B (31 patients) were on a hypertransfusion program (maintaining the Hct at around 30% at all times) and desferrioxamine treatment (subcutaneous injection of 20–40 mg/kg per dose, 2–5 doses/week), and 25 patients of group C who had a Hct >25% and were not on desferrioxamine. The duration of desferrioxamine treatment was 3.7 ± 2.3 years (mean ± SD). The criteria for selection of patients for desferrioxamine and hypertransfusion treatments were not based on clinical severity, but on their financial status. Table 1 summarizes the demographic data of patients and controls. There was no significant difference in the proportion of patients with and without splenectomy amongst the three groups. None of the patients were positive for human immunodeficiency virus antibody and 2 patients were positive for hepatitis B surface antigen antigen.

Forty-one patients (39%) had a body weight <3rd percentile for age. Height and weight SDS are shown in Table 1. Blood urea nitrogen and serum creatinine were within normal limits in all except 1 patient from group B, who had a serum creatinine of 1.1 mg/dl (estimated glomerular filtration rate (GFR) using Schwartz's formula [20] 69 ml/min per 1.73 $m^2$). Using Schwartz's formula to estimate the GFR, all groups had values within the normal limit (89–165 ml/min per 1.73 $m^2$). Serum electrolytes were all within normal limits.

Urine osmolarity in all groups except group B was lower than normal controls ($P$ = 0.0005 for group A and $P$ = 0.02 for group C). Group A had the lowest urine osmolarity (Table 1). The urine urea level was lower than controls in all groups. Splenectomy did not affect urine osmolarity ($P$ = 0.08). Urine protein and sugar by Labstix were negative. Microscopic hematuria (10 red blood cells per high-power field) was also found in 8 patients (5 in group A, 2 in B, and 1 in C), but no renal casts were found. Generalized aminoaciduria was found in 32 of 102 patients. No difference in the proportion of patients with aminoaciduria was found amongst the three groups (chi-squared, $P$ = 0.6559).

All three groups had significantly higher levels of urine NAG than controls ($P$ < 0.05), but no difference was found amongst patients. The LMW proteinuria/creatinine in groups A, B, and C was significantly higher than controls ($P$ = 0.0002, 0.0390, 0.0062, respectively). Severely anemic (A) and mildly anemic (C) groups had significantly higher total urinary protein/creatinine ratios ($P$ = 0.0017 and

0.0398), but there was no difference between group B and controls ($P = 0.0595$). There was no evidence of glomerular proteinuria by electrophoresis. Urine MDA in groups A and C was significantly higher than controls ($P < 0.0001$ and 0.0003), but there was no difference between group B and controls ($P = 0.2132$) (Table 1).

## Discussion

We report a high frequency of renal abnormalities in a group of β-thal patients with disease of varying severity. The key abnormalities included: increased levels of proteinuria, especially LMW fraction, aminoaciduria, increased urinary NAG and MDA. Urine osmolarity was significantly lower in patients than controls. The data suggest some degree of proximal tubular dysfunction. Increased urine NAG [21, 22], aminoaciduria [23], and LMW proteins [24–27] are indicators of proximal tubular damage.

When patients were divided into three groups according to their clinical severity, patients receiving multiple blood transfusions and desferrioxamine treatment (group B) had lower levels of LMW proteinuria, urinary MDA, and higher urine osmolarity. The most pronounced abnormalities were found in group A, the group with the most-severe anemia (Hct <25%). MDA, the end product of lipid peroxidation, was highest in groups A and C, and correlated well with the clinical classification. This suggested that oxidative stress might be an important factor responsible for the damage. In thal, the imbalanced synthesis of Hb leads to the presence of excess unpaired globin chains and a high intracellular non-Hb iron content. The unstable Hb subunits generate free oxygen radicals, which start a chain of oxidative events leading to disintegration to denatured globin chains, heme and iron which bind to different membrane proteins, altering their normal structure and function [28]. The excess free iron is a catalyst of lipid peroxidation via participation in the Fenton reaction [29, 30]. The high urine MDA in groups A and C, and the normal levels in group B supports the hypothesis that lipid peroxidation occurs in untreated groups and can be reduced or reversed by desferrioxamine treatment [31]. The reduction of urinary MDA and milder renal manifestations in group B may also be due to the direct suppressive effect of desferrioxamine on peroxidation [32, 33]. Group B responded favorably to desferrioxamine treatment and frequent transfusions, despite high levels of serum ferritin. The latter indicates that the treatment given to this group was not adequate, i.e., not able to significantly reduce tissue iron. The improvement in renal function in group B might be due to the direct suppressive effect of desferrioxamine on peroxidation [28]. This is significant, since a comprehensive program of desferrioxamine chelation is not usually achieved in developing countries, due to economic constraints.

We found no detrimental effects of desferrioxamine on renal function, as reported by Koren et al. [34] and Cianciulli et al. [35]. The desferrioxamine dosage in our study is lower than in others in which nephrotoxicity has been reported. A decrease in the ability to concentrate urine was correlated with clinical severity. The group with severe anemia (A) had the lowest urine osmolarity after overnight fasting and the patients on desferrioxamine and hypertransfusion (B) had the highest urine osmolarities, which were not statistically different from controls (Table 1). These data are in accordance with those reported by others [6, 10–12]. The cause of this defect is not known. However, it is possible that malnutrition might play a role [36, 37], since two-thirds of the patients in group A were underweight. Paniagua et al. [38] demonstrated that malnutrition by itself did not cause the abnormalities, but other insults, such as electrolyte imbalances and infections, were responsible for the defects. All three groups had lower levels of urine urea (which generally reflects the degree of malnourishment) than controls, but patients in group B had a comparable ability to concentrate urine. This indicates that malnutrition is not the key factor leading to abnormal concentrating ability. Another possible contributory factor is the hyperperfusing effect of anemia [6]. The estimated GFR in groups A and C was high, although within normal limits; these findings support the above hypothesis. The fact that urine osmolarity was higher in patients on iron chelation suggests that the concentration defect is reversible and deposited iron and anemia might play a role in the pathogenesis of the defect.

Our findings are in accordance with previous studies in thal patients. Two studies have identified some degree of medullary fibrosis and suggested that the pathology might contribute to the abnormal concentrating ability [6, 10]. Generalized aminoaciduria was also reported by Hyman et al. [39].

In conclusion, our data indicate that there are proximal tubular dysfunctions in patients with homozygous β-thal and β-thal/HbE disease. The cause of this dysfunction is not known, but anemia and iron deposition may be key factors. The role of desferrioxamine as a protective agent for tubular damage is also suggested by our study.

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