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Original Research Article

Role of Urinary Microalbumin in Thalassemia Major Patients

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Abstract

Beta thalassemia is one among the commonest hereditary haemolytic disorders that result from defective synthesis of haemoglobin and ineffective erythropoiesis. Very less data is available in the early identification of renal involvement in patients with thalassemia. Renal impairement in thalassemic patients may be due to chronic anemia, chronic hypoxia, iron overload and iron chelators toxicity. The objective of the study was to estimate renal parameters in pediatric age group with transfusion dependent beta thalassemia major and to correlate the outcome with the degree of iron overload. The study consists of 30 diagnosed cases of beta thalassemia major aged between 3-18 yrs admitted in Vani Vilas Hospital attached to BMC&RI, Bangalore and 30 healthy age and sex matched controls. Blood samples were analyzed for hemoglobin, serum urea, creatinine and spot urine sample for microalbumin. Data analysis was done by Pearson's Correlation analysis and Student's t test. Mean age of cases was 8.9 years (SD=4.14) among whom 50% were females and 50% were males. Mean age of controls was 9.06 years (SD=3.5). Mean hemoglobin levels were decreased significantly ($p<0.01$) in cases when compared to controls. Mean urinary microalbumin excretion was significantly increased ($p<0.01$) in cases as compared to controls and positively correlated with duration of chelation, suggesting renal damage. Therefore microalbuminuria is important to assess the risk of renal dysfunction in these patients.

Key words: Beta Thalassemia major, hemoglobin, microalbumin, anemia, chelation, iron overload, renal failure.

INTRODUCTION

Beta-thalassemia is a hereditary blood disorder in which there will be abnormalities in haemoglobin synthesis where in mutations of the β-globin gene gives rise to various degrees of defective β chain production, an imbalance in α/β-globin chain synthesis, ineffective erythropoiesis, and a spectrum of anemia[1]. Patients with β-thalassemia major present in the first year of life with profound anemia and subsequently require regular blood transfusions for their survival, as well as iron chelation therapy to treat transfusional iron overload and prevent end-organ damage. Patients with β-thalassemia intermedia present later in life with a milder form of anemia and are not transfusion dependent[2].

Due to multiple transfusions iron overload is becoming a common complication of thalassaemia syndromes. This leads to multiple organ damage and increased mortality [3,4]. Iron deposition in the various parenchymal tissues begins within 1 year of start of blood transfusion. Even though blood transfusions is mandatory in these patients with anemia, repeated transfusions is leading to iron overload as body cannot remove excess iron.

Due to the severity of thalassaemia, transfusions may have to be given regularly during acute exacerbations. Iron overload may be a consequence of both the transfused iron and increased absorption of iron by intestine from ineffective erythropoiesis. If left untreated, the progressive effects of iron overload lead to significant morbidity and mortality [5]. A single unit of red blood cells transfusion contains approximately 250mg of iron [6], while the body cannot excrete more than 1mg of iron per day. A patient who receives 25 units per year, accumulates 5 grams of iron per year in the absence of chelation therapy[7].

With increased lifespan of β-thalassemic patients, the effects of iron overload in the various organs like liver, pancreas, and heart is severe but the effects on kidney is not exactly known [8]. Renal impairement due to improper functioning of kidneys may occur in β-thalassemic patients without any absurd clinical symptoms or even before the

complications set in [9]. Renal impairement in thalassemic blood transfused patients may be due to chronic anemia, chronic hypoxia, iron overload and chelator toxicity[10].

Effective management of iron overload in these patients requires monitoring both for iron toxicity and the effects of excessive chelation [11]. Variant studies have shown that microalbuminuria is the early marker of kidney disease in which albumin traces is excreted in urine due to glomerular damage [12,13]. Microalbuminuria is an early indicator of renal dysfunction. Early diagnosis of patients with renal failure should be given utmost importance as it specify us to undertake specific measures that delays the renal damage progression in turn reducing the incidence of renal impairment[13,14].

The aim of this study was to determine the urinary microalbumin excretion along with serum levels of urea, creatinine and to correlate their levels with urinary microalbumin in β-thalassemia major patient, to correlate urinary microalbumin levels with blood transfusion and duration of chelation.

MATERIALS AND METHODS

The study included total of 60 subjects which included 30 cases of beta thalassemia major and 30 age and sex matched controls. Beta thalassemia was diagnosed by clinical examination and haemoglobin electrophoresis in the age group of 3-18 years who are on regular blood transfusions for two or more years admitted for blood transfusion to Thalassemia ward in the department of Paediatrics of Vani Vilas Hospital attached to BMC&RI, Bangalore. Exclusion criteria include other hemoglobinopathies such as sickle cell anemia, type1 diabetes mellitus, acute febrile illness and patients on diuretic medications. Ethical committee approval was obtained from the institution before starting the study. Written informed consent from parents/guardian of thalassemic children was obtained before collecting blood and urine samples. The investigations done were hemoglobin, serum urea, and creatinine and spot urine microalbumin. The same investigations were done in controls also and compared.

Venepuncture was done under aseptic precautions. 3ml venous blood was drawn prior to blood transfusion. The blood drawn was collected in 2 vacutainers, out of which 1ml was added in an EDTA vacutainer which was used for haemoglobin estimation using Beckman Coulter cell coulter 5 part and the remaining 2ml was collected in a plain vacutainer. It was allowed to clot and centrifuged at 5000 rpm for five minutes. The serum separated was then used to estimate serum urea, creatinine by fully automated random access chemistry analyzer Beckman Coulter AU480. 5ml of spot urine sample was collected in sterile containers without preservative. It was assayed for microalbumin in Mispai2 analyzer.

STATISTICAL ANALYSIS

Descriptive statistics was done to calculate the levels of haemoglobin, serum urea, creatinine, electrolytes and urinary microalbumin in both cases and control groups. Student's t-test was used to test the significance of difference in the parameters among the cases and controls. Pearson's correlation was applied to find correlation between renal impairement with duration of blood transfusion and chelation therapy in the study. The analysis of data was performed using IBM SPSS 21.0 and a p value of < 0.05 was considered significant.

RESULTS

The study was conducted in 30 patients with β-thalassemia major and 30 healthy controls aged 3-18 years. The mean age in cases was 8.9± 4.14 years and in controls was 9.06 ±3.5years. Cases consisted of 15 males and 15 females. Control group consisted of 16 males and 14 females.

The details of the findings are elaborated in Table1. As mentioned in the table, mean haemoglobin levels were significantly decreased in cases as compared to controls. There was no statistical significant difference in the mean values of urea levels between study groups. Mean serum creatinine levels were significantly decreased in cases when compared to controls.

Mean values of spot urinary microalbumin in cases was significantly increased when compared to controls, suggesting microalbuminuria in these patients.

Table 1: Mean±SD of different parameters among study groups

Parameters	Cases (30) M=15; F=15 (Mean±SD)	Controls (30) M=16; F=14 (Mean±SD)	P value
Age (years)	8.9 ± 4.14	9.06 ±3.5	
Hemoglobin (g/dL)	7.52±2.09	12.18±0.74	0.00001***
Serum urea (mg/dL)	22.97±7.67	24.0±8.57	0.313
Serum creatinine (mg/dL)	0.29±0.15	0.38±0.09	0.0076***
Spot urinary Microalbumin (mg/L)	11.34±10.07	6.15±2.62	0.0041***

Microalbuminuria is positively correlated with number of blood transfusions ($r = 0.054$; $p < 0.05$) and duration of chelation ($r = 0.106$; $p<0.05$).

Table-2: Pearson's correlation of urinary microalbumin levels

Urinary microalbumin vs	r value	p value
Number of blood transfusions	0.054	<0.05*
Duration of chelation	0.106	<0.05*

SD= Standard Deviation
p = test of significance
*- p value <0.05- significant
**- p value <0.01- highly significant
***- p value < 0.001 – very highly significant

DISCUSSION

Renal involvement in β-thalassemia patients is related to the patient's age, severity of anemia, and frequency of blood transfusion. Urinary microalbumin is considered to be one of the early indicators of kidney disease which is inexpensive[13]. Hence, in this study we evaluated the levels of urinary microalbumin in β-thalassemia major patients and correlated the levels with other renal markers.

The normal glomerular filtration barrier is size and charge dependent and thus prevent albumin, globulin and other large plasma proteins to pass. Proteins with a molecularweight < 20,000 daltons pass in minute amounts through the glomerular capillary and get reabsorbed by the proximal tubular cells. Small molecules such as alpha2-microglobulin, β_2-microglobulin, apoprotein, certain enzymes, and peptides, are normally excreted in small amounts into the urine. Normally individuals excrete <150 mg/d of total protein and <30 mg/d of albumin [15-17]. Microalbuminuria is a condition in which urinary albumin excretion exceeds the normal limits but is less than clinical albuminuria (20 – 200 µg/min or 30 – 300 mg/24 h), which is 30 – 140 µg albumin/ml urine[18].

The underlying mechanism for renal dysfunctions in β-thalassemic patients may be caused by many factors like chronic anemia, chronic hypoxia, iron overload and deferoxamine toxicity[19]. Iron deposit in the kidneys cause glomerular damage leading to an increased filtration of large molecular weight (> 20.000 daltons) plasma proteins which exceeds the tubular capacity to reabsorb leading to microalbuminuria in β-thalassemia patients.

In the present study, there was no significant difference in mean serum urea levels in β-thalassemia major patients as compared to controls as shown in Table 1. Studies done by Aldudak *et al.* has shown that there was no significant difference between serum urea values in cases and controls which goes in accordance with our study[20].

The mean serum creatinine levels in cases was within the normal range as compared to controls but it was lowered significantly. This is in accordance with other studies [20-22]. The same results were obtained when compared between Thalassemia major and thalassemia intermedia patients [23,24]. Other studies [25, 26] reported that serum creatinine was within the normal range among Thalassemia major patients; but it was higher when compared to that of the controls. El-Alfyet *et al*. [27] found that patients with thalassemia major had significantly higher serum creatinine and blood urea nitrogen values, possibly due to higher iron deposition in their kidneys.

In this study, there was significant increase in excretion of the spot urinary microalbumin in cases as compared to controls which was in accordance with prior studies [21,22,28]. The probable mechanism of microalbuminuria may be an increased filtration of large molecular weight (> 20.000 daltons) plasma proteins which exceeds the tubular capacity of the kidney to reabsorb and increased free radical production and lipid peroxidation due to iron deposition in renal parenchyma leading to dysfunction. This suggests that glomerular dysfunction occurs in an earlier stage of the disease process.

In this study it was seen that microalbuminuria is positively correlated with number of blood transfusions and duration of chelation which indicates renal failure may be due to increased iron overload with each successive transfusion. This go hand in hand with other studies which shows that the renal dysfunction increase in β-thalassemia major patients with increased duration of blood transfusion and Desferoxamine usage [21,29]. Microalbuminuria is considered as a predictor of worse outcomes for kidney patients.

CONCLUSION

β-thalassemia major patients who are receiving regular blood transfusions revealed iron overload leading to renal failure. The high incidence of microalbuminuria is due to defective ability of the proximal tubular cells to reabsorb protein besides glomerular dysfunction. Therefore urinary microalbumin may be introduced as a screening test for early detection of enal disease in β thalassemia major patients.

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