

Study of biochemical parameters in beta thalassemia major patients

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Abstract

Children with thalassemia have various skeletal changes and effect on liver, lungs and kidneys. All these effect are due to ineffective erythropoiesis and hemochromatosis. All this leads to impairment of functions of various organs. The present study was conducted to assess the kidney functions with blood urea, serum creatinine, serum phosphates, serum sodium, serum potassium, urinary total protein and urine specific gravity in children's with beta thalassemia major.

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INTRODUCTION

Beta thalassemia is common form of hemoglobinopathy in India. Homozygotes for β thalassemia, which account for about one third of patients, β globin synthesis is absent. This condition is called as Beta thalassemia major. The synthesis of Hb A ($\alpha_2\beta_2$) is markedly reduced or absent, the imbalance of α and β globin production is more severe. Therefore free α – globin accumulates, and unpaired α –chains aggregate and precipitate to form inclusion bodies, destruction of immature developing erythroblasts within the bone marrow (ineffective erythropoiesis).¹ Studies of the renal involvement in thalassemic syndromes have shown few renal abnormalities and interstitial iron deposition and hemosiderin deposits in tubules.² This may lead to impairment of renal function which in this study was assessed with the biochemical parameters like blood urea, serum creatinine, serum phosphates, serum sodium,

serum potassium, urinary total protein and urine specific gravity.

AIMS AND OBJECTIVES

To assess the impairment in renal function in children with beta thalassemia major by basic biochemical parameters with effect of chelation therapy on it.

MATERIALS AND METHOD

The present study was carried out in Department of Biochemistry, Government Medical College, Nagpur from June 2004 to June 2006.

Selection of Cases

Cases were selected amongst the patients admitted in pediatric wards (wd. 3,5,6) of Government Medical College, Nagpur.

Inclusion Criteria

We selected 25 patients diagnosed as beta-thalassemia major by Hb electrophoresis. The patients were aged between 1 to 15 years. Patients were included irrespective of chelation therapy and splenectomy. Patients on regular blood transfusion therapy (at least one).

Exclusion Criteria

- Patients with history of hospitalization for more than 10 days due to fever and / or having convulsions.
- Patients with past history of renal involvement like acute glomerulonephritis, nephritic syndrome, acute renal failure and chronic renal failure.

- Patients with urinary tract infection.
- Patients with anemia due to other cause.
- Patients with past history suggestive of liver cell dysfunction.

Selection of Controls

25 healthy, normal children with age group of 1-15 years were selected.

COLLECTION OF SAMPLE

Fasting blood sample was collected in sterile plain bulb just before blood transfusion under all aseptic precaution

with informed consent. Sample was allowed to stand for clotting for 25 to 30 mins. Then serum was collected after centrifuging the sample for 10 min. Early morning urine sample was collected in sterile jar. Hemoglobin was estimated by Sahli's hemoglobinometer from finger prick. Serum Urea was estimated by Berthlot's method, serum creatinine by Modified jaffe's method, serum phosphorus by UV molybdate method, serum sodium and serum potassium by flame emission photometry and urinary total protein by End-Point Method (Dye binding method using pyragallol red).

Table 1:

Parameters Mean \pm SD	Controls	Patients	P Value
Hemoglobin levels(gm%)	12.1 \pm 0.97	6.42 \pm 0.96	<0.001***
Blood urea	33.72 \pm 6.58	34.76 \pm 6.93	>0.05
Serum creatinine	0.486 \pm 0.15	0.42 \pm 0.15	>0.05
Serum uricacid (mg%)	2.82 \pm 1.06	6.72 \pm 2.58	<0.001***
Serum phosphate(mg%)	4.71 \pm 0.49	2.48 \pm 1.59	< 0.001***
Sodium	138.24 \pm 4.74	158.6 \pm 17.02	<0.001***
Potassium	4.30 \pm 0.29	4.89 \pm 0.58	<0.001***
Urine Total Protein	202.05 \pm 104.34	901.84 \pm 554.80	<0.001***
Urine Ph	6.6 \pm 0.24	6.4 \pm 0.48	<0.05**
Urine Specific gravity	1.021 \pm 0.004	1.025 \pm 0.003	<0.001***

Table 2: Urinary protein by dipstick method in study subjects

Urine protein dipstick	Controls	Patients	p value
Nil	N=16(64%)	N=6 (24%)	0.004**
Trace	N=9(36%)	N=19 (76%)	

Table 3: Urine for RBC's in study subjects

Urine for RBC's	Controls	Patients	p value
Present	N=0	N=4 (56%)	<0.001***
Absent	N=25(100%)	N=11 (44%)	

DISCUSSION

Beta thalassemia represents a serious health problem because of its heterogeneous frequency and existing endogamy system. Anemia in Beta thalassemia is caused by ineffective erythropoiesis and premature hemolysis of red blood cells in peripheral circulation. Shortened RBC life span, rapid iron turnover and tissue deposition of excess of iron were major factors responsible for functional and physiological abnormalities found in Beta thalassemia major. Half of homozygous Beta thalassemia patients die before the age of 12 years, due to severe infection, anemia and multiple organ failure.^{3,4}

In the present study the basic renal function parameters were investigated, e.g. blood urea, serum creatinine, phosphates, uric acid, sodium, potassium, and malondialdehyde, urinary total protein, and urinary protein by dipstick method, urine specific gravity and urine for RBC's. These chemical tests are indicative of impaired kidney function. Thalassemic patients in the present study had mean hemoglobin level of 6.42 \pm 0.96

gm/dl, while controls had 12.1 \pm 0.97 gm/dl. This indicated that cases enrolled were anemic due to hemolysis of immature RBC's and RBC's with inclusion bodies. Out of 25 patients, 23 thalassemic patients with moderate anemia had 5-8gm% hemoglobin whereas only 2 patients had very severe anemia <5 gm% hemoglobin. The findings in the present study were similar to those of Oktenli C *et al*⁵ and Cetin T.⁶ They also found hemoglobin levels being significantly reduced in cases as compared to controls. Aperia AC *et al*⁷ provided experimental evidence that anemia, even under normal blood flow conditions could lead to kidney hypoxia. In the present study the mean blood urea level in thalassemic patients was 34.76 \pm 6.93 mg/dl and in controls was 33.72 \pm 6.8 mg/dl. There was no statistical difference in thalassemic patients as compared with controls. Similar results were observed by Khalifa AS *et al*⁸, Sumboonnanda A *et al*,⁹ and Aldudak B *et al*.¹⁰ Creatinine is more sensitive marker of kidney function test than blood urea. Mean level of creatinine in patients

was found to be 0.42 ± 0.15 mg/dl, which was within normal limits. In controls the mean value was 0.48 ± 0.15 mg/dl. Our reports are similar to Khalifa A S *et al*⁸ and Aldudak B *et al*.¹⁰ They did not find any marked difference in serum creatinine in patients population. In the present study we found significant decrease in serum phosphate level in patients (2.48 ± 1.59 mg/dl) as compared to controls (4.71 ± 0.49 mg/dl). Our results are in agreement with the reports of Cetin T *et al*,⁶ they found hypophosphatemia and hypomagnesaemia with renal magnesium wasting in thalassemic patients. Lapatsanis P *et al*¹¹ found no difference in serum phosphorus levels with markedly high excretion of urinary phosphates and they explained this normal serum phosphate level by rapid erythrocyte turnover in patients with beta thalassemia. According to Litchman AM *et al*¹² and Jacob H *et al*¹³ hypophosphatemia may reduce the 2,3-DPG (diphosphoglycerate) and ATP content of red cells causing hypoxic stress and lead to severe hemolytic anemia.^{14,15} According to Lapatsanis P *et al*¹¹ the high incidence of negative phosphorus balance in thalassemic children may be due to decreased absorption from intestine or increased urinary excretion. They have studied that there was no evidence of malabsorption hence the thalassemic patients have an abnormally high phosphaturia, which may lead to phosphorus deficit. We found significantly high level of mean serum sodium (158.6 ± 17.02 mmol/L) in patients as compared to controls (138.24 ± 4.74 mmol/L). In contrast to our study, Aldudak B *et al*¹⁰ and Ong-ajyooth L *et al*¹⁶ did not find statistical difference in mean sodium levels in the patients as compared to controls while Sumboonnanda A *et al*⁹ found mean sodium levels within normal limits. According to Granner DK *et al*¹⁷ hypophosphatemia may be the stimulatory factor for 1 alpha-hydroxylase which is responsible for the conversion of 25-hydroxycholecalciferol [25(OH)D₃] to 1, 25-dihydroxycholecalciferol [1,25 (OH)₂ D₃]. According to Tenenhouse H *et al*¹⁸ increase in 1, 25-dihydroxycholecalciferol [1,25 (OH)₂ D₃] may lead to increased transcription of Type IIa protein receptor located in the apical plasma membrane of kidney tubules which in turn is responsible for sodium dependant phosphate co-transport. Type IIa sodium phosphate co-transport is electrogenic (i.e. it involves the inward flux of a positive charges), with three sodium ions and one phosphate ion (preferentially divalent) being transported. As a consequence there is increase in serum sodium level. Mean serum level of potassium was significantly higher (4.89 ± 0.58 mmol/L) in patients as compared to controls (4.30 ± 0.29 mmol/L), which may be due to massive blood transfusion and hemolysis responsible for increased retention of K⁺. Our finding supports the reports of

Aldudak B *et al*¹⁰ and Ong-ajyooth L *et al*¹⁶ who found increased mean potassium levels in patients than in controls but the difference was not statistically significant while Sumboonnanda A *et al*⁹ found serum potassium within normal limits. Urine protein by dipstick method is often used to measure protein in urine semiquantatively. Dipstick methods are more sensitive to albumin than to other plasma proteins. These are therefore excellent screening tests for glomerular proteinuria but unsatisfactory for detection of tubular or overload proteinuria. We observed traces of protein in urine 19 patients and in 6 patients there was no protein in urine by dipstick method, while in controls only 9 subjects had in traces in their urine. In the present study urinary total protein was significantly increased in thalassemic patients (901.84 ± 554.80 mg/day) as compared with controls (202.05 ± 104.34 mg/day). This indicates that the proteinuria may be glomerular or tubular, however urine protein by dipstick method (indicating glomerular proteinuria) found traces in thalassemic patients. If the proteinuria would have been glomerular for such high values of urine total protein (901.84 ± 554.80 mg/day), there would be parallel increase in protein by dipstick method, which was not so, suggesting that the proteinuria may be tubular. When tubular proteinuria occurs albumin excretion is increased slightly which explains the traces of protein present in urine of thalassemic patients. Our findings are in agreement with the previous study by Aldudak B *et al*.¹⁰ According to Sumboonnanda A,⁹ significantly high levels of protein, specifically low molecular weight protein indicates proximal tubular damage. Ong-ajyooth L *et al*¹⁶ found significant increase in urine protein in thalassemic patients than controls and specifically significant high levels of low molecular weight protein/creatinine ratio. These all reports may suggest proximal tubular damage, which might be secondary to oxidative lipid peroxidation by iron overload. We found increased levels of urinary total protein in control groups (n=9). (Normal value 28-141 mg/day) which may be due to physical exertion of the control subjects belonging to age groups 2 to 15 yrs. The average pH of urine is 6.0, reflecting the renal excretion of non-volatile acids produced by metabolic processes. In the present study though there was significant difference in urinary pH of patients (6.4 ± 0.48) as compared to controls (6.6 ± 0.24), the urinary pH was within normal limits. This suggests the ability of kidney to excrete variable amounts of acid or base was normal. Our findings were in agreement with Ong-ajyooth *et al*¹⁶ who found significant reduction in urinary pH (5.8 – 5.4) as compared to controls. Specific gravity can provide an indication of the concentration of urine. Concentration of glucose and protein contributes to the specific gravity in

urine. In the present study mean specific gravity in thalassemic patients (1.025 ± 0.003) was significantly elevated as compared to controls (1.021 ± 0.004). But the mean specific gravity in both, patients and controls was within normal limits. In the present study the urinary total proteins were significantly elevated in patients as compared to controls, which should contribute, to parallel increase in specific gravity in patients, which is not present. This may indicate the concentrating ability defect found in beta thalassemia. According to Keitel HG *et al*¹⁹ and Statius Van Eps LW *et al*²⁰ who proposed hypothesis concerning the pathogenesis of tubular defects such as the theory of increased blood flow through vasa recta which will disturb the effectiveness of countercurrent system described in anemia. The blood flow in vasa recta could cause a “wash-out” of medullary hyperosmolality with hypotonic urine. However, the increased amount of medullary fibrosis which was also observed in some renal biopsies of Cooley’s anemia could be an explanation for the poor urine concentrating ability. According to Landing BH *et al*²¹ and Sonakul D *et al*²² the defect in concentrating ability in thalassemic patients may be due to organic lesions of the nephron segments which regulate the countercurrent mechanism. In four thalassemic patients RBC’s were present in urine and absent in urine of all control subjects, which showed highly significant difference. Due to hemolysis present in β thalassemia there may be hemolysed RBC’s in urine. Urine is often dark brown reflecting the excretion of products of heme catabolism. Khalifa AS *et al*⁸ also found hematuria in 67% patients. In the present study, due to hemolysis in beta thalassemia major there was moderate anemia. The severe anemia was prevented in patients by frequent blood transfusions. Hypophosphatemia found in these patients may be secondary to renal tubular wasting. Increased levels of serum sodium may be a compensatory effect of hypophosphatemia. Whereas increase in levels of serum potassium may be due to increased erythrocyte turnover. Thus the present study indicates that renal damage may occur to certain extent in beta thalassemia major. Further studies are warranted and means of prevention of these defects should be urgently sought in view of protection of the thalassemic patients.

CONCLUSION

Thalassemia major although may not overtly damage the kidneys leading to renal failure but may induce certain subtle changes which will have a long-term impact on the kidneys.

The reason for these subtle changes may be:

- The oxidative stress at renal tubular level secondary to severe anemia, as demonstrated by higher MDA levels in thalassemia.

- Uric acid is most abundant aqueous antioxidant and contributes as much as two thirds of all free radical scavenging capacity in plasma. This may probably be the reason along with increased hemolysis for significant elevation of serum uric acid levels in thalassemia.
- Renal wasting of phosphate may be responsible for hypophosphatemia in thalassemia.
- The renal compensatory mechanism to correct hypophosphatemia may result in increase in activation of Na- dependant / phosphate co-transporter type IIa resulting in increased serum sodium levels in thalassemia.
- Premature hemolysis found in thalassemia may be responsible for elevated potassium levels.
- Low molecular weight proteinuria attributed to increased total urinary protein in thalassemia.
- Defect in urine concentrating ability resulted in significant difference in specific gravity in thalassemia as compared to controls.

Further studies are needed with special emphasis on tests for specific tubular dysfunction helping early diagnosis of renal damage and guiding the line of management of thalassemia patients.

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