

Oxidative stress in patients with beta thalassemia major

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Abstract

Thalassemia major is cause of severe anemia. Anemia is treated with repeated blood transfusion. All this leads to ineffective erythropoiesis and hemochromatosis. The present study was conducted to assess the severity of anemia and iron overload due to repeated blood transfusion leading to oxidative stress.

Keywords: Oxidative stress, Beta thalassemia major, Malondialdehyde (MDA).

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INTRODUCTION

Beta thalassemia major is the inherited defect in β – globin chain synthesis. Homozygotes for β thalassemia account for about one third of patients. This condition is called as defective β – globin synthesis and it exerts at least three distinct yet interlocking effects. Those are Ineffective erythropoiesis, Hemolytic anemia and Hypochromia with microcytosis, which reduces the oxygen carrying capacity of those few red cells that do survive. This leads to compensatory erythroid hyperplasia causing massive bone marrow expansion and exerts numerous adverse effects on the growth, development, and functions of critical organ systems. Iron toxicity is due to increased iron absorption which can result in rapid iron accumulation of 2 to 5 gm per year, and blood transfusion doubles this rate of accumulation. The patients presents with consequences of iron toxicity to liver, heart and pancreas. The oxidative injury hypothesis postulates that iron overload in vivo can result in formation of free radicals with resultant damage to cellular function. Many breakdown products result from lipid peroxidation, including the reactive aldehydes

malondialdehyde (MDA) and 4 –hydroxynonenal (HNE). Our aim in the present study was to assess the oxidative stress which may also damage to liver kidney through free radicals.

AIMS AND OBJECTIVES

- The aim of this study was to assess the oxidative stress with respect to anemia and number of blood transfusions in beta thalassemic patients in India.
- We also tried to assess the hypoxia induced increased free radical generation in patients with beta thalassemia major.

MATERIAL AND METHODS

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 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 any major illness.
- Patients with anemia due to other cause.

- Patients with past history suggestive of kidney or 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. Hemoglobin was estimated by Sahli’s hemoglobinometer from finger prick. Malondialdehyde (MDA) Double heating method using Thiobarbituric acid. Uric Acid by Enzymatic method.

OBSERVATIONS AND RESULTS

Table 1: Distribution of patients with respect to number of blood transfusion

Group	No of blood transfusions	Patients	Percentage
Group I	1-50	15	60
Group II	50-100	6	24
Group III	100-150	4	16
Total		25	100

Above table shows that amongst the studied thalassemia patients who belonged to Group I, 60% of the patients received blood transfusions between 1 to 50 times; Group- II, 24% received between 50 to 100 times; Group

III, 16% received between 100-150 times. This shows that the major group of thalassemic patients belonged to group I receiving less number of blood transfusions.

Table 2: Mean Hb levels in cases and controls

Parameters Mean ± SD	Controls	Patients	P Value
Hemoglobin level (gm%)	12.1 ± 0.97	6.42 ± 0.96	<0.001***

Table 3: Mean MDA and Uric acid levels in study subjects

MDA (nmol/gm%Hb)	Controls	Patients	p value
Mean ± SD	7.11 ± 2.25	14.58 ± 3.46	<0.001***
Serum uric acid (mg%)	2.82 ± 1.06	6.72 ± 2.58	<0.001***

DISCUSSION

Beta thalassemia is common form of hemoglobinopathy in India. Beta thalassemia represents a serious health problem because of its heterogeneous frequency and existing endogamy system. An excess pool of unpaired alpha hemoglobin chains in beta thalassemia can lead to red blood cells damage by oxidative means which may be further potentiated by heme.¹ Anemia in Beta thalassemia is caused by ineffective erythropoiesis and premature hemolysis of red blood cells in peripheral circulation. Furthermore, Beta thalassemia patients are under continuous blood transfusions leading to iron overload. The abnormal synthesis of globin chains or the productions of abnormal Hb have wide impacts on the physiological function of virtually every major organ. 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.^{2,3} Clinical manifestation of thalassemia major usually becomes apparent during 6 months of life and the diagnosis is usually evident by 2 years of age. In the early reports death occurred in late infancy and childhood as a consequence of severe anemia. In a retrospective review

from Italy, the average survival of children with untreated thalassemia major was less than 4 years and more than 80% died in the first 5 years of life, hence transfusions are necessary to prevent early death; in 50% of cases transfusion therapy is initiated in first year of life.⁴ As the clinical manifestation becomes evident, the patients come to pediatric OPD for diagnosis (Hb electrophoresis) and the treatment in form of blood transfusion is given. Initially in first year of life the requirement for blood transfusion was less, as growth was slow from 1 year to 5 years of life. After 5 years the growth rate rises and there is increased metabolic demand. Therefore patients need frequent blood transfusion. The present study supported this fact, as maximum patients enrolled were in the range of 5-10 years. According to Ehlers KH *et al*⁵, iron deposition was directly proportional to number of blood transfusions. Each 500 ml of transfused blood resulted in tissue deposition of about 250 mg of iron that cannot be eliminated by physiologic process. Excess iron can be stored as ferritin and hemosiderin in both the cytoplasm and lysosomes. Our study showed that 60% of thalassemic patients belong to group I (number of blood transfusions between 1 to 50). The 24% of thalassemic cases belong to group II (number of blood transfusions between 50 to 100) while only 16% belong to group III (number of blood transfusions between 100 to 150). This

indicates less percentage of cases were present in group III. The majority thalassemic patients in the present study received less number of blood transfusions (group I). Thalassemic patients in the present study had mean hemoglobin level of 6.42 ± 0.96 gm/dl, while controls had 12.1 ± 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 thalassemia patients with moderate anemia had 5-8 gm% 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.*⁸ and Cetin T. They also found hemoglobin levels being significantly reduced in cases as compared to controls. In the present study the mean uric acid level was significantly elevated in patients (6.72 ± 2.58 mg/dl) as compared to controls (2.82 ± 1.06 mg/dl). The significant increase in uric acid concentration may suggest impaired renal function or increased hemolysis. Increase in uric acid levels in thalassemic patients may be due to increased hemolysis of immature RBC's and shortened RBC's life span, which causes increased degradation of nucleic acid product, which in turn results in increased uric acid levels. Uric acid is also most abundant aqueous antioxidant in humans and contributes as much as two thirds of all free radical scavenging capacity in plasma. It is particularly effective in quenching hydroxyl superoxide and peroxy nitrite radicals, and may serve a protective physiological role by preventing lipid peroxidation.⁶ The increased uric acid concentration might be a compensatory mechanism that confers protection against increased free radical activity.⁷ In the present study serum MDA was significantly raised in patients (14.58 ± 3.46 nmol/gm Hb) as compared to controls (7.11 ± 2.25 nmol/gm Hb). Raised levels of MDA in thalassemic patients suggest that there may be oxidative stress in thalassemic patients. Our reports are in agreement with the results of Ong-ajyooth *et al.*¹⁰, Naithani R *et al.*¹¹ who found increase levels of MDA in thalassemic patients. Livrea *et al.*⁹ also observed about two fold increase in malondialdehyde as compared to controls. Increased mean level of serum MDA in thalassemic patients was suggestive of oxidative stress resulting in generation of free radicals. Elevated levels of mean serum uric acid levels in patients of thalassemia major might be a compensatory mechanism that confers protection against free radicals.

CONCLUSION

In Thalassemia major, due to anemia the patients are subjected to repeated blood transfusions which lead to

subtle changes. 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.

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