OXIDATIVE STRESS AND DISTURBANCE IN ANTIOXIDANT BALANCE IN BETA THALASSEMIA MAJOR

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ABSTRACT

Repeated blood transfusion in beta thalassemia major patients may lead to peroxidative tissue injury by secondary iron overload. In the present study, 72 children with beta thalassemia major were included. Serum levels of total lipid peroxides, Iron, Total Iron Binding Capacity, Copper, Zinc, Vitamin E, plasma Total Antioxidant Capacity, activity of Erythrocyte Superoxide Dismutase, were measured. The findings were compared with 72 age matched healthy controls irrespective of sex. A significant increase in the levels of lipid peroxide and Iron ($p<0.001$), whereas, significant decrease in the levels of vitamin-E, Total Antioxidant Capacity and Total Iron Binding Capacity ($p<0.001$) was observed. Serum Zinc was significantly increased ($p<0.001$) with significant decrease in the levels of copper ($p<0.001$). Non Significant increase in the activity of Erythrocyte Superoxide Dismutase ($p>0.05$) was found in the patients when compared with controls. This suggest that oxidative stress and reduced antioxidant defense mechanism play an important role in pathogenesis of beta thalassemia major.

KEY WORDS

Beta thalassemia major, Oxidative stress, Antioxidants.

INTRODUCTION

Beta thalassemia major is the most prevalent type of thalassemia as it is common in certain populations. In India, every year more than 10,000 children are born with thalassemia major. It produces severe anemia in its homozygous state (1). About 190 million people throughout the world have genetic mutations associated with different hemoglobinopathies and more than 90 million of them carry defective genes leading to thalassemia (2,3).

The disease is associated with profound anemia, jaundice, splenomegaly, expanded bone marrow space, siderosis and cardiomegaly. These symptoms appear after about 2-4 months of age. Impaired erythropoiesis, hemolysis in the peripheral

circulation and deposition of excess iron in the tissues, are some of the causes of clinical manifestations(2).

Earlier studies have shown that, in thalassemia there is excess production of reactive oxygen intermediates, such as superoxide anion (O_2^-), hydroxyl radical ($OH\cdot$), singlet oxygen and hydrogen peroxide (H_2O_2) within the erythrocytes, all these events lead to oxidative stress. This oxidative stress and a possible consequential accelerated apoptosis may contribute to shortened life span of erythrocytes. Malondialdehyde (MDA), a product of lipid peroxidation is generated in excess amounts in supporting the fact that large amount of membrane bound iron is present in thalassemic erythrocytes (2,4). Trace metals, especially Iron are implicated as causative agents in excessive generation of free radical which are capable of causing oxidative damage to erythrocytes (1). Antioxidants are complex and diverse group of molecules that protect key biological sites from oxidative damage. They scavenge free radicals and other reactive oxygen species (ROS) (5).

The present study was initiated to evaluate the role of oxidants, antioxidants and trace elements in beta thalassemia major.

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MATERIALS AND METHODS

This study was carried out in the Department of Biochemistry, Dr.V.M.Government Medical College, Shree Chattrapati Shivaji Maharaj General Hospital and Gopabai Damani Blood Bank, Thalassemia Center, Solapur (Maharashtra). On the basis of area of study and prevalence, statistically present study included 144 subjects whose age were 7-12 years. Out of 144 subjects, 72 subjects were regarded as healthy controls and remaining 72 were patients with beta thalassemia major. They were clinically diagnosed on the basis of severe anemia and haemoglobin electrophoresis. All the patients were examined regularly once or twice a month by clinicians. They were regularly receiving erythrocytes transfusions every month and were not receiving any iron chelating drugs. Transfusion characteristics and duration of transfusion were similar in all the patients. All the patients were thalassemia jaundiced (15). The control group include 72 healthy children. None of these children had history of anemia, abnormal complete blood counts and abnormal hemoglobin electrophoresis results.

10ml of venous blood was collected from the subjects under aseptic condition, 6ml of this blood was collected in plain bulb and the remaining 4ml of blood were poured in heparinised bulb. Serum and plasma were separated by centrifugation at 3000 rpm for 10 min at room temperature. Analysis of all the biochemical parameters was done manually using the chemicals from Qualigens Fine Chemicals Co., Mumbai. The parameters were run on visible Spectrophotometer. Serum lipid peroxide was measured by precipitating lipoproteins with trichloroacetic acid and boiling with thiobarbituric acid which reacts with Malondialdehyde to get pink colour as per the method of Kei Satho (6). For estimation of Iron, serum was deproteinized first and 2-2' dipyridyl in acetic acid was added which reacted with ferrous ions to give pink colour as described in Ramsay's Dipyridyl Method (7). TIBC was determined by Ramsay's Dipyridyl Method (8). Erythrocyte SOD activity was measured by Kajari Das which is based on superoxide radicals generated by photoreduction of riboflavin which react with hydroxylamine hydrochloride to produce nitrite. The nitrite in turn reacts with sulphanilic acid to produce diazonium compound, which subsequently reacts with naphthylethylene-diamine, a red azo-compound (9). Serum Vitamin-E was determined by Baker and Frank Method which is based on reduction of Ferrous ions which forms a red colored complex with α - α^1 dipyridyl (10). Total Antioxidant Capacity (TAC) was estimated by Ferric Reducing Ability of plasma (FRAP Assay) where antioxidant power converts ferric to ferrous ion reduction at low pH causing a coloured ferrous tripyridylfrazine complex (11). Serum copper and Zinc were estimated by Atomic

Absorption Spectrophotometer (AAS) (12). Blood from thalassemia patients was collected just before the transfusion. The samples were stored at 4°C before analysis and analysis of the biochemical parameters were performed immediately on the same day.

All the results were expressed as mean $\pm$ SD. Student 'Z' test was applied for the comparison of data. $p>0.05$ was considered as non-significant whereas $p<0.001$ was considered as highly significant.

RESULTS AND DISCUSSION

There was a significant ($p<0.001$) increase in the level of serum MDA in patients with beta thalassemia major as compared to controls (2). Absence of beta globin chains lead to accumulation of unpaired alpha globin chains. Excess presence of the alpha globin chains is a primary reason for the cellular oxidative damage and also iron overload. As a result of both high plasma iron and high intracellular non-hemoglobin iron in beta thalassemia, there is an enhanced generation of ROS(13-16). Moreover, repeated blood transfusion causes iron overload which increases free radical production and peroxidative damage of tissues. In such condition, depletion of endogenous antioxidants may be expected. Peroxidative damage of lipids is indicated by the increase in serum MDA levels.

A significant increase ($p<0.001$) in serum iron whereas decrease in serum TIBC ($p<0.001$) was observed in beta thalassemia major when compared with controls. The patients have severe anemia due to ineffective erythropoiesis which is primary reason for iron overload and blood transfusion is secondary to it. Thus, increased iron may increase the potential of oxidative injury to erythrocytes and cell organelles (1,5,13).

During the course of metabolism, superoxide anion is converted to H_2O_2 by ubiquitous enzyme superoxide dismutase. Normally H_2O_2 is converted to innocuous compounds by the action of catalase and peroxidase. But if free iron is available, it reacts with H_2O_2 to form hydroxyl radicals which are extremely reactive species leading to depolymerisation of polysaccharide, DNA strand breakage, inactivation of functional proteins etc. (15).

Highly significant depletion ($p<0.001$) in serum vitamin E was observed in present study. Vitamin E plays a key role in protecting cells against oxidative damage. The antioxidant role of vitamin E is attributed to its ability in quenching highly reactive lipid peroxide intermediate by donating hydrogen and

this prevents extraction of hydrogen from PUFA. This assists in restricting self perpetuated lipid peroxidation chain reaction (2,5).

In the present study, the activity of Erythrocyte SOD was non-significantly ($P>0.05$) increased as compared to controls. Erythrocyte SOD scavenges superoxide radicals to form hydrogen peroxide and protects the cell membrane from its damage. Increased Erythrocyte SOD activity may be due to blood transfusion and increase in the proportion of younger erythrocytes, as a compensatory mechanism after increased oxidative stress (15).

The decreased serum zinc levels in beta thalassemia major has been attributed to hyperzincuria which may be due to cirrhotic changes owing to hemosiderosis or to an increased rate of glomerular filtration of zinc seen in chronic hemolysis. There was significant depleted level ($p<0.001$) of serum zinc (1).

Copper is present largely in the form of organic complexes, many of which are metalloproteins acting as enzymes. Serum copper level was found to be significantly increased ($p<0.001$) in patients when compared with controls. The increased level of copper could be explained by the antagonistic effect of the zinc, as zinc deficiency in beta thalassemia major could greatly increases copper absorption via the gastrointestinal tract(1).

Significantly lower levels of TAC were observed in patients when compared with controls($p<0.001$). The iron overload and increased production of ROS might be associated with increased hemolysis in beta thalassemia major patients. Antioxidants play important role in defense mechanism that

protects the body from free radical or promoting their decomposition. Lowered level of TAC might be due to its utilization for nullifying the action of ROS and iron overload.

Thus, the present study shows alterations in iron metabolism, intense production of oxygen free radicals with subsequent consumption of antioxidants.

Our study indicates that oxidative stress may be a major cause of hemolysis in beta thalassemia major. The administration of selective antioxidants such as vitamin E might represent a promising way of counteracting the oxidative damage.

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Table 1 : Showing levels of oxidants and antioxidants in controls and beta thalassemia major patients (Mean $\pm$ SD)

Parameter	Group I	Group II
Serum MDA (nmol/ml)	1.24 $\pm$ 0.23	2.48 $\pm$ 0.65*
Serum Iron (mg/dl)	111.14 $\pm$ 16.28	164.55 $\pm$ 14.30*
Serum TIBC (μ g/dl)	277.19 $\pm$ 16.28	224.15 $\pm$ 20.43*
Serum Vitamin E (mg/dl)	1.43 $\pm$ 0.16	0.81 $\pm$ 0.22**
Erythrocyte SOD(Unit/mg Hb)	2.92 $\pm$ 0.36	3.47 $\pm$ 0.22**
Serum Copper (μ g/dl)	145.88 $\pm$ 19.25	90.92 $\pm$ 15.09*
Serum Zinc (μ g/dl)	94.06 $\pm$ 21.20	46.97 $\pm$ 8.40*
Plasma TAC (mMol/L)	1.50 $\pm$ 0.14	0.90 $\pm$ 0.17*

Statistical comparison was done between Group I and Group II

* $p<0.001$ - Highly significant; ** $p>0.05$ - Non significant;

Group I - Healthy Children; Group II - Beta thalassemia major patients.

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