ATTENUATION OF SERUM FERRITIN AND IRON BURDEN BY INTAKE OF ANTIOXIDANTS IN BETA THALASSEMA MAJOR

SONALI S. BHAGAT¹, PURNIMA DEY SARKAR², ADINATH N. SURYAKAR³, RAMCHANDRA K. PADALKAR¹, RAHUL A. GHONE¹, SANGITA M. PATIL¹ AND PRAKASH S. HUNDEKAR⁴

¹Department of Biochemistry, PDVVPF’s Medical College, Ahmednagar, (MS), India
²Department of Biochemistry, MGM Medical College, Indore, (MP), India
³Registrar, Maharashtra University of Health Sciences, Nashik, (M.S.), India
⁴Department of Biochemistry, Ashwini Rular Medical College, Solapur, (M.S.), India

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Abstract: It has been anticipated that iron and ferritin burden in patients with beta thalassemia major is associated with enhanced free radical formation and blemished antioxidant defense system. The goal of study was to scrutinize impact of serum iron, total iron binding capacity (TIBC), ferritin and erythrocyte catalase in patients with beta thalassemia major. 140 beta thalassemia major patients were studied before and after supplementation of antioxidants for one month, and status was compared with 140 age and sex matched healthy controls. A significant elevation was found in the levels of serum iron and ferritin (P<0.001) with concomitant decrease in erythrocyte catalase (P<0.001) in patients when compared with controls. After one month supplementation of antioxidants, catalase was elevated significantly (P<0.001) and marginal rise in serum TIBC concentration increased marginally while iron and ferritin were decreased marginally (P>0.05) when compared with controls and baselines values. Beta thalassemia major children receive multiple blood transfusions, and are at risk of secondary iron overload induced oxidative stress. These effects may be help to minimize with supplementation of antioxidants.

Key words: beta thalassemia major serum ferritin oxidative stress iron overload

INTRODUCTION

β–Thalassemia major is a genetic disorder caused by mutations in the HBB gene in chromosome 11 (β) which leads to defective synthesis of β-globin subunits of hemoglobin HbA (α₂β₂) (1).

In beta thalassemia major, due to repeated blood transfusion, the patients shows
the symptoms like, profound anemia characterized by extreme pallor, jaundice, accompanied by poor feeding, irritability, decreased activity. Hepatosplenomegaly, expanded bone marrow, cardiomegaly, impaired erythropoiesis, hemolysis in peripheral circulation and deposition of excess iron in the tissue are usually present (2, 3).

Worldwide, there are 240 million carriers of β-thalassemia in which 1,00,000 children born with thalassemia major are reported. In India 10,000 children are born with beta thalassemia major with mean prevalence of 3.3% (4).

The pathology is characterized by decreased hemoglobin production and red blood cells (RBCs) survival rate, which results in excess formation of unaffected α-globin chains. This forms unstable homotetramers, which precipitate as inclusion bodies. The free α-globin chains are highly unstable and readily precipitate with release of iron in reactive form (1). The symptoms appear after about 2-4 months duration of (3, 5).

Today, different studies point out that high levels of oxygen with hemoglobin containing iron attacks on the non- saturated fatty acids and RBCs which result in production of free radicals and reactive oxygen species (ROS) in the erythrocytes of thalassemic patients. This alters the redox status of the patients and intensification of oxidative stress with gross depletion of antioxidant nutrients (6, 7). This escorts towards congestive heart failure and is one of the most prevalent cause of death in beta thalassemia major (7, 8, 9). Thalassemia major manifests itself with severe anemia (3-7 gm/dl of Hb) and lifelong depends on lifelong blood transfusion to sustain life. Repeated blood transfusions and increased gastrointestinal iron absorption causes iron surplus in the body (1, 8).

In patients with iron burden, total iron binding capacity of transferrin and non-transferrin bound iron exceed, which cause tissue toxicity leading to increased lipid peroxidation with subsequent consumption of antioxidants (8, 9). The combined interaction between vitamins as well as minerals and their effects on antioxidant status in β-thalassemia major has been least studied. In this research we intend to study the antioxidants intake and its affiliation with iron burden on the body by means of serum ferritin, iron and TIBC in children with beta thalassemia major.

MATERIAL AND METHODS

This study was conducted in the Department of Biochemistry, NSCB Govt. Medical College, Jabalpur, (M.P.) and Department of Biochemistry PDVVPF’s Medical College, Ahmednagar, Maharashtra. The Institutional Ethical Committee clearance was obtained and utmost care was taken during experimental procedure according to the Declaration of Helsinki 1975.

The duration of the study was 12 months. The study has been performed on total of 280 subjects which included 140 age and sex matched (96 males and 44 females) healthy controls and 140 (87 males and 53 females) α- thalassemia major children who were previously diagnosed by High Performance Liquid Chromatography (HPLC) and electrophoretic patterns. They were under
the strict supervision of medical professionals during this period. The patients were blood transfusion dependent, and aged between 3-12 years. All the patients having history of cardiovascular diseases, hypertension, thyroid dysfunction, diabetes mellitus, which induce oxidative stress, were excluded from the study.

After obtaining a written consent, total of 5 mL blood was withdrawn aseptically from the antecubital vein from each subject, out of this approximately 2 mL blood in EDTA (0.47 mol/l K3-EDTA) container and 3 mL blood in plain blub. The samples were centrifuged at 3000 rpm for 10 min to separate serum and RBCs respectively. The separated serum was collected for further analysis in polythene tube with cork and stored at –20°C.

Serum ferritin concentration was performed by Assay Max Human Ferritin ELISA kit (Catalog No. EF2010-I, Batch No. 120897) in batches of ten each along with healthy controls. For serum iron and TIBC estimations, Ramsay’s Dipyridyl method were used (10,11). Catalase activity in RBCs was determined as per the L Goth method (12).

The analyses of serum iron, TIBC and erythrocyte catalase were done manually using the chemicals of Qualigens Fine Chemicals Co., Mumbai. The parameters were run on UV visible Spectrophotometer (Systronix).

The assessment of the above parameters from patient groups only were conducted before and on 30th day of the antioxidant supplementation in the form of an antioxidant tablet A-Z b.i.d., which was predominantly composed of antioxidant vitamins, minerals and trace elements.

Statistical analysis

The study type is prospective crosssectional before after comparison study. The statistical analysis was made by the SYSTAT software, version 12 for Windows. The Student ‘Z’ test was applied to assess the difference between means of intervention and controls and pre and post intervention data. The results were expressed in mean±SD. P values (P<0.001) were considered as highly significant and (P>0.05) as non significant.

RESULTS

Table I shows significantly elevated (P<0.001) levels of serum ferritin and Iron and the mean value of erythrocyte catalase along with TIBC were depleted (P<0.001) in cases than healthy controls. After one month of antioxidant supplementation to patients group it was observed that, a non significant decrease (P>0.05) in the levels of ferritin and iron when compared with baseline results. Similarly erythrocyte catalase level was determined to be higher (P<0.001) and TIBC was elevated marginally (P>0.05) on the 30th day of the antioxidants supplementation when compared with baseline results.

DISCUSSION

There are two main mechanisms by which iron overload develops in beta thalassemia major, increased iron absorption due to ineffective erythropoiesis and repeated blood transfusion (2, 3). The goal of transfusion includes correction of anemia, suppression
Our findings are in accordance with Kuldeep K. Gupta et al and Rafaella Mariani et al. There were significantly increased serum iron and depleted TIBC levels in patients with beta thalassemia major as compared with healthy controls (1, 13).

Ferritin is the main iron storage protein in the body. Our findings are in agreement with the other researchers like Nadeem Ikram et al, Fliliz Simsek et al and Livrea MA et al (8, 14, 15) that iron indices were markedly increased and the mean concentration of serum ferritin was elevated more than 20 times than healthy controls.

They found that most patients had hyperferritinemia with high oxidative stress and low vitamin C and E levels and after vitamins supplementation, plasma vitamins

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n=140)</th>
<th>Group I Beta thalassemia major patients (n=140) Before supplementation of antioxidants.</th>
<th>Group II Beta thalassemia major patients (n=140) After 30th day supplementation of antioxidants.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ferritin (ng/ml)</td>
<td>152.51±77.23</td>
<td>3869.4±996.06 p&lt;0.001</td>
<td>3703.27±546.3 p&gt;0.05</td>
</tr>
<tr>
<td>Serum Iron(µg%)</td>
<td>128.58±13.85</td>
<td>197.81±31.53 p&lt;0.001</td>
<td>207.31±13.57 p&gt;0.05</td>
</tr>
<tr>
<td>Serum TIBC(µg%)</td>
<td>320.28±13.85</td>
<td>280.19±50.14 p&lt;0.001</td>
<td>282.54±40.82 p&gt;0.05</td>
</tr>
<tr>
<td>Erythrocyte Catalase (KU/L)</td>
<td>202.37±43.83</td>
<td>124.27±26.75 p&lt;0.001</td>
<td>161.57±21.04 p&lt;0.001</td>
</tr>
</tbody>
</table>

Values are expressed in mean with standard deviation (mean±SD). p<0.001 – considered as highly significant and p>0.05 – considered as non significant. n = number of subjects.

Statistical comparison was done between controls, group I and II by applying student ‘Z’ test for individual comparison.

Group I – Beta thalassemia major patients before supplementation of antioxidants.

Group II – Beta thalassemia major patients after supplementation of antioxidants.

This oxidative stress and possible consequential accelerated apoptosis may contribute to shortened erythrocyte life span, primary or secondary amenorrhoea, hypogonadism, osteoporosis, and other endocrine disorders (5, 6, 7).
and glutathione were significantly increased with mild decrease in total bilirubin. They concluded that vitamins supplementation improve antioxidant status and enhance liver function.

Catalase hydrolyzes potent $\text{H}_2\text{O}_2$ at low concentration to the water molecule. Our investigations shows significantly depleted activity of erythrocytic catalase in patients as compared to the controls and baseline results. This supports the hypothesis made by various studies Fliliz Simsek et al and Livrea MA et al Nandita Das et al that, enhanced production of hydrogen peroxide by the activity of superoxide dismutase which can inhibit various peroxidase enzyme activities, it may contribute to further augmentation of oxidative stress (14, 15, 16).

Elevated activity of erythrocytic catalase on 30th day after the supplementation of antioxidants was seen in patients group. Increased activity of catalase suggests the decreased formation of $\text{H}_2\text{O}_2$ and reduces ROS. Supplementation of antioxidants neutralizes as well as reduces formation of superoxide radical and $\text{H}_2\text{O}_2$.

Our study support to the idea that, supplementation of antioxidants enhances the liver enzymes this might be due to increase in vitamin A level, which has a role in improvement of liver enzymes. Vitamin A protects against chemical induced lipid peroxidation in the heart, brain and liver. Vitamin C has been proved to suppress this mechanism of hemolysis. Vitamin E, particularly $\alpha$-tocopherol is a potent peroxyl radical scavenger. Vitamin E and glutathione are the red cell protective antioxidants, which decrease intravascular hemolysis.

**Conclusion**

The essence of the current study lies in the fact that, there was enhanced oxidative stress in the form of serum iron, TIBC, ferritin and reduced catalase activity in patients before treatment. Because of repeated blood transfusions for survival results in increased free iron overload, which leads to development of abnormalities in the body. Till date the treatments available for this genetic disorder are iron chelation is a treatment for iron overload and not for the genetic disorder chelation, bone marrow transplantation, and stem cell therapy. But these treatments are burdensome, very expensive and hence a person depends on regular blood transfusion throughout life. One of the ways to increase the survival rates of such patients is by inhibiting the effect of oxidative stress. Combined antioxidant therapy with iron chelator in beta thalassemia major may improve the antioxidant status by neutralizing the free radicals formation. It also facilitates to trim down the hemolytic rate and recover the hepatic status by both decrease in the liver iron concentration and the degree of liver fibrosis.

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