Metalloprotein status in Indian patients with beta thalassemia trait

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ABSTRACT

Introduction and Aim: Beta Thalassemia trait (BTT) is the most prevalent heterozygous hemoglobinopathy in Asian population. The current study aims to evaluate plasma antioxidant metalloproteins like SOD, ceruloplasmin, ferritin and correlate them with the trace elements in patients with BTT.

Materials and Methods: The subjects included in the study were divided into two groups comprising of 40 BTT patients in group I and 40 age and sex matched normal individuals in group II. Cation exchange HPLC was used for hemoglobin variant analysis. Plasma iron, copper, ceruloplasmin and SOD were assayed using the spectrophotometric method while zinc was determined by atomic absorption spectrometry and ferritin by ECLIA.

Results: There was a marked reduction in plasma trace elements in BTT compared to healthy controls. The decrease in zinc (p<0.001) and copper (p<0.04) was statistically significant. Both SOD and ferritin levels were significantly lower in BTT patients (p<0.001). However, an apparent decrease was observed in ceruloplasmin levels. Iron and ferritin showed a significant positive correlation (r=0.84 p=0.01), similarly copper and ceruloplasmin correlated positively (r=0.92 p=0.001) in BTT patients. Interestingly HbF correlated negatively with all the three trace elements.

Conclusion: It can be concluded that decreased antioxidant metalloproteins may lead to free radical toxicity in BTT. The study highlights the indirect role of trace elements in oxidative stress and draws attention on the requirement for regular and timely assessment of metalloenzymes and periodic administration of trace elements in reduction of free radical damage and associated complications in BTT patients.

Keywords: Beta thalassemia trait; Ceruloplasmin; Copper; Ferritin; SOD.

INTRODUCTION

Beta thalassemia is the most prevalent hemoglobinopathy that has emerged as a major public health problem worldwide, with prevalence of 3.3% in India. Beta thalassemia trait (BTT), is a heterozygous haemoglobinopathy with the characteristic feature of microcytosis, hypochromia and low grade hemolysis (1). In thalassemia, expression of normally silenced gamma globin gene results in excess production of fetal hemoglobin and hemoglobin A2 in adults to compensate for decreased production of beta globulins. The presence of excess alpha chains creates a prooxidant condition leading to oxidative stress which may damage the red blood cells (2). Several enzymes like SOD, catalase, ceruloplasmin which fight against free radicals are metalloproteins. Moreover, the biochemical role of micro elements like iron, zinc and copper in heme biosynthesis is well established. Our earlier studies revealed a significant reduction in plasma levels of these elements in BTT patients compared to healthy subjects (3). Reports on metalloenzymes like catalase, superoxide dismutase showed variable results in thalassemia major patients. However, studies on metalloproteins in BTT patients are scanty.

As an extension of our previous research, the current study was designed to evaluate plasma antioxidant metalloproteins like SOD, ceruloplasmin, ferritin and correlate them with trace elements, thereby assess the importance of trace elements in oxidative stress of BTT patients.

MATERIALS AND METHODS

The subjects included in the study were divided into two groups. Group I comprised of 40 BTT patients and Group II consisted of 40 age and sex matched normal individuals. Institutional ethics committee approval was taken prior to conducting the study. Informed consent was obtained from all the subjects. Random blood sample collected in heparin vacuum tube was centrifuged for 10 minutes at 3000g. Biochemical investigations were performed in the plasma sample. Plasma copper estimation was carried out using bathocuproine disulfonate as the chromogen. Iron was estimated by ferrozine method spectrophotometrically and plasma zinc was determined by atomic absorption spectrometry (3). Ferritin was estimated by ECLIA based on sandwich principle (4), ceruloplasmin by paraphenylenediamine oxidation (5) and SOD by pyrogallol method.
(6). Cation exchange HPLC was used for hemoglobin variant analysis in EDTA blood samples (7). Subjects were labelled as BTT, only when HbA2 was greater than 3.5% or HbF >2.0%. Non-anemic patients with Hb greater than 14.0 g% in males and greater than 12.0 g% in females were considered for the study. Further, patients without any nutritional deficiency, hepatic or renal diseases or any chronic infections were included in the study. None of the subjects were on micronutrient supplementation prior to or at the time of blood sampling. Statistical analysis was done by student t test using SPSS version 20. p < 0.05 was considered statistically significant. Correlation between trace elements and metalloproteins were tested by Pearson’s correlation.

RESULTS

Demographic and HPLC findings of BTT patients are depicted in Table 1. Significantly low Hemoglobin A1 level with concomitant increase in HbA2 and Hb F was hallmark of BTT patients. There was a significant reduction in plasma zinc and copper levels in patients with BTT compared to controls, however, drop in iron was not statistically significant (Table 2).

Table 1: Demographic and HPLC findings in BTT patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Males – 22 (55%)</td>
<td></td>
</tr>
<tr>
<td>Females – 18 (45%)</td>
<td></td>
</tr>
<tr>
<td>HbA1 (g %)</td>
<td>90.90 ±2.3</td>
</tr>
<tr>
<td>HbA2 (g %)</td>
<td>5.27 ±0.24</td>
</tr>
<tr>
<td>HbF (g %)</td>
<td>1.49 ±1.26</td>
</tr>
</tbody>
</table>

Table 2: Comparison of plasma micro minerals in control and patients with BTT

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=40)</th>
<th>BTT (n=40)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (µg/dl)</td>
<td>96 ± 14</td>
<td>86 ± 15</td>
<td>0.04</td>
</tr>
<tr>
<td>Zinc (µg/dl)</td>
<td>83 ± 5</td>
<td>40 ± 9</td>
<td>0.001</td>
</tr>
<tr>
<td>Iron (µg/dl)</td>
<td>74 ± 11</td>
<td>63 ± 27</td>
<td>NS</td>
</tr>
</tbody>
</table>

A marked reduction in plasma SOD was seen in BTT patients (p<0.001) compared to healthy controls. Further, the decrease in ferritin was also equally significant in these patients compared to normal individuals. Furthermore, an apparent decline in ceruloplasmin levels was observed patients with BTT (Table 3).

Table 3: Plasma metalloproteins in control and beta thalassemia trait

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=40)</th>
<th>BTT (n=40)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/ml)</td>
<td>3.08 ± 0.72</td>
<td>2.41±0.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ceruloplasmin (mg/dl)</td>
<td>30.15 ± 7.4</td>
<td>28.22 ± 6.22</td>
<td>0.41</td>
</tr>
<tr>
<td>Ferritin/ng/dl</td>
<td>88.12 ± 2.85</td>
<td>19.59 ±11.42</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

A statistically significant positive correlation was seen between ferritin and iron (r= 0.84, p= 0.01). However, iron showed negative correlation with ceruloplasmin and SOD. Both copper and zinc showed a positive correlation with all the metalloproteins studied. Positive correlation between copper and ceruloplasmin was statistically significant (r= 0.92, p=0.01) (Table 4).

Table 4: Correlation of trace elements with hemoglobins and other metalloproteins

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Iron</th>
<th>Copper</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1</td>
<td>0.68</td>
<td>0.50</td>
<td>0.82</td>
</tr>
<tr>
<td>Hb A2</td>
<td>0.06</td>
<td>0.92</td>
<td>0.03</td>
</tr>
<tr>
<td>Hb F</td>
<td>-0.76</td>
<td>-0.39</td>
<td>0.08</td>
</tr>
<tr>
<td>Ferritin</td>
<td>0.84</td>
<td>0.50</td>
<td>0.82</td>
</tr>
<tr>
<td>SOD</td>
<td>-0.39</td>
<td>0.92</td>
<td>0.03</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>-0.50</td>
<td>0.92</td>
<td>0.82</td>
</tr>
</tbody>
</table>

* Significant

DISCUSSION

BTT is the most prevalent heterozygous haemoglobinopathy with hypochromic microcytic anemia due to low grade hemolysis. Though most of the patients are asymptomatic and require no treatment, deficiency of dietary micronutrients may add to the morbidity. Copper acts as a cofactor for several enzymatic reactions and deficiency of which hampers transportation and utilization of serum iron. Ceruloplasmin through its ferroxidase activity may decrease the availability of ferrous iron for Haber-Weiss reaction that generates reactive oxygen species and acts as an antioxidant. Our study showed markedly low plasma trace elements viz., copper, zinc and iron in patients with BTT compared to normal subjects. However, Fahmy et al. observed zinc and copper deficiency in only a small percentage of pediatric thalassemia major patients (8).

Copper along with zinc is a central molecule of antioxidant enzyme SOD. Waseem et al, (9) reported 85% reduction in RBC membrane SOD in copper deficient thalassemia major patients, resulting in oxidative stress and decline in the RBC survival time. In agreement with this, there was a significant decline in plasma SOD levels in BTT patients in the present study, however, mean ceruloplasmin level showed only an apparent decrease. The thalassemic RBCs are susceptible to oxidant damage due to unpaired alpha chains. Excess superoxide generated following autoxidation of isolated alpha chains may be an important contributor of hemolytic processes in BTT. Further, release of heme due to mild hemolysis can trigger redox reactions leading to surge in superoxide radicals. SOD may be overconsumed to mitigate damage caused by these ions. Further, marked decline in SOD may be linked to the
significant reduction in both plasma copper and zinc in patients with BTT. Moreover, the decrease in zinc was more pronounced than copper and iron in BTT. Dangi et al., opined that ratio of trace elements were found to be more valuable indicators of microelement imbalance than individual elements (10). Decrease in SOD, ceruloplasmin and ferritin may be attributed to low plasma trace elements. It may be noted that hypozincemia observed in BTT patients may be attributed to urinary excretion of zinc, released by hemolysis.

The decrease in SOD and ceruloplasmin may have contributed to the oxidative stress in BTT. In addition to these copper containing antioxidants, defense proteins against reactive oxygen species mediated damage comprise of the iron binding proteins such as apotransferrin and apoferritin (11). These proteins sequester iron and restrict its availability for Fenton reaction that leads to formation of toxic hydroxyl radicals from hydrogen peroxide. A well-defined iron overload is seen in thalassemia major and intermedia patients, due to frequent blood transfusions. In these patients, iron concentration can surpass the detoxification and storage capacity of ferritin and fully saturate transferrin leading to the accumulation of ferrous iron in the tissues and blood (12). However in thalassemia minor, an asymptomatic disease, significant iron storage is not seen as blood transfusion is not a therapeutic tool. This fact is proved by low plasma ferritin in patients with BTT observed in the current study.

Fonseca et al., (13) opined that serum ferritin was the best index for evaluation of the iron status in thalassemia minor patients, though no statistical difference was observed in plasma ferritin in female BTT patients. Further, there was no correlation between plasma iron and hemoglobin in thalassemia trait patients (14). However, serum ferritin was significantly elevated in children with thalassemia major (15). Lipid peroxidation, nitric oxide, antioxidant enzyme SOD correlated positively with serum ferritin levels and not with serum iron in thalassemia major patients (16), underlining the importance of estimation of ferritin rather than iron. In contrast to the present study, one of the studies observed increased erythrocyte superoxide dismutase, catalase, and glutathione peroxidase in beta thalassemia trait and almost normal values of these enzymes in erythrocytes of beta thalassemia major patients (17).

Interestingly, in the current study, hemoglobin F correlated negatively with zinc, iron and copper which are in confirmation with the study on sickle cell disease patients with high HbF (18). Increased fetal hemoglobin may have a role in modulation of trace elements levels in plasma and may act as a contributor to oxidative stress.

CONCLUSION
It can be concluded that oxidative stress in BTT may be a consequence of decreased antioxidant metalloproteins that may be linked to low plasma trace elements. It also draws attention on the need for regular assessment and timely administration of these microelements in the reduction of oxidative stress and related complications in patients with BTT.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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476


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