A Cross Sectional Research Study on Vitamin C Deficiency and Oxidative Stress Levels in Children with Transfusion-Dependent Thalassemic Disorders

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Abstract
Aim: study of Vitamin C Deficiency and Oxidative stress Levels in Children with Transfusion-Dependent -Thalassemia. Methods: A cross-sectional study was conducted in the Department of Paediatrics, Patna Medical College and Hospital, Patna, Bihar India for 18 months. All children (below 18 years of age), with TDT and receiving regular transfusions at the centre were included in the study group. Any child who was already receiving vitamin C prior to enrolment was excluded. In the control group, 50 asymptomatic children were included in this study. Results: Plasma vitamin C levels were low in 84 (84%) children in the study group, while all children in control arm had normal plasma vitamin C levels despite comparable dietary deficiency of vitamin C (P<0.001). The correlation of dietary deficiency with low plasma vitamin C levels in the 2 groups. Age (P=0.76), number of transfusions received (P=0.57), chelation (P=0.74), and associated infections (HIV, P=0.61, anti-HCV antibody positive, P=0.53) did not have any correlation with vitamin C levels, while increasing serum ferritin values correlated with vitamin C deficiency (r=0.4, P=0.03). There was a correlation between higher serum ferritin values and MDA levels done prior to administration of vitamin C (r=0.37, P=0.04). On administration of vitamin C, the mean (SD) levels of vitamin C rose from 0.2 (0.1) mg/dL to 0.7 (0.2) mg/dL in those with low plasma levels of vitamin C (P<0.001). The mean (SD) level of MDA dropped from (7.5) nmol/mL to 8.5 (2.7) nmol/mL after 15 days of administration of vitamin C (P<0.001). Conclusions: Low levels of vitamin C are common in children with thalassemia. Dietary counselling along with supplementation with vitamin C, in those with low levels may prevent oxidative stress.
Keywords: Vitamin C, Thalassemia, Children.

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Introduction

Thalassemic are a group of hereditary anemia which happen as a result of genetic disorders that affect the synthesis of normal hemoglobin (Hb), in which a reduced rate of synthesis of one or more of the globin chains leads to defective Hb production, and damage to the red cells or their precursors[1]. β-Thalassemic is more common in Mediterranean countries and islands including Cyprus, Sardinia, and Malta[2]. However both α and β Thalassemic types are common in Africans and Black Americans[3]. Thalassemic is usually associated with many complications such as hepatosplenomegaly, artherosclerosis infections, gall stones and bone deformities that alter facial features and result in pathogenic fractures[4]. Studies on β-thalassemic patients suggest that they may develop symptoms of iron loading that includes chelating therapy complications, heart and liver diseases, and endocrinopathies[5]. Normally, erythrocytes degrade reactive oxygen species (ROS) via the actions of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). In β-Thalassemic major, intra erythrocyte release of heme induces a glutathione (GSH)-dependent self-amplifying and self-propagating Hb oxidation pathway, resulting in injury to the thalassemic cell[6]. In addition, iron can serve as a potent catalyst of lipid peroxidation[7]. The presence of hypochromia may facilitate oxidation of the red cell membrane by reducing the amount of Hb available for buffering protection[8]. The peripheral red cells of patients with β-Thalassemic demonstrate a variety of morphological, biochemical and metabolic changes, which specifically contribute to the extent and severity of lipid per oxidation and hemolysis[9]. Unpaired α-Hb chains may denature and bind to the cell membrane, thus giving rise to cytoskeleton alterations and lipid peroxidation[10]. The free α chain in β-Thalassemic increase autoxidation rates by about two times faster than normal Hb A7. It has been reported that the accumulation and autoxidation of the unpaired α-globin chains in severe β-Thalassemic would generate ROS; superoxide (O2-) and hydrogen peroxide that would cause accelerated apoptosis and ineffective erythropoiesis[11].

Materials and methods

A cross-sectional study was conducted in the Department of Paediatrics, Patna Medical College and Hospital, Patna, Bihar India for 18 months. after taking the approval of the protocol review committee and institutional ethics committee.

All children (below 18 years of age), with TDT and receiving regular transfusions at the center were included in the study group. Any child who was already receiving vitamin C prior to enrolment was excluded. In the control group, 50 asymptomatic children were included in this study.

Methodology

A detailed history including age, gender, number of transfusions received till date, chelation therapy, dietary history and examination findings were entered in a predesigned proforma after taking informed consent. All children in the study group underwent the following investigations: complete blood count with RBC indices, liver and renal function tests, HIV antibody by ELISA, hepatitis B surface antigen (HBsAg), anti-HCV antibody, serum ferritin levels and baseline vitamin C levels prior to transfusion. Dietary assessment was done by oral questionnaire method by recalling food eaten in last 48 hours and during weekends[12] and comparing it with the ICMR food composition tables[13]. Nutritional assessment was done by calculating weight for height/mid upper arm circumference in less than 5 years, and as per body mass index in children more than 5 years using the WHO growth charts[14]. In the control group, a detailed dietary history with clinical examination was documented in the proforma, and their
blood samples were collected for vitamin C estimation. All children with low levels of vitamin C were administered vitamin C orally in therapeutic doses of 200mg per day for a period of 15 days, while counselling to improve dietary content of vitamin C was also done. The dose 200 mg was chosen as non-heme iron absorption enhanced by vitamin C occurs above this dose[15]. Plasma vitamin C and MDA levels were repeated in these 36 children after completion of 15 days of oral administration of vitamin C.

Vitamin C estimation in plasma was done using 2, 6- dichlorophenol indophenol dye method[16]. A level of 0.3 mg/dL was considered as deficient according to this method. MDA estimation was done by modified method of Sadasavidu, et al.[17].

**Results**

A total of 100 children with TDT were included in this study. Of these, 65 (65%) were males (median age 9.5 years, IQR 7-14 years). In control group, of the 50 children enrolled, 25(50%) were males (median age – 9.5 years, IQR 7.2-12.5 years). There was no statistically significant difference between the age and gender distribution in these two groups (P=0.61 and 0.21, respectively).

The mean (SD) number of transfusions was 215 (115.5) and serum ferritin level was 4734.5 (3080.3) ng/mL. There was no statistically significant difference in the nutritional status between the study and control group (P=0.3); however, there were higher percentage of under- nourished children in the study group (92% vs 66%).

Bone pains (5 children) and gum bleeds (4 children) were seen only in the study group (P=0.74). Signs of scurvy were seen in 5 (5%) (Gum hypertrophy in 3 and typical skin changes in 4 children) of the children in study group whereas in none in control group (P=0.41). 88 children (88%) were on regular chelation, of which 60 (60%) children were on deferasirox, while 28 were receiving deferiprone (28%). 3 (3%) children with TDT were HIV-1 infected, 20(20%) were positive for anti-HCV antibodies and none were HBsAg positive. The mean (SD) value of vitamin C in study group was 0.2 mg/ dL (0.1) and in controls was 0.7 (0.2) mg/dL (P<0.001).

Plasma vitamin C levels were low in 84 (84%) children in the study group, while all children in control arm had normal plasma vitamin C levels despite comparable dietary deficiency of vitamin C (P<0.001).

**Table 2** depicts the correlation of dietary deficiency with low plasma vitamin C levels in the 2 groups. Age (P=0.76), number of transfusions received (P=0.57), chelation (P=0.74), and associated infections (HIV, P=0.61, anti-HCV antibody positive, P=0.53) did not have any correlation with vitamin C levels, while increasing serum ferritin values correlated with vitamin C deficiency (r=0.4, P= 0.03). There was a correlation between higher serum ferritin values and MDA levels done prior to administration of vitamin C (r=0.37, P=0.04). On administration of vitamin C, the mean (SD) levels of vitamin C rose from 0.2 (0.1) mg/dL to 0.7 (0.2) mg/dL in those with low plasma levels of vitamin C (P<0.001). The mean (SD) level of MDA dropped from (7.5) nmol/mL to 8.5 (2.7) nmol/mL after 15 days of administration of vitamin C (P<0.001).

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>Female</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>
Table 2 Vitamin C Dietary Deficiency and Plasma Levels in Children with Transfusion-Dependent Thalassemia and Controls Dietary Deficiency

<table>
<thead>
<tr>
<th>Study group</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal level=16 (≥0.3 mg/dL)</td>
<td>31 (62)</td>
</tr>
<tr>
<td>Low level=84 (&gt;0.3 mg/dL)</td>
<td>76 (76)</td>
</tr>
<tr>
<td>Total</td>
<td>107 (107)</td>
</tr>
</tbody>
</table>

Discussion

In addition to occasional case reports of scurvy occurring in children with thalassemia, a few studies have also described vitamin C deficiency in these children[18,19]. We determined the magnitude of vitamin C deficiency in Indian children with TDT and its impact on oxidant (MDA) levels. Clinical symptoms and/or signs of scurvy were seen in 8% of patients in the study group and none in the control group, and vitamin C deficiency was associated with iron overload and higher oxidant (MDA) levels.

Previous studies from various countries have reported vitamin C deficiency in 64-100% of patients with thalassemia[18-20], similar to 84% reported in this study. Hussien, et al.[19] reported suboptimal plasma levels of vitamin C in all children with TDT, despite a diet sufficient in vitamin C. We also found low levels of vitamin C in 76% of children with TDT without dietary deficiency, though it was higher in those with dietary deficiency (92%). In the control group, irrespective of dietary deficiency, all children had normal vitamin C levels, probably due to lower or no oxidant stress in them. Similar to our findings, a relation between iron overload and vitamin C deficiency has also been reported by Hussien, et al.[19].

The levels of oxidants and lipid peroxides are high in children with TDT due to the accumulation of free iron radicals and production of reactive oxygen species. A MDA higher level signifies per oxidative damage to lipid membranes in children with TDT[21,22]. Our results are similar to other studies done in patients with transfusion-dependent-thalassemia, which have also found a marked imbalance in the oxidant and antioxidant status with reduction in the antioxidants and increase in the oxidant level with vitamin C deficiency[21,23]; although, few authors have not reported such an association[22]. A significant reduction in the MDA levels oxidant load was observed after administration of vitamin C, suggesting higher oxidative stress in children with vitamin C deficiency. This also confirmed that supplementation of vitamin C does not further increase the oxidative stress and hence is safe to be given in children who are deficient the present study had some limitations. Only plasma vitamin C and MDA levels were measured out of numerous antioxidants and oxidants that are present in the body. Iron overload was estimated using serum ferritin alone which may also be elevated due to infections and inflammation. Tissue iron overload was not estimated using T2*weighted magnetic resonance imaging.

Besides regular packed red cells and adequate iron chelation, maintaining vitamin C homeostasis is the key to reducing the oxidative stress, thereby protecting these children from myocardial damage and consequent mortality. Despite the fact that there was no statistically significant difference in nutritional status between the two groups, the proportion of undernourished children with TDT was higher; hence, improved dietary intake through counseling and supplementing vitamin C in those children with TDT with low plasma vitamin C levels, will improve outcomes in these children.

Conclusions

Low levels of vitamin C are common in children with thalassemia. Dietary
counseling along with supplementation with vitamin C, in those with low levels may prevent oxidative stress.

Reference

20. Wapnick AA LS, Krawitz P, Seftel HC, Charleston RW, Bothwell TH. Effects of