RESEARCH PAPER

Vitamin C Deficiency and Oxidant Levels in Children With Transfusion-Dependent β-Thalassemia

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Objectives: To study vitamin C levels in children with transfusion-dependent β-thalassemia and correlate with age, transfusions received and iron overload; and to study the effect of administering vitamin C on its levels and Malondialdehyde (MDA) in deficient patients. **Methods**: This case-control study enrolled 100 children with transfusion-dependent β-thalassemia and 30 healthy controls. MDA levels before and after administration of vitamin C were performed randomly in 36 children with low vitamin C levels. **Results**: 81/95 (85.3%) study subjects vs none in control group, had low plasma vitamin C levels (*P*<0.001). Vitamin C levels were low in 64 of 71 (74.7%) subjects with dietary deficiency, while none of the 19 (63.3%) controls with dietary deficiency had low levels (*P*=0.04). Increasing serum ferritin values correlated with vitamin C deficiency (*P*=0.02). The mean level of MDA reduced (*P*<0.001) with vitamin C supplementation. **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.

Keywords: *Iron overload, Oxidative stress, Thalassemia*, Scurvy, Supplementation.

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Children with transfusion-dependent β-thalassemia (TDT) require frequent blood transfusions resulting in iron overload [1]. Dietary vitamin C is destroyed through irreversible oxidation by ferric iron deposits, thus leading to its deficiency causing scurvy [2,3], given the fact that dietary deficiencies are common in Indian children [4]. However, the risk of vitamin C supplementation is that excess of vitamin C enhances iron absorption and also iron-mediated peroxidation of membrane lipids, causing an increased iron-induced membrane damage in cultured myocardial cells [2]. This study was thus designed to assess the plasma levels of vitamin C in Indian children with TDT and correlate them with various patient and disease factors, including overload and oxidant levels.

METHODS

This was a cross-sectional study conducted in the day-care thalassemia centre of a tertiary hospital between December, 2011 to May, 2012. 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, 30 asymptomatic children who visited the pediatric outpatient department were enrolled.

A detailed history including age, gender, number of transfusions received till date (using record maintained by the patients), chelation therapy, dietary history and examination findings (with special reference to signs of scurvy) 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 [5] and comparing it with the ICMR food composition tables [6]. 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 [7]. 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.

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All children with low levels of vitamin C were administered vitamin C orally in therapeutic doses of 200

mg 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 [8]. In randomly selected children (*n*=36) with low levels of vitamin C, blood was also collected for oxidant malondialdehyde (MDA) levels prior to administration of vitamin C and prior to transfusion. 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 [9]. 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. [10].

Sample size was calculated using Stata Version 15.1 (StataCorp) based on the 64% incidence of vitamin C deficiency in a previous study [12] of individuals with thalassemia. With an alpha of 0.05, power of 80%, and delta of 0.14, we estimated the sample size to be 98. Thus, we recruited 100 participants.

Data analyses: Data was entered in MS Excel (Microsoft Corp.) and converted to Stata Version 10 (Stata Corp) for analysis. The differences in the categorical outcomes were tested using the chi square test or Fisher exact test and the differences in means of the continuous variables were tested using the *t* test. We calculated the correlation coefficient (r) between vitamin C levels, MDA and ferritin levels. A *P* value of <0.05 was considered statistically significant.

RESULTS

A total of 100 children with TDT were enrolled. Of these, 95 were evaluable of which 61 (64.2%) were males (median age 9 years, IQR 7-13 years). In control group, of the 30 children enrolled, 16 (53.3%) were males (median age - 9 years, IQR 7.2-12 years). There was no statistically significant difference between the age and gender distribution in these two groups (*P*=0.56 and 0.29, respectively). The mean (SD) number of transfusions was 205 (111.5) and serum ferritin level was 4634.5 (2980.3) ng/mL. There was no statistically significant difference in the nutritional status between the study and control group (*P*=0.4); however, there were higher percentage of under-nourished children in the study group (90% vs 64%).

Bone pains (4 children) and gum bleeds (3 children) were seen only in the study group (*P*=0.69). Signs of scurvy were seen in 5 (5.3%) (Gum hypertrophy in 2 and typical skin changes in 3 children) of the children in study group whereas in none in control group (*P*=0.45). Eighty three children (87.4%) were on regular chelation, of which 54 (65.1%) children were on deferasirox, while 29 were receiving deferiprone (34.9%). Two (2.1%) children with TDT were HIV-1 infected, 18 (19%) 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 0.8 (0.2) mg/dL (*P*<0.001).

Plasma vitamin C levels were low in 81 (85.3%) 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 I** depicts the correlation of dietary deficiency with low plasma vitamin C levels in the 2 groups. Age (*P*=0.86), number of transfusions received (*P*=0.67), chelation (*P*=0.84), and associated infections (HIV, *P*=0.55, anti-HCV antibody positive, *P*=0.63) did not have any correlation with vitamin C levels, while increasing serum ferritin values correlated with vitamin C deficiency (r=0.3, *P*= 0.02) (**Fig.1**). There was a correlation between higher serum ferritin values and MDA levels done prior to administration of vitamin C (r=0.35, *P*=0.03). On administration of vitamin C, the mean (SD) levels of vitamin C rose from 0.2 (0.1) mg/dL to 0.8 (0.2) mg/dL in those with low plasma levels of vitamin C

Table I Vitamin C Dietary Deficiency and Plasma Levels in Children With Transfusion-Dependent Thalassemia and Controls

Dietary deficiency	*Study group, n=95*			*Control group,[a] n=30*
	Normal level n=14 (>0.3 mg/dL)	*Low level n=81 (≤0.3mg/dL)*	*Total*	*Normal level n=30 (>0.3 mg/dL)*
Present	7 (50)	64 (79)	71 (74.7)	19 (63.3)
Absent	7 (50)	17 (21)	24 (25.3)	11 (36.7)

All values in no. (%). [a]None had low vitamin C level. P=0.04 for low vitamin C levels in diet deficient children in study and control group.

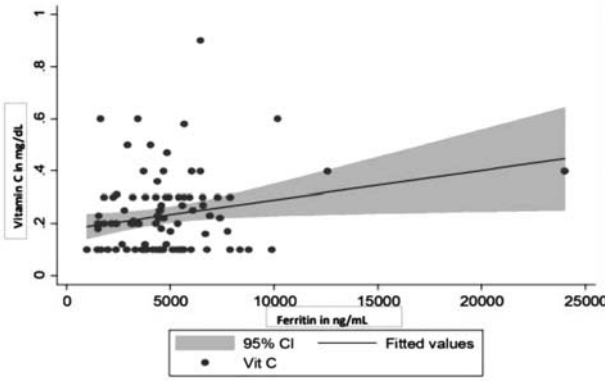

Fig. 1 Scatter diagram depicting increasing serum ferritin values significantly correlated with vitamin C deficiency.

WHAT THIS STUDY ADDS?

- Children with transfusion-dependent thalassemia are deficient in vitamin C and are more likely to develop scurvy, besides posing a risk of oxidative stress.

(P<0.001). The mean (SD) level of MDA dropped from 17.1 (7.0) nmol/mL to 8.1 (2.5) nmol/mL after 15 days of administration of vitamin C (P<0.001).

DISCUSSION

In addition to occasional case reports of scurvy occurring in children with thalassemia [3], a few studies have also described vitamin C deficiency in these children [11,12]. 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 7% 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 [11-13], similar to 85.3% reported in this study. Hussien, et al. [12] 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 70.8% of children with TDT without dietary deficiency, though it was higher in those with dietary deficiency (90.1%). 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. [12].

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 peroxidative damage to lipid membranes in children with TDT [14,15]. Our results are similar to other studies done in patients with transfusion-dependent β-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 [14,16]; although, few authors have not reported such an association [15].

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.

Ethics clearance: Institutional Ethics Committee of LTMM College and LTMG Hospital, Mumbai; No. PS/IECHR/DISS/105(11/10).
Contributors: VB: conducted the study and prepared the draft of the manuscript; RS: helped in management of the patients, monitored the outcomes and helped in analysing the results; SS: helped in collecting data and managing the patients; PJ: performed the tests in the laboratory; BD: supervised the laboratory testing of the samples and helped in correlating with the clinical findings; MS: helped in planning the study and did the statistical analysis; NS: helped in revising the draft; MM: conceptualized the study, guided throughout the study and finalized the draft. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
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