RESEARCH PAPER

Serum Micronutrients and Antioxidant Levels in Children With Transfusion- Dependent Thalassemia

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Objectives: To estimate serum zinc, copper, magnesium and antioxidant in children with transfusion-dependent thalassemia (TDT). **Methods**: Cross-sectional study, enrolling children with TDT aged 3-14 years and age-matched healthy children without thalassemia. Serum zinc, copper, magnesium and total antioxidant capacity were estimated by direct colorimetric method and ELISA, respectively. **Results**: 72 children (24 females; mean (SD) age 8.5 (3.2) years) were enrolled. Mean (SD) values of micronutrients in the study group and control group children were: serum zinc [89.4 (26.9) vs 93.5 (41.6) mg/dL; *P*=0.496], copper [118.3 (36.6) vs 123.3 (29.8) mg/L; *P*=0.133], magnesium [1.9 (0.3) vs 2.0 (0.2) mg/dL; *P*=0.015]. Total oxidant capacity level was not different in both the groups [median (range) – 124.8 (16.0 – 501.7) vs 146.8 (14.0 – 641.7) mg/mL; *P*=0.605]. 24 (33%) children with TDT had low serum zinc levels (<65 mg/dL), and 31 (43%) had high serum copper levels (≥121 mg/L). **Conclusions**: Children with TDT were found to have significantly lower magnesium levels compared to healthy children.

Keywords: *Free radical damage, Growth, Nutrition, Oxidation.*

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Micronutrients and antioxidants have been studied in transfusion-dependent thalassemia (TDT) to understand the pathophysiology of end organ damage [1-3]. Previous studies have indicated that oxidative stress in patients with TDT is caused by the alteration in serum trace elements and antioxidant levels [4,5]. Zinc, copper and magnesium are few of the important microelements having multiple roles at cellular level reducing the free iron induced cellular damage. Apart from the known chelating effect of desferrioxamine on zinc, no other chelators have any proven effect on these micronutrients. As the findings of previous studies on various micronutrients and antioxidants in children with TDT have not shown uniform results globally [1-6], we planned to study micronutrient and antioxidant levels among children with TDT in this region.

METHODS

This cross-sectional study was conducted at the thalassemia day care centre of a tertiary care pediatric hospital, between November, 2020 and October, 2021. All children with TDT aged between 3-14 years, who were registered at our centre and receiving regular blood transfusions and chelation were enrolled.

Children with TDT who had poor compliance to transfusion, less than six months of transfusion duration, and taking zinc or multivitamin supplementation during the last 3 months were excluded. The study was approved by the institutional ethic committee, and informed consent was obtained from parents and assent was taken from children older than 7 years. Healthy controls were taken from children visiting hospital's outpatient department either for routine immunization or for minor illnesses like upper respiratory infection. Those who had taken multivitamin or zinc supplementation at any time in last three months were excluded from the study. Detailed history and examination were done for all enrolled children. Children were asked to come after overnight fasting, and 4 mL of venous blood was collected (2 mL each for complete blood count, and for estimation of zinc, copper, magnesium, and antioxidant levels). Serum zinc and copper were estimated by direct colorimetric method with Diasys reagent and magnesium by xylidyl blue method (Beckman Coulter AU 680 biochemistry analyzer). Serum total antioxidant level was estimated by double antibody sandwich ELISA technique (Qaybee-Bio life sciences total antioxidant capacity kit). We measured the total antioxidant level in blood and body fluids (total antioxidant capacity, T-AOC); however, individual antioxidants were not analyzed separately. The normal level of serum zinc, copper and magnesium were defined as 65-118 mg/dL, 63.7-140.12 µg/dL and 1.8-2.5 mg/dL, respectively [7-9].

Statistical analysis: Data were entered in a Microsoft Excel sheet and analyzed using Statistical Package for Social Sciences (SPSS) Version 25.0 for windows. Student *t* test was used to compare differences between two means. Chi-square test or Fisher exact test were used for comparing categorical variables. *P* value <0.05 was taken as significant.

RESULTS

Eighty-five children with TDT were screened, out of which 72 aged 3-14 years (mean (SD) age 8.5 (3.2) years; 24 females) were included as study group and matched 83 healthy children were enrolled as controls (mean (SD) age 8.6 (2.8) years; 49 females). Comparison of baseline parameters of children with TDT and healthy controls is shown in **Table I** and **Table II**.

Children with TDT had lower serum zinc, copper, magnesium and antioxidant levels compared to healthy children, but the difference was statistically significant only for serum magnesium levels. 33% of the children with TDT had low serum zinc levels (*P*=0.003), while 43% had high serum copper levels (*P*=0.337). Around 22% of our study population was magnesium deficient (**Table I**). The antioxidant level was comparatively higher in TDT patient of age <5 years but not statistically significant.

There was no significant correlation between zinc and ferritin level or frequency of transfusion. The mean body weight and height were significantly lower among TDT patients with low zinc level (*P*=0.020 and *P*=0.021), which indicates that children of TDT with poor nutritional status had low zinc level. Serum copper value did not have any correlation with above mentioned parameters. The mean body weight and height were significantly higher among TDT children with hypermagnesemia (*P*=0.042 and *P*=0.010, respectively). Mean magnesium value had no correlation with height, weight or ferritin value. There was no correlation between anthropometric parameters including weight, height, BMI and antioxidant status in cases. The VLDL (very low density lipoprotein) and TG (triglyceride) level were negatively correlated with antioxidant level of children with TDT, where high VLDL level was seen with decreasing antioxidant level (RR -0.244; *P*=0.039).

Table I Baseline Characteristics of Children With Transfusion-Dependent Thalassemia

Parameters	*Outcome*
Age at diagnosis (mo)	11.8 (1.5)
Age at first transfusion (months)	11.4 (1.4)
Number of transfusions/y	17.5 (3.9)
Ferritin level (µg/L)[a]	1500 (123-8543)
Duration of chelation	
Deferasirox, *n*=72	68.6 (37.9)
Deferiprone, *n*= 57	17.1 (9.5)

Values in mean (SD or [a] median (range).

DISCUSSION

In this study involving 72 children with TDT, serum zinc, copper, magnesium and antioxidant levels were compared to healthy children.

Similar to our results, another Indian study of 35 thalassemia major patients, found 65% patients were zinc deficient [10]. A study from Egypt evaluated the prevalence of zinc deficiency in TDT patients where 98% were found to be zinc deficient [11]. On the contrary, low zinc level in only 2.6 % patients [3], and no zinc deficiency [12] in TDT have also been reported. Copper deficiency has been infrequently reported in TDT [2,15], and one study reported excess copper levels in up to 20% children [13]. Most of the studies on zinc level in TDT children showed low levels while few studies showed normal levels, and copper level was normal or elevated in most of the similar studies. Studies on magnesium are too less for predicting a trend in children with TDT.

The findings of our study on zinc and copper correlate with many of the similar studies done previously on TDT children, but the low magnesium value in children with TDT has been uncommonly reported, as very few studies have looked at the magnesium levels of these patients.

Table II Serum Levels of the Study Variables in Children With Transfusion-Dependent Thalassemia and Controls

Parameter	*Case (n=72)*	*Control (n=83)*
Age (y)	8.5 (3.2)	8.6 (2.8)
Weight (kg)[c]	23.1 (7.0)	26.4 (8.5)
Height (cm)	124.7 (1.3)	127.4 (18.3)
Body mass index (kg/m^2)[d]	14.6 (1.3)	15.7 (2.0)
Serum zinc (ug/dL)	89.4 (26.9)	93.5 (41.6)
Low (<65 µg/dL)[a,c]	24 (33.3)	41 (49.4)
High (>118 µg/dL)[a,c]	9 (12.5)	19 (22.9)
Serum copper (µg/L)	118.3 (36.6)	123.3 (29.8)
Low (<51 µg/L)[a]	1 (1.4)	0
High (>121 ìg/L)[a]	31 (43.1)	43 (51.8)
Serum magnesium (mg/dL)[c]	1.9 (0.3)	2.0 (0.2)
Hypomagnesemia (<1.8 mg/dL)[a]	16 (22.2)	12 (14.5)
Hypermagnesemia (>2.5 mg/dL)[a]	2 (2.8)	2 (2.4)
Total antioxidant status[b]	124.8 (16.0-501.7)	146.8 (14.0-641.7)

Value in mean (SD), [a] no. (%) or [b] median (range). [c]P<0.05 ; [d]P<0.001.

WHAT THIS STUDY ADDS?

- Children with transfusion-dependent thalassemia had lower magnesium levels than children without thalassemia.

The study has certain limitations including lack of detailed dietary evaluation, and not studying the effect of supplementation of the studied micronutrients. Lower than expected hemoglobin in children with TDT can be explained by the inability to maintain 3-4 weekly transfusion policy during the pandemic due to lack of blood donors, and inability to reach the hospital.

No significant differences were found between serum zinc, copper and anti-oxidant levels between children with TDT and healthy controls; though, serum magnesium levels were significantly lower in these with TDT. The pathophysiological basis for this difference, and its clinical implications still need to be demonstrated.

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