

PLASMA TRACE ELEMENT STATUS IN BETA-THALASSEMIA TRAIT PATIENTS

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ABSTRACT

Objective: The study aims to evaluate the plasma trace element status in beta-thalassemia trait (BTT) patients and demonstrate the correlation between trace elements and hemoglobin (Hb)-A1, HbA2, and HbF.

Methods: The study population consisted of 20 normal individuals and 40 patients with BTT aged between 25 and 55 years of both the sex. Hemoglobin variant analysis was performed in blood samples using cation exchange high-performance liquid chromatography. Patients were labeled as beta-thalassemia trait only if they had HbA2 >3.5% or HbF >2.0%. Plasma zinc was estimated by atomic absorption spectrometry, copper by bathocuproine disulfonate method, and iron by ferrozine method spectrophotometrically. Data were analyzed statistically by Student's *t*-test and Pearson's correlation using software SPSS version 20. $p < 0.05$ was considered statistically significant.

Results: Plasma zinc and copper decreased significantly in BTT patients compared to healthy controls. There was an apparent decrease in plasma iron in these patients. Degree of decline in zinc ($p < 0.001$) was much greater than that of copper ($p < 0.04$). Moreover, there was significant elevation of copper-zinc ratio and iron-zinc ratio ($p < 0.001$) in BTT patients compared to controls. Both HbA1 and HbA2 correlated positively with both copper and iron. Interestingly, HbF showed negative correlation with all the three trace elements in BTT patients.

Conclusion: The study highlights marked deficiency of plasma trace elements, hence, the need for periodic assessment and prompt administration of these micronutrients to reduce the complications in BTT patients. Further, ratios are more valuable markers in the determination of trace element status than individual elements.

Keywords: Beta-thalassemia trait, Copper, Iron, Zinc, Trace elements.

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INTRODUCTION

Beta-thalassemia trait (BTT) is the most common single-gene inherited hemoglobinopathy in India with no gender discrimination. Among all the health problems in India, though the burden of hemoglobinopathies is significant, little attention has been given compared to malnutrition and other communicable diseases [1]. In beta-thalassemia, there is reduced production of beta-globin chains and increased synthesis of alpha and gamma chains. Increased alpha chains make the developing erythrocytes more fragile, leading to ineffective erythropoiesis, early damage, and anemia. Some of the patients may exhibit other symptoms such as impaired immune status, osteoporosis, and infections [2]. Deficiency of various micronutrients such as minerals and vitamins adds to the complications in these patients. Trace elements, namely iron, copper, and zinc, are required for heme biosynthesis. Copper plays a key role in several enzymatic reactions in RBC and its deficiency interferes with iron transport and therefore heme synthesis. Zinc is another trace element that is essential for cell-mediated immunity, activation of ALA dehydratase, and general growth [3]. Most of the trace element studies have been done on beta-thalassemia major patients and data are scarce on trait patients. Hence, our study aims to evaluate the plasma trace elements and demonstrate the correlation between trace elements and HbA1, HbA2, and HbF in BTT patients.

METHODS

The study population consisted of 20 normal individuals and 40 patients with BTT aged between 25 and 55 years of both the sex. Random blood samples were collected in EDTA vacutainers and centrifuged at 3000 g for 10 min, plasma separated was used for the estimation of zinc by atomic absorption spectrometry and copper by bathocuproine disulfonate method where cuprous ions react with

bathocuproine disulfonate to give a red-colored product [4]. Plasma iron was estimated by ferrozine method, in which ferrous ions formed by reduction using ascorbate combines with ferrozine to produce colored complex which is then measured spectrophotometrically [5]. Hemoglobin variant analysis was performed in blood samples using cation exchange high-performance liquid chromatography. Patients were labeled as beta-thalassemia trait only if they had HbA2 > 3.5% or HbF > 2.0%. Patients with anemia (Hb <14.0 g% in males and <12.0 g% in females) with no evidence of "nutritional deficiency," renal or hepatic derangement, or chronic infection were considered for the study. None of the participants had taken mineral supplements before blood sampling. The study was approved by the institutional ethical committee and informed consent was taken from all the patients. Data were analyzed statistically by Student's *t*-test and Pearson's correlation using software SPSS version 20. $p < 0.05$ was considered statistically significant.

RESULTS

Hemoglobin A1 level was significantly lower with concomitant increase in HbA2 and HbF in BTT patients compared to normal individuals (Table 1). There was an apparent decrease in iron values in BTT patients and the decrease in zinc and copper in BTT patients were statistically significant compared to healthy individuals. Degree of decline in zinc level ($p < 0.001$) was much greater than that of copper ($p < 0.04$) (Table 2). Further, there was a marked elevation in iron-zinc ratio and copper-zinc ratio ($p < 0.001$) in BTT compared to controls. There was an insignificant negative correlation between zinc and different hemoglobins. Both HbA1 and HbA2 correlated positively with both copper and iron. Interestingly, HbF showed negative correlation with all the three trace elements in BTT patients (Table 3).

Table 1: Comparison of hemoglobin levels in BTT patients and normal controls

Parameter	Normal(n=20)	BTT(n=40)	p value
HbA1 (g %)	96.36±1.3	90.90±2.28	<0.001
HbA2 (g %)	2.94±0.24	5.27±0.24	<0.001
HbF (g %)	0.73±0.49	1.49±1.26	0.081

n: Number of samples. BTT: Beta-thalassemia trait, Hb: Hemoglobin

Table 2: Comparison of plasma trace elements in normal and BTT

Parameter	Normal(n=20)	BTT(n=40)	p value
Copper (µg/dl)	96.41±13.66	85.66±14.77	0.04
Zinc (µg/dl)	83±5	40±9	<0.001
Copper: zinc	1.16±0.21	2.34±0.84	<0.001
Iron (µg/dl)	74.07±10.6	62.64±28.8	0.26
Iron: copper	0.77±0.13	0.72±0.05	0.17
Iron: zinc	0.89±0.11	1.56±0.27	0.001

n: Number of samples. BTT: Beta-thalassemia trait

Table 3: Correlation of trace elements with hemoglobins in BTT

	Cu		Zn		Fe	
	r	p	r	p	r	p
HbA1	0.18	0.29	-0.45	0.79	0.68	0.09
HbA2	0.05	0.73	-0.01	0.95	0.06	0.88
HbF	-0.20	0.43	-0.84	0.75	-0.76	0.13

BTT: Beta-thalassemia trait, Hb: Hemoglobin

DISCUSSION

Trace elements play a key role in metabolism within erythrocytes by participating in heme biosynthesis and other enzymatic reactions. BTT is a common heterozygous hemoglobinopathy characterized by hypochromia, microcytosis, and low-grade hemolysis [6]. Deficiency of trace elements like zinc is implicated in immune dysfunction. Moreover, impaired immune function is one of the salient features of BTT. Thus, alterations in these essential minerals seem to be related to different clinical complications associated with BTT.

The present study demonstrated a significant decrease in both copper and zinc in plasma of BTT patients compared to healthy controls. In accordance with our study, thalassemia major patients on iron chelation therapy and Jordanian thalassemia patients also exhibited decreased plasma copper and zinc [7,8]. However, thalassaemic natives of Iran showed high copper but low plasma zinc [9]. In contrast, plasma copper remained normal in thalassemia major children but high in adult patients [10,11]. Increase in copper level has been attributed to recurrent infections in thalassemia major patients. Decreased plasma zinc in adult thalassemia major patients was also true for thalassemia minor patients as noted in our results. Decrease in plasma zinc may be due to increased excretion of zinc in urine released from hemolyzed red cells and due to malabsorption. Deficiency of zinc both in erythrocytes and plasma was a common feature of other hemoglobinopathies such as sickle cell disease and Hb E [12,13]. Further, copper and zinc deficiency may lead to a significant decline in erythrocyte membrane SOD, leading to increased oxidative stress and decreased RBC survival time [14]. Sickle cell anemia patients treated with zinc showed improvement both in skeletal growth and immune status [15]. Interestingly, zinc correlated negatively with all types of hemoglobins. Further, BTT patients exhibited marked elevation in copper-zinc ratio than individual elements in the present study, highlighting the fact that this ratio may be a more valuable indicator of imbalance in the microelement status in hemoglobinopathies [9]. Decrease in plasma iron in BTT patients as observed in the present study may be due to low ceruloplasmin that interferes in iron transport and utilization. Moreover, Babu *et al.*

observed significant variation in complete hemogram in BTT patients compared to healthy individuals justifying our findings [16]. Iron-zinc ratio doubled in BTT patients compared to normal signifying the importance of ratios in assessing the trace element status in these patients. In RBC, free iron is most likely redox active element that leads to the production of hydroxyl radical which initiates membrane lipid peroxidation and protein oxidation thereby hemolysis. On aerobic incubation of RBCs, release of free iron was greater in BTT patients than controls [17]. High levels of HbF in BTT patients correlated with iron release, suggesting that persistence of HbF favors iron release and excess alpha chains act as prooxidants [18]. Moreover, trace elements (iron and copper) correlated positively with Hbs (A1 and A2) and a negatively with HbF in BTT patients. On the contrary, earlier reports showed no correlation between the iron state and the hemoglobin levels in beta-thalassemia trait subjects [19].

The study highlights marked deficiency of plasma trace elements, hence, the need for periodic assessment and prompt administration of these micronutrients to reduce the complications in BTT patients.

AUTHORS' CONTRIBUTION

Prabhakaran: Biochemical analysis, Dr. Sudha K: Study design and manuscript preparation, Dr. Reshma K: Manuscript revision, and Mr.Durgarao: Statistical analysis.

CONFLICTS OF INTEREST

There are no conflicts of interest among the authors.

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