

Quantitative Assessment of Serum Copper Status in Children with Thalassemia Major

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Authors' contributions

This work was carried out in collaboration among all the authors. Authors TRC, AKMAM designed the study, wrote the protocol, supervise the work and review the manuscript. Authors TRC, SN managed the analyses of the study. Authors SN, SB, TIC prepared the first draft of manuscript. Authors SI and BKP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objective: Support of ordinary degrees of copper, has a significant influence in reducing the morbidities related with thalassemia major. Levels of this component has been seen as modified in this incessant transfusion subordinate infection from everywhere throughout the world, however with

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broadly factor results. Other than rehashed blood transfusions and utilization of chelating operators, the level of copper in thalassemia patients are influenced by hereditary and dietary factors likewise, convincing us to embrace this investigation in our population. The Objectives of this study was to appraise of serum levels of copper in children with beta-thalassemia major.

Methods: In this observational comparative research design, all children between 5 to 15 years affected by beta thalassemia major (30 patients) and 30 normal children were evaluated for serum copper levels in the Department of Pediatrics, DMCH and attended at Thalassemia Center, DSH (Dhaka Shishu Hospital). Serum measurements for copper were performed using Atomic Absorption Spectrophotometer (Varian, AA 240 FS, USA) in Analytical Chemistry Laboratory, Chemistry Division, Atomic Energy Centre, Dhaka, Bangladesh.

Results: The minimum, maximum, and the mean concentration of serum copper in patients were 103.50, 239.94, and 147.55 ± 35.17 $\mu\text{g/dl}$ and in control group, those were 58.75, 192.43, and 123.85 ± 33.19 $\mu\text{g/dl}$ respectively. There was no significant difference in serum copper level in the β thalassemia group and the control group ($p=0.54$) ($P=0.3$).

Conclusion: This investigation revealed that there is no copper inadequacy. Further assessment in such manner is suggested.

Keywords: Thalassemia; pediatrics; serum; atomic absorption spectrophotometer.

1.INTRODUCTION

Thalassemia is one of the most common inherited disorders of hemoglobin in the world [1]. It has been estimated that there are 270 million carriers of mutant globin alleles worldwide which can cause severe forms of hemoglobinopathy and thalassemia [1]. This disease was initially described by Cooley and Lee [2]. The most common types of this disease are the alpha and beta thalassemia. Thalassaemia is an inherited hemoglobin disorder resulting in chronic hemolytic anaemia. In this disease, β – globin fibers are not enough (β^+) or do not exist (β^0). More than 200 mutations can cause β – thalassaemia but 20 identical alleles bring 80% of thalassaemia in the world. The gene prevalence of thalassaemia has been reported all over the world on average of 3% [3]. WHO has estimated that about 1.5% of the world's population might be carriers of β – thalassaemia, and it is predicted that annually 70,000 children are born with various types of thalassemia, the majority of which are β -thalassemia, which is the most severe form, commonly presenting with common symptoms of anemia and requiring regular blood transfusions [4,5]. These individuals mostly originate from the Mediterranean, Middle East, Central Asia, India & Southern China [6]. The Maldives has the highest incidence of thalassemia in the world with a carrier state of 18% of the population. The estimated prevalence is 3-8% among populations from Bangladesh, China, India, Malaysia & Pakistan. A very low prevalence has been reported from the pupil in Northern Europe (0.1%) and Africa (0.9%).

Beta thalassemia major is the most severe form requiring repeated blood transfusions and desferrioxamine injections. Although such treatments increase the patients' life span, on the other hand a variety of complications, including endocrine, metabolic, skeletal, and growth disorders are being observed due to the high contents of iron storage in the body [7,8].

Trace elements and other minerals play asignificant role in thebody. These elements are presented in appropriate amounts and reacted with other elements to form key biomolecules as well as participatingin chemical reactions in the body. Insufficiency or abundance of any elementcauses severe metabolic abnormalities and leads to toxicity. The variation in the element level of serum can be potentially used for the diagnosis and monitoring of various disorders, environmental exposure and nutritional deficiencies in the tissues and body fluids [9,10]. Moreover, copper is viewed as a significant micronutrient with the most noteworthy sum in liver, cerebrum, heart, and kidneys. Copper is also an essential structural co-participant of many enzymes acting as a cofactor in a majority of enzymatic reactions including those of cytochrome C oxidase, lisyloxidase, superoxide dismutase, and thyrosinase [11,12]

Also some reports are indicating a change in serum level of copper in patients with thalassemia major [13,14]. We have conducted this study to evaluate the serum copper level in β thalassaemia major and to compare it to the normal subjects.

2. MATERIALS AND METHODS

2.1 Sample Collection

In this prospective observational research study all children between 5 to 15 years affected by beta-thalassemia major (30 patients) and 30 number of age and sex-matched normal healthy children that were covered by Department of Pediatrics, DMCH and attended at Thalassemia center, DSH (Dhaka Shishu Hospital), were evaluated for copper levels. Inclusion criteria were: 1) Children suffering from β thalassaemia major, diagnosed by Hbelectrophoresis. (HbA – absent or decreased, Hb A-2-variable. HbF-increased > 90%) 2) Age in between 5-15 years 3) Patients who received at least 10 times blood transfusion. Children less than 5 years and above 15 years old and other hemoglobinopathies were excluded from the study. Demographic and anthropometric data and history of disease and treatment were collected by physical examination, medical records, and laboratory measurements.

Weight and height were measured by using standard methods by corresponding pediatrician. There is standard international serum values copper in children. The normal value ranges for copper were 70–150 $\mu\text{g/dl}$ [15]. Written permission of the parents to take a 5 ml blood sample for determination of serum levels copper was obtained following a clear full oral explanation of the subject of research. After collection of the whole blood, the blood was transferred into labeled acid-washed Cu-free tubes and allowed to clot by leaving it undisturbed at room temperature. This usually takes 15–30 minutes. The clot was removed by centrifuging at 2500 rpm for 10 minutes in a centrifuge.

The resulting supernatant is designated serum. Following centrifugation, it is important to immediately transfer the liquid component (serum) into a clean polypropylene tube using a Pasteur pipette. The samples were maintained at 2–8°C while handling. When the serum is not analyzed immediately, the serum sample was apportioned into 0.5 ml aliquots, stored, and transported at –20°C or lower. Later, the samples were transferred to the Analytical Chemistry Laboratory, Chemistry Division at Atomic Energy Center, Bangladesh Atomic Energy Commission, Dhaka.

2.2 Solvents and Reagents

High-purity de-ionized water (resistivity 18.2M Ω cm⁻¹) was obtained using a Milli-Q water purification system (Thermoscientific, MA USA). All reagents were of analytical-grade. All solutions were prepared in a pre-cleaned hood. Standard Cu solution (1000 $\mu\text{g/L}$; Spectropure, USA) was obtained from Agilent Technologies (Santa Clara, CA, USA). Polypropylene bottles and tubes were soaked overnight in 10% (v/v) HNO₃, and cleaned by rinsing three times with high-purity deionized water and dried. All operations were performed in a cleanhood to prevent contamination of samples.

2.3 Sample Preparation and Analysis

After receiving the serum samples in Analytical Chemistry Laboratory, dilution of samples were tendered to 10 mL using ultrapure deionized water if required. Analytical calibration standards and spiked samples with matrix from known standards were prepared from standard Cu stock solution of 1000 $\mu\text{g/L}$. A calibration curve of 0.1–10 $\mu\text{g/L}$ was constructed. The serum content of copper was measured using Flame Atomic Absorption Spectrophotometer (FAAS) (Varian AA 240FS) equipped with hollow cathode lamp having a Lamp current 4 mA, wavelength of 324.8 nm and a slit width of 0.7 nm and operated according to the conditions recommended by the manufacturer. The type of flame was Air/Acetylene, the flow rate of Air and Acetylene was 13.50 L/min and 2.90 L/min respectively. Moreover, the purity of Acetylene gas was 99.99% pure used for the flame.

In order to monitor the result whether it is reliable enough to be released or not, internal quality control activity was performed. Furthermore, analysis of blank, duplicate, and spike recovery of the analyte were measured during the analysis as a piece of the inward quality control rehearses.

The data were recorded and analyzed by the t-test. P-value < 0.05 was considered as significant and Chi-square and one-way ANOVA test has been used to find out the significance of the study parameters on a categorical scale between two or more groups. All parents were given clear explanations regarding the methodology of the research. The children were included in the study if their parents agreed and signed the consent form.

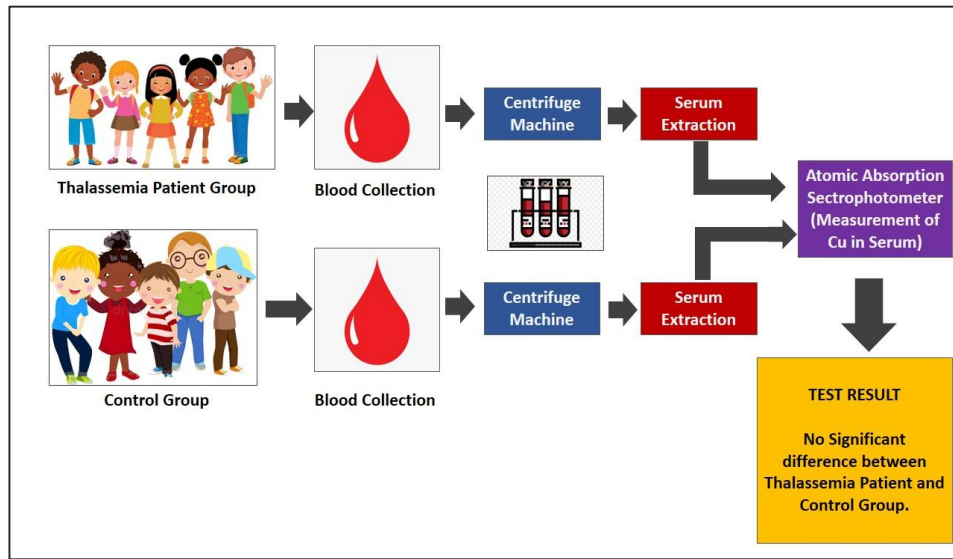


Fig. 1. A conceptual diagram of serum copper status in children with Thalassemia major and healthy control group

3. RESULTS

A total of 30 children samples were collected from β -thalassemia major where 15 (50%) cases acquired from males and 15 (50%) cases from females. The age range of collected 30 samples of β -thalassemia patients and healthy samples was 60-168 months. Total of sixteen children (26%) came from a family with consanguinity of marriage while 44 (74%) came from a family of non-consanguinity of marriage. In β thalassemia group 11(36%) children were from a family of consanguinity while it was only in 5(16%) in the control group. In β thalassemia group twenty-three (76%) cases had palpable liver and in 7(24%) cases liver was not palpable. In the case of the spleen, 100% of cases came with the palpable spleen.

Regarding the nutritional status of the study population, among 30 thalassemia patients, 2 patient was severely wasted and 5 patient was

moderately wasted. In the control group no children were severely wasted, 2 children were moderately wasted and rests are normal. There was no significant difference in weight for height between the two groups.

In this study, the hematological parameters of both groups are extremely variable (Table-1& Fig. 2). In β thalassemia major, the mean hemoglobin level, Mean Corpuscular Volume (MCV) of RBC, Mean Corpuscular Hemoglobin (MCH) , Corpuscular Hemoglobin Concentration (MCHC) were significantly lower than that of the control group ($p=.001$).

The results of minimum, maximum, and the mean concentration of serum copper in patients and the control group were shown in Table 2. There was no significant difference in serum copper level in the β thalassemia group and the control group ($p=0.54$). (Table-2).

Table 1. Hb and RBC indices of the study population (n= 60)

Haematological parameters	β thalassemia major group				Control group				p value
	Mean	SD	Min	Max	Mean	SD	Min	Max	
Haemoglobin (gm/dl)	6.2	1.4	3.5	8.5	10.62	2.9	8.0	12.5	.001
MCV(fl)	68.03	2.19	55	80	83.39	5.0	74	92	.001
MCH(pgm)	21.76	3.37	17	27	27.23	1.6	25	30	.001
MCHC(gm/dl)	27.63	2.32	23	32	31.74	1.5	28	34	.001

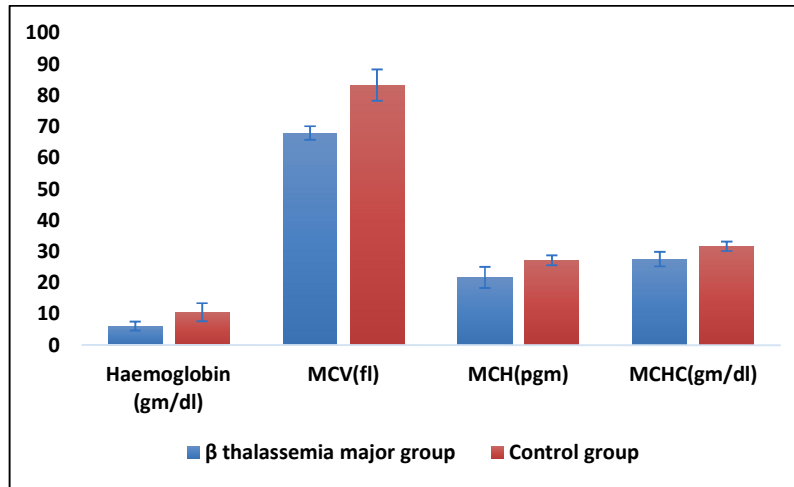


Fig. 2. Distribution of hematological parameters inthalassemia patients and control group

Table 2. Serum copper level of the children

Group	Serum copper (µg/dl)				p value
	Mean	SD	Min	Max	
B Thalassemia group	147.55	35.17	103.50	239.94	0.54
Control group	123.85	33.19	58.75	192.43	

4. DISCUSSION

In this study, male and female patients were equal in number, which was consistent with the study conducted in Bangladesh by Rahman & Jamal [16] where the sex ratio was roughly 1:1. The mean age in the study group was approximately 106.12 months with standard deviation of 30.76 months. The youngest and the oldest children were of 60 and 168 months respectively. These findings are almost consistent in previous studies [17,18].

These results of low Hb among patients can be explained by the limited health education of the parents about the disease, so that, blood transfusion was used only when the patient showed clinical symptoms caused by severe anemia or simply just to sustain life [19,20]. Whereas reports from other countries focused on a super transfusion program (maintaining Hb level above 12 g/dl) or hyper transfusion program (where the Hb level never allowed dropping below 9 g/dl) [21].

Other haematological parameters of both groups were extremely variable. Mean MCV, MCH and MCHC in thalassemic group were significantly lower than those in their control counterpart ($p = 0.001$ in all parameters). These findings are favorably comparable with those of Vichinsky

[22]. These findings are comparable with Iranian and Thai studies where mean height for age was $96.2 \pm 4.7\%$ and $96 \pm 4.0\%$ respectively [23,24].

Copper is one of the essential micronutrients of the human body mainly attached to albumin and ceruloplasmin. This trace element acts as the cofactor for at least 30 enzymes and many manifestations of copper deficiency and toxicity are associated with irregularities in these enzymes [11,12]. Some studies showed that there was an increase in the serum level of copper in patients experiencing thalassemia major [14,24,25]. Al-Samarrai et al. concluded that the etiology of hypercupremia is hemochromatosis, which is a principal complication of thalassemia [26]. However, reports of Bekheirnia [27], Tabatabaei [28], Naser [29] and Eshghi [30] revealed a reduction in serum level of copper, although a study by Kassab-Chekir showed no change in copper concentration of serum [31].

The serum concentration of copper in patients with thalassemia major depends on several factors including the amount of copper intake in the daily diet, intestinal uptake of copper, iron accumulation, kidney function, copper to zinc ratio, and administration of Desferal [14,25]. Shamshirsaz et al. found the deficiency of serum copper concentration while Kajanachumpol et al.

found the higher copper concentration in thalassemia patients [13,14]. Fortunately none of our thalassemia patients had copper deficiency. This shows that the factors that influence the copper levels are under control.

5. CONCLUSION

This study revealed that but there was no copper deficiency. Further evaluation in this regard is recommended. There was no significant difference between β thalassemia major and normal control in terms of serum copper level. The limitations of our study were the small group, local investigation, and unavailability of data concerning diet regimens. We recommend the repetition of this study on a larger scale.

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CONSENT AND ETHICAL APPROVAL

The aims and objective of the study along with its procedure, alternative diagnostic methods, risk and benefits were explained to the patient's parents in detail, in easily understandable local language and then voluntary informed written consents were taken from the parents before collecting data. Privacy, anonymity and confidentiality were maintained during the procedure. It was assured that the procedure was helpful for both the physician and patients in making rational approach regarding management of the case. The Ethical Committee of Dhaka Medical College approved this research protocol.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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