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Pattern of Transfusion Related Zinc and Copper Derangements in Beta Thalassemia Major Patients

Kehkshan Waheed, Zujaja Hina Haroon, Afshan Bibi, Muhammad Usman Munir, Sobia Irum Kirmani, Muhammad Amir

Department of Chemical Pathology, Armed Forces Institute of Pathology/ National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To assess serum zinc and copper levels in beta thalassemia major patients as compared to healthy subjects.
Study Design: Comparative cross-sectional study.
Place and Duration of Study: Chemical Pathology and Endocrinology Department Armed Forces Institute of Pathology, in collaboration with Armed Forces Institute of Transfusion, Rawalpindi Pakistan, from Jan 2021 to Jun 2021.
Methodology: The patients of transfusion dependent thalassemia major aged between 4 to 35 years were included in the study along with healthy subjects in 1:1. After collection of blood samples in plain serum tubes, serum Zinc (Zn) and copper (Cu) levels of all subjects were analyzed on atomic absorption spectrophotometer. Zn and Cu levels were expressed as Mean±SD. Independent sample t-test with significant *p*-value of ≤0.005 was used to compare Zn and Cu concentration of patients with thalassemia major with that of healthy controls.
Results: Study included 80 subjects (40 patients and 40 controls) with mean age of 13.33±7.69 years. The mean value of serum Zn and Cu in beta thalassemia major patients were 8.62±1.77 µmol/L and 14.46±5.92µmol/L respectively as compared to 15.08±2.8 µmol/L and 13.45±2.80 µmol/L in healthy controls. Zn levels showed a statistically significant difference (p=0.005) between two groups while Cu levels also showed statistically significant difference (p=0.03) between the two groups.
Conclusion: Beta thalassemia major patients showed significantly lower levels of serum zinc and higher levels of serum copper as compared to healthy controls which should be taken into consideration for continuous monitoring and prompt correction.

Keywords: Atomic Absorption Spectrophotometry, Beta Thalassemia Major, Serum Zinc, Serum Copper.

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INTRODUCTION

The most commonly inherited single gene disorder, Beta-Thalassemia major is caused by approximately 200 mutations in the β-globin genes resulting in abnormal synthesis of hemoglobin chain causing anemia. Patients in this situation need repeated blood transfusions in order to survive.[1] Approximately 3% of the entire world's population carries this gene and there are an estimated 60000 beta thalassemia babies born every year around the world.[2] The carrier rate in Pakistan is between 5 - 8%, so there are 9.8 million carriers and around 5000 new cases of beta thalassemia are detected every year.[3] The Thalassemia Federation of Pakistan registers almost 25,000 children which is actually a small number compared to the actual number which may be about hundred thousand as many people who are living in villages aren't enumerated in any thalassemia centers. Beta thalassemia major is caused by a lack of knowledge and consanguineous marriages that is why among first degree relatives, thalassemia is most prevalent.[4]

As a result of ineffective erythropoiesis, patients with thalassemia have faster red blood cell (RBC) turnover, which causes RBCs to die sooner, which increases energy and nutritional requirements in order to keep up with normal erythropoiesis. Getting proper treatment will allow these patients to live longer and with a better quality of life.[5,6] Even though iron overload in transfusion dependent beta thalassemia major patients has been widely studied, there is little information about other trace element levels.[7]

Zn is an essential micronutrient for human body, works as a cofactor for approximately 300 enzymes, and has an essential role in human growth and development.[8] Moreover, Zn contributes significantly to the synthesis of heme through activation of certain enzymes.[9] Among patients with transfusion dependent beta thalassemia major, Zn deficiency is considered to be a major cause of growth and puberty disorders. Shamshirsaz *et al.*, has found that about 80% of thalassemic patients were deficient in zinc and 68% in copper in his study of beta thalassemia major patients.[10]

Correspondence: Dr Kehkshan Waheed, Department of Chemical Pathology, Armed Forces Institute of Pathology Pakistan
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Copper (Cu) is also one of the important trace element; it is present in high levels in heart, kidney, and brain. Furthermore, Cu plays significant role in the growth of connective tissues, nerves and bones. Also, it is involved in a variety of enzymatic reactions that reduce molecular oxygen function like superoxide dismutase, cytochrome oxidase, and other oxidases.[11]

Due to lack of studies in local population this study was planned to determine derangements of serum Zn and Cu in thalassemia major patients. The aim of study was to assess levels of serum zinc and copper in transfusion dependent thalassemia major patients before the harmful effect of deranged levels of these elements occur.

METHODOLOGY

This comparative cross sectional research work was conducted at the Chemical Pathology & Endocrinology Department of Armed Forces Institute of Pathology (AFIP) Rawalpindi, in collaboration with Armed Forces Institute of Transfusion (AFIT) Rawalpindi Pakistan, from January 2021 to June 2021 following approval from the Ethical Committee of the Institute under the IRB No (FC-CHP-3/READ-IRB/21/148). Non probability consecutive sampling technique was employed for sample collection.

World health organization calculator was used for sample size estimation, based on prevalence of Beta thalassemia major in Pakistan taking 95% confidence interval and 5% margin of error. Pakistan has beta thalassemia trait frequency between 5-8%, thus there are more than 10 million carriers in country and every year, around 5000 children are diagnosed as beta thalassemia major in Pakistan,[12] A total of 80 participants (40 patients and 40 healthy controls) aged between 4 and 35 years including both genders were inducted by consecutive non-probability sampling. Patients were enrolled after taking informed consent with fulfillment.

Inclusion Criteria: Of having Beta thalassemia major confirmed on hemoglobin electrophoresis and undergoing transfusion for longer periods than 6 months. Age and gender matched subjects having Zn and Cu within reference limits were enrolled as healthy controls for comparison purpose. Subjects taking Zn/Cu supplementations or having chronic illnesses affecting serum Zn and Cu levels like chronic liver disease (CLD), chronic kidney disease (CKD), ischemic heart disease (IHD)or any malignancy were put under.

Exclusion Criteria: Each subject had 5ml of whole blood drawn in a plain tube with clot activator gel to analyze serum Zn and Cu levels. As soon as the samples were collected, the gel tubes were allowed to clot at 25ºC (room temperature) and centrifuged for 3 minutes at 3000 rotations per minute (RPM) within 2 hours to obtain serum. The serum was separated and frozen at -20ºC until analysis. Serum Zn and Cu levels were measured by flame atomic absorption spectrophotometer by Agilent Technologies USA and 95% Nitrogen was used as fuel along with room air to achieve temperatures of 2000C.Two level quality control materials were run with each batch of samples. Quality control was validated by plotting the results on Levy Jennings (LJ) chart and applying Westgard rules. For evaluating the deficiency of Zn among the study population, the reference ranges were searched in literature and serum Zn levels of 10.7-18.4umol/l were taken as optimal levels and for copper 11-24umol/l were taken into account as optimal levels. The values below and above these ranges were taken as abnormally low and high respectively.

Data was evaluated using statistical package for social sciences (SPSS) version 21. Variables were found to be having normal distribution according to the Kolmogorov-Smirnov test. Hence, to compare serum Zn and Cu concentrations in transfusion dependent Beta thalassemia major patients and normal healthy controls, paired sample t-test was applied. For quantitative variables, mean and standard deviation were calculated, whereas frequencies were calculated for the qualitative variables. Statistically, p-value≤0.05 was considered as significant.

RESULTS

Our study included a total number of 80 participants having mean age of 13.3±7.6 years ranging from 4 to 35 years including 30(40%) females and 50(60%) males. Participants were divided into two groups depending on presence or absence of beta thalassemia major. Out of 80 participants 40(50%) had beta thalassemia major disease and were dependent on repeated blood transfusion for survival and rest 40(50%) participants were taken as normal healthy controls. All the participants having beta thalassemia major disease were undergoing blood transfusion for more than six months on regular intervals to maintain their hemoglobin level hence survival.

Both the groups were matched for age and gender. Blood hemoglobin level was found significantly lower (p-value=0.001) and serum ferritin

significantly higher (*p*-value=0.001) in transfusion dependent beta thalassemia (TDT) major patients compared to that of healthy controls as shown in (Table-I). This difference could be attributed to the disease process including fast RBC turnover and excessive iron overload due to repeated blood transfusions.

The means of the studied nutrients among the participants are shown in (Table-II). As compared to the healthy controls, beta thalassemia major patients had significantly lower serum Zn levels (*p*=0.001) and significantly higher serum Cu levels (*p*=0.030). The difference could be attributed to exhaustion of these divalent ions during metabolic processes to cope with energy requirement and oxidative stress in beta thalassemia major patients.

Table-I: Descriptive Statistics of Quantitative Parameters (n=80)

Parameter	Healthy group (n=40) Mean±SD	Transfusion Dependent Thalassemia Major (n=40) Mean±SD	*p*-value
Age (years)	13.42±7.54	13.3±7.69	
Haemoglobin (g/dl)	11.76±1.04	7.71±0.89	0.001
Ferritin (ng/ml)	52.25±43	3128.62±1787.89	<0.001

Hb (haemoglobin). TDT (transfusion dependent thalassemia

Table-II: Serum Zinc and Copper Levels in TDT Patients and Healthy Controls (n=80)

	Thalassemia Major Patients (Mean±SD)	Healthy Controls (Mean±SD)	*p*-value
Serum zinc(µmol/L)	8.62±1.77	15.02±2.82	0.001
Serum copper(µmol/L)	14.46±5.92	13.45±2.80	0.030

DISCUSSION

Zinc and copper are important divalent ions required for many metabolic processes in the body. Their main source is dietary intake and deficiency is attributed to many metabolic diseases. In already compromised patients of beta thalassemia major, this can lead to severe consequences and lead to metabolic crisis. A simple intervention in the form of dietary supplementation of these micronutrients can overcome this deficiency.

In our study, out of total eighty patients, 40(50%) were transfusion dependent beta thalassemia major (TDT) patients and 40(50%) were age and gender matched healthy individuals. Mean serum Zn levels in TDT patients was found to be 8.62±1.77 (µmol/L) while in healthy people it was found to be 15.02±2.82 (µmol/L).We did not stratify the study population in groups of male and female genders as it is only significant in elderly groups. A study done in Geriatric Medicine department of The Queen's University in Belfast UK also found that gender differences in serum Znare present in elderly, with males exhibiting elevated levels as compared to females of age matched geriatric patients.[13]

Patients with thalassemia major enrolled in the study had a significantly lower values of serum Zn as compared to the controls. Out of 40 beta thalassemia major subjects 33(82.5%) had Zn levels <10.7 µmol/L which is the lower limit of optimal serum Zn levels. While among healthy individuals out of 40 subjects none were having serum Zn levels <10.7µmol/L. Findings of present study are consistent with a study done in Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand which revealed that Zn deficiency seemed to be positively associated with thalassemia major status which probably resulted from combined low dietary zinc intake, repeatedencountersto red blood cell lysis and iron chelation therapy.[14]

Patients with beta thalassemia major on transfusion are thought to develop abnormal glucose tolerance due to distorted oxidant-antioxidant function. A micronutrient essential for insulin synthesis, storage, and secretion, zinc is an important antioxidant. It can reduce hemolysis and iron overload while providing other health benefits. A study published by Mahmoud R *et al*., in May 2020 revealed that Zn as a supplement when given to beta thalassemia major patients having DM reduced hyperglycemia, also caused an increase in secretion of insulin, and their glycemic control was improved without causing any undesirable effects.[15]

The results of our study also showed that there was a significant difference in serum Cu levelsamong transfusion-dependent beta thalassemia major patients and controls in our study. Beta thalassemia major patients had significantly raised Cu levels as compared to healthy controls. Out of 40 thalassemia major subjects 4(10%) had Cu levels above the upper reference limit. These results are inconsistent with Sharif Y *et al*.,[16] whoshowed that serum copper levels were lower in thalassemic patients than in healthy controls. This can be attributed to the lysis of RBCs during repeated transfusion and exhaustion of these divalent by consumption in oxidative phosphorylation

and cytochrome systems. In a study by Fahmya E Metal done in Sohag University Hospital, Egypt in 2019, no significant correlation was seen between serum Cu and Zn levels and serum ferritin level.[17] Study by Zekavat *et al.,* (2018) also had similar results.[18] The inconsistent results might be due to the fact that serum copper levels are also influenced by various other factors, such as over-supply of iron, copper-to-zinc ratio, copper consumption, renal function and use of iron chelation therapy like deferoxamine.[19,20]

This study emphasizes the need to assess serum zinc and copper concentrations in transfusion dependent beta thalassemia patients for prompt management and improvement in their quality of life, and also decreasing chances of complications.

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LIMITATIONS OF STUDY

Zinc and copper levels may differ with individual's diet and medications.Still there are some possible limitations that must befocused upon. Because the sample size in this cohort is small and not illustrative of the actual population of TDT patients, the generalizability of the current findings may be limited. Furthermore, the serum values might not be the best predictor of total body reserves of the above-mentioned trace elements.

RECOMMENDATIONS

Regular determination of zinc and copper levels in these patients may help prevent the adverse effects of altered micronutrient concentrations such as cardiovascular atherosclerotic disease and can aid in improving quality of life and health status. More research is needed to better understand Zn and Cu deficiency and its consequences on such patients with bigger sample size, longitudinal study & multi-centric approach. Zn supplementation may be a useful public healthintervention for transfusion dependent beta thalassemia major patients in improving their quality of life by replacement therapies.

CONCLUSION

Serum Zn levels were found significantly lower in patients of thalassemia. While Cu levels were significantly raised. Transfusion dependent patients of Thalassemia Major should be monitored for serum Zinc and Copper as they may develop deficiency of zinc and overload of copper.

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Authors' Contribution

Th following authors have made substantial contributions to the manuscript as under:

KW & ZHH: Data acquisition, data analysis, critical review, approval of the final version to be published.

AB & MUM: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

SIK & MA: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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