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Serum Antioxidant Levels in Children with Beta-Thalassemia Major

Farzin L[1]*, Sajadi F[1], Kupai L[1]
1- Environmental Laboratory, Nuclear Science Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran (AEOI), Tehran, Iran.

***Corresponding Author:** Farzin L, Email: LFarzin84@yahoo.com*

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Abstract

Background: Thalassemia represents a serious health problem in Iran because of its heterogeneous frequency and the existing endogamy system. It is an inherited blood disease characterized by the under production of normal hemoglobin, the oxygen-carrying protein in red blood cells.

Materials and Methods: In this study, serum antioxidants including selenium (Se), zinc (Zn) and copper (Cu) were measured using atomic absorption spectrometry (AAS) in children with beta-thalassemia major (n=40) and compared with the control group (n=40).

Results: There were significant differences in the values of Se, Zn and Cu between the two groups. Se and Zn levels were 41.78±22.87 µg/l and 61.70±13.25 µg/dl in thalassemia, but 108.63±25.79 µg/l and 114.72±23.70 µg/dl in healthy children. Meanwhile, Cu levels were 156.60±22.62 and 102.88±31.15 µg/dl among patients and controls, respectively.

Conclusion: This study shows that Se, Zn and Cu may play a role in the pathogenesis of beta-thalassemia major.

Key words: Beta-thalassemia, antioxidants, children

Introduction

Red cell genetic abnormalities are chronic diseases with no cure. They often require lifelong care and management strategies. Among these genetic abnormalities, thalassemia constitutes a major public health problem in many parts of the world, particularly in the Mediterranean region, Middle East and Southeast Asia. Iran is in the middle of the so-called thalassemia belt and has a high thalassemia carrier rate which exceeds 10% in some parts of the country [1]. Thalassemia is more prevalent in the northern (Caspian Sea coast) and southern (Persian Gulf and Oman Sea coasts) parts of the country [1].

Beta-thalassemia major (β-TM) arises as a consequence of decreased synthesis of beta hemoglobin chains. Consequently, the concentration of the beta hemoglobin tetramers is substantially reduced and an excess pool of unpaired alpha hemoglobin chains is present, leading to red blood cells damage by oxidative means, which may be further potentiated by the heme [2]. Anemia in β-TM is caused by a combination of ineffective erythropoiesis and premature hemolysis of red blood cells in peripheral circulation. Furthermore, β-TM patients are under continuous blood transfusion leading to iron overload.

To guard against oxidative hemolysis, some protective antioxidant agents exist in the body[3]. Antioxidants play a part in the synthesis and structural stabilization of both proteins and nucleic acids and some of them, such as Se, Zn and Cu are part of enzymes [4]. The formation of free radicals able to damage proteins, lipids and nucleic acids is a continuous physiological reaction [5]. Recently, more attention has been paid to the role of antioxidants and the free radicals has proven as being involved in aging and a number of chronic diseases such as Beta-thalassemia.

Atomic absorption spectroscopy (AAS) is currently a preferable method for the quantitative analysis of a significant number of biological samples [6].

The aim of this study was to evaluate the level of antioxidants (Se, Zn and Cu) in children with beta-

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thalassemia major living in Tehran in comparison with normal subjects.

Materials and Methods

Patients and controls

This study was carried out among 80 children from Tehran, Iran, belonging to different social levels, whose age was less than 12 years. We examined two groups of children, one with beta-thalassemia major (n=40), and the other one as controls (n=40). Patients were receiving regular transfusion therapy, but were not receiving any chelation therapy. Twenty (50%) of the 40 children with β-TM were male and 20 (50%) were female. In the control group, there were 19 (47.5%) male and 21 (52.5%) females. To avoid the effects of concurrent infections on serum Se, Zn and Cu concentrations, children who had an infection as recently as 2 weeks before the study were excluded. Moreover, children with malnutrition were not included in the study. The purpose of this study was explained to all the participants and a written informed consent was obtained.

Sample collection

We took blood samples (10ml) at 8-9am after fasting and collected it in polipropilen tubes containing lithium heparin (Vacuette, Geiner Labortechnik, Kremsmünter, Austria). Serum was separated within two hours, and if the samples were not going to be evaluated at the same day, they were stored at –30 ºC [7].

All laboratory ware including pipette tips and autosampler cups were cleaned thoroughly with detergent and tap water, rinsed with distilled water, soaked in dilute nitric acid then rinsed thoroughly with deionized distilled water.

Determination of antioxidants in the serum

The serum samples were diluted five times in chloric acid (0.1 N), with the addition of double distilled water for Zn and Cu measurements. Flame AAS (Spectra AA 220, Varian, Australia), equipped with deuterium background correction was used [8]. Se was measured by the direct graphite furnace AAS (AA 220, GTA 110, Varian, Australia) equipped with pyrolytically coated graphite tubes and deuterium background correction after a further dilution of serum with Triton X-100 (0.1% v/v). Direct determination of Se in body fluids by graphite furnace AAS may suffer from problems like severe background, matrix effects, preatomization losses, and spectral interferences. So, the mixture of Pd+Mg (NO3)2 was used as matrix modifier in graphite furnace AAS for the direct determination of Se in the serum [9]. The corresponding hollow cathode lamps were used as light sources, each instrument operated under the optimized conditions indicated in Table 1 and 2. The accuracy of the measurement was evaluated based on recovery studies and analysis of quality control material (QCM).The QCM (SeronormTM Trace Elements Whole Blood, Level 1, Art. No. 201405, Norway) was supplied freeze-dried and reconstituted by adding 3 mL of water. Accuracy was 97.5% for Se, 98.8% for Zn and 99.4% for Cu.

Statistics

Statistical evaluation was carried out using SPSS software version 16 for windows. Summary statistics (n, mean, standard deviation) were calculated. Values were statistically compared using one-way analysis of variance (ANOVA), also taking into account the sex as a grouping variable. All results were expressed as mean values ± SD. Statistical significance was defined as $p<0.05$.

Results

Biochemical data are presented in Table 3. A significant difference in Se Zn and Cu levels was

Table1: Instrument settings for determining the zinc and copper levels in human serum using FAAS.

Element	Calibration mode	Measurement mode	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Fuel gas	Oxidant gas
Zn	Concentration	Integration	213.9	1	5	Acetylene	Air
Cu	Concentration	Integration	324.8	0.5	4	Acetylene	Air

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observed between patients and the control group ($p<0.05$). Se level was found to be 41.78±22.87 µg/dL among beta-thalassemia major cases, and this value was found to be statistically lower compared to the controls (108.63±25.79 µg/dl). The Cu level was higher in patients than those of controls (156.60±22.62 vs. 102.88±31.15 µg/dl). Serum Zn level was lower in thalassemia patients (61.70±13.25 µg/dl) than controls (114.72±23.70 µg/dl).

The significant increase of serum iron in patients indicated an existing iron overload in our patients. The mean concentrations of iron were found to be 178.10±26.81 and 98.84±24.12 µg/dl in the patients and control group, respectively.

There was no significant relation between Se, Zn and Cu levels with age and sex.

Discussion

Patients with thalassemia are known to have poor growth, altered puberty, and immune function as well as reduced bone mineral acquisition [10]. The etiology of these comorbidites is typically ascribed to the toxic effects of transfusion-related iron-overload. We observed a significant increase ($p<0.01$) in serum iron and a decrease in serum Se and Zn ($p<0.001$ and $p<0.0001$, respectively), in beta thalassemia major patients compared to controls. People with severe forms of thalassemia often suffer from anemia, so they often require blood transfusions. The transfusion therapy can cause iron overload in patients, but they also suffer from iron overload independent of blood transfusions. Thalassemia patients overproduce a protein called GDF15, which suppresses the production of a liver protein, which in turn leads to an increase in the uptake of dietary iron in the gut [11]. The iron overload can generate oxygen-free radicals and promote peroxidative damage to cell and organelle membranes in organs that accumulate the excess iron including liver, pituitary gland, pancreas and heart. In such condition, depletion of endogenous antioxidants may be expected. In addition, iron overload in beta-thalassaemia major could greatly decrease selenium and zinc absorption via the gastrointestinal tract.

Zn is the second most abundant trace element in the body. Our data showed that in patients with β-TM there was a significant decrease in the levels of serum Zn. These results are in agreement with other studies published elsewhere in [12-15]. Zn deficiency in thalassemia may not only be due to high iron levels, but may also be due to multi-factorial causes such as, hyperzincuria, hepatic

Table2: Optimized instrument parameters and working conditions for selenium analysis in human serum.

A- Instrument settings for determining of selenium in serum by GFAAS.

Calibration mode	Measurement mode	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Sample volume (µL)	Modifier volume (µL)
Concentration	Peak height	196	1	10	10	5

B- Furnace optimized parameters for analysis of selenium in serum by GFAAS.

Element	Step	Temperature (°C)	Time (s)	Argon Flow-rate (L/min)
Se	Drying	85	5.0	3
	Pre last drying	95	40.0	3
	Post last drying	120	10.0	3
	Ashing	900	5.0	3
	Ashing	900	1.0	3
	Gas stop	900	2.0	0
	Ramp stop	2600	0.8	0
	Atomization	2600	2.0	0
	Tube clean	2800	2.0	3

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Table3: Biochemical data (mean±SD) in children with Beta-thalassemia major and the control group.

Element	Thalassemia Major group (n=40)	Control group (n=40)	p value
Selenium (µg/l)	41.78±22.87	108.63±25.79	< 0.001
Zinc (µg/dl)	61.70±13.25	114.72±23.70	<0.0001
Copper (µg/dl)	156.60±22.62	102.88±31.15	< 0.0001
Iron (µg/dl)	178.10±26.81	98.84±24.12	<0.01

Statistical significance was defined as p<0.05

dysfunction and impaired Zn absorption. It is possible that these trace metals are also chelated with iron and are removed by urine. So, if the trace metals are measured in the urine of the patients, they may be increased.

Recently, a study in Iran showed that serum zinc deficiency is prevalent among nearly 80% of thalassemic patients and suggested that Zn deficiency in patients could be attributed to a high prevalence of deficiency of this trace element in the Iranian general population [16].

Cu is present largely in the form of organic complexes, many of which are metalloproteins acting as enzymes. In our study serum Cu level was found to be significantly increased in patients when compared with controls (p<0.0001). The increased level of Cu could be explained by the antagonistic effect of the Zn, as Zn deficiency in beta thalassemia major could greatly increase the Cu absorption via the gastrointestinal tract [17].

The limitations of our study were the small population, local investigation and unavailability of data concerning diet regimens.

Conclusion

This study shows that Se, Zn and Cu may play a role in the pathogenesis of beta-thalassemia major.

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