

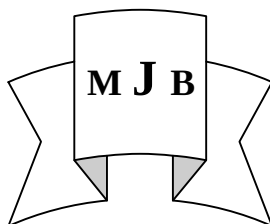
Effects of Iron Overload and Treatment Methods on Serum Levels of Zinc (Zn) and Copper (Cu) in Beta Thalassemia Major (BTM) Patients

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Abstract

This study is aimed to measure the serum levels of Zn and Cu in BTM patients with respect to their iron overload, and methods of treatment. A total of 81 BTM patients were enrolled in the study among them 12 patients were just diagnosed and received no treatment to serve as a second control group beside the first one which was composed of the healthy subjects. Patients on scheduled blood transfusions and those on subcutaneous (Sc.) Desferrioxamine (DFO) infusions beside blood transfusion were compared firstly with the healthy controls and secondly with the newly diagnosed thalassemics. A third type of comparisons was done among the study groups themselves. The results showed that in comparing the patients on blood transfusion with the healthy controls, a highly significant increase in Cu serum levels ($p<0.01$) were found. Comparison of the patients on DFO infusions with the healthy controls showed a highly significant increase in serum Cu ($p<0.01$), and a significant decreases in Zn serum levels ($p<0.05$). In comparing the splenectomized patients with the healthy controls a highly significant decrease were found in Zn serum levels ($p<0.01$), and a significant increase in Cu serum levels ($p<0.05$), and so did the non splenectomized patients.. In comparing the newly diagnosed thalassemic patients with the healthy controls, a highly significant increase was found in Cu serum levels ($p<0.01$), no significant correlation was found between serum levels of ferritin and either Zn or Cu. From this study it was concluded that treatment methods can significantly affect serum levels of both Zn and Cu.

الخلاصة

تهدف هذه الدراسة لقياس مستويات مصل الدم من الزنك و النحاس لدى مرضى التلاسيميا الكبرى نوع بيتا مع الأخذ بنظر الاعتبار مستوى زيادة الحديد وطريقة العلاج. كان العدد الكلي للمرضى الداخلين في الدراسة ٨١ مريضاً من بينهم ١٢ مريضاً تم تشخيصهم حديثاً و لم يستقبلوا أي علاج من أي نوع ليكونوا فريق المقارنة الثاني إلى جانب الفريق الأول و المكون من أشخاص أصحاء. المرضى المعالجين بنقل الدم المنتظم، و المرضى المعالجين بنضح الديفروكسامين تحت الجلد بجانب نقل الدم المنتظم تمت مقارنتهم بالأشخاص الأصحاء أولاً، و مع المرضى المشخصين حديثاً ثانياً، إضافة إلى نوع ثالث من المقارنات بين مجاميع الدراسة نفسها. تم أخذ عينة دم من كافة المشاركين للحصول على المصل. أظهرت النتائج إن في مقارنة المرضى المعالجين بنقل الدم المنتظم مع الأصحاء لوحظ وجود فرق معنوي في مستوى النحاس ($p<0.01$) و الزنك ($p<0.05$). في مقارنة مستأصلي الطحال مع الأصحاء لوحظ وجود فرق معنوي في مستوى الزنك ($p<0.01$) و النحاس ($p<0.05$) وكذلك الغير مستأصلي الطحال. في مقارنة المشخصين حديثاً مع الأصحاء لوحظ وجود فرق معنوي في النحاس ($p<0.01$). لم توجد علاقة ملحوظة بين الفيريتين و أي من مستوى مصل الدم من الزنك أو النحاس. من هذه الدراسة نستنتج أن طريقة العلاج تؤثر بطريقة ملحوظة في مستويات الزنك و النحاس.

Introduction

The term "thalassaemia" refers to a group of blood diseases characterized by decreased

synthesis of one of the two types of polypeptide chains (α or β) that form the normal adult human haemoglobin

molecule (HbA, $\alpha_2\beta_2$), resulting in decreased filling of the red cells with haemoglobin, and anemia [1]. Regular red blood cell (RBC) transfusions eliminate the complications of anemia and compensatory bone marrow expansion will permit normal development throughout childhood and extend survival [2]. In parallel, blood transfusions result in a "second disease" while treating the first, that has resulted from the inexorable accumulation of tissue iron, further altering the prognosis of thalassemia major over the last 20 years has been progressed in the development of iron-chelating therapy for iron overload [3].

In patients with β -thalassemia in whom yearly transfusion requirements exceed 200 ml packed cells per kilogram body weight, splenectomy should significantly diminish RBC requirements and iron accumulation [4].

Copper and Zinc are essential micronutrients involved in many metabolic processes [5]. Zinc is involved in the metabolism of energy, proteins, carbohydrates, lipids and nucleic acids and it is essential for tissue accretion [6]. There is less interaction between iron and Zn in human than in experimental animals, however, some researchers reported a modest but significant fall in serum ferritin in a group of volunteers taking (50 mg/day) Zinc without additional iron [7]. The increase of Zn and magnesium in the liver and spleen following iron overload was probably a result not only of increased intestinal

absorption but also of increased uptake from the cell [8]. Copper is a component of several enzymes, including cytochrome oxidase, superoxide dismutase (Cu/Zn SOD), monoamine oxidase and lysyl oxidase [6]. Copper concentration in plasma and cells as well as Cu metalloenzymes are indicative of Cu status [9]. The intimate relationship between Cu and Fe metabolism has been recognized for many years. In fact, Cu was identified as an 'anti-anemic' factor, when studies demonstrated that Cu could facilitate Hb formation [10]. Indeed, the Cu-Fe connection had been acknowledged for at least 100 years before this finding [11]. It is only relatively recently that the molecular basis for the biological interactions between these two metals has begun to be understood. The discovery of caeruloplasmin, the Cu-dependent ferrioxidase, formed the initial bridge between Fe utilization and Cu status. However, in recent years it has become apparent that Cu-Fe interactions occur at the dietary and intestinal level. The molecular mechanisms underlying these interactions suggest that the Cu-Fe relationship may be more complex than it was first thought [12]. Caeruloplasmin has subsequently been shown to act as a ferrioxidase, converting ferrous to ferric [13], and to increase the rate of loading of Fe onto transferrin. There is good evidence for a role for Cu in intestinal Fe absorption [14].

Materials and Methods

This is a case-control study that was done in The Inherited Blood Diseases Center in Babylon Maternity and Children Hospital.

A total of 81 β -thalassemia major patients (42 males and 39 females) were enrolled in this study. age range

(1.5 to 19 years) (8.37 ± 5.63 years). They were subdivided according to the method used in their treatment into: the newly diagnosed thalassemic patients who still did not receive blood transfusion nor DFO therapy, the patients treated by scheduled blood

transfusion to maintain their Hb levels around 9.5 mg/dl, the patients treated by scheduled blood transfusion and to treat their transfusional iron overload they receive DFO (Desferal®) in vials that contain 500mg from Novartis, Switzerland, as Sc. infusions, the mean dose of DFO was 20-40 mg /kg / infusion over 8-12 hour, 5-6 days per week.

Patients were excluded from the study if they had one or more of the following conditions: hepatitis B or C, a history of positive HIV test, chronic renal failure, heart failure, and endocrine complications. 5 ml of blood were taken from each participant to obtain the serum by centrifugation to assess the serum levels of Zn and Cu using the commercially available kits from LTA, Italy, which are based on a colorimetric reaction.

Serum levels of ferritin were measured by minividas using commercially available kits from

Biomerieux, France. The assay principle combines a one-step enzyme immunoassay sandwich method with a final fluorescent detection.

The serum samples were frozen at -20°C until used. All values were expressed as means $\pm$ standard deviation ($\mu \pm \text{S.D}$). The data were analyzed by using a computerized SPSS program. Student's *t*-test was used to examine the differences between any two groups while ANOVA test were used to examine the relations within the groups.

Also a study for the regression and correlation was also done between serum ferritin and serum levels of Zn and Cu. A ($p < 0.05$) denoted by (*) is considered to be significant and a ($p < 0.01$) denoted by (**) is considered to be highly significant [14]. clinical features of the study groups are illustrated in table (1).

Table 1 clinical features of the study groups

The study group	Sample size	Age(years)			Sex	
		Range	Mean	SD	Male	Female
1-Healthy controls	10	2-20	10.8	6	5	5
2-Newly diagnosed thalassemics	12	1.5-2	1.2	1	4	8
3-Blood infused thalassemics	19	1.5-4	3.2	2.5	8	11
4- DFO infused thalassemics	50	4-20	10.6	5.1	30	20
4a-Splenectomized	24	5-19	12.6	5.5	15	9
4b-non splenectomized	26	4-19	8.8	4.4	13	13

Results

In comparing the newly diagnosed thalassemics with the healthy controls a highly significant increase in serum levels of Cu ($p < 0.01$), and a non significant decrease in serum levels of Zn were found, while in comparing the blood transfused thalassemics with the

healthy controls a highly significant increase ($p < 0.01$) in serum Cu and a non significant decrease in serum levels of Zn were found. Comparison of the DFO infused thalassemics with the healthy controls showed a highly significant increase ($p < 0.01$) in serum

Cu levels and a significant decrease in serum levels of Zn ($p < 0.05$). These results are illustrated in table (2). All values were expressed as means $\pm$ standard deviation ($\mu \pm S.D$).

By comparing blood transfused thalassemic patients, and thalassemic patients treated with DFO with the newly diagnosed thalassemic patients, both of the blood transfused thalassemic patients, and thalassemic

patients treated with DFO showed a non significant decrease in serum levels of Cu and Zn. These results are illustrated in table (2).

DFO infused thalassemics showed a non significant decrease in serum levels of Cu, and Zn when compared with the blood transfused thalassemics as shown in table (2).

Table 2 Comparison of the serum levels of Zn and Cu of the study

Serum levels ($\mu\text{g/dl}$)	healthy controls N=10	newly diagnosed thalassemics N=12	blood transfused thalassemics N=19	DFO infused thalassemics N=50
Cu	145.88 $\pm$ 15.09	214.32 $\pm$ 82.73 A**	194.04 $\pm$ 98.98 A**	187.37 $\pm$ 63.01 A**
Zn	100 $\pm$ 8.4	99.7 $\pm$ 40.1	85.63 $\pm$ 35.37	85.55 $\pm$ 20.58 A*

(A) means a significant difference between the indicated group and the healthy controls, (*) means $p < 0.05$, (**) means $p < 0.01$.

The patients receiving Sc. DFO infusions were also subdivided according to the spleen state into splenectomized and non splenectomized. By comparing these two groups with the healthy controls, the splenectomized patients showed a significant increase ($p < 0.05$) in serum levels of Cu, and a highly significant decrease in serum levels of Zn ($p < 0.01$), and so did the non splenectomized patients as illustrated in table (3). In comparing the splenectomized and the non splenectomized patients with the

newly diagnosed thalassemics, the splenectomized patients showed a non significant decrease in serum levels of Cu, and Zn, and so did the non splenectomized patients as illustrated in table (3).

The splenectomized patients showed a non significantly lower serum levels of Cu and a non significantly higher serum levels of Zn when compared with the non splenectomized patients, All these results are illustrated in table (3).

Table 3 Comparison of the serum levels of Cu and Zn of the study groups

Serum levels ($\mu\text{g/dl}$)	the healthy controls n=10	The newly diagnosed thalassemics N=12	splenectomized n=24	non splenectomized n=26
Cu	145.8 $\pm$ 15.0	214.3 $\pm$ 82.7	175.34 $\pm$ 47.10 A*	195.89 $\pm$ 74.00 A*
Zn	100 $\pm$ 8.4	99.7 $\pm$ 40.1	87.9 $\pm$ 17.6 A**	83.3 $\pm$ 23.3 A**

(A) means a significant difference between the indicated group and the healthy controls, (*) means $p < 0.05$, (**) means $p < 0.01$.

A study of regression and correlation of serum levels of Zn and Cu with the level of serum ferritin had shown no

relationship with Cu and a non significant positive relationship with Zn as shown in table (4).

Table 4 Regression and correlation study of serum ferritin with serum levels of Zn and Cu.

The Serum levels of ferritin versus (A) and (B) ($\mu\text{g/dl}$)	r value	significance
(A) Cu	0.034	0.88
(B) Zn	0.347	0.173

Discussion

In the comparison with the healthy controls, the newly diagnosed untreated thalassemic patients showed a non significant decrease in serum levels of Zn and a highly significant increase in serum levels of Cu, this group was reported to have decreased serum Zn compared to the healthy controls associated with an increased urinary Zn excretion [15].

Zinc deficiency is always accompanied with an increased copper absorption from the gut due to the antagonistic effect of Zn [16], this can explain the significant increase in Cu serum levels as well.

Blood transfused thalassemics when compared with the healthy controls showed a non significant decrease in serum Zn levels with a highly significant increase in serum Cu levels, this could be explained on the basis of the above reciprocal relationship between those two trace elements.

Zinc deficiency in this group occurs due to the hyperzincurea resulting from the release of Zn from the hemolysed red cells [17], they also mentioned that many hypercupremia in blood transfused thalassemic patients occurs due to acute, chronic infections and hemochromatosis which is a principal complications in thalassemia.

DFO infused thalassemics when compared with the healthy controls showed a highly significant increase in serum Cu levels and a significant decrease in serum levels of Zn, this agrees with the results found by the other researchers [16].

Our results can be explained depending on the fact that beside the chelation of iron, DFO can also chelate Zn, Cu and Co especially in the presence of low iron burden since the effect of iron chelation treatment on trace elements in patients with iron overload depends on the relative affinity of the chelator to these metals [18].

Some previous studies, have mentioned an increased urinary excretion of zinc and decreased Leukocyte Alkaline Phosphates (LAP) activity, which is a zinc-dependent enzyme, during DFO infusion in small doses and it is concluded that chelation of trace elements, including Zinc, may be related to the low iron burden [19].

Another explanation for the lower Zinc levels in thalassaemic patients without any relationship between the mean zinc level and the dosage and duration of DFO treatment, is that other factors, such as anorexia, nutritional status, psychological problems (such as depression) and different metabolic and endocrine complications have led to zinc deficiency [20].

In this comparison a highly significant increase in serum levels of Cu was found in DFO infused thalassemics, this agrees with the findings of other studies [20,16]. This result could be attributed to:

1-An impaired Cu utilization in tissues accounting for the pathogenesis of thalassemia [21].

2-The blood transfusion from healthy people and increased Cu absorption via the gastrointestinal tract [20].

3-Another possible cause is the abnormal trace elements metabolism or impaired kidney function [22].

4- The high level of Cu may be caused by the parenchymal hepatic damage, which is a common side effect in blood transfused patients [20].

Serum levels of Cu in the blood transfused thalassemics when compared with the newly diagnosed thalassemics showed a non significant decrease with a non significant decrease in serum levels of Zn. The decrement of Zn in those patients occurs due to hyperzincurea resulting from the release of Zn from the hemolysed red cells[17].

Patients (on DFO) when compared with the newly diagnosed thalassemics also showed a non significant decrease in serum levels of Cu with a non significant decrease in serum levels of Zn, We should mention in this place the possible ability of DFO to chelate Zn, Cu , Co especially in the presence of low iron burden as explained before [20].

In comparing the blood transfused thalassemics with the DFO infused thalassemics there was a non significant decrease in serum levels of Cu with a non significant decrease in serum levels of Zn which might indicate the fact that DFO could reduce the serum levels of Cu as well as Zn to a non significant degree.

Comparing the splenectomized patients with the healthy controls a significant increase in Cu levels found which can be due to blood transfusions [20], while Zn levels showed a highly significant decrease, and this further reflects the reciprocal relationship between Zn and Cu discussed by [16] in addition to the other mentioned factors.

The non splenectomized patients showed the same results, this was explained earlier by the fact that the repeated blood transfusions [20] or the

impaired tissue utilization of Cu [21] could be responsible for the elevated Cu levels when we compare the thalassemic patients with the healthy controls.

Therefore, according to our results we suggest that this disturbance in serum levels of Cu and Zn is not related to the presence or absence of trace elements. The non splenectomized patients showed a non significant decrease in serum levels of Zn and Cu and as we said this is an effect caused by DFO.

As we explained before, the alterations in trace elements are not related to the absence or presence of the spleen but is related to the thalassemia condition. Cu and Zn serum levels showed no differences between the splenectomized and the non splenectomized patients groups.

No correlation was found between ferritin and Zn levels this is a well documented fact [15,18,23], where ferritin does not interfere by any means

the spleen but is related to thalassemia condition.

Comparing the splenectomized patients with the newly diagnosed thalassemics a non significant decrease in serum levels of Cu, and Zn was found and again this refers to the ability of DFO to chelate those two

with the metabolism or the glomerular filtration rate of Zn as well as Cu which also showed no correlation. From this study it was concluded that treatment methods can significantly affect serum levels of both Zn and Cu, since in the stage of blood transfusion there is an increase in Cu serum levels which is compensated by a decrease in Zn serum levels due to the reciprocal relationship between these two trace elements, then on the initiation of DFO Sc. infusions both Zn and Cu significantly decreased due to chelation by DFO.

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