The Effects of Chelating Therapy on the Levels of Serum Ferritin, Zinc, Copper & its relation with Malondialdehyde in Patients with B-Thalassemia Major

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Abstract

Background: ß-Thalassemia is a hereditary hemolytic anemia, which needs to be treated by repeated and regular blood transfusions. Multiple transfusions could result in iron overload which is treated by chelating agents. This chelating agent chelates iron and may also chelates zinc and copper which are important components of antioxidant enzymes system.

Objectives: Evaluating the effects of chelating therapy on the serum levels of ferritin, Zinc and Cu in B-thalassemia patients and correlate such effects on the antioxidant activity by determining the levels of malondialdehyde (MDA).

Patients & Methods: This study was performed during the period from 1st of June to 31st of August 2008. A total of 50 B-thalassemia patients were enrolled in the current study. Patients were from the thalassemia center in Sulaimani city & 50 healthy children consulting Shaheed Hersh Health Center for different reasons were taken as a control group. The blood samples were collected from 50 patients, 25 males and 25 females with different ages, ranging from (2 - 15) years, 37 (74%) of them were taking regular blood transfusions and chelating agent therapies, and 13 (26%) were not taking chelating agent therapies (poor compliance). The determination of the serum levels of copper, zinc, MDA was determined by spectrophotometer method &the level of serum ferritin levels was determined by enzyme immunoassay.

Results: Analysis of the data obtained from the 50 ß-thalassemia patients showed that the mean concentration of serum ferritin and MDA was significantly higher, and the mean concentration of serum zinc and copper was significantly lower with respect to the data obtained from the 50 control children. Further analysis of the data were obtained to study the effects of chelating therapy, age, sex and history of splenectomy on the serum levels of ferritin, zinc, copper and MDA among the ß-thalassemic patients which demonstrated that there were no significant differences in the levels of the parameters investigated between those receiving chelating therapy and those not receiving chelating therapy, between different age groups, between males and females and between those with positive history of splenectomy and those without splenectomy, except in the level of MDA in relation to the sex of patients, which was significantly higher in male compared to female ß-thalassemic patients.

Conclusion: Significantly low levels of serum zinc and copper were demonstrated among B-thalassemia patients as compared to their levels in normal children. In contrast the serum levels of ferritin and MDA in ß-thalassemia patients were significantly higher with respect to their levels in controls. And this further confirms that oxidative stress may be an important feature of ß-thalassemia patients.

Key Words: Thalassemia, Chelating agents, Serum zinc, Copper, Malondialdehyde.

Introduction

Cooley and lee first described a severe form of anemia associated with splenomegaly and bone change occurring in young children, the condition was later named Thalassemia [1].

Thalassemia is a heterogeneous hereditary anemia caused by absent or defective synthesis of one or more of the polypeptide chains of globin for hemoglobin. Usually, the synthesis of either the alpha or beta chains of HbA (α 2 ß2) is impaired; thalassemia is named according to the chain with reduced or absent synthesis [2, 3, 4].

Most patients with ß-thalassemia major are severely anemic and many of the distressing symptoms of the disease are directly related to the anemia, so regular blood transfusions are the essential import therapy for such patients [5].Since human beings lack the ability for effective excretory route of excess iron, these patients are at risk for iron overload, which could be deposited as ferritin and as insoluble hemosiderin in various tissues including liver, heart and endocrine organs [6, 7].

Such problem of iron overload creates the necessity to the use of chelating therapy which involves the administration of drugs that bind heavy metals such as iron to form soluble products that can be excreted through urine or bile, so it prevents their deposition and interaction with vital organs [8, 9].

Most iron chelating therapies are highly selective for iron binding and chelation; however they have variable affinity for other trace elements such as zinc and copper and may lead to variable reductions in tissues and serum concentration of these essential trace elements [10].

One of the vital functions of copper and zinc is their role as antioxidant enzymes system. These enzymes neutralize excessive reactive oxygen and prevent these harmful derivatives of oxygen from causing serious damage to the cellular structure. There are two types of antioxidants in the human

body: enzymatic antioxidants and non-enzymatic antioxidants [11, 12].

The most effective antioxidant enzyme is called super oxide dismutase which is considered as the first line of defense that body has to act against the free superoxide radicals which is the first reactive oxygen formed in the body when exposed to oxidative stress and from which other reactive oxygen species can be formed, the cytosolic isoenzyme of superoxide dismutase contains copper & zinc in its structure [13, 14].

Malondialdehyde as biomarker for oxidative stress is only formed by fatty acids with three or more double bonds and is used as a measure of lipid per oxidation. The reaction is initiated by an existing free radical or by metal ions [15].

The aim of study is to evaluate the effects of chelating therapy on the serum levels of ferritin, Zinc, Cu in B-thalassemia patients and correlate such effects on the antioxidant activity by determining the levels of (MDA) in such patients.

Material & Methods:

A total of 50 B-thalassemia patients were enrolled in the current study, patients were from thalassemia center in Sulaimani city. The study was performed for the period from 1st of June 2008 to 31st of August 2008. Personal and medical information was collected by using questionnaire forms of the patient, and 50 children consulting Shaheed Hersh health center for different reasons, were taken as a control group.

The blood samples collected from all 50 patients, 25 (50%) males and 25(50%) females with different ages, ranging from (2- 15) years, 37(74%) of them were taking regular blood transfusions and chelating agent therapies (Deferiprone) orally four days in week and (desferoxamine) by injection two days in week, and 13 (26%) were not taking chelating agent therapies after every blood transfusion (poor compliance).

The blood samples collected from 50 healthy children, 25 (50%) males and 25 (50%) females as control group.

Estimation of serum ferritin had been performed by enzyme immunoassay sandwich method using kit promoted by bioMeeieux ®, Francais. REF 30 411, The level of serum zinc was determined by colorimetric test using Zinc kit (GIESSE DIAGNOSTICS, CAT.NO.3119, Italy), the level of serum copper was determined by colorimetric test using Copper kit (GIESSE DIAGNOSTICS, CAT.NO.3119, Italy) and the level of serum MDA, as indicator of lipid peroxidation, was determined by a modified procedure described by Guidet B et al (16)

Data were translated into codes using a specially designed coding sheet, and then converted to computerized database. An expert statistical advice was sought and statistical analysis was done using SPSS (Statistical Package for Social Science) version 13 and STATGRAGH PLUS (version 4) computer software.

The statistical significance of difference in means of a quantitative continuous variable between two Independent groups was assessed by Mann-Whitney test T test. Wilcox on signed rank test used between two paired groups. When P value is less than 0.05, was considered significant.

Results

The values of serum ferritin, Zn, Cu and MDA in B- thalassemia patients (n=50) compared with control group (n=50) revealed that the levels of serum zinc and copper were significantly lower in thalassemic patients as compared to the control group. ($P<0.001$and $p<0.05$ respectively) **(Table 1).**While the serum levels of ferritin and MDA were significantly higher in thalassemic patients $p <0.001$. The effects of chelating therapy on serum levels of Ferritin, Zinc, Copper and MDA on thalassemic patients, out of 50 patients with B-thalassemia, 37 patients (74%) were receiving chelating therapy and 13 patients (26%) were not receiving chelating therapy **table (2).**

The serum Ferritin, Zn, Cu and MDA in patients receiving chelating therapy were not significantly differing from patients who were not receiving chelating therapy $p> 0.05$.

Table (1): Comparison of serum levels of Ferritin, Zinc, Copper and MDA between B- thalassemia patients and a control group.

Parameter	Mean serum level ± SD		P-value
	B-Thalassemia major (n= 50)	Controls (n= 50)	
Serum Ferritin (ng/ml)	1220.35 ± 367.15	45.25 ± 5.8	P < 0.001
Serum Zinc(µg/dl)	71.63 ± 19.28	94.62 ± 20.87	P < 0.001
Serum Copper (µg/dl)	95.68 ± 49.29	112.68 ± 26.14	P <0.05
Serum MDA (µ mole/L)	4.34 ± 1.99	1.88 ± 0.59	P < 0.001

Table (2): Effects of chelating therapy on serum levels of ferritin, Zn, Cu and MDA in B-thalassemia patients

Parameter	Mean serum levels ± SD		P-value
	Male n=25 (50%)	Female n=25(50%)	
Serum Ferritin (ng/ml)	1350.76 ± 240.88	1297.37 ± 275.66	P > 0.05
Serum Zinc (µg/dl)	73.58 ± 19.48	69.68 ± 19.27	P > 0.05
Serum Copper (µg/dl)	96.4 ± 49.45	96.01 ± 52.50	P > 0.05
Serum MDA (µ mole/L)	4.88 ± 2.52	3.79 ± 1.09	P < 0.05

Table (3): Comparison of serum levels of Ferritin, Zinc, Copper and MDA in patients with ß-thalassemia

Ages	Mean serum levels ± SD				
	Serum Ferritin (ng/ml)	Serum Zinc (µg/dl)	Serum Copper (µg/dl)	serum MDA (µ mole/L)	
< 5 yrs (n=12) (24%)	1260.53±311.16	79.26 ± 24.09	108.33 ± 40.96	3.80 ± 0.97	**P > 0.05**
5 –10 yrs (n=17) (34%)	1195.65±370.115	70.36 ± 14.81	94.67 ± 53.60	4.32 ± 1.14	**P > 0.05**
> 10 yrs (n=21) (42%)	1250.22±270.85	68.30 ± 19.17	90.52 ± 53.81	4.66 ± 2.81	**P > 0.05**

Table (3) shows the age distributions of patients with ß-thalassemia were classified into three age groups, less than 5 years which account for 12 patients (24%), between 5-10 years, which account for 17 patients (34%), more than 10 years, which account for 21 patients (42%)

There was no significant difference in serum levels of Ferritin, Zn, Cu and MDA in the three age groups.

Table (4) shows the distribution of thalassemic patients according to sex, 50% were males & 50% were female. The serum levels of ferritin, Zn, Cu were not significantly different in both sexes, the (p> 0.05).While the level of MDA significantly differed. It was higher in males, the (P<0.05).

In an attempt to correlate serum levels of Ferritin, Zn, Cu and MDA with respect to history of splenectomy, thalassemic patients were classified into two groups, group one including 23 (46%) patients had splenectomy and 27 (54%) non- splenectomized patients **table (5).** There were no significant differences in serum levels of these parameters in both groups.

Table (4): Comparison of serum Ferritin, Zinc, Copper & MAD in B-thalassemia patients according to sex.

Parameter	Mean serum levels ± SD		P-value
	Splenectomized n=23 (46%)	Non-Splenectomized n=27 (54%)	
Serum Ferritin (ng/ml)	1310.25 ± 215.66	1345.11 ± 298.17	**P > 0.05**
Serum Zinc(µg/dl)	73.69 ± 25.59	69.87 ± 11.80	**P > 0.05**
Serum Copper (µg/dl)	82.58 ± 41.91	107.81 ± 54.87	**P > 0.05**
Serum MDA (µ mole/L)	4.41 ± 2.78	4.27 ± 0.98	**P > 0.05**

Table (5): Comparison of serum levels of Ferritin, Zinc, Copper & MDA in patients with & without spleen.

Discussion

ß-thalassemia is a genetic disorder and

Parameter	Mean serum levels ± SD		P-value
	Receiving Chelating Therapy 37(74%)	Non- Receiving chelating Therapy 13 (26%)	
Serum Ferritin (ng/ml)	1290.68 ± 311.75	1185.45± 385.38	**P > 0.05**
Serum Zinc (µg/dl)	78.50 ± 23.23	69.22 ± 17.40	**P > 0.05**
Serum Copper (µg/dl)	104.92 ± 41.10	93.14 ± 53.55	**P > 0.05**
Serum MDA (µ mole/L)	3.77 ± 0.93	4.54 ± 2.23	**P > 0.05**

encompasses a wide variety of clinical phenotypes, ranging in severity from clinically silent heterozygous ß-thalassemia to severe transfusion-dependent thalassemia major. Patients who have ß-thalassemia major have to receive multiple blood transfusions, and if without iron chelating therapy, iron overload occurs [1, 2, 3, 5].

The present study showed that the levels of serum zinc and copper decreased significantly in thalassemic children compared with the matched healthy controls, while serum ferritin and MDA levels were significantly higher in children with ß-thalassemia major as compared to controls (table 1). Similar observations were made by Shamshirsaz et al [17] and Nasr et al [18], who showed significantly low levels of serum zinc and copper in ß-thalassemia patients. In contrary, Bashir [19] reported that the serum levels of copper and zinc were significantly increased in ß-thalassemia. With regard to serum ferritin and M.D.A, similar results were obtained by Simsek et al [20], who found significantly increased levels of serum ferritin and MDA in ß-thalassemia.

The zinc and copper status of thalassemic patients were previously reported by several studies. Arcasory et al [21] showed that there was a marked zinc deficiency in the presence of hyperzincuria in the thalassemic patients. Suthipark and Colleagues [22] reported low levels of zinc and copper in thalassemic patients compared with non thalassemic controls and they related that to abnormalities of trace elements metabolism in thalassemic patients. Shamshirsaz et al [17] stated that the deficiency of zinc and copper in patients with ß-thalassemia major has been under debate and seems that the deficiency of copper and zinc could be attributed to high prevalence of poor dietary habits in thalassemic patients .In addition to that, Giardina [23] suggested that the chronic haemolysis in ß-thalassemia might be the cause of low levels of these trace elements in such patients.

There is extensive evidence of in vivo oxidative damage as well as enhanced sensitivity to exogenous oxidant stress in red cells of ß-thalassemia [24]. Clemens [25] has postulated that the biochemical and metabolic changes of ß-thalassemic red blood cells are associated with a constant oxidative stress within the cells caused by the precipitation of excess alpha-globin chains, iron decompartimentalization, and release of free iron. Several studies reported that plasma MDA increased in ß-thalassemia [26, 27, and 28], MDA is a good indicator of oxidative damage, and it has been found that both free and total MDA are higher in regularly transfused thalassemia major patients [29].

Although several studies reported that the serum copper and zinc were significantly decreased in ß-thalassemia [17, 21, 22, and 23], and these trace elements are important cofactors for the antioxidant enzyme: copper- zinc superoxide dismutase (Cu-Zn SOD) [30].

However Simsek et al [20], Meral et al [26] and Yenchitsomanus et al [31] reported that the activity of erythrocytes superoxide dismutase was found to be increased in patients with ß-thalassemia. Both ß-thalassemia and accompanied iron overload in vivo lead to lipid peroxidation and compensatory increase in the antioxidant enzymes activity [26, 32]. So the results of this study which revealed a high level of MDA in ß-thalassemia patients are unlikely to be due to the impaired activity of the antioxidant enzyme but rather than high level of MDA is likely to be the result of frequent blood transfusions, the frequent blood transfusions might subjected the ß-thalassemia patients to peroxidative tissue injury by the secondary iron overload. These findings might support the idea that iron overload in ß-thalassemia leads to an enhanced generation of the reactive oxygen species and oxidative stress.

Table (2) shows no significant difference ($p>0.05$) in the serum ferritin, zinc, copper and MDA in ß-thalassemia patients receiving chelating therapy and those not receiving chelating therapy. Nasr and associates (18) studied the correlation between serum levels of ferritin and certain trace elements in Egyptian children with ß-thalassemia

They found that there was no significant correlation between serum ferritin and serum levels of zinc and copper. Also the same study demonstrates no significant correlation was found between the serum levels of zinc and copper, and the duration of transfusion treatment, or with the duration of chelating treatment. In addition to that Al Refaie et al [33] and Bartakkes et al [34] failed to demonstrate any significant relationship between the doses and duration of chelating therapy and urinary zinc excretion. Also Bartakkes and co-workers [34] were unable to demonstrate significant lowering of plasma zinc in ß-thalassemia patients receiving chelating therapy.

The serum ferritin & MDA were not different significantly in those who received & those who not received chelating therapy ($P>0.05$), however their levels being high in both groups.

Simsek et al [20] indicated that the increased level of plasma MDA in ß-thalassemic patients might be the result of continuous blood transfusions, which might subjects these patients to peroxidative tissue injury by the secondary iron overload. In our study, all the ß-thalassemia patients received regular blood transfusions. The finding of high level of MDA was probably due to iron overload as reflected by the high level of serum ferritin in those receiving and those not receiving chelating therapy [35].

In relation to the present observation that all the parameters investigated do not differ significantly in those receiving and those not receiving chelating therapy, it should be pointed out that chelating therapy in ß-thalassemia patients could have no effects on the serum levels of these parameters, or could have effects but missed to be detected because of the small number of ß-thalassemia patients especially those not receiving the chelating therapy, so it is reasonable to suggest further detailed investigations of this matter.

Table3 showed no significant difference in all parameters investigated among the three age groups. The present finding is in agreement with that of Livrea et al [35], who stated that the variation in the antioxidants and peroxidation parameters evaluated in their study was not correlated with the age. In addition to that, they also suggested that the measurement of peroxidation products, matched with the evaluation of antioxidants, may be used as a simple measure of iron toxicity in thalassemia, in addition to the conventional indices of iron status. Similarly Livrea et al [28], Nielsen et al [15] reported that no correlation was found between the age of thalassemic patients and plasma MDA.

So far, an attempt was made to compare the serum levels of ferritin, zinc, copper and MDA obtained in all thalassemic patients classified according to their sex (table 4). Although the values of these parameters were higher than normal children, however there is no significant difference between males and females in the levels of serum ferritin, zinc and copper ($p>0.05$). In contrary, the level of MDA was significantly higher in males as compared with females ($p<0.05$), similarly Nielsen and associates [15] showed an independent effect of gender on the level of the biomarker of oxidative stress.

Table 5 demonstrated that there was no significant difference ($p>0.05$) in the parameters investigated between splenectomized & non splenectomized patients. This result was in agreement with the finding of Sumboonnanonda et al [36]. They studied the oxidative stress in 10 splenectomized thalassemic patients, 24 non-splenectomized thalassemic patients, and compared the results with 15 normal children. Their results revealed that the levels of MAD were significantly higher in both splenectomized and non-splenectomized patients as compared with levels of MDA in normal children. They concluded that the iron overload is the possible cause of increased oxidative stress, since the levels of MDA were increased in both splenectomized and non-splenectomized patients.

Significantly low levels of serum zinc and copper were demonstrated among patients with ß-thalassemia as compared to their levels in normal children. In contrast the serum levels of ferritin and MDA in ß-thalassemia patients were significantly higher with respect to their levels in controls. And this further confirms that oxidative stress may be an important feature of ß-thalassemia patients.

This study which measured the parameters investigated at the time of study, and since there is no any idea about the base line values. Therefore, it is recommended to conduct a cohort study which involves the measurement of base line values of each parameter investigated, and then following up the patient for years to determine the effects of blood transfusion chelating therapy, age and other factors on the level of these parameters.

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