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Copper and Zinc Status in Jordanian Patients with â-Thalassemia Major Treated with Deferoxamine

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Abstract: The present study aims at evaluating the serum copper and zinc in Jordanian thalassaemic patients. Forty two patients (age from 4-14 years) with â-thalassemia major (20 males and 22 females) that undergo periodical blood transfusion and they are on Deferoxamine (DFO) as chelating agent were involved in this study. All patients were free from HBV, HCV and HIV. The diagnoses of â-thalassemia major were made based on the clinical, hematological and hemoglobin electrophoresis profiles for the patients. Hb electrophoresis for the father and mother and genetic study of the b globins genes in some disputable cases were also done. Forty controls of matched age and gender (20 males and 20 females) were also, included in the study. Results showed that the copper and zinc levels were significantly ($p<0.05$) increased in â-thalassaemic patients (189.92±73.7, 220.8±11.3 Ug dL^{-1}, respectively) compared with controls (108.61±13.33, 105.30±12.01 Ug dL^{-1}, respectively). The results revealed also that non-significant differences ($p>0.05$) appeared between males and females in the control and experimental groups concerning the level of copper and zinc. These finding may be explained by the decreasing rate of glomerular filtration of zinc seen in chronic hemolysis and the disturbance in the metabolism of zinc and copper in thalassaemic patients due to the increasing serum zinc. Moreover, the high level of copper could be explained by the increase in copper absorption via the gastrointestinal tract. But they prove clearly the unnecessary supplementaion of zinc for the regularly transfused thalassemic patients.

Key words: â-thalassemia major, desferrioxamine, copper, zinc, iron overload

INTRODUCTION

â-thalassemia major is the most prevalent type of thalassaemia as it is common in certain populations. It produces severe anemia in its homozygous state (Widad *et al*., 2003). About 190 million people throughout the world have genetic mutations associated with different hemoglobinopathies and >90 million of them carry defective genes leading to thalassaemia (Ambekar *et al*., 2001; Das *et al*., 2004). According to Yesilipek (2007) there are over 200,000 â-thalassemia patients in the Mediterranean area alone. In Jordan, according to the Jordan Ministry of Health data, there are 1500 patients registered with â-thalassaemia in Jordan and about 150000 carriers. The disease is associated with profound anemia, jaundice, splenomegaly, expanded bone marrow space, siderosis and cardiomegaly. These symptoms appear after about 2-4 months of age. Impaired erythropoeisis, hemolysis in the peripheral circulation and deposition of excess iron in the tissues, are some of the causes of clinical manifestations (Das *et al*., 2004). Transfusion therapy, which is the mainstay of treatment when Bone Marrow Transplantation (BMT) is not possible permit normal growth and suppress the ineffective and the extramedullary erythropoeisis but in the same times like transfusion-transmitted infections (primarily hepatitis B and C), which are an important cause of death in countries where, proper testing is not available and iron overload, which results both from transfusional hemosiderosis and excess gastrointestinal iron absorption (Hoffbrand *et al*., 2003). Iron deposition in the heart, liver and multiple endocrine glands results in severe damage to these organs, with variable endocrine organ failure. However, the most serious complication of iron overload is life-threatening cardiotoxicity. Cardiac events due to iron overload are still the primary cause of death

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(Papanikolaou *et al*., 2005). Both transfusion iron overload and excess gastrointestinal absorption are contributory. Paradoxically, excess gastrointestinal iron absorption persists despite massive increases in total body iron load. Uncertainties as to the optimal age for the start of chelation therapy continue to exist. Reports of abnormal linear growth and metaphyseal dysplasia observed in children treated with deferoxamine before the age of 3 years (Rachmilewitz and Stern, 2005) have prompted recommendations for later therapy. Iron overload causes most of the mortality and morbidity associated with thalassemia. Iron-chelating therapy is largely responsible for doubling the life expectancy of patients with thalassemia major: It has been proven to prevent liver and heart damage, allow for normal growth and sexual development in children with thalassemia and increase life span (Rund and Rachmilewitz, 2005). In the absence of chelating therapy, the accumulation of iron results in progressive dysfunction of the heart, liver and endocrine glands. In the last 30 years, conventional treatment of â-thalassaemia major, based primarily on regular blood transfusions and iron chelation therapy with Desferrioxamine (DFO) has markedly improved the prognosis of the disease. Adequate administration of parenteral DFO reduces or prevents iron accumulation and iron-mediated organ damage, resulting in a consistent decrease of morbidity and mortality (Wong and Richardson, 2003). DFO is a powerful chelator of zinc, cobalt and copper in addition to serum iron (Cohen *et al*., 2002). These trace metals are more likely to be chelated in the presence of reduced iron levels. Zinc deficiency is associated with delayed skeletal maturation and a reduction in growth, as well as bone matrix and collagen synthesis. Zinc supplements have been shown to increase growth in some thalassaemic patients (Arcasoy *et al*., 2006). There is no record of low serum copper in chelated patients; although, it is postulated that chelation of copper within the metaphysic may result in deficient collagen formation and skeletal lesions (Arcasoy *et al*., 2006; Tyler *et al*., 2006). Another fact states that iron chelation therapy with desferrioxamine proved to increase zinc and copper urinary excretion (Wolfgang and Sandstead, 2006; Mehdizadeh *et al*., 2008). However, other studies showed that serum zinc level is less than normal in 80-100% of the patients (Wolfgang and Sandstead, 2006), while others showed that serum zinc level was not statistically different. This study aimed to evaluate, the levels of copper and zinc in the serum of Jordanian patients with â-thalassaemia major treated with deferoxamine for evaluating the effect of DFO in chelating trace elements as copper and zinc.

MATERIALS AND METHODS

Experimental subjects: This study was carried out in the thalassaemia center at princess rahma teaching hospital in Irbid-Jordan. The study population included 42 patients with â-thalassaemia major (20 males and 22 females) that undergo periodical blood transfusion and DFO as chelating agent. All patients are free from HBV, HCV and HIV. The tested group aged from 4-14 years. The diagnoses of â-thalassaemia major were made based on the clinical, hematological and hemoglobin electrophoresis profiles and genetic study in some cases. All patients were interviewed and filled out standardized questionnaires during the first visit of study and their medical histories were obtained from the hospital files. All patients were transfusion dependent at a rate ranged from 1-3 times monthly. None of the subjects was treated with vitamin E supplementation before the study. The control group includes 40 healthy children. None of these children had history of anemia, abnormal complete blood counts and abnormal hemoglobin electrophoresis results. The study was approved by the institutional review board of princess Rahma educational hospital.

Blood collection: Five milliliters of venous blood was collected just before the transfusion from the patients under aseptic condition, 3 mL of this blood was collected in plain bulb and the remaining 2 mL of blood were poured in heparinised bulb for some hematological analysis. The samples were stored at 4°C before analysis and separated by centrifugation at 3000 rpm for 10 min at room temperature using a bench top centrifuge (Cenformix). The analysis of the copper and zinc were performed immediately on the same day.

Hematological analyses, Hb, hematocrit, WBC were examined using Automate blood cell counter. Serum feritin was examined using commercial analytical kits from Sigma (St. Louis, Mo, USA). Copper and zinc were examined were estimated by Atomic Absorption Spectrophotometer (AAS).

Statistical analyses: All the results were expressed as mean±SD. Student 'Z' test was applied for the comparison of data, $p>0.05$ was considered as non-significant whereas $p<0.001$ was considered as highly significant.

RESULTS

The hematological and ferritin concentration results of the examined patients and the control group are shown in Table 1. It is clear from the results that a significant decrease ($p<0.05$) of hemoglobin concentration (9.2±1.6,

Table 1: Hematological data of â-thalassemia major patients

	Male		Female	
Parameters	Control (n = 20)	Experimental (n = 20)	Control (n = 20)	Experimental (n = 22)
Hematocrit (%)	35%±2.6%	29%±2%*	36%±3.8	27%±4.2%*
Feritin (μg L^{-1})	62±22	2564±1362*	58±14	2389±1484*
Hemoglobin (g dL^{-1})	12.9±0.7	9.2±1.6*	11.1±0.9	8.4±2.2*
White blood cell $\times 10^9$ L^{-1}	8650±2940	11200±4360	10400±2481	9950±2150

*The mean difference is significant in comparison with control untreated group ($p<0.05$)

Table 2: Means, SD and t-test for copper and zinc according to experimental and control groups

Variables			Mean			
(ug dL^{-1})	Group	N	(ug dL^{-1})	SD	t-value	Sig.
Copper	Exp	42	189.92	73.70	2.51	0.02*
	Cont	40	108.61	13.33		
Zinc	Exp	42	220.00	11.30	6.53	0.000*
	Cont	40	105.30	12.01		

Table 3: Means, SD and test for copper and zinc according to gender in the control group

Mineral			Mean			
(ug dL^{-1})	Gender	N	(ug dL^{-1})	SD	t-value	Sig.
Copper	Male	20	109.30	13.72	0.33	0.745
	Female	20	107.90	13.25		
Zinc	Male	20	104.05	12.40	-0.65	0.518
	Female	20	106.55	11.70		

Table 4: Means, SD and t-test for copper and zinc according to gender in the experimental group

Mineral			Mean			
(ug dL^{-1})	Group	N	(ug dL^{-1})	SD	t-value	Sig.
Copper	Male	21	175.6	47.50	0.33	0.745
	Female	21	273.7	49.30		
Zinc	Male	21	228.6	137.8	-0.65	0.518
	Female	21	214.5	89.70		

8.4±2.2 g dL^{-1}, respectively) in both males and females was noticed in comparison with controls (12.9±0.7, 11.1±0.9 g dL^{-1}, respectively). On the other hand, ferritin concentration was significantly ($p<0.05$) higher in both males and females (2564±1362, 2389±1484 ng mL^{-1}, respectively) in comparison with controls (62±22, 58±14 ng mL^{-1}, respectively). As shown in Table 2 the level of copper and zinc significantly increased ($p<0.05$) in patients with â-thalassaemia major (189.92±73.7, 220.8±11.3 ug dL^{-1}, respectively) compared to controls (108.61±13.33, 105.30±12.01 ug dL^{-1}, respectively). Table 3 and 4 show the means and standard deviations for the copper and zinc in both groups according to the gender. The results revealed that non-significant differences ($p>0.05$) appeared between males and females in the control and experimental groups concerning the level of copper and zinc.

DISCUSSION

In patients with â-thalassaemia major, impaired biosynthesis of the beta-globin leads to accumulation of unpaired alpha-globin chain. Shortened red cell lifespan and iron overload cause functional and physiological abnormalities in various organ systems. Thus, in patients with â-thalassaemia major the most important cause of mortality and morbidity is organ failure due to deposits of iron. The present study investigated the status of copper and zinc in patients with â-Thalassaemia major treated with DFO as chelating agent. A rise in iron indices observed in the â-thalassaemic patients may be due to erythrocyte hyperhemolysis and to chronic blood transfusion. Similar results were found by Kassab *et al.* (2003). The significant increase of serum ferritin in the patients indicated an existing iron overload. The acute iron overload found in â-thalassemia can lead to the accumulation of an abnormal molecular iron form (non-transferrin-bound: NTBI). NTBI has hepato and cardio-cytotoxic properties. Furthermore, NTBI contributes to the formation of free radicals and increases hemolytic process (Das *et al.*, 2004). The released iron could play a central role in the oxidation of membrane cells and senescent cell antigen formation, one of the major pathways for erythrocyte removal. Zinc and copper are key component necessary to maintain cellular homeostasis. The primary reason for this necessity is associated with the fact that hundreds of metalloenzymes require a metal element, as cofactors, to be functional (Kietzmann, 2000). Zinc has many important roles in the body and its deficiency causes broad and nonspecific signs and symptoms, including suppressed immunity (Fraker *et al.*, 1987, 2000; Wapnir, 2000), decreased growth velocity, delayed sexual maturity and dysguesia (Cousins and Hempe, 1990; Belton, 2005). Recently, a study in Iran showed that serum zinc deficiency is prevalent among nearly 80% of thalassaemic patients and suggested that zinc deficiency in these patients could be attributed to a high prevalence of deficiency of this trace element in the Iranian general population and the Zn deficiency was thought to have important role in the growth retardation and sexual development in thalassemia (Shamshirsaz *et al.*, 2003). In addition, zinc deficiency can cause other disorders in thalassaemic patients, such as inadequate protein-energy status (Simsek *et al.*, 2005) and it also plays a role in the pathogenesis of deferoxamine neurotoxicity (Naithani *et al.*, 2006). Different levels of trace elements (zinc and copper) in thalassaemic patients compared with non-thalassaemic controls due to abnormal trace element metabolism in patients were reported (Mehdizadeh *et al.*, 2008). The results showed higher serum zinc and copper levels in the thalassaemic group

compared to the control group (Table 4), which supports the results of some other studies (Tanphaichitr *et al*., 1995; Oktekin and Gokmen, 2000). Bashir (1995) found that the Copper levels were significantly increased in â-thalassaemia and Sickle Cell Anaemia (SCA) and the zinc levels were significantly increased in â-thalassaemia but significantly decreased in SCA. The regular blood transfusion donated by healthy donors seems somehow to prevent such deficiency (Alexander *et al*., 2000) and it may explain the normal and even higher serum zinc or copper values in our patients compared with the control group. The increased serum zinc levels in â-thalassemia major has been attributed to cirrhotic changes owing to hemosiderosis or to an abnormal rate of glomerular filtration of zinc seen in chronic hemolysis. One study found increased amount of both zinc and copper in the erythrocyte of the thalassaemic patients and suggested that it may reflect the impairment of zinc and copper utilization in tissues in the pathogenesis of these thalassaemic patients (Mehdizadeh *et al*., 2008). Although, Nasr *et al.* (2002) in their study showed the low serum zinc and copper level in Egyptian thalassaemic patients, such a finding has not been concomitant with clinical manifestations including cutaneous signs in their patients. Arcasoy *et al.* (2001) have showed that while, serum zinc level decreased and serum Zinc Binding Capacity (ZnBC) increased in nutritional zinc deficiency, in thalassaemic patients serum zinc level decreased but ZnBC did not increase simultaneously. The high levels of iron can affect the zinc and copper in blood and lowering the levels of zinc in thalassemia. Though, there are wide variations in different reports, these discrepancies can be attributed to differences in patient's ages, rather than to difference in treatment protocols, including differing transfusion rates or chelation therapies (Eleftheriou, 2003). Some reports indicate that desferrioxamine increases urinary zinc excretion and may decrease zinc body content (Faranoush *et al*., 2008). Hyman *et al.* (1989) indicated in their study that the desferal induced moderate aluminum excretion in urine but had no effect on copper or zinc excretion. Although serum zinc levels were somehow lower in patients, who were on higher doses of desferrioxamine (50 mg/kg/day. Some other researchers found a significant negative correlation between zinc level and the duration and dosage of deferoxamine treatment (Moghadam *et al*., 1998). De-Virgiliis *et al.* (1988) reported a reduction in hair and leukocyte zinc levels and LAP activity associated with a significant increase in urinary zinc excretion after one month of deferoxamine treatment in those patients, who received it by daily subcutaneous infusion simultaneously with the beginning of the transfusion compared with patients, who received it after three years of age at similar doses and with those who were treated with intramuscular small doses. They concluded that chelation of trace elements, including zinc, may be related to the low iron burden. Considering the lower zinc level in thalassaemic patients without any relationship between the mean zinc level and the dosage and duration of deferoxamine treatment, it is suggested that other factors, such as anorexia, nutritional status, psychological problems (such as depression) and different metabolic and endocrine complications have led to zinc deficiency (Ugsal and Akar, 1993). Suthipark *et al.* (1991) reported different levels of trace elements (zinc and copper) in thalassaemic patients compared with non-thalassaemic controls due to abnormal trace element metabolism in thalassaemic patients. These antioxidant trace elements are important cofactors for several antioxidant enzyme systems: Copper-Zinc Superoxide Dismutase (Cu-Zn SOD), which is found in the extracellular fluid (Bunker, 1992). Few reports have mentioned normal serum zinc levels (Rea *et al*., 1984) or hair zinc content in thalassaemic patients (Donma and Gunbey, 1990). Kwan *et al.* (1995) reported that only 3 of their thalassaemic patients had zinc deficiency in their study population. In previous studies, some researchers (Schilir *et al*., 1987) have mentioned increased urinary excretion of zinc and decreased Leukocyte Alkaline Phosphates (LAP) activity, zinc-dependent enzyme, during deferoxamine infusion in small doses and they concluded that chelation of trace elements, including zinc, may be related to the low iron burden.

Desferal induced moderate aluminum excretion in urine but had no effect on copper or zinc excretion as mentioned in previous study in this field (Hyman *et al*., 1989). Other study found that low levels of zinc and copper can be related to nutritional problems in thalassaemic patients. Malnutrition was primarily caused by inadequate nutrient intake, as indicated by the capacity to gain weight appropriately when provided with nutrition support and by the absence of intestinal malabsorption. Plasma zinc, depressed in half the children on admission, improved, as did alpha-tocopherol, while copper decreased (Fuchs *et al*., 1996). Another study by Klevay (2001) was interested in copper deficiency and found that the iron can interfere with copper utilization without anemia and the dietary requirement for copper of people with iron overload (such as thalassaemic patients) may exceed that of the general population and there is deficiency among patients in zinc and copper levels concerned in bone mineral density in thalassemia. Copper is present largely in the form of organic complexes, many of which are metalloproteins acting as enzymes. Serum copper level was found to be significantly increased ($p<0.001$) in our patients when compared with controls. Blood transfusion and increase copper absorption via the gastrointestinal tract could explain this finding. Our

finding may be explained by the decreasing rate of glomerular filtration of zinc seen in chronic hemolysis and the disturbance in the metabolism of zinc and copper in thalassaemic patients due to the increasing serum zinc and copper. Moreover, the high level of copper could be explained by the increase in copper absorption via the gastrointestinal tract or is probably due to parenchymal hepatic damage, which is a common side effect in blood transfused patients.

CONCLUSION

Our data suggest that the increased level of copper and zinc, which was noticed in all our thalassaemic patients is in concordance with other studies and confirm the unnecessary supplementation of zinc or other metal elements for thalassaemic patients on regular blood transfusion except in certain situations like clear under nutrition or a known deficient population.

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