Original Article

Zinc and Copper in Transfusion-Dependent Thalassemia Patients on Different Iron Chelators in Basrah: A Case-Control Study

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Abstract

Background: Disturbances induced by chelating agents in the essential metal ions, including zinc and copper, can adversely impact the health of patients with transfusion-dependent thalassemia (TDT). **Objectives:** The aim of this study was to investigate the effect of different iron chelators on the levels of zinc and copper among patients with TDT. **Materials and Methods:** This case-control study involved 94 patients with TDT and 59 healthy controls, their ages ranged from 5 to 37 years. Patients with TDT were subdivided into two groups: 51 patients were on deferasirox and 43 were on deferoxamine. Blood samples were collected from all participants for complete blood counts and serum ferritin, iron, zinc, and copper levels, which were determined by spectrophotometry. **Results:** Serum iron, ferritin, and zinc levels were significantly higher among patients with TDT, while the Hb level was significantly lower than that in the healthy population ($P < 0.05$). Serum copper levels did not differ significantly between the groups. A high frequency of zinc deficiency was reported among patients with TDT on deferasirox (56.9%) and control group (47.5%), compared to 16.3% for patients with TDT on deferoxamine ($P < 0.001$). For serum copper, most of them had normal levels (81.4%–90.2%). Patients on deferoxamine had significantly higher serum zinc and copper levels than those on deferasirox and control group, although the mean serum values were within the normal range of values for the three groups ($P < 0.01$). **Conclusions:** Low serum zinc was reported in a considerable percentage of patients with TDT receiving deferasirox compared to those receiving deferoxamine, while serum copper was within normal range in the majority of patients with TDT.

Keywords: Copper, iron chelators, thalassemia, zinc

Introduction

Thalassemia is a group of heterogeneous hemolytic anemia of autosomal recessive inheritance characterized by impaired hemoglobin (Hb) synthesis. It is classified according to the type of hemoglobin chain affected, the most common are α- and β-thalassemia.[1,2] Approximately 70,000 babies are born annually with different types of thalassemia, mainly β-thalassemia.[2]

β-Thalassemia can be further subdivided based on the phenotype severity and the need for transfusions into transfusion-dependent thalassemia (TDT) and nontransfusion-dependent thalassemia (NTDT).[3] Patients with TDT, the most severe form of β-thalassemia, require regular blood transfusions throughout their lives in order to survive and maintain organ function, although this strategy is complicated by chronic iron overload (IOL).[3] Secondary IOL adversely affects vital organs and structures, causing various life-threatening complications in the long term, including cardiotoxicity, hepatic, endocrine, metabolic, and growth disorders. Ineffective erythropoiesis can also increase iron absorption from the gastrointestinal tract in patients with TDT and NTDT.[4,5] In addition to decreasing the chronic hemolysis, chronic blood transfusions in TDT or other hemolytic anemias, such as sickle cell disease, could change the micronutrient status of these patients.[6]

Access this article online

Quick Response Code:

Website: www.medjbabylon.org

DOI: 10.4103/MJBL.MJBL_294_22

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Submission: 25-Nov-2022 **Accepted:** 21-Dec-2022 **Published:** 27-Apr-2023

How to cite this article: Hasan BK, Saud WH. Zinc and copper in transfusion-dependent thalassemia patients on different iron chelators in Basrah: A case-control study. Med J Babylon 2023;20:124-9.

Iron chelation therapy is a mandatory, life-long treatment for TDT patients with IOL that has greatly improved both their survival and quality of life. The use of chelating agents can induce disturbances of the homeostatic equilibria of various essential metals which subsequently will lead to serious health consequences. The ideal iron chelator should have a high selectivity to remove the iron without interfering with the levels of other essential metals.[7]

Zinc (Zn), an essential element, has a paramount effect on the growth, development, and the function of the cells mediating immunity. Zinc deficiency in TDT patients can be attributed to increased urinary zinc excretion, high ferritin levels, hepatic IOL, and hepatic dysfunction.[8] Zinc deficiency has been reported to play an important role in many common complications, such as retarded growth, hypogonadism, delayed puberty, and depression, which are commonly reported in patients with TDT, especially during adolescence.[9]

Copper (Cu) is an important component of hemoglobin and many antioxidant enzymes (antioxidant superoxide dismutase and ceruloplasmin) that protect the cells from free radical injury.[10]

Although zinc and copper deficiencies have been reported in patients with TDT, the clinical effects of reduced zinc and copper levels and the role of iron chelators on their levels are controversial.[6,11,12] Therefore, this study was carried out to evaluate serum Zn and Cu levels in patients with TDT and the effect of iron chelation on their levels.

Materials and Methods

Patients

This was a case-control study that has been carried during the period of April 1, 2021 till the end of June 2022. A total of 94 patients with TDT registered at the Basrah Center for Hereditary Blood Diseases were recruited, and their ages ranged from 5 to 37 years.

The term TDT was used to label patients with thalassemia who require lifelong regular blood transfusions for survival starting before the age of 2 years.[13]

Data were collected from the patients and/or one of their parents and their medical records. Data included date of birth, age at diagnosis of the disease, and details of the transfusion therapy (age at first transfusion, frequency of blood transfusion, and other details about the transfusion therapy). Data about iron chelation therapy (ICT) was collected, including the type (deferasirox or deferoxamine), age of starting ICT, daily dose (mg) of each therapy, and frequency/week. The patients were divided into two subgroups in relation to the type of ICT.

Patients using other NTDTs and those who were on zinc supplements were excluded from this study.

Control group

It included 59 age- and sex-matched persons who do not have a history of any hemoglobinopathy, anemia, or family history of hemoglobinopathy.

Methods

Blood samples were withdrawn from all subjects and sent for the following investigations:

- Complete blood count using hematology analyzer Mindray-BC 5300 (Shenzhen, China) within 30 min of sample collection.
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- Serum ferritin was measured quantitatively by a chemiluminescent microparticle immunoassay technique (CMIA; Architect, Abbott, i1000, USA).
- Serum iron, zinc and copper

Serum iron, zinc, and copper were quantified by spectrophotometry (Architect, Abbott, C4000 System), with kits provided by Abbott Laboratory Inc. (Abbott Park, Illinois). The normal-range values for the serum zinc, copper, and iron levels according to this instrument and corresponding kits are as follows:

- Zinc normal range (µg/dL): males: 0–16 years: 66–144, ≥17 years: 75–290; females: 0–16 years: 66–144, ≥17 years: 65–256.
- Copper normal range (µg/dl): males and females; 4 months–4 years: 27–153, 5–16 years: 67–147, 17–60 years: 80–155, >60 years: 63–140.
- Iron normal range (µg/dL): males: 65 to 176; females: 50 to 170.

Statistical analysis

The Statistical Package of the Social Sciences (SPSS) software program, version 20.0 was used for data tabulation and statistical analysis (IBM Corp., Armonk, New York). Categorical variables (presented as numbers and percentages) were evaluated by the χ^2 test. For continuous variables; the Student *t* test (for two variables) and ANOVA (for more than two variables) were used for parametric variables, and Mann–Whitney and Kruskal–Wallis tests, were used for nonparametric variables. For assessing the significance of the means of multiple comparisons a post hoc test was used. The scale variables are presented as the means and standard deviations (SDs). Probability (*P*) values of less than 0.05 were regarded as statistically significant.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and

analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the (4S/272 in 29/6/2021) to get this approval.

Results

A total of 94 patients with TDT and 59 healthy subjects were recruited for this study. There was no significant difference in age or sex between the two groups [$P > 0.05$, Table 1].

Patients with TDT were subdivided into two groups by the type of iron chelator: 51 patients were on deferasirox, and 43 were on deferoxamine. There were no significant differences in the age of the patients at diagnosis, age at first transfusion, interval between blood transfusions, or mean pretransfusion Hb in either group, $P > 0.05$ [Table 2]. However, the age of the patients on deferoxamine, age at starting chelation therapy, and duration of chelation therapy were significantly higher than those on deferasirox [$P < 0.001$, Table 2].

Patients and healthy subjects were compared regarding their hemoglobin, iron, ferritin, zinc, and copper levels. Serum iron, ferritin, and zinc levels were significantly higher among the 94 TDT patients (regardless of the type of iron chelator), while their mean Hb level was significantly lower than that in the healthy population ($P < 0.05$). The serum copper level did not differ significantly between the groups [Table 3].

A high frequency of zinc deficiency was reported among TDT patients on deferasirox (56.9%) and the control group (47.5%), compared to only 16.3% in TDT patients on deferoxamine [$P < 0.001$, Figure 1]. None of the studied participants had high serum zinc levels. Most of the serum copper levels were normal (81.4–90.2%).

The levels of Hb, iron, ferritin, zinc, and copper were evaluated in the two groups of TDT patients (deferasirox and deferoxamine groups) and compared with the control group. The levels of the studied parameters were significantly different among the three groups. Post hoc analysis revealed that the Hb levels were significantly lower among patients on deferasirox and deferoxamine, while serum iron and ferritin levels were higher among patients on deferasirox and deferoxamine than among healthy subjects [$P < 0.001$, Table 4]. The post hoc tests also revealed that patients on deferoxamine had significantly higher serum levels of zinc and copper than those on deferasirox and the control group, although the mean serum values were within the normal range of values for the three groups, $P < 0.01$. There were no significant differences in serum zinc and copper levels between patients on deferasirox and the control group, $P > 0.05$. Hemoglobin and iron levels were not significantly different between patients on deferoxamine and deferasirox [Table 4].

Table 1: Selected demographic variables of TDT patients and the control group

Variables	TDT patients	Control group	*P* value
	Total (94)	Total (59)	
	N (%)	*N* (%)	
Age (years)			
5–10	42 (44.7)	20 (33.9)	0.389*
>10–15	22 (23.4)	18 (30.5)	
>15	30 (31.9)	21 (35.6)	
Mean ± SD	14.2 ± 8.0	14.8 ± 7.7	0.517**
Gender			
Male	41 (43.6)	25 (42.4)	0.880*
Female	53 (56.4)	34 (57.6)	

*Chi squared test

**Mann–Whitney test

Discussion

This study investigated the serum zinc, copper, and iron levels of patients with TDT in Basrah on different iron chelators. This study showed that patients on deferasirox had a higher frequency of Zn deficiency and a lower mean serum Zn level than TDT patients on deferoxamine ($P < 0.01$) and healthy subjects, although the difference was not significant ($P > 0.05$). Serum copper was significantly higher among those on deferoxamine therapy.

Table 2: Clinical characteristics of patients with TDT in relation to type of iron chelator

Variables	Deferasirox	Deferoxamine	Total	*P* value*
	N 51	*N* 43	*N* 84	
	Mean ± SD	Mean ± SD	Mean ± SD	
Age at diagnosis (months)	10.07 ± 9.46	10.58 ± 11.25	10.30 ± 10.26	0.811
Age of patients (year)	9.39 ± 2.98	20.00 ± 8.45	14.24 ± 8.08	<0.001
Age at 1st transfusion (months)	10.52 ± 9.67	10.73 ± 11.21	10.64 ± 10.34	0.990
Age at starting chelation therapy	3.25 ± 1.62	8.64 ± 4.51	5.71 ± 4.23	<0.001
Interval between blood transfusions (weeks)	3.00 ± 0.92	3.07 ± 1.84	3.03 ± 1.41	0.822
Duration of chelation therapy	5.82 ± 3.27	10.34 ± 6.95	7.88 ± 5.71	<0.001
Mean pretransfusion Hb (g/dl)	7.34 ± 0.94	7.55 ± 0.92	7.43 ± 0.93	0.277

*Student *t* test/Mann–Whitney test

Table 3: Zinc and copper in patients in the TDT and control groups

Variable	Control group	TDT patients	*P* value*
	Total (59)	Total (94)	
	Mean ± SD	Mean± SD	
Hb (g/dl)	12.52±1.46	7.40±1.05	<0.001
Iron (µg/dl)	67.36±29.16	156.10±41.52	<0.001
Ferritin (ng/ml)	39.39±34.51	4592.04±4023.03	<0.001
Zinc (µg/dl)	51.89±11.31	58.90±14.53	0.002
Copper (µg/dl)	90.29±28.80	96.48±31.88	0.216

*Student *t* test/Mann–Whitney test

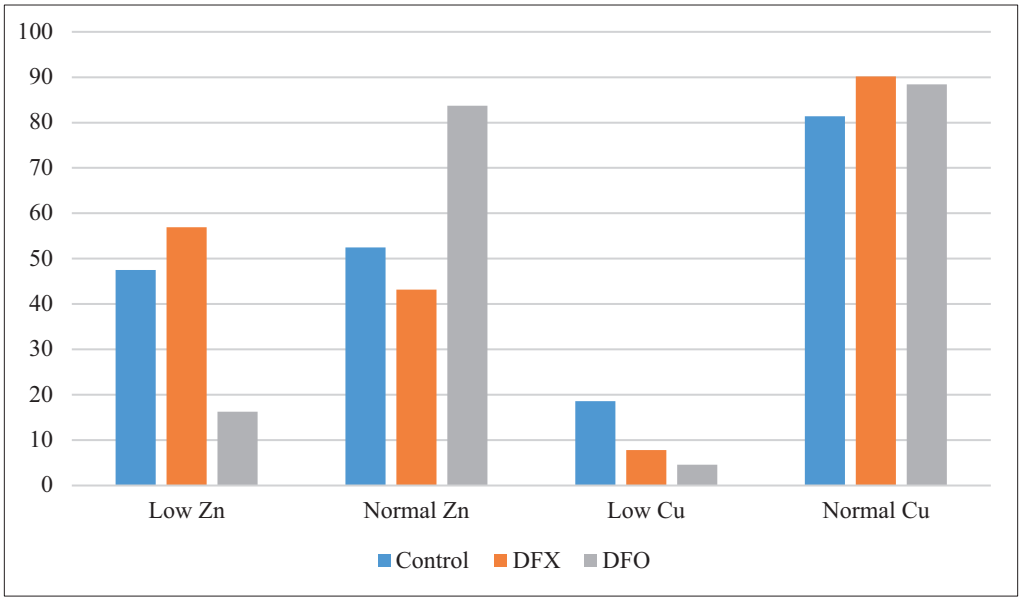

Figure 1: Frequency of low and normal serum zinc and copper in the studied populations. DFX: deferasirox, DFO: deferoxamine, Zn: zinc, Cu: copper

Table 4: Zinc and copper in patients on DFX and deferoxamine and in the control group

Variable	Deferasirox group	Deferoxamine group	Control group	*P* value*
	Total (51)	Total (43)	Total (59)	
	Mean ± SD	Mean ± SD	Mean ± SD	
Hb (g/ml)	7.40±0.99	7.39±1.12	12.52±1.46	<0.001
Iron (µg/dl)	160.78±50.58	150.53±26.68	67.36±29.16	<0.001
Ferritin (ng/ml)	2751.04±2058.54	6775.56±4668.63	39.39±34.51	<0.001
Zinc (µg/dl)	51.21±11.56	68.03±12.29	51.89±11.31	<0.001
Copper (µg/dl)	84.48±27.43	110.72±31.19	90.29±28.80	<0.001

*ANOVA/Kruskal–Wallis test

Zinc, copper, and iron are trace elements that are essential for the maintenance of the cell structure, proliferation, and function. Different proteins play an important role in the maintenance of a stable balance of these essential metals through a well-coordinated regulation of their uptake, intracellular storage and excretion.[14]

Although the mean age at diagnosis and the mean pretransfusion Hb were not significantly different between the groups of TDT patients, patients on deferoxamine were significantly older than those on deferasirox and had started chelation therapy at an older age.

Serum ferritin was significantly higher among TDT patients on deferoxamine, which could be attributed to the older age of starting chelation therapy reported in our patients and to less compliance with this injectable iron chelator in comparison with deferasirox.[15,16]

Zinc and copper status in TDT patients have been studied by many researchers, but the results are conflicting. In this study, zinc levels were higher among TDT patients on deferoxamine than those on deferasirox and healthy people. Our results are in agreement with those found by Şahin *et al.* in Turkey.[17] However, Genc *et al.* did

not report a significant difference in serum zinc between patients and healthy groups.[18] The median zinc level was lower among patients than healthy subjects in a study by Zekavat *et al.*[12], and Al-Thamir *et al.*[19] in Babylon, which could be due to the ability iron chelators to chelate zinc in addition to iron. However, Zardkhoni *et al.*, in Iran, did not find any association between serum zinc levels and the type of iron chelation.[11]

The differences between different studies can be attributed to the differences in the number of patients recruited in these studies, different socioeconomic statuses, differences in dietary intake, the duration and dose of the iron chelating agent, increased urinary excretion of Zn, different cutoff values and different definitions of zinc deficiency.[20,21] A previous study in Iraq by Al-Timimi *et al.* reported that children had the lowest mean serum zinc level compared to adolescents and adults,[22] which could partly explain the lower mean serum zinc level in TDT patients on deferasirox in our study as they were younger than those on deferoxamine.

Concerning serum copper, the results of different studies were also conflicting. We found significantly higher serum copper among patients on deferoxamine compared to those on deferasirox and the control group, although most TDT patients (89.4%) and the control group (90.2%) had normal copper values. There were no significant differences in serum copper levels between patients on deferasirox and the control group. Higher serum copper levels in β-thalassemia major patients than in healthy subjects were also reported by Awjagh IS in Kirkuk, Iraq[23] and Zekavat *et al.*[12] Şahin *et al.* did not report significant changes in copper between patients and controls.[17] Many factors affect the level of serum copper in TDT patients including the daily dietary copper intake, intestinal uptake of copper, rate of iron accumulation, the presence of renal dysfunction, copper/zinc ratio, and iron chelation therapy.[24]

The current study has many limitations. The first limitation is the relatively small size of the control group. The other limitation is that serum zinc and copper were not evaluated in TDT patients not receiving iron chelation.

From this study, it can be concluded that low serum zinc was present in a considerable percentage of TDT patients receiving deferasirox compared to those receiving deferoxamine, while serum copper was within the normal range in the majority of TDT patients. Additional studies on the effect of zinc supplementation for TDT patients on iron chelation need to be conducted.

Acknowledgments

We would like to thank Dr. Ahmed Qasim, Ph.D. Community Medicine for his great assistance in conducting the statistical analysis of the data.

Authorship

BK and WH designed and planned the study. WH collected the data. Both authors contributed to the analysis and writing of the manuscript, and both have read and approved the final manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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