Original Article

Blood Lead and Cadmium Levels in Transfusion-Dependent Thalassemia Patients

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Abstract

Background: The risk of heavy metal toxicity, including lead and cadmium, is high in individuals with diseases requiring multiple blood transfusions like thalassemia. **Objectives:** To evaluate blood lead levels and serum cadmium in transfusion-dependent thalassemia (TDT) patients and the impact of iron chelation therapy (ICT) on their levels. **Materials and Methods:** This case–control study involved 110 patients with TDT and 70 healthy individuals, their ages ranged from 4 to 37 years. Patients with TDT were subdivided into those on deferasirox (60 patients) and deferoxamine (50 patients). Blood samples were collected from all participants for complete blood counts of serum ferritin and iron. Serum cadmium and lead levels were estimated using an AA-7000 Atomic Absorption Spectrophotometer. **Results:** Mean hemoglobin level was significantly lower while serum iron and ferritin were significantly higher in TDT patients compared to the control group. Serum levels of lead and cadmium were not significantly different between healthy subjects and TDT patients, $P > 0.05$. Serum lead and cadmium levels were also not significantly different between patients on different iron chelators, $P > 0.05$. Pearson correlation did not reveal any significant correlation between lead (*R** = 0.05 and 0.176) and cadmium (*R** = -0.012 and -0.075) with each of serum iron and ferritin, respectively. **Conclusions:** Although serum levels of lead and cadmium were not significantly different between TDT patients and healthy individuals, the relatively high lead levels among studied participants need further studies to identify risk factors and to put an immediate action plan.

Keywords: Cadmium, lead, transfusion-dependent thalassemia

Introduction

Patients with thalassemia present with different clinical phenotypes which are related to the severity of the globin gene mutation and co-inheritance of other genetic determinants. Patients are classified as transfusion-dependent thalassemia (TDT) and non–transfusion-dependent thalassemia (NTDT) depending on the degree of transfusion dependence.[1]

Iron overload (IOL) is one of the inevitable complications in TDT patients develops from increased intestinal iron absorption due to ineffective erythropoiesis and also due to regular transfusions.[1] IOL is associated with increased morbidity and mortality in TDT patients due to cardiac siderosis, hepatic and various endocrine complications.[1-3]

Both unstable hemoglobin and IOL stimulate the production of excess free radicals and increased oxidative stress. The symptoms aggravated by oxidative stress include increased hemolysis, ineffective erythropoiesis, and functional failure of vital organs mainly the heart and liver.[4]

Furthermore, divalent metal-ion transporter 1 (DMT1) exhibits reactivity with a broad range of metal ions might increase in patients with that is, due to the increased activity of DMT1 protein leading to heavy metals accumulation.[5]

Both lead (Pb) and cadmium (Cd) are widely distributed in the environment and exposure to these toxic heavy

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metals is considered a global health problem even with minimal amounts.[6]

Lead accumulation can affect the central nervous system (CNS), reproductive organs, skeletal, renal, and cardiovascular systems.[7] Hematopoietic system is also a target of lead. Lead interferes with heme biosynthesis and influences the generation and function of red blood cells (RBCs).[8]

Cadmium exposure in minimal doses may result in nephrotoxicity, osteoporosis, cardiovascular diseases, and cancer.[9,10] It can decrease antioxidant compound levels of human cells by inactivating enzymes and various antioxidant molecules.[10]

Human beings acquire heavy metals from many resources through water, air, food, or industrial settings. The increase in population, urbanization, and industrialization have caused an increase in the production of waste without proper disposal systems. Increasing mining activities, illegal refining, use of leaded petrol, airborne dust, burning of toxic waste, and absorption of production industries in populated areas, have all led to heavy metal pollution.[6]

Blood transfusion has been considered as a source of heavy metals, including lead and cadmium, from donors with high blood concentrations of these heavy metals. The risk of heavy metal toxicity is especially high in individuals with diseases requiring multiple blood transfusions.[11]

Iron chelation therapy (ICT) used to prevent and treat IOL in TDT patients can effectively lower serum iron and normalize iron stores. The available and widely used iron chelators are Deferasirox (DFX), Deferoxamine (DFO), and Deferiprone (DFP).[12] ICT might also have a protective effect in patients with ineffective erythropoiesis against heavy metal toxicity.[5]

Information about heavy metal toxicity including lead and cadmium are underestimated in TDT patients, therefore this study was carried to evaluate blood lead levels (BLL) and serum cadmium in TDT patients and the impact of ICT on their levels.

Materials and Methods

Patients

A total of 110 patients with TDT registered at the Basrah Center for Hereditary Blood Diseases, aged 4–38 years, were included in this case–control study that was carried out from the first of October 2022 till the end of April 2023.

Transfusion dependence refers to patients with thalassemia who require lifelong regular blood transfusions for survival that starts before 2 years of age.[13]

Selected demographic and clinical data such as age, 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) were obtained from the patients and/or one of their parents and medical records. ICT data, including the type (deferasirox or deferoxamine), age of starting ICT, the daily dose (milligram) of each therapy, and frequency/week was obtained.

Patients with other types of thalassemia or hemoglobinopathies were excluded from this study.

Control group

It included 70 age- and sex-matched persons who do not have a history of any hemoglobinopathy, anemia, or family history of hemoglobinopathy.

Methods

Blood samples withdrawn from patients and control groups were sent for the following investigations:

- Complete blood count (CBC): using hematology analyzer Mindray-BC 5300 (Shenzhen, China) within 30 min of sample collection.
- Serum ferritin:

Serum ferritin was measured quantitatively by a chemiluminescent microparticle immunoassay technique (CMIA; Architect, Abbott, i1000, USA).

- Serum iron was quantified by spectrophotometry (Architect, Abbott, C4000 System, USA), with kits provided by Abbott Laboratory Inc., Abbott Park, IL, USA. The normal range of values for iron levels according to this instrument and corresponding kits are 65–176 µg/dL for males and 50–170 µg/dL for females.
- Serum cadmium and lead levels were estimated using an AA-7000 Atomic Absorption Spectrophotometer, Shimadzu, Japan. The principle of this analysis has involved the absorption of light by the atomic vapor of the metal of interest, which is generated by heating the sample in the graphite furnace.
- The reference range for serum cadmium levels is <5 µg/L for all ages, with an average range of 0.1–4 µg/L.[14,15] For blood lead; levels should be ≤3.5 µg/dL for children[16,17] and <5 µg/dL in adults.[18,19]

Statistical analysis

The Statistical Package of the Social Sciences (SPSS) version 20 was used for data tabulation and statistical analysis (IBM Corp., Armonk, NY, USA). Categorical variables (presented as numbers and percentages) were evaluated by the Chi-squared test. For continuous variables; the Student's *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). Correlation between cadmium and lead with serum iron and ferritin was studied using Spearman's correlation coefficient. Probability (*P*) value of <0.05 was considered 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 the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by the local Scientific and Ethics Committee (Ref. number 030401-113-2022) according to the 15S on June 19, 2022, to get this approval.

Results

A total of 110 patients with TDT and 70 healthy subjects were enrolled in this study. There was no significant difference in age or sex between the two groups ($P > 0.05$) [Table 1].

All TDT patients were on iron chelators; 60 patients were on deferasirox and 50 were on deferoxamine. Patients on deferasirox were significantly younger (mean age 9.0 ± 2.97 years) and were started on chelation therapy at younger age group (mean 3.2 ± 1.4 years) than those on deferoxamine (19.0 ± 8.16 years) and (8.4 ± 4.3 years) respectively, $P < 0.001$. However, the study did not reveal significant differences in sex distribution and age of first transfusion (9.9 ± 9.8 vs. 11.1 ± 9.8 months) among patients on deferasirox and deferoxamine, respectively, $P > 0.05$.

Table 1: Selected demographic variables of TDT patients and healthy control

Variables	Control group Total: 70 *N* (%)	TDT patients Total: 110 *N* (%)	*P* value
Age (years)			
<5–10	28 (40)	48 (43.6)	0.890*
>10–15	21 (30)	31 (28.2)	
>15	21 (30)	31 (28.2)	
Mean ± SD	13.9 ± 7.49	13.57 ± 7.74	0.760**
Gender			
Male	30 (42.9)	50 (45.5)	0.723*
Female	40 (57.1)	60 (54.5)	

*Chi-squared test.
**Student's *t* test

Hemoglobin, serum iron, ferritin, lead, and cadmium levels were compared for both the control group and TDT patients. Mean hemoglobin level was significantly lower while serum iron and ferritin were significantly higher in TDT patients compared to the control group ($P < 0.001$) [Table 2]. Serum levels of lead and cadmium were not significantly different between healthy subjects and TDT patients, $P > 0.05$.

The two groups of patients on ICT (deferasirox and deferoxamine) and healthy subjects were compared regarding their hemoglobin, serum iron, ferritin, lead, and cadmium levels. The levels of hemoglobin, serum iron and ferritin were significantly different among the three groups. *Post hoc* analysis revealed that hemoglobin 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 3]. The *post hoc* tests also revealed that patients on deferasirox had significantly lower serum levels of ferritin compared to those on deferoxamine, $P < 0.001$, whereas hemoglobin and serum iron levels were not significantly different among both patient's groups, $P > 0.05$. Serum lead and cadmium levels were not significantly different among the three groups ($P > 0.05$) [Table 3].

Pearson correlation did not reveal any significant correlation between lead (*R** = 0.05 and 0.176) and cadmium (*R** = -0.012 and -0.075) with each serum iron and ferritin, respectively [Figures 1 and 2].

Discussion

The current study investigated the serum iron, lead, and cadmium levels of TDT patients compared with healthy individuals. This study did not show significant differences in serum lead and cadmium levels in patients with TDT and control group, $P > 0.05$.

Table 2: Lead and cadmium in patients with TDT and control group

Variable	Control group Total: 70 Mean ± SD	TDT patients Total: 110 Mean ± SD	*P* value*
Hb (g/dL)	12.56 ± 1.43	7.47 ± 1.11	<0.001
Iron (µg/dL)	67.17 ± 28.19	158.24 ± 41.57	<0.001
Ferritin (n/mL)	40.09 ± 33.70	4535.35 ± 3899.57	<0.001
Lead (µg/dL)	8.49 ± 5.13	9.96 ± 7.85	0.165
Cadmium (µg/L)	3.11 ± 2.89	2.46 ± 2.37	0.101

*Student's *t* test/Mann–Whitney's *U* test

Table 3: Lead and cadmium in patients on DFX and DFO and control group

Variable	DFO group Total: 50 Mean ± SD	DFX group Total: 60 Mean ± SD	Control group Total: 70 Mean ± SD	*P* value*
Hb (g/dL)	7.51 ± 1.16	7.44 ± 1.08	12.56 ± 1.43	<0.001
Iron (µg/dL)	155.18 ± 29.50	161.49 ± 51.56	67.17 ± 28.19	<0.001
Ferritin (n/mL)	6865.31 ± 4376.59	2593.72 ± 1936.80	40.09 ± 33.70	<0.001
Lead (µg/dL)	10.96 ± 5.08	9.13 ± 9.53	8.49 ± 5.13	0.148
Cadmium (µg/L)	2.15 ± 0.98	2.71 ± 3.08	3.11 ± 2.89	0.140

*ANOVA/Kruskal–Wallis test

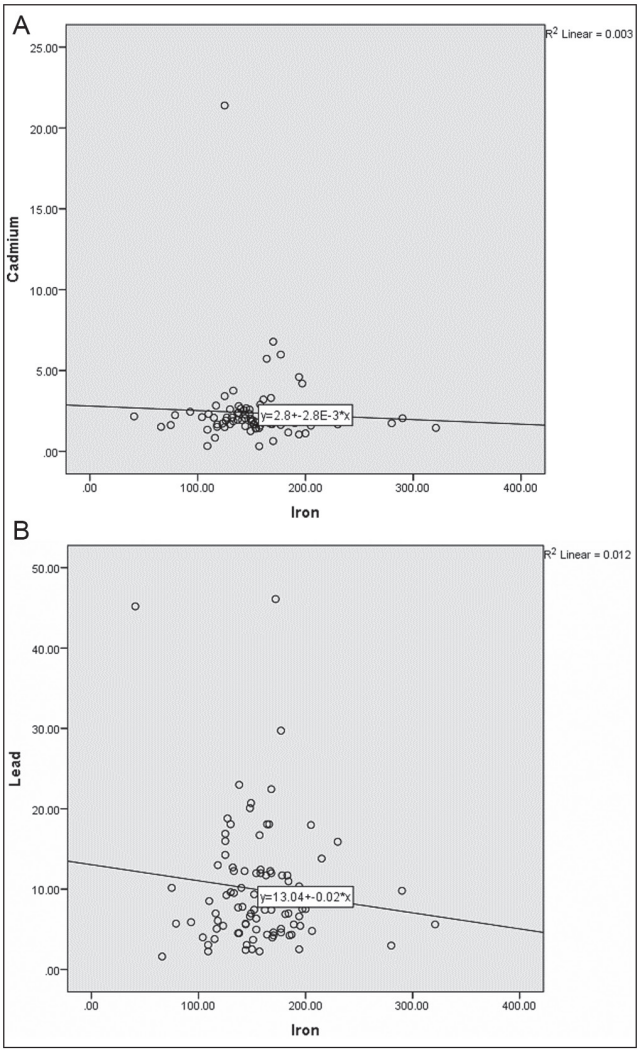

Figure 1: (A) Correlation between cadmium and iron in TDT patients. (B) Correlation between lead and iron in TDT patients

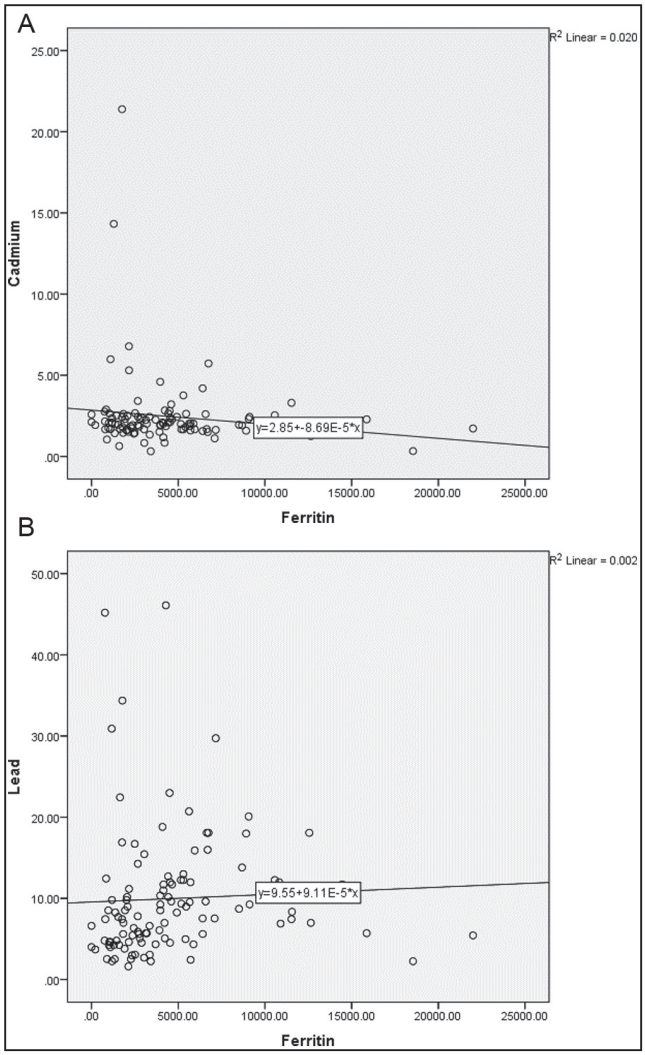

Figure 2: (A) Correlation between cadmium and ferritin in TDT patients. (B) Correlation between lead and ferritin in TDT patients

Exposure to lead and cadmium can cause severe illness in the human beings including renal system dysfunction, gastrointestinal and liver diseases, anemia, lung cancer, and nervous system diseases,[20] can damage the immune system (cellular and humoral), immune responses, and cytokine production through the induction of reactive oxygen species (ROS), thereby leading to damage to different organs and increased susceptibility to infections and cancer.[21]

Humans are exposed to toxic metals through different routes of entry including blood transfusions. The risk of toxicity is especially high in at-risk populations such as neonates and individuals requiring multiple blood transfusions, this is more in developing countries. A universal routine screening of donated blood for heavy metals has been recommended to reduce toxic metal exposure in vulnerable individuals, especially children

because of the dangerous and irreversible effects of heavy metals on the developing brain.[11,22]

Although BLL were not significantly different between TDT patients (9.96 ± 7.85) and healthy controls (8.49 ± 5.13), $P > 0.05$, these levels were lower than that reported in a previous study in Basrah by Al-Naama *et al*.[23] (12.62 ± 3.85 µg/dL in those <15 years and 11.20 ± 3.4 µg/dL in individuals older than 15 years and that reported in Nasiyria (13.9 ± 4.8 µg/dL for children, and 14.4 ± 3.6 µg/dL for adults).[24] However, our results were comparable to that reported by Al Dosky *et al*.[25] in Duduk (7.3 ± 2.8 µg/dL). The relatively high levels of lead in Iraq can be attributed to higher contents of lead in some foods,[26] drinking water,[27] lead acid battery recycling and manufacture, metal mining and processing, and electronic waste.[28]

An important finding of the current study is that cadmium levels were within normal limits for TDT patients and the control group. Our results are lower than that reported in healthy subjects in Basrah (14.02 ± 21.68 in males and 21.41 ± 15.45 in females),[29] and comparable to that reported in Salahuddin (0.24 ± 0.18) and Wasit (0.25 ± 0.007),[30] these variations can be attributed to increased levels of cadmium in groundwater as a result of widespread effects of plant irrigation and low free chlorine in drinking water.[30]

Among TDT patients, we reported neither a significant difference in the levels of lead and cadmium on different iron chelators (deferasirox and deferoxamine) nor a significant correlation between serum ferritin levels with each lead and cadmium. Different studies concerning the levels of lead and cadmium among TD patients by other researchers showed conflicting results; our results are similar to those found by Iyen *et al*.[31] in Malaysia, who did not find a significant difference between lead levels between thalassemia patients and healthy subjects and suggested that the reduced lead level in thalassemic patients can be attributed to the iron chelators therapy. Aliyev *et al*.[32] in Turkey reported a significantly lower lead and cadmium levels among thalassemia patients (37.53 ± 17.71 ppb and 0.92 ± 0.71 ppb), than among the control group (45.43 ± 18.82 ppb and 1.30 ± 0.88 ppb), respectively, with significant positive correlation between serum iron and lead levels and negative correlation with cadmium ($P < 0.05$). Another study, by Bayhan *et al*.[5] in Turkey also, reported that TDT patients were found to have lower lead and higher cadmium levels compared to the control group but no significant correlation between cadmium and ferritin and patients treated with deferasirox had lower lead compared with the control group and concluded that deferasirox therapy may have protective against heavy metals. On the other hand, Yüksel *et al*.[33] have reported a significantly higher blood lead level in β-thalassemia patients (71.20 ± 43.38 µg/L) than in the control group (27.58 ± 8.18 µg/L) ($P < 0.05$), with a statistically negative correlation between iron and lead among β-thalassemia patients (r = –0.424, $P < 0.05$).

The non-significant differences between cadmium and lead levels in our study can be attributed to ICT,[31,34] and low zinc levels reported among TDT patients on deferasirox in Basrah,[35] which have been reported to be associated with lower cadmium levels.[36]

One limitation of this study is that all studied TDT patients were on iron chelators, hence we were unable to assess lead and cadmium levels among patients not receiving ICT. Another limitation is that it was a cross-sectional study, therefore, we were not able to assess these two heavy metals of TDT patients at baseline, and then after a period of starting ICT.

From this study, it can be concluded that although serum levels of lead and cadmium were not significantly different between TDT patients and healthy individuals, the relatively high lead levels among our studied participants signify other risk factors as environmental risk factors and needs further studies to identify risk factors and to put an immediate action plan for early detection and possibly screening of children.

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Conflicts of interest

There are no conflicts of interest.

Authorship

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

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