

Article

Evaluation of Lead and Nickel Levels in Blood Serum Samples of Beta-Thalassemia Patients

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Abstract: β -Thalassemia major (β -TM) is a genetic disorder characterized by severe anemia, requiring regular blood transfusions, which may lead to the accumulation of heavy metals such as lead (Pb) and nickel (Ni). Although previous research has highlighted the potential for increased heavy metal accumulation in β -TM patients, there is limited understanding of Pb and Ni levels in these individuals compared to healthy controls. This study aimed to assess and compare the concentrations of Pb and Ni in the blood serum of β -TM patients and healthy individuals residing in Baghdad Governorate, Iraq, using flame atomic absorption spectroscopy (FAAS). The study included 150 participants, with 100 β -TM patients and 50 healthy controls. Statistical analysis was conducted using SPSS software, employing a One-way independent test to compare metal concentrations, ANOVA to assess differences in age and smoking habits, and Spearman correlation to evaluate the relationship between Pb and Ni levels. The results demonstrated significantly higher mean concentrations of Pb (81.19 ± 8.05 ppb) and Ni (16.67 ± 3.853 ppb) in the serum of β -TM patients compared to the control group (Pb: 27.35 ± 3.941 ppb; Ni: 2.49 ± 0.359 ppb) with p-values < 0.05 . Additionally, a significant positive correlation ($r = 0.424$, $p < 0.001$) was observed between Pb and Ni levels in β -TM patients. These findings suggest that β -TM patients are at an elevated risk of heavy metal accumulation, potentially contributing to their clinical complications. This study underscores the importance of monitoring heavy metal levels in β -TM patients and may inform future therapeutic strategies to mitigate heavy metal toxicity in this vulnerable population.

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1. Introduction

β -thalassemia is one of the most prevalent genetic diseases brought on by an inability to synthesize beta globin chains. In the world, the estimated yearly occurrence of this trait is one per million [1]. Because (β -TM) major patients are dependent on safe blood transfusions for survival, their blood becomes iron-rich [2], which can Pb to several other complications, including parathyroid, diabetes mellitus, thyroid insufficiency, growth retardation, and cardiac failure. In addition, it changes the levels of elements in serum [3].

Heavy metals play a fundamental role in the body's organs to perform their proper basic functions. The human body contains appropriate amounts of heavy elements that can be reacted together and form macromolecules and participate in all critical chemical reactions [4].

Heavy metals that have no biological effective function affect human health by replacing basic minerals by catalyzing many oxidative stress series reactions [5]. Heavy

metals with low toxicity can have long-term toxicity due to their continuous accumulation in the bodies of living organisms as well as characterized by an imbalance between reactive oxygen concentrations [6].

Lead (Pb) is a recognized toxicant that can induce oxidative stress by generation the reactive oxygen species (ROS) [7], Where the ROS pathway has pathogenesis in many diseases such as increasing the diabetes risk by pathways unrelated resistances of insulin [8]. Accumulation of lead in the human body cannot be degraded or destroyed even at low levels, causing chronic health effects, so Pb has been considered a predictor of the risk of many diseases [9].

Nickel (Ni) is a natural element that is present earth's crust and the solid material as soil and igneous rocks. In addition, it is present in water of sea, river, and even well water, as well as present in air and biological samples. [10]. Because of its special physical and chemical characteristics, Ni is employed in a wide range of contemporary metallurgical operations, including the creation of Ni-cadmium batteries, which pollute the environment [11]. The compounds of Ni are carcinogenic action and have the ability of Ni+2 to get oxidized to Ni+3 rendering it's potential to generate reactive oxygen species (ROS) in the system leading to oxidative stress and causing hemotoxic [12].

Different techniques are employed to identify blood serum heavy element levels that are elevated. Heavy element poisoning can be identified by looking for tiny blood cell alterations or the lack of thick lines in the child's X-rayed bones [13]. Nonetheless, testing blood samples for heavy element concentrations is the most crucial method for identifying high heavy element levels in the body [14].

2. Materials and Methods

Blood samples were collected through venipuncture, and these two groups were placed in a jet tube. They were distinguished by the numbers assigned to the study participants to calculate heavy element concentrations.

The total serum samples for the (β -TM) patient and control group were 150. Initial approvals for the study protocol were obtained by volunteers who participated in the study.

The questionnaire included data on medical history through an interview, taking into account exposure to sources of toxic elements such as smoking habits.

Three ml of blood was drawn from all the experimental persons directly from the vein at the start of the experiment. Tubes containing anticoagulant were used for blood parameters.

The study was conducted in Baghdad governorate, between the dates of March 1, 2023, and April 1, 2024. During this period, blood samples were collected from a total of 150 individuals who were divided into two groups. The control group consisted of 50 samples, while the β -TM group included 100 samples. The Flame Atomic Absorption Spectroscopy was employed to ascertain the levels of heavy element concentrations in the blood samples of the participants.

Heavy element measurement

The Flame Atomic Absorption Spectroscopy (FAAS) was employed to ascertain the levels of heavy element concentrations in the blood samples of the participants. Laboratory tests were conducted by collecting blood in gel tubes without anticoagulant and subsequently subjecting them to centrifugation at 3000 cycles for a duration of 20 minutes to separate the serum. Twenty ml of the sample was injected directly into the electric oven, and the absorbance was measured and converted into concentrations. The device automatically drew the parameters curve and then extracted the slope equation.

Statistical analysis

The statistical analysis of the data of the experiment was measured by using the SPSS, One-way independent test was used for the experiment, and the least significant difference was performed to assess significant differences between group means, results were expressed as mean \pm standard error and $P < 0.05$ was considered statistically significant. In addition, the relationship between Pb concentrations and Ni concentrations was determined by the Spearman correlation test, as well as results were expressed as $R=1$ exactly, the β -TM patient group and control group are perfectly correlated. Positive correlation is stronger as the R approaches +1, and negative correlation is stronger as the R approaches -1.

3. Results and Discussion

The data collected was analyzed using SPSS to examine the participants' samples. The statistical difference between measured elements for each group (β -TM and control) was found using the one-way independent test. Additionally, the relationship between Pb and Ni concentrations was determined by the Spearman correlation test.

Blood serum samples were obtained from 100 patients with β -TM and a control group of 50 healthy volunteers. Table 1 shows some descriptive statistics of elements such as the number of samples, minimum, maximum value, and mean \pm standard error for the patient and control groups.

Table 1. Statistical description of Pb and Ni levels (ppb) in blood serum samples of the study groups.

Statistical Value	Pb Concentrations (ppb)		Ni Concentrations (ppb)	
	β-TM Group	Control group	β-TM Group	Control group
No. of Samples	100	50	100	50
Minimum	14.061	5.733	1.462	0.250
Maximum	261.049	90.754	86.006	6.687
Mean± Std. Error	81.19±8.05	27.35±3.941	16.67±3.853	2.49±0.359
P- Value	0.040		0.000	
Spearman correlation test	R=0.424			

Table (1) shows that the minimum value of Pb and Ni levels of the β -TM group is (14.061 ppb) and (1.462 ppb) respectively. As well as, the maximum value of Pb and Ni levels of the β -TM group is (261.049 ppb) and (86.006 ppb) respectively. The mean values of Pb level in the β -TM group and control group are (81.19 ppb) and (27.35 ppb) respectively, while the mean values of Ni level in the β -TM group and control group are (16.67 ppb) and (2.47 ppb) respectively. The increase in the mean value of both Pb and Ni levels in the β -TM group compared to the control group is statistically significant (>0.05).

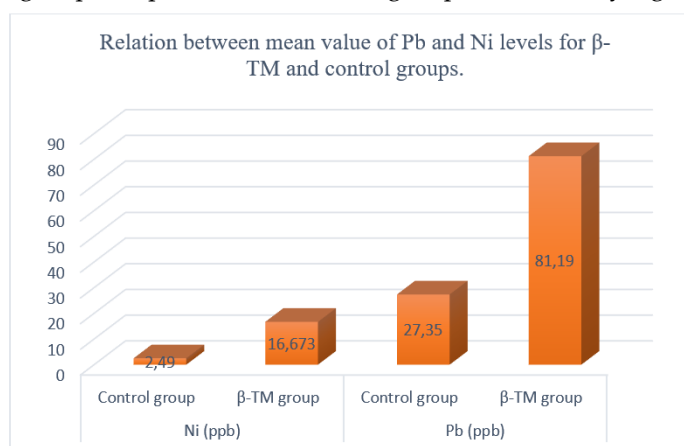


Figure 1. The mean value of Pb and Ni concentrations for β -TM and control groups.

Figure (1) shows the β -TM patients were found to have higher Pb and Ni levels about 0.75 and 0.87 compared to the control group. Long-term exposure to Pb and Ni can iron deficiency and anemia [15]. This is because the consumption of Pb can decrease the viability of red blood cells and inhibit heme production, which are both factors contributing to anemia [16] [17].

Table (1) also shows the correlation between Pb and Ni levels, that is a moderate positive correlation between Pb and Ni was found in the (β -TM) patients ($r = 0.424$ with $p < 0.001$). Thus, as a consequence of unintentional exposure is an increase of Pb with Ni level. According to many previous studies, the iron (Fe) level in β -TM is higher than the normal level when compared to healthy people [18], The high levels of Pb, Ni and Fe are causes of severe anemia and morphological variation at the level of blood cells. This indicates the importance of studying and monitoring the proportion of these elements continuously in people with β -TM diseases.

Tables 2, 3, and 4 classify β -TM patient samples according to age and smoking habits. The statistical differences between measured elements for the β -TM group were found using the One-Way ANOVA test.

The age groups of β -TM patient samples and the control group were divided into five subgroups: (1-10), (11-20), (21-30), (31-40), and (41-50), as shown in the table (2) and figure (2).

Table 2. The mean value of Pb and Ni levels for β -TM in blood serum samples as a function of age (years).

Age (years)	Mean value \pm Std. Error	
	Pb	Ni
(1-10)	66.19 \pm 3.685	25.36 \pm 15.805
(11-20)	68.20 \pm 7.446	21.29 \pm 8.762
(20-30)	75.69 \pm 22.047	9.55 \pm 2.612
(31-40)	78.88 \pm 19.446	18.83 \pm 10.738
(41-50)	60.04 \pm 5.057	4.16 \pm 0.625
P-value	0.341	0.378

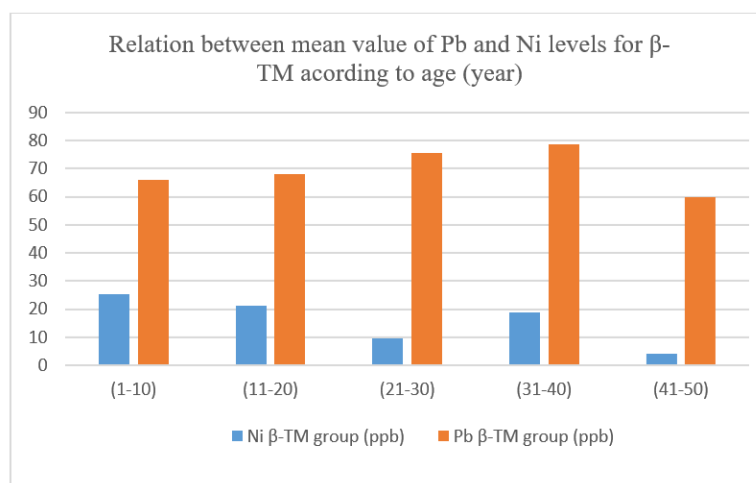


Figure 2. Pie chart of mean values of Pb and Ni concentration (ppb) for β -TM as a function of age groups.

There was no statistical difference between β -TM groups in terms of age and heavy metal exposure because this study includes unintentional exposure.

Table (3) presents the mean value of Pb and Ni elements as a function of the smoking habits parameter. In this study, the smoking habits were classified into three sub-groups which are (5-10), (11-15) and (16-20), as show in table (3) and figure (3).

Table 3. The mean value of Pb and Ni level for β -TM in blood serum samples as a function of the time period of smoking (year).

Time period of smoking (year)	Mean value \pm Std. Error	
	Pb	Ni
(5-10)	47.64 \pm 3.164	2.69 \pm 0.220
(11-15)	67.65 \pm 2.046	7.52 \pm 0.667
(16-20)	124.67 \pm 16.839	43.12 \pm 8.831
P-value	0.000	0.000

The results showed that there were a statistically significant increases in the Pb and Ni levels with the time period of smoking (year) (p-value< 0.05).

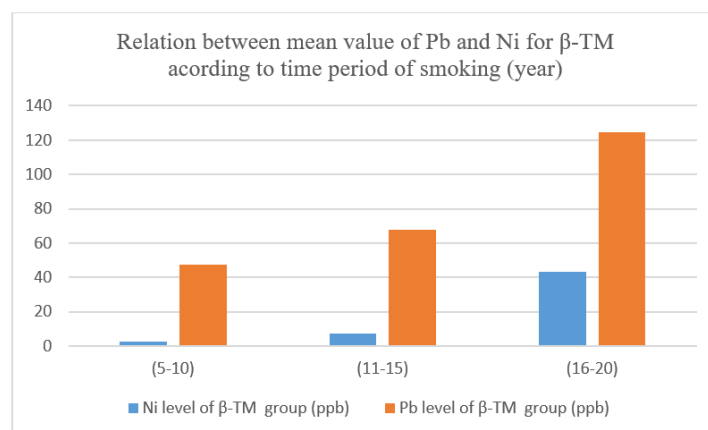


Figure 3. The statistical histogram for the mean value of Pb and Ni levels in blood serum samples of β -TM according to the time period of smoking (year).

It was observed that the mean values of Pb and Ni levels are directly proportional to the smoking habits, as they increase with the time period of smoking (year), as shown in Figure (3). This means that Pb and Ni accumulation increases with the increase in smoking, as the results showed a statistically significant increase in the mean values with smoking habits. This agreement with many previous studies which showed that Pb and Ni are related to smoking habits[19].

4. Conclusion

This study demonstrates that continuous blood transfusions for β -TM patients not only cause an increase in iron levels but also lead to elevated levels of lead and nickel. These findings highlight the importance of continuously studying and monitoring the levels of these elements in individuals with β -TM disease, as well as assessing the presence of other heavy metals.

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