Effect of Desferrioxamine Injection on Some Metal Ions in β-Thalassemia Major Patients

Rawa Ratha, 1 Dler M. salh, and Dlzar D. Ghafoor. 2
1Dept. of Clinical Pharmacy, College of Pharmacy, Sulaimani University
2Dept. of Chemistry, Faculty of Science and Science Education, Sulaimani University

Abstract-- In this study, 50 patients with β-thalassemia major, and 20 apparently healthy control subjects were investigated for: hemoglobin, serum iron, serum copper, serum magnesium, total iron binding capacity, transferrin saturation, and serum ferritin. The present study, which used the sensitive and accurate ICP instrument revealed that the mean serum Fe and Cu levels in thalassemia major patients are significantly higher than in healthy subjects, whereas the mean serum Mg levels in the patients are significantly lower compared to control groups. Total iron binding capacity, transferrin saturation, and serum ferritin was higher in patients compared to healthy individuals. Hemoglobin level was decreased in comparison to control groups.

Index Term-- Desferrioxamine, β-Thalassemia, metal ions, iron chelations.

1. INTRODUCTION

Thalassemia is a genetic blood disorder. This disease was described by Cooley and Lee[1,2]. Alpha and beta types thalassemia are the most common types of the disease. β-Thalassemia, which is the most prevalent type of thalassemia is a group of inherited autosomal recessive blood disorder characterized by genetic mutations resulting in reduced synthesis of β-globin chains [3]. β-thalassemia is widespread throughout the Mediterranean region, Africa, the Middle East, and the Southeast Asia [4]. Three main forms of thalassemia have been described which are thalassemia major, thalassemia intermedia and thalassemia minor. Beta thalassemia major which is the case under study is the most severe form in which individuals often suffer severe transfusion-dependent anemia within the first two years of life requiring regular red blood cell (RBC) transfusions and desferrioxamine injections [5].

According to the Ministry of health data, there are more than 2500 registered thalassemia patients in Kurdistan region among these AsSulaimaniyah has a high prevalence of thalassemia, as more than 500 patients suffer from this disease. Iron overload in the organs is one of the complication followed multiple blood transfusion. desferrioxamine chelation therapy with blood transfusion may chelate other metal ions beside iron. This study aimed to estimate the levels of copper, iron and magnesium in the serum of patients with β-thalassemia major treated with desferrioxamine for evaluating the effect of the drug in chelating trace elements as copper and magnesium.

Iron overload in transfusion-dependent β-thalassemia patients is associated with progressive dysfunction of the heart, liver, and endocrine glands, so iron chelation therapy is essential for the optimal management of this disease [6, 7]. Iron chelation treatment is necessary to prevent iron overload and is the only available method for preventing early death due to iron toxicity [8]. Iron overload of tissues if not treated will lead to severe complications. Thalassemia major patients can live longer if they have access to proper treatment [9]. Desferrioxamine is the most commonly used compound in transfusion-dependent β-thalassemia patients [6, 7].

Desferrioxamine has a strong affinity toward trivalent ions such as ferric ion with binding constants 10^30 [10]. The stability constants of desferrioxamine are lower when binding to divalent ions such as Cu^{2+}, Mg^{2+} and Fe^{3+} ranging from (10^{14} \cdot 10^{9} and 10^{7}), respectively. Ferrioxamine which is produced from chelton of Fe^{3+} with desferrioxamine is stable and non-toxic and it is excreted through urine and feces [11]. The use of desferrioxamine is recommended in patients who have a serum iron concentration greater than 500 µg/dl [12]. Desferrioxamine binds to both free iron and bound iron from ferritin and removes only a small amount from transferrin while the iron from hemoglobin, and cytochromes are inaccessible to desferrioxamine [13].

Beside the chelation of iron, desferrioxamine can chelate other metals such as copper and magnesium. Copper, iron and magnesium are essential micronutrients involved in many metabolic processes [14] and are all altered in blood diseases including thalassemia [15]. copper is an essential structural coparticipant of many enzymes acting as cofactor in the majority of enzymatic reactions, including those of cytochrome C oxidase, superoxide dismutase (Cu/Zn SOD), lysyl oxidase, monoamine oxidase and thyrosinase [16]. Iron present in almost all cells of the body and it is required for the synthesis of hemoglobin and myoglobin. It is also used in the connective tissues, some of the neurotransmitters in the brain, and to maintain the immune system. Magnesium which is the second most abundant intracellular metal after potassium is essential for maintaining proper body functions such as body’s immune system, cardiovascular, and musculoskeletal systems [17].

2. MATERIALS AND METHODS

We had studied 50 β-thalassemia major patients in which desferrooxamine was indicated, 25 of each sex, with a mean age (13.26 ± 5.4 years) aged between (6 and 28 years) (BMI 19.1±3.4) and attending the Sulaimany Thalassemia Center, 20 healthy subjects were taken from both sex as controls, their mean age (15.9 years) aged between (7 and 30 years) were included in this case. Serum levels of zinc, copper and
magnesium of all groups were measured using Inductive coupled plasma – Optical emission spectrometer (ICP-OES) by Perkin Elmer (model Optima 2100 DV). The hematological data of beta thalassemia major patients compared to control groups were measured. Serum total iron binding capacity was estimated by TIBC kit in semiautoanalyzer. Serum ferritin was estimated by Immunoassay method, transferrin saturation was calculated as Serum iron × 100 / TIBC. All patients have been receiving recurrent blood transfusion and deferoxamine at a dose of 40 mg/kg/day (8 h, 6 days a week) by subcutaneous infusion.

2.1 Exclusion Criteria
Patients who are shifting between Desferoxamine injection and Deferasirox tablet, pregnancy or expected pregnancy within six months, lactation, presence of active infection, history of tuberculosis, HIV, hepatitis B or C, severe pancreatitis, malignancy, and any inflammatory or infectious disease that induced high false serum ferritin levels were excluded from this study.

2.2 Blood collection
5ml of venous blood sample was collected under aseptic precautions in a sterile bulb from selected subjects. Part of the sample was used for hematological analysis and the remaining part was immediately centrifuged and the separated serum was used for determination of Fe, Cu, and Mg concentration in the blood by using ICP (Inductive coupled plasma) instrument. Serum, total iron binding capacity, ferritin and transferrin saturation percentage were estimated.

2.3 Statistical analysis
Data were expressed as mean ± standard deviation. Student t-test of two independent samples was used to compare between means. P values ≤ 0.05 was considered as statistically significant. The statistical package for social sciences (SPSS version 17) was used to perform statistics.

3. RESULTS
Serum iron, copper and magnesium concentrations in both control and thalassemia major patients are shown in table 1. The comparison of the concentration of each metal ion in patients and healthy subjects is shown in figure 1. It is clear from the results that a significant increase (p=0.0001) of iron concentration (4035.412 ±1514.3 µg/L) was noticed in comparison to control healthy subjects (1542.53± 654.23 µg/L). Serum copper concentration was increased significantly (p= 0.011) in patients (1397.31± 243.04 µg/L) when compared to control group (1100.467 ±340.07 µg/L). On the other hand, magnesium was decreased significantly (p=0.022) in β-thalassemia major patients (18829.41 ± 3824.548 µg/L) in comparison to healthy subjects (22045 ± 3013.785 µg/L).

<table>
<thead>
<tr>
<th>Minerals</th>
<th>G1(Control), N=20</th>
<th>G2 (thalassemia patients), N=50</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Cu (µg/L)</td>
<td>1100.46±340.07</td>
<td>1397.312± 243.04</td>
<td>0.011</td>
</tr>
<tr>
<td>Fe (µg/L)</td>
<td>1542.53± 654.23</td>
<td>4035.412±1514.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mg (µg/L)</td>
<td>22045 ± 3013.78</td>
<td>18829.41 ± 3824.54</td>
<td>0.022</td>
</tr>
</tbody>
</table>

As shown in table II, the level of hemoglobin decreased in β-thalassemia major patients receiving multiple blood transfusion with chelation therapy (9.5 ± 1.2 g/dl) compared to control group (12.7 ± 1.5 g/dl). Ferritin levels increased more than 25 folds in patients (3529.12 ± 1099.61 ng /ml) when compared to healthy controls (135.4 ± 29.4 ng /ml). TIBC and Transferrin saturation was also increased in patients (871.5 ± 16.5 µg/dl, 46.3 ± 13.8%, respectively) in comparison to healthy subjects (453.5 ± 32.9 µg/dl, 34.0 ± 8.6%, respectively).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb (g/dl)</th>
<th>TIBC (µg/L)</th>
<th>Ferritin (ng /ml)</th>
<th>Transferrin Sat%</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (Control)</td>
<td>12.7 ± 1.5</td>
<td>453.5 ± 32.9</td>
<td>135.4 ± 29.4</td>
<td>34.0 ± 8.6</td>
</tr>
<tr>
<td>G2 (thalassemia patients)</td>
<td>9.5 ± 1.2</td>
<td>871.5 ± 16.5</td>
<td>3529.12 ± 1099.61</td>
<td>46.3 ± 13.8</td>
</tr>
</tbody>
</table>

4. DISCUSSION
Iron overload is one of the most frequently reported complications of multiple blood transfusion therapy in patients with β-thalassemia major. Iron absorption is increased in β-thalassemia major patients so they may have the greatest iron overload. Excess iron deposits in body organs, causing organ damage [18].
The present study investigated the status of iron, copper and magnesium in β-thalassaemia major patients. Haematological data were measured to reveal the level of iron storage and transporting proteins as well as hemoglobin percentage.

Our study revealed that copper and iron levels were significantly increased and magnesium was significantly decreased in β-thalassaemia major patients with desferrioxamine therapy compared to control healthy subjects. ICP instrument was used to measure the concentration of trace metal ions which is much more sensitive and accurate method compared to AAS. An elevated level of copper in β-thalassaemia major patients are consistent with previous studies [19, 20, 21].

Hypercupremia in β-thalassemia major patients may be due to hemochromatosis, chronic infections, which is excluded in this study, the amount of copper intake in daily diet, intestinal uptake of copper, iron accumulation, kidney function, copper to zinc ratio and the administration of desferrioxamine therapy [22]. However, some researchers found lower serum copper concentration in β-thalassemia major patients compared to healthy controls [23].

Hypomagnesemia in β-thalassemia major patients were shown in some studies [17], while other studies reported significantly higher levels of serum magnesium in patients compared to control groups [21, 24]. Some other studies reported normal levels of magnesium in patients with β-thalassemia major [25]. The fluctuation of serum magnesium concentration in the human body may result from diet or the chelation effect of desferrioxamine.

Serum ferritin, serum iron in conjunction with total iron binding capacity and transferrin have been measured to assess the efficiency of chelation therapy [26, 27]. According to the results, iron overload is prohibited as transferrin saturation does not exceed 60% in males or 50% in females [28, 29]. Serum iron concentration does not exceed iron binding capacity which means that chelation therapy after desferrioxamine was successful in preventing iron poisoning. If more transferrin becomes saturated, which is the case of iron overload, there will be an increase in non-transferrin bound iron (NTBI) [30, 31]. Non-transferrin-bound iron, which is a highly toxic form of iron, is formed when the iron binding capacity of transferrin has been exceeded.

REFERENCE

[19] Sabah N, Sherien M. Effects of iron overload and treatment methods on serum levels of zinc (zn) and copper (Cu) in beta thalassemia major(BTM) patients. medical journal of Babylon, 2011; 8(2).
[22] Zahraa MA Naji, Serum trace elements (zinc, copper and magnesium) in Iraqi patients with thalassemia major receiving desferrioxamine and its relation with growth state, IRAQI J MED SCI, 2012; 10(4).