Biomarkers and trace elements in beta thalassemia major

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Abstract:

**Introduction:** Beta thalassemia is one of the most common inherited single gene disorders result from absent or reduced β - chain production. Regular blood transfusions are required to correct anemia for normal growth and development, this cause secondary iron overload which is responsible for peroxidative damage. Iron overload causes high concentrations of serum ferritin which is a protein that plays a key role in iron metabolism by binding and storing excess iron within cells. In β-thalassemia major, liver damage accounts for the changes in lipid profile including total-cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TGL). Trace elements including zinc (Zn), copper (Cu) and magnesium (Mg) play a vital role in the body to perform its functions properly and should present in the body in appropriate amounts and must be available for reacting with other elements to form critical molecules as well as to participate in various important chemical reactions.

**The aims:** to evaluate serum ferritin and trace elements (Copper, zinc and magnesium) and lipid profile in beta thalassemia major patients.

**Patients, material and methods:** Patients: sixty patients were diagnosed as beta thalassemia major, they were on blood transfusion program, and thirty healthy medication free volunteers matched for age and sex were studied as control. Blood samples were taken after an overnight fast (12-14-hours.) and serum was obtained for estimation of ferritin levels by ELISA and for TC and TG that were determined enzymatically. The HDL-C was measured after precipitation of other lipoproteins. Copper; zinc and magnesium were estimated by Atomic Absorption Spectrophotometer.

**Results:** A high ferritin level (747.55 ± 158.62 mg/l) was observed. The serum cholesterol and high density lipoprotein levels were found to be lower than normal control (94.4±7.48 and 40.83±4.64 mg/dl respectively), while serum triglyceride level was significantly higher than normal control (177.83±15.91). There was no correlation between serum ferittin and any of the studied lipids. Serum Copper and magnesium levels were significantly higher than normal control (152±6.4 mg/dl and 2.28±0.17 mg/dl respectively), while Serum zinics levels were significantly lower than normal (67.55±2.7 mg/dl)

**In conclusion:** assessment of serum ferittin, trace elements and lipid profile in patients with beta thalassemia major revealed different levels in different studies suggesting variation in the factors influencing their levels in each patients and could be useful in follow up patients with beta thalassemia major.

**Key words:** beta thalassemia, serum ferritin, lipid profile, total cholesterol, triglycerides, high density lipoprotein, Zinc, Copper, Magnesium.

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**Introduction:**

Beta thalassemia is one of the most common inherited single gene disorders that are widely distributed throughout the world, with considerable frequencies in the Eastern Mediterranean countries, including Iraq (1). Results from absent or reduced β - chain production while α - chain synthesis is unaffected. The imbalance in globin chain production leads to an excess of α - chains. The free α - globin chains are highly unstable and precipitate in red cell precursors, forming intracellular inclusions that interfere with red cell maturation cause a variable degree of intramedullary destruction of erythroid precursors (i.e. ineffective erythropoiesis). The anaemia of

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\[ \beta \text{ thalassaemia results from a combination of ineffective erythropoiesis and haemolysis. It stimulates erythropoietin production, which causes expansion of the bone marrow and may lead to serious deformities of the skull and long bones. Splenomegaly, together with bone marrow expansion, causes a major increase in plasma volume, which also contributes to the anaemia.} \]

Regular transfusions are required to correct anaemia for normal growth and development and to suppress the abnormal erythroid hyperplasia, to stop bony distortion, limits excessive iron absorption and reduces extramedullary haemopoiesis.(2)

Recurrent blood transfusions in beta thalassemia major lead to accumulation of excess iron in the body tissues. This secondary iron overload is responsible for oxidative damage by increased production of reactive oxygen species within the erythrocytes leading to oxidative stress. This oxidative stress will cause growth failure as well as liver, cardiovascular, endocrine, and neurological complications in beta thalassemia major.(3)

Ferritin is a protein that plays a key role in iron metabolism by binding and storing excess iron within cells. It is an intracellular hollow protein shell composed of 24 subunits surrounding an iron core that may contain as many as 4000-4500 iron atoms. The concentration of serum ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation (5, 6) and used for the assessment of iron stores. During the first months of life, mean serum ferritin concentrations change considerably, reflecting changes in storage iron concentration. Concentrations are lower in children (<15 years) than in adults and from puberty to middle life are higher in men than in women. In most normal adults, serum ferritin concentrations lie within the range of 15-300 mg/l in male and 15-200 mg/l in female (5,7,8) Iron overload causes high concentrations of serum ferritin, but these may also be found in patients with liver disease, infection, inflammation or malignant disease and in some rare inherited causes of increased ferritin production unlinked to iron stores (8).

Iron-induced liver injury is often characterized by the development of fibrosis and eventually, cirrhosis. In \( \beta \)-thalassemia major, liver damage accounts for the changes in lipid profile including total-cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TGL). (9,10)

Some studies have suggested that low blood cholesterol values may occur as a result of an increase of erythropoiesis in patients with \( \beta \)-thalassemia major and increase of LDL uptake by macrophages and histiocytes that exist in reticuloendothelial system.(10)

Trace elements and the minerals play a vital role in the body to perform its functions properly and should present in the body in appropriate amounts and must be available for reacting with other elements to form critical molecules as well as to participate in various important chemical reactions. Here we are interested to study trace elements like copper, zinc, and magnesium in beta thalassemia major.

Copper is the major component of hemoglobin which is a protein responsible for oxygen transport in blood cells. It is antibacterial and bears important antioxidant properties, and also helps in the formation of protein called ceruloplasmin thereby protecting the cells from free-radical injury. Copper is also required for the production of hormones like nor adrenaline and prostaglandins. Deficiency of this trace element will lead to anaemia, neutropenia, and growth impairment, abnormalities in glucose and cholesterol metabolism, and increased rate of infections. On the other hand, an accumulation of copper in body leads to Wilson’s disease with copper accumulation and cirrhosis of liver (11). Normal range of serum copper is 70-150 µg/dL (11-24µmol/L) (12)

The next essential trace element present in the body is zinc. It is vital for many biological functions and plays a crucial role in more than 300 enzymes in the human body. The adult body contains about 2-3 grams of zinc. (13) It takes part in various important body functions including protein and DNA synthesis and cellular growth. It is found almost in every cell and plays a vital role in body’s immune system affecting innate and acquired immunity. Zinc also has significant antioxidant properties thereby protecting the cells from damage due to free radicals. Deficiency of zinc which is one of the factors responsible for growth and puberty disorders in thalassemic patients (11). Normal range of serum zinc is 60,72-110,130 µg/dL(9.2,11-17,20 µmol/L)(12).

Magnesium is the second most abundant intracellular metal after potassium which is essential for maintaining proper body functions. Cell Magnesium is an important modulator in cell volume regulation and affects the activity of various membrane cation transport pathways.(14)

It is vital for body’s immune system, cardiovascular, and musculoskeletal systems. Deficiency of this element will lead to hypertension, diabetes, and cardiovascular diseases.(11) Normal range of serum magnesium is 1.5, 1, 7,2-2,3 mEq/L or mg/dL(0.6,0.7-0.82,0.95 mmol/L)(12)

The study aimed to evaluate the serum level of ferritin, lipids (cholesterol, triglycerideand high density lipoprotein), and trace elements(copper, zinc and magnesium) in beta thalassemia major patients.

**Patients, Material and Methods:**

**Patients:** sixty patients were diagnosed as beta thalassemia major, 29 male and 31 female, their ages was ranged from 2 to 11 years. All of them were on blood transfusion program in Al-Krama center of thalassemia.

**Control:** thirty healthy medication free volunteers matched for age and sex were studied as control.

**Samples:** Blood samples were taken after an overnight fast (12-14-hours.) and serum was obtained by centrifugation. Ferritin levels were performed by enzyme-Linked immuno-sorbent assay (ELISA) using a kit from Human from Human company (Germany).

Concentrations of total cholesterol (TC) and triglycerides
(TG) and high density lipoprotein (HDL) were determined by using electromagnetic reflectron form Roche company (Germany).

Copper and zinc were estimated by colorimetric spectrophotometer kit from LTA company (Germany), and magnesium was estimated by colorimetric spectrophotometer kit from Human company (Germany).

Statistical analyses: Statistical analyses were carried out using SPSS software version 17. Values were reported as the mean (SD), and independent sample t tests were used to compare study groups. The associations between variables were assessed by using Pearson’s correlation coefficient. All statistical tests were 2-tailed. P value of <0.05 was considered as statistically significant.

**RESULTS:**

There was significantly higher serum ferritin, copper and magnesium than normal control, and significantly lower serum zinc as shown in table 1.

**Table 1:** serum ferritin, zinc, copper and magnesium in beta thalassemia major patients and in control group.

<table>
<thead>
<tr>
<th>parameters</th>
<th>Patients n=60</th>
<th>Control n=30</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Mean</td>
</tr>
<tr>
<td>Serum ferritin ng/ml</td>
<td>747.55</td>
<td>158.62</td>
<td>52.36</td>
</tr>
<tr>
<td>Zinc µg/dl</td>
<td>67.55</td>
<td>2.70</td>
<td>81.03</td>
</tr>
<tr>
<td>copper µg/dl</td>
<td>152.01</td>
<td>6.48</td>
<td>101.63</td>
</tr>
<tr>
<td>Magnesium µg/dl</td>
<td>2.28</td>
<td>0.17</td>
<td>2.11</td>
</tr>
</tbody>
</table>

Regarding lipid profile, serum total cholesterol and high density lipoprotein levels were found to be significantly lower than normal control, while serum triglyceride level was higher as shown in table 2.

**Table 2:** lipid profile in beta thalassemia major patients

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>Patients n=60</th>
<th>Control n=30</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Mean</td>
</tr>
<tr>
<td>Total cholesterol mg/dl</td>
<td>94.41</td>
<td>7.48</td>
<td>116.93</td>
</tr>
<tr>
<td>triglyceride mg/dl</td>
<td>177.83</td>
<td>15.91</td>
<td>109.93</td>
</tr>
<tr>
<td>High density lipoprotein mg/dl</td>
<td>40.83</td>
<td>4.64</td>
<td>34.33</td>
</tr>
</tbody>
</table>

There was no correlation between serum ferritin and any of the studied lipids as shown in table 3.

**Table 3:** Correlations between serum ferritin and total cholesterol, triglycerides and High density lipoprotein levels.

<table>
<thead>
<tr>
<th>Serum ferritin versus total cholesterol, triglycerides and High density lipoprotein levels.</th>
<th>Pearson Correlation</th>
<th>Significance (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.18</td>
<td>0.15</td>
</tr>
<tr>
<td>triglycerides</td>
<td>-0.03</td>
<td>0.80</td>
</tr>
<tr>
<td>High density lipoprotein</td>
<td>0.11</td>
<td>0.38</td>
</tr>
</tbody>
</table>
Discussion:

In this study the majority of the patients revealed a high ferritin levels (747.55 ± 158.62 mg/dl), which is expected as a result of repeated blood transfusion, and the same results were mentioned by many studies (15, 16, 17, 18, 19).

The serum cholesterol and high density lipoprotein levels were found to be lower than normal control, while serum triglyceride level was higher. The decreases in total cholesterol, HDL-cholesterol were also mentioned in other studies (10, 20). However, the exact cause of lower lipid level in beta thalassemia major is not exactly known.

Triglycerides were significantly higher in patients compared to controls. The increased concentrations of TG were observed in most published studies on lipid profiles of thalassemic patients.(10,20,21,22,23,24,25).

Whereas the triglyceride level does not exhibit significance increase in some studies (26, 27).

There was no correlation between serum ferritin and any of the studied lipids, but there was a significant positive correlation between triglyceride and serum ferritin levels according to Arca V et al study. (10).

Changes in lipid profile may be due to Iron-induced liver damage (9, 10), an increased erythropoiesis in patients with beta thalassemia major and increase of LDL uptake by macrophages and histiocytes that exist in reticuloendothelial system. (10).

Serum Cupper levels were significantly higher than normal control, some studies showed that there was an increase in serum level of copper in patients with thalassemia major. (28,29,30,31,32).

The etiology of hypercupremia is thought to be hemochromatosis, which is a principal complication of thalassemia (22) and also occurs in acute and chronic infection (11). However reduction in serum level of copper was reported in other studies (33, 34, 35).

The serum concentration of copper in patients with thalassemia major depends on several factors including the amount of copper intake in daily diet, intestinal uptake of copper, iron accumulation, kidney function, copper to zinc ratio, and administration of desferrioxamine (Desferal). (36)

Serum zinc levels were significantly lower than normal control. zinc deficiency may be due to hyperzincuria resulted from the release of zinc from hemolysed red cells (10) and desferrioxamine therapy (36) and other factors un related to thalassemia such as nutritional status that may be responsible for hypozincemia. (37).

Although many studies revealed low serum zinc in thalassemia patients (10, 36, 37, 38), at least one study by Mehdizadeh M et al revealed significantly higher serum zinc in the thalassemic group with no significant correlation between serum zinc level and serum ferritin level so indicates zinc deficiency in thalassemic patients who are on regular blood transfusion is rare (39).

Serum magnesium levels were significantly higher than normal control, same finding was described in Al-Samarrai AH et al study, (31) but magnesium was found to be at normal levels according to Arcasoy A. et al. (40) Other studies revealed abnormalities of Mg metabolism have been described in β-thalassemia, and low serum Mg has been reported in children affected by the homozygous form of the disease, (41, 42) also reduced serum or erythrocytes Mg have been reported in human beta thalassemia, (14,43) therefore magnesium supplementation used to stabilize damaged red blood cell by its specific interactions with K-Cl cotransport and effects on the red blood cell membrane and improved erythrocyte survival and morphology. (14)

In conclusion:

Assessment of serum ferritin, trace elements and lipid profile in patients with beta thalassemia major revealed different levels in different studies suggesting variation in the factors influencing their levels in each patients like dietary intake, supplementation and the regular use of blood transfusion and chelating programs. So we recommend a regular check up for those parameters to each patient and use them as indirect indicator to follow up patients with beta thalassemia major.

References:


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المؤشرات الحيوية والعناصر النادرة في أنيميا البحر المتوسط الكبرى نوع بيتا

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1. المقدمة:
يعتبر فقر دم البحر المتوسط (ثلاسيميا) نوع بيتا من أكثر الأمراض الوراثية شيوعاً وهو ينجم عن غياب أو انخفاض وتواتر الكريات الحمراء مسببة فقر دم و يتطلب نقل الدم بشكل منتظم لضمان النمو والتقدم الطبيعي للمريض، وهذا يتطلب تأقلم الزائد حصول الإجهاد الذي يؤدي إلى التأكسد كما يسبب الضرر في الكبد والقليل والأوعية الدموية في العضلات المعرضة للفرط. يمكن قياس الزيادة في الحديد الزائد في الدم في حالات تورم الدم مضاعفات مزمنة و ise في الكبد و الأوعية الدموية وتلف الكبد، مما يسبب التهاب في معدن الدهون (إجمالي الزيت الدهني الفعال) والكبد و النحل و المغنسيوم ضعيف جداً في الدم. يجب أن تكون مستويات مختلفة من النحاس و المغنيسيوم و الزنك و النحاس و الدهون في الدم متغيرة في مرضى الثلاسيميا الكبرى نوع بيتا.

2. الأهداف:
قياس وتقييم معدلات النحاس و الزنك و المغنيسيوم و الدهون في مرضى الثلاسيميا الكبرى نوع بيتا.

3. المرضى والمعدات:
تم تشخيص 60 مريضاً معذراً بالثلاسيميا الكبرى نوع بيتا حيث تم استنتاج عينة دم من جميع المرضى بعد صيام و فحص الدهون و استخدام جهاز الاستشعار لقياس النحاس، الزنك، المغنيسيوم و الدهون.

4. النتائج:
لقد تم قياس و تقويم معدلات النحاس و الزنك و المغنيسيوم و الدهون في مجموعةtiles مريضاً مصاباً بالثلاسيميا الكبرى نوع بيتا. كما تم قياس معدلات الدهون التدريبية في مصل الدم و العناصر النادرة في مرضى الثلاسيميا الكبرى.

5. الملاحظات:
توجد تغيرات في الدهون قد تكون بسبب النحاس في الدم، و المغنيسيوم في مرضى الثلاسيميا الكبرى نوع بيتا، كما تم استنتاج مستويات النحاس و الزنك و المغنيسيوم و الدهون في الدم من مرضى الثلاسيميا الكبرى نوع بيتا.

6. المخارطة:
يمكن استخلاص نتائج هذه الدراسة في تأكيد نتائج تراكم الحديد وما يقوم به من تأثيرات ضخمة في الدم، و المغنيسيوم في مرضى الثلاسيميا الكبرى نوع بيتا. يمكن استخلاص نتائج هذه الدراسة في تأكيد نتائج تراكم الحديد وما يقوم به من تأثيرات ضخمة في الدم، و المغنيسيوم في مرضى الثلاسيميا الكبرى نوع بيتا. يمكن استخلاص نتائج هذه الدراسة في تأكيد نتائج تراكم الحديد وما يقوم به من تأثيرات ضخمة في الدم، و المغنيسيوم في مرضى الثلاسيميا الكبرى نوع بيتا. يمكن استخلاص نتائج هذه الدراسة في تأكيد نتائج تراكم الحديد وما يقوم به من تأثيرات ضخمة في الدم، و المغنيسيوم في مرضى الثلاسيميا الكبرى نوع بيتا.