Assessment of Liver Functions in Thalassaemia

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Abstract
Thalassaemias are a heterogenous group of genetic heritable disorders of haemoglobin synthesis, considered as the most common monogenic disorder in the world. This study was conducted to find out the role of iron overload- without concomitant viral hepatitis as a cause of liver dysfunction in patients with β-thalassaemia major. One hundred six (106) patients with homozygous β-thalassaemia major and forty four (44) apparently healthy subjects were analyzed at the Thalassaemic Centre of Ibn Al-Atheer hospital in Mosul city in Iraq for the assessment of liver functions as reflected by the serum levels of bilirubin, AST, ALT and ALP and iron status as reflected by serum ferritin. The mean pretransfusional Hb was (87.33±13.84 g/L and 85.85±13.21 g/L) for thalassaemia males and females respectively. All thalassaemic patients showed elevated serum ferritin with a mean level of (3799.30±2343.68 ng/ml and 4100.61±1977.28 ng/ml) for thalassaemia males and females respectively. A significant increase in the serum levels of AST, ALT and ALP and iron status as reflected by serum ferritin. Serum ferritin showed a significant positive correlation with ALT in male and female patients. Hepatomegaly was found in 71.70% of the studied cases. A significant alteration in liver functions of thalassaemic patients shown by changes in biochemical markers and hepatomegaly, both with the close association between elevated ALT and iron overload, necessate re-evaluation of transfusion, Desferal doses and therapies other than blood transfusion.

توحشم وظائف الكبد لدى مرضى الثلاسيميا

ترجمة

الملخص
التلسماطيا، هي مجموعة غير متصلة من الاضطرابات الجينية الموروثة لتوليف الهيموغلوبين، يعتبر أضرارًا أحادي الجينات الأكثر شيوعًا في العالم. أجريت هذه الدراسة لمعرفة دور الحم المعالج في النزاع النقوي الكبد الفيروسي. كسب للخلل الوظيفي في كبد مرضى قررتم البحر المتوسط الأكبر نوع بيتا . حلت مخاطرة وست مرضي مصابين يقدر بكم البحر المتوسط الأرضي نوع بيتا المتماثل وأربع وأربعين شخصًا كمجموعة ضابط في مركز التلسماطيا لمسحتي أدنى الأثير في مدينة الموصل في العراق لتقييم وظائف الكبد مما يعكس من مسارات أنزيم ناقل الأمين الأسبارتيت، أنزيم ناقل الأمين النائلي، أنزيم الفوسفاتيز القاعدي وبلاتين carrera في المصل ووضع الحديد على نحو ما يعكس حديد الكبد. كان معدل الهيموغلوبين قبل نقل الدم (87.33 ± 13.84 غرام / لتر و 85.85 ± 13.21 غرام / لتر) لأي ذكور ونائلي مرضي الثلاسيميا على التوازي. جميع مرضى الثلاسيميا أظهروا ارتفاع حديد الكبد مع مستوى عدمه (30.30 ± 2343.68 نانوغرام / مل و 4100.61 ± 1977.28 نانوغرام / مل) لدى ذكور ونائلي مرضي الثلاسيميا على التوازي. تم إعداد ارجات معنوي في مستوى أنزيم ناقل الأمين الأسبرتيت، أنزيم ناقل الأمين النائلي، أنزيم الفوسفاتيز القاعدي وبلاتين carrera في مصل مرضى الثلاسيميا بالمقارنة مع المجموعة الضابطة. أظهرت أوراق الدين في المصل أرجات معنوي مع أنزيم ناقل الأمين النائلي لدى ذكر ونائلي مرضي الثلاسيميا. وجد تضخم الكبد في 71.70% من الحالات التي تم تجربتها. استنتج أن هناك تغير معنوي في وظائف الكبد لدى مرضى الثلاسيميا. أفاد التغير في المتغيرات البيوكيميائية وتضخم الكبد، مع كل من ارتقاء وثيق بين ارتفاع أنزيم ناقل الأمين النائلي والحم. الحمدي النزاع، توجب إعادة تقييم نقل الدم وجرعة الأدوية الدوائية والعلاجات الأخرى بخلاف نقل الدم.
Introduction
The thalassaeemias are a heterogeneous group of genetic heritable disorders of haemoglobin (Hb) synthesis\textsuperscript{1}, considered as the most common monogenic disorder in the world\textsuperscript{2}, affecting men and women equally\textsuperscript{3} and poses a severe health and economic burden to patients and families at risk\textsuperscript{4}. Thalassaemias are classified into alpha (α) and beta (β) thalassaemia according to the globin chain whose synthesis is adversely affected\textsuperscript{5}. Beta thalassaemia major, also known as Cooly's anemia, is the most severe form of β-thalassaemia\textsuperscript{6}, characterized by severe anaemia beginning in the first year of life and patients require maintenance red cell transfusions every 4–6 weeks\textsuperscript{7}. Over the last three decades, the development of regular transfusion therapy and iron chelation has dramatically improved the quality of life and transformed thalassaemia from a rapidly fatal disease to a chronic disease compatible with prolonged survival\textsuperscript{8}. Despite the increased life expectancy of thalassaemia patients, complications keep arising\textsuperscript{9}. Frequent blood transfusions necessary for the treatment of thalassaemia major, unfortunately carry the adverse side effect of iron build up in the body\textsuperscript{10}. Thus, chronic blood transfusion in thalassaemic patients is a double-edged sword\textsuperscript{11}. Iron overload may damage the liver, heart and endocrine glands leading to debilitating and life-threatening problems\textsuperscript{12}. It is well known that cardiac disease is responsible for 70% of deaths in thalassaemia major patients\textsuperscript{13,14}. During the last years, liver disease has emerged as a major cause of mortality in patients with β-thalassaemia major. In spite of its clinical relevance, thalassaemia-associated liver damage has been insufficiently characterized\textsuperscript{15}. Liver disease in these patients can manifest as hepatomegaly, increased aspartate and alanine transaminase activities, hepatitis B and C. Significant fibrosis is frequent and its progression is mostly influenced by iron overload which may be attributable to hypertransfusion, inadequate chelation, erythrocyte catabolism and excessive iron absorption from the gut as a consequence of ineffective erythropoiesis. Hepatocytes are the major storage site for body iron, so with iron overload, these cells are relentlessly bombarded by reactive oxygen species and eventually die. Damage to these cells (hepatocytes) start to accumulate within a year of commencing transfusion therapy after as few as 10–20 transfusions\textsuperscript{12,16}. To the best of the researcher's knowledge, the role of iron overload -without concomitant viral hepatitis B/C- as a cause of liver dysfunction has never been studied in detail in thalassaemic patients of Mosul. In the present study we, therefore, investigated the relationship between the extent of hepatocellular injury as reflected by serum levels of biochemical markers "Liver function test" and iron status as reflected by serum ferritin.

Subjects and Methods
The present study represents a case-control study, conducted during the period from the 2\textsuperscript{nd} of November 2010 to the 2\textsuperscript{nd} of April 2011 at the Thalassemic Centre of Ibn Al-Atheer hospital in Mosul city in Iraq. A total of 106 patients with homozygous β- thalassaemia major and 44 subjects -apparently healthy, non thalassaemic, with no family history of thalassaemia or history of liver disease, who were referred from a physician- were enrolled in this study.

Each of the patients and control subjects were divided into male and female groups Table (1). As far as possible the control subjects were of similar ages as the patients to assure group matching. Diagnosis of β- thalassemia major was made on the basis of Hb- electrophoresis findings and only thalassaemic patients with negative quantitative test for hepatitis B/C viruses RNA by PCR were included in this study. The subjects of the two groups were thoroughly interviewed and clinically examined especially for
abdominal tenderness and hepatomegaly. Hepatomegaly was diagnosed based on clinical grounds and proved by Ultrasound (U/S) of the abdomen. The general information and other relevant information was taken from the patients and their parents mainly regarding any previous history of hepatic disease. 7.0 ml of venous blood were collected from all the subjects included in this study and distributed in the following manner:

i. One (1)ml of the blood sample was collected into ethylene diamino tetracetic acid (E.D.T.A) tube, with gentle shaking for proper mixing with anticoagulant, this was used for measurement of packed cell volume (PCV) and Hb.

ii. The remaining 6.0 ml of blood was transferred into a sterile test tube, kept in incubator for 30 min. and then centrifuged for serum separation. Part of the clear serum was separated into a sterile epindorf tube and preserved at -20°C for estimation of serum ferritin. The remaining serum was used for measurement of total serum bilirubin (T.S.B), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) at the same day.

Packed cell volume and Hb was measured by the well known (Microhematocrit Method)\textsuperscript{17} using plain capillary tube and calculated using (Janetzki P.C.V measuring instrument). Ferritin level in the serum of all subjects included in this study was determined by (EIA)\textsuperscript{18} by using a kit supplied from (Monobind, USA) and measured by ELISA. Colorimetric method (Reitman and Frankel)\textsuperscript{19} was used for determination of serum activities of AST and ALT by a kit supplied by (Randox, United Kingdom), while a kit from (Biomerieux, France) was used for colorimetric determination of serum ALP activity\textsuperscript{20}. Serum activity up to 12 U/L was considered normal for the former two enzymes. Haemolyzed sera were excluded. Estimation of T.S.B was done colorimetrically depending on Malloy-Evelyn principle\textsuperscript{21} by Biolabo kit (France).

Table (1):

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of patients or subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>Group 1 (Thalassaemic group)</td>
<td>52</td>
</tr>
<tr>
<td>Group 2 (control)</td>
<td>24</td>
</tr>
<tr>
<td>Total number</td>
<td>76</td>
</tr>
</tbody>
</table>

Results
In this study, the age ranged from 7 years to 24 years. The mean age for thalassaemic males was (14.06 ± 4.83) years and (13.88 ± 3.90) years for control males. For thalassaemic females, the mean age was (13.31 ± 4.17) years and (13.20 ± 5.39) years for control females. There were 52 (49.1%) males and 54 (50.9%) females in the study cases. Seventy one and seventy (71.70%) of thalassaemic patients had hepatomegaly according to the U/S and
clinical examination and the percentage was higher in males (82.7%) than females. The mean pretransfusional Hb was (87.33±13.84 g/L and 85.85±13.21 g/L) for thalassaemic males and females respectively. In comparison to control subjects, there was a highly significant (p≤0.001) decrease in the level of Hb both in male and female patients Tables (2,3). All the thalassaemic patients exhibited a highly significant (p≤0.001) increase in the level of serum ferritin when compared to control subjects with a mean level of (3799.30±2343.68 ng/ml) for serum ferritin of thalassaemic males and (4100.61±1977.28 ng/ml) for serum ferritin of thalassaemic females Tables (2,3). The serum activities of the liver enzymes (AST, ALT and ALP) were found to be elevated (p≤0.001) with respect to control subjects and the mean levels for thalassaemic males were (25.54±12.35 U/L, 25.63±15.58 U/L and 113.71±37.13 U/L) for the three enzymes respectively, while for thalassaemic females the mean levels were (28.1±10.47 U/L, 27.9±15.57 U/L and 110.85±41.82 U/L) for the three enzymes respectively Tables (2,3). At the same time, T.S.B was abnormally elevated (p ≤0.001) in the serum of male (25.88±21.08 µmol/L) and female (19.12±11.85 µmol/L) thalassaemic patients when compared to control groups Tables (2,3). Serum ferritin had a significant positive correlation (p≤0.05) with ALT in thalassaemic males and females Figures (1,2).

Table (2): Comparison of measured parameters between thalassaemic males and control males.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Thalassaemic (n=52)</th>
<th>Control (n=24)</th>
<th>p-value ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/L)</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>87.33 ± 13.84</td>
<td>131.58 ± 16.61</td>
<td></td>
</tr>
<tr>
<td>PCV (L/L)</td>
<td>0.27 ± 0.04</td>
<td>0.41 ± 0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>3799.30 ± 2343.68</td>
<td>54.71 ± 41.27</td>
<td>0.001</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>25.54 ± 12.35</td>
<td>7.96 ± 2.58</td>
<td>0.001</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>25.63 ± 15.58</td>
<td>8.17 ± 3.27</td>
<td>0.001</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>113.71 ± 37.13</td>
<td>82.75 ± 36.99</td>
<td>0.001</td>
</tr>
<tr>
<td>T.S.B (µmol/L)</td>
<td>25.88 ± 21.08</td>
<td>8.23 ± 3.23</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Using unpaired t-test
Table (3): Comparison of measured parameters between thalassaemic females and control females.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Thalassaemic (n=54)</th>
<th>Control (n=20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/L)</td>
<td>85.85 ± 13.21</td>
<td>123.65 ± 11.98</td>
<td>≤0.001</td>
</tr>
<tr>
<td>PCV (L/L)</td>
<td>0.26 ± 0.05</td>
<td>0.38 ± 0.04</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>4100.61 ± 1977.28</td>
<td>53.68 ± 59.18</td>
<td>≤0.001</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>28.1 ± 10.47</td>
<td>9.85 ± 2.08</td>
<td>≤0.001</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>27.9 ± 15.57</td>
<td>8.45 ± 2.52</td>
<td>≤0.001</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>110.85 ± 41.82</td>
<td>73.70 ± 36.85</td>
<td>≤0.001</td>
</tr>
<tr>
<td>T.S.B (µmol/L)</td>
<td>19.12 ± 11.85</td>
<td>7.88 ± 3.51</td>
<td>≤0.001</td>
</tr>
</tbody>
</table>

Using unpaired t-test

Discussion

Thalassaemias are inherited disorders characterized by abnormal production of haemoglobin, associated with low haemoglobin production and excessive destruction of red blood cells. In current study, the number of affected females (54) were slightly higher than affected males (52) and this is similar to what has been noticed in Al-Saffar study (2002) in Tikrit, however, the reported difference in the current study is not appreciable and deserves further investigations considering thalassaemia as a single-gene disease transmitted by a recessive mode of inheritance. Hepatomegaly was found in (71.70%) of the patients in this study and males were found to be affected more than females. It seems that the females in the Thalassaemic Centre in Mosul were more compliant with the treatment than males and this may be explained further by the higher number of thalassaemic females involved in this study. However, the percentage of hepatomegaly reported by the present study was lower than (94%) documented by Al-Haj Ahmad (1992) in Mosul, which indicates improvement in transfusion regimens and chelation therapy received by the patients in Thalassaemia Centre in Mosul. Enlargement of the liver in thalassaemic patients occurs as a result of extramedullary haemopoiesis and iron overload. The low levels of Hb and PCV in thalassaemic patients are expected when compared with control subjects, Tables (2,3), because β-thalassaemia major is a hereditary disorder of Hb synthesis that results in severe anemia. This result was in agreement with other studies. Serum ferritin results reported by many studies done in developing countries show nearly the same or even higher than the mean level reported by the present study, Table (4). This indicates that in our part of the world patients with β-thalassaemia major have levels of serum ferritin far more than the patients in developed countries. In our set up only a few patients get their serum ferritin levels done once a year. Some of the patients do not have their own infusion pumps and in those patients who have the pumps, some are faced with the problem of it's malfunction for few months leading to interruption of the chelation therapy and sometimes even stopping it. In the present study, there was a significant rise in the serum activities of the liver enzymes in thalassaemic patients compared with the
control groups Tables (2,3). At the same

time, this abnormality elevated levels of
de liver enzymes was accompanied by a

significant elevation of T.S.B in

thalassaemic patients compared to control

subjects, as shown in Tables (2,3). Injury

to the liver cells causes leakage of the

enzymes into the circulation, in addition,

the elevations of ALT and bilirubin are

used largely to determine if the liver has

been damaged and it's function is

impaired. Liver disease associated with

chronic blood transfusions in thalassaemic

patients is caused by hepatotropic

infections or hepatic siderosis. Both

factors may act either synergistically or

independently in promoting chronic liver
disease, inducing cellular damage through

similar oxidative pathways. The role of

iron overload as a cause of liver
dysfunction in thalassaemic patients of the

present study is suggested to be clear.

Ferritin in the present study showed a

significant positive correlation with ALT

in thalassaemic males and females Figures

(1,2). This result was in accordance with a

study done in Northern Iran (2008),

which also means that liver function in

thalassaemic patients, as reflected by

elevated iron overload, as reflected by

elevated serum ferritin.

Table (4):- Comparative Serum Ferritin Levels

<table>
<thead>
<tr>
<th>Study</th>
<th>Serum ferritin levels (mean)</th>
<th>Reference Range:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cunningham MJ et al, 2004</td>
<td>1696 ng/ml</td>
<td>Male: 16-220 ng/ml</td>
</tr>
<tr>
<td>Choudhry VP et al, 2006</td>
<td>6723 ng/ml</td>
<td>Females: 10-124 ng/ml</td>
</tr>
<tr>
<td>Arshad MS et al, 2009</td>
<td>4718 ng/ml</td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>3799 ng/ml (♂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4100 ng/ml (♀)</td>
<td></td>
</tr>
</tbody>
</table>
Figure (1): Relationship between serum ferritin and ALT in male thalassaemic patients.

Figure (2): Relationship between serum ferritin and ALT in female thalassaemic patients.
References
22. Ul-Ain Q, Ahmad L, Hassan M, Mahboob SH, Jabeen R, Jabeen F. Prevalence of β-thalassemic patients associated with consanguinity and