DIAGNOSTIC SIGNIFICANCE OF LIVER & RENAL FUNCTION TESTS (LFT& RFT) IN IRON OVERLOAD IN PATIENTS WITH β-THALASSEMIA MAJOR

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ABSTRACT

Background: β-Thalassemia results from a defect in β-globin chain production and ranges from clinically silent heterogeneous thalassemia minor to severe transfusion dependent homozygous thalassemia major. After approximately one year of transfusion, iron begins to deposit in parenchymal tissues, which might cause damage to the parenchyma and organ function impairment. So, iron overload may be develop liver, kidney, heart and endocrine abnormalities.

Objectives: The aim of study was to correlate the early hepatic and renal complications in condition of β-thalassemia with serum iron and total iron binding capacity (TIBC) levels.

Method: In study 50 β-thalassemia major patients were compared with 50 normal healthy subjects as controls. Fasting serum iron, TIBC, LFT and RFT were estimated using standard kit methods. Data were statistically evaluated by Student’s t-test and Pearson’s correlation coefficient (r).

Result: A significant increase in the levels of serum iron and a significant decrease in TIBC in β-thalassemia patients were observed. Serum levels of liver enzymes (Aspartate transaminase, Alanine transaminase and alkaline phosphatase) were significantly higher in β-thalassemia patients as compared to controls. A significant decrease in total proteins and increase in serum bilirubin level was found in β-thalassemia patients. Serum urea and creatinine levels were significantly higher, but no change in levels of serum uric acid was found in β-thalassemia patients when compared to controls. There was significant negative correlation between serum iron and TIBC, total protein among patient group.

Conclusion: Iron overload due to frequent transfusions in β-thalassemia results in abnormal organ function tests. Proper and timely screening of these parameters can help in early diagnosis & prevention of iron overload.

Key Words: β-thalassemia major, Iron, TIBC, SGOT, SGPT, ALP, Total protein, Urea, Creatinine, Uric acid

INTRODUCTION

Thalassemia were first described by Dr. Cooley in 1925, is a life threatening anemia which is characterized by ineffective erythropoiesis, bone marrow expansion, and increased destruction of defective red blood cells (RBCs)1-3. It is more prevalent in the Mediterranean region, North and West Africa & Middle East. About 150 million people worldwide carry β-thalassemia gene4. Thalassemia are one of the most common single gene disorders in our country. Every year around 100000 children were born with thalassemia major in the world and around 10000 children are born in India alone. The carrier rate of β-thalassemia gene varies between 1-3% in south India and 5-15% in north India4-6. The resultant anemia and other complications can be corrected with repeated regular blood transfusion program7.

Frequent blood transfusion causes progressive iron overload, which is major clinical complication for the treatment. As a result of iron overload, patients develop liver, kidney, heart and endocrine abnormalities8-9. Iron overload was introduced as the main reason responsible for renal malfunctioning10. 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 transfusions11-12. To judge the extent of the liver cells damage caused by iron
accumulation, we need to assess the serum transaminase enzymes.

So, the aim of this study to investigate relationship between the extent of hepatocellular injury as reflected by serum levels of biochemical markers “Liver function test” and iron status and any correlation between the early hepatic and renal complications in condition of β-thalassemia with serum iron and total iron binding capacity (TIBC) levels.

MATERIAL AND METHOD

In this study 50 patients with homozygous β-thalassemia major patients receiving regular blood transfusion were included. Thirty seven were males and 13 females, with age ranging from 1 to 16 years, selected from OPD and IPD of SRG Hospital & Medical College, Jhalawar, Rajasthan, India. Based on following exclusion and inclusion criteria, a random selection of the subjects for the study was made on the basis of detailed history and proper clinical examination. Patients with age>16 years, liver, renal, endocrine, cardiac disease were excluded and age 1-16 years, pediatric patients (β-thalassemia major patients) without any disease were included in this study. Fifty age and sex matched subjects apparently healthy, non thalassemic, with no family history of thalassemia or liver and renal diseases were enrolled in this study.

Biochemical analysis: - 5 ml of blood was collected in plain vial from each subject and serum was separated after 20 minutes clotting. Fasting serum iron level, TIBC, liver function tests (Bilirubin, SGOT, SGPT, ALP, and TP), renal function tests (BUN, Creatinine, Uric acid) were estimated with commercial kits on MURA 200 fully automatic analyzer.

STATISTICAL ANALYSIS

Statistical analysis was carried out using Medical 14.0.0 version software. Student’s t-test was used for group comparison and Pearson’s correlation coefficient(r) was used to correlate serum iron overload with LFT and RFT parameters. All data were expressed as mean±SD and p value of <0.05 were taken as significant.

RESULTS

In this study, the mean age for thalassemic patients was (7.01 ±3.95 years) and mean age for control subjects was (11.12 ± 3.44 years) with 37 males and 13 females in patients & 24 males and 26 females in control group in patients. A significant increase in the level of serum iron in β-thalassemic patients as compared to controls (270.09 ± 179.03 µg/dl & 126.62 ± 10.88 µg/dl respectively) whereas a significant decrease in TIBC (267.54 ± 80.40 µg/dl & 292.74 ± 41.26 µg/dl respectively) was found (Table 1).

| Table 1: Serum iron levels in homozygous β- thalassemia and normal subjects. |
|----------------------------------|----------------|----------------|----|
| Parameters                      | Cases (n=50) Mean±SD | Controls(n=50) Mean±SD | p-value |
| Serum Iron(µg/dl)               | 270.09±179.03     | 126.62 ± 10.88     | 0.000* |
| TIBC (µg/dl)                   | 267.54 ± 80.40    | 292.74 ± 41.26     | 0.054* |

The liver function tests of β-thalassemic patients and controls were compared statistically. The activities of the liver enzymes in serum (ALT, AST and ALP) were significantly higher in β-thalassemic patients as compared to controls (36.56 ± 22.05U/L & 26.1 ± 7.63U/L respectively in ALT; 40 ± 23.41U/L & 22.54 ± 7.07U/L respectively in AST and 92.26 ± 32.15U/L & 83.3 ± 16.8U/L respectively in ALP). The decrease in total proteins was found to be significant in β-thalassemic patients as compared to controls (5.08 ± 0.08g/dl & 6.72 ± 0.47g/dl respectively). A significant increase in serum bilirubin level in β-thalassemic patients as compared to controls (0.95 ± 0.62mg/dl & 0.6 ± 0.17mg/dl respectively) was found (Table 2).
Table 2: Comparison of liver function test (LFT) of β-thalassemic cases & controls.

<table>
<thead>
<tr>
<th>LFT Parameters</th>
<th>Cases (n=50) Mean±SD</th>
<th>Controls (n=50) Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT(U/L)</td>
<td>36.56 ± 22.05</td>
<td>26.1 ± 7.63</td>
<td>0.002*</td>
</tr>
<tr>
<td>SGOT(U/L)</td>
<td>40±23.41</td>
<td>22.54 ± 7.07</td>
<td>0.000*</td>
</tr>
<tr>
<td>ALP(U/L)</td>
<td>92.26 ± 32.15</td>
<td>82.2 ± 17.25</td>
<td>0.004*</td>
</tr>
<tr>
<td>TP(g/dl)</td>
<td>5.07±0.87</td>
<td>6.72 ± 0.46</td>
<td>0.000*</td>
</tr>
<tr>
<td>Bilirubin(mg/dl)</td>
<td>0.95 ± 0.62</td>
<td>0.6 ± 0.172</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Table-3 shows urea and serum creatinine levels were significantly higher in β-thalassemic patients as compared to controls (20.52 ± 12.27mg/dl & 16.67 ± 3.74mg/dl respectively in Urea and 0.99 ± 0.51mg/dl & 0.68 ± 0.17mg/dl respectively in creatinine). There is no significant difference was found in serum uric acid in β-thalassemic patients as compared to controls (4.33 ± 2.23mg/dl & 4.06 ± 0.57mg/dl respectively). In this study, serum iron is significantly negatively correlated with TIBC and total protein in β-thalassemic patients. There was no significant correlation between serum iron and LFT, RFT parameters except serum total proteins in thalassemia major patients. (Table 4; Fig. 1& 2).

Table 3: Comparison of Renal function test (RFT) of β-thalassemic cases & controls.

<table>
<thead>
<tr>
<th>RFT Parameters</th>
<th>Cases (n=50) Mean±SD</th>
<th>Controls (n=50) Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea [mg/dl]</td>
<td>20.52 ± 12.27</td>
<td>16.67 ± 3.74</td>
<td>0.038*</td>
</tr>
<tr>
<td>Creatinine(mg/dl)</td>
<td>0.99 ± 0.51</td>
<td>0.68 ± 0.17</td>
<td>0.000*</td>
</tr>
<tr>
<td>Uric acid(mg/dl)</td>
<td>4.33 ± 2.23</td>
<td>4.06 ± 0.57</td>
<td>0.403</td>
</tr>
</tbody>
</table>

*p<0.05 considered as significant. Where n= number of subjects.

Table 4: Correlation between Serum Iron and Other Biochemical Parameters in thalassemia patients.

<table>
<thead>
<tr>
<th>Serum Iron (n=50)</th>
<th>r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIBC</td>
<td>-0.4761</td>
<td>0.000*</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>-0.1058</td>
<td>0.464</td>
</tr>
<tr>
<td>SGPT</td>
<td>0.1189</td>
<td>0.410</td>
</tr>
<tr>
<td>SGOT</td>
<td>0.1150</td>
<td>0.426</td>
</tr>
<tr>
<td>ALP</td>
<td>0.0426</td>
<td>0.768</td>
</tr>
<tr>
<td>Total protein</td>
<td>-0.3625</td>
<td>0.009*</td>
</tr>
<tr>
<td>Urea</td>
<td>-0.1344</td>
<td>0.352</td>
</tr>
<tr>
<td>Creatinine</td>
<td>-0.2210</td>
<td>0.122</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.1076</td>
<td>0.457</td>
</tr>
</tbody>
</table>

*Correlation is significant at 0.05 level (2-tailed)
DISCUSSION

Thalassemias are inherited disorders characterized by abnormal production of hemoglobin, associated with low hemoglobin production and excessive destruction of red blood cells. Although an increasing number of patients are now treated with bone marrow transplantation, the majority of the patients still depend on regular blood transfusions. So they develop iron overload by regular blood transfusions.

The present study investigated the relationship between iron overload and some biochemical parameters in blood of children affected with beta thalassemia major. It was found that the majority of the patients with beta-thalassemia major had significant increase in serum iron level and significant decrease of total iron binding capacity (Table 1). Ghone and colleagues (2008) showed a significant increase in serum iron level and significant decrease in total iron binding capacity in beta-thalassemia major. The patient with beta-thalassemia have severe anemia due to ineffective erythropoiesis which is primary reason for iron overload and blood transfusion is secondary to it. Moreover, because of regular blood transfusion, all beta-
thalassemic patients had very high levels of serum iron, indicating that these patients have iron overload, probably due to multiple blood transfusion, increased dietary iron absorption or in adequate chelating therapy with desferal\textsuperscript{16}.

In the present study, there was a significant rise in the serum activities of the liver enzymes in thalassemic patients compared with the control group (Table 2). At the same time, this abnormally elevated levels of liver enzymes was accompanied by a significant elevation of serum bilirubin in patients with thalassemia major compared to controls (Table 2).

Injury to the liver cells causes leakage of the enzymes into the circulation, in addition, the elevation of ALT and bilirubin are used largely to determine if the liver has been damaged and its function is impaired\textsuperscript{17}. Liver disease associated with chronic blood transfusions in thalassemic patients is caused by hepatotropic infections or hepatic siderosis. Both factors may act either synergistically or independently in promoting chronic liver disease, including cellular damage through similar oxidative pathways. The role of iron overload as a cause of liver dysfunction in thalassemic patients of the present study is suggested to be clear\textsuperscript{18}.

A fore said finding was in accordance with a study done in Northern Iran (Ameli et al, 2008). Which also means that liver function in thalassemic patients, as reflected by elevated ALT, is affected or deteriorated by increasing serum iron overload, as reflected by elevated serum iron\textsuperscript{19}. According to Mohammad et al, (2011) a significant increase in activity of AST, ALT and bilirubin level was found in thalassemic patients as compare to control subjects. A significant alteration in liver functions of thalassemic patients shown by changes in biochemical markers (elevated ALT & AST) hepatomegaly, both with the close association with iron overload\textsuperscript{20}. The results showed a significant decrease of total protein in β-thalassemia major as compare to control (Table 2). Malik et al, (2010) also found a significant decrease in the total protein and albumin levels in sera of both major and minor thalassemic patients compared to normal groups\textsuperscript{21}.

In our study, we also revealed serum urea and serum creatinine levels in thalassemic patients were significantly increased (P≤0.01) when compared with controls. Whereas no significant difference in serum uric acid level in β-thalassemic patients compared to controls (Table 3). Younus et al, (2012) reported that a significant decrease in serum creatinine level and a significant increase in serum urea level in β-thalassemic patients, receiving only regular blood transfusion, when compared with controls\textsuperscript{22}. In study by Jafari (2011) on β-thalassemia major, a significant decrease in serum creatinine level in β-thalassemic patients compared to control healthy subjects was found\textsuperscript{23}. In patients with β-thalassemia major, the most important cause of mortality and morbidity is organ failure due to deposits of iron.

In this study, we also concluded that serum iron is significantly negatively correlated with TIBC & serum total protein (Table 4; Fig. 1 & 2). Iron overload was suggested by some investigators as an important contributing factor in lowering serum total protein level\textsuperscript{24}.

**CONCLUSION**

The present study reflects that iron overload due to frequent transfusions in β-thalassemia results in abnormal organ function tests as reflected by variations in LFT and KFT parameters. Proper and timely screening of these parameters can help in early diagnosis & prevention of iron overload.

**REFERENCES:**