Iron overload status in β thalassemia major

Aashka M Shah¹, Akash M Shah², Mahendrakumar R Shah³, Mukesh Gohel⁴*

¹Resident Doctor, ⁴Assistant Professor, Department of Biochemistry, B.J. Medical College, Ahmedabad.
²Intern Doctor, Smt. NHL Municipal Medical College, Ahmedabad.
³Assistant Professor, Department of Physiology, AMC MET Medical College, Ahmedabad.

INTRODUCTION
β thalassemia major is an important inherited hemoglobinopathy in which disproportion in the synthesis of alpha and beta chains of hemoglobin is observed resulting in microcytic hypochromic anemia. These affected individuals need repeated, regular and lifelong blood transfusion therapy. Such repeated blood transfusions and ineffective erythropoiesis lead to accumulation of iron in the body. One unit of PCV (packed cell volume) contains 200-250 mg of iron.¹ This is stored in our body, because iron is not excreted from our body. This way iron metabolism is unique. Iron homeostasis in our body is maintained by its regulation at the level of absorption and not by excretion.² Thus, we can understand that repeated blood transfusions and ineffective erythropoiesis lead to accumulation of iron in the body. This increased serum iron leads to decrease in TIBC (Total Iron Binding Capacity) of serum.

ABSTRACT
BACKGROUND: β thalassemias are a group of inherited blood disorders caused by reduced or absent synthesis of the beta chains of hemoglobin. These affected individuals need repeated, regular and lifelong blood transfusion therapy. Such repeated blood transfusions and ineffective erythropoiesis lead to accumulation of iron in the body. Aim of this study is to assess iron overload status and to determine its severity in patients of β thalassemia major by measuring levels of Serum Iron, TIBC, % transferrin saturation and Serum Ferritin. METHODS: 60 cases of β thalassemia major (without hepatitis) and 60 matched controls were recruited for study. Serum Iron and TIBC were determined by Ferrozine method. Serum % Transferrin saturation was calculated as the ratio of Serum Iron and TIBC. Serum Ferritin levels were estimated by direct ELISA method. RESULTS: Serum Iron, % Transferrin saturation and Serum Ferritin levels were found to be above biological reference interval in almost all the cases with significant p values compared to controls (p value <0.001). CONCLUSIONS: Almost all cases were in iron overload status reflected by increased Serum Iron, % Transferrin saturation, ferritin levels and reduced TIBC compared to control. Serum Ferritin levels reflect the tissue iron deposits and thus, the total body iron stores. Serum Ferritin assessment methods are easy and relatively inexpensive as well as provide an early detection of iron overload status in patients of β thalassemia major.

Keywords: thalassemia, iron overload, Serum Iron, TIBC, % Transferrin saturation, Ferritin
may be manifested as growth failure, diabetes and hypogonadism. "Iron overload" has rightly been referred to as "second disease" during the treatment of the first. So it is important to assess iron overload status.

Ferritin is storage form of iron in our body. Normal Serum Ferritin levels for six months to fifteen years of age are 7-140 ng/ml. Thus, we can see that there is little Ferritin in blood under normal conditions. However, in patients with excess of iron, amount of Ferritin in plasma is markedly elevated. Thus, Serum Ferritin levels serve as an index of body iron stores.

**MATERIAL AND METHODS**

This study was a hospital based cross sectional study conducted on 60 cases of β thalassemia major who were admitted at Civil hospital, Ahmedabad during the period of January 2013 to December 2013 for regular blood transfusions and 60 age and sex matched healthy control subjects. Known cases of β thalassemia major who had not received iron chelation therapy irrespective of age and sex were included in study. β thalassemia major cases who developed hepatitis were excluded from the study.

The objectives of study were explained to all eligible subjects for this study. Informed consent of all subjects included in the study was obtained for involvement in study and for venipuncture. Emphasis was given that participation in this study was voluntary. The clinical details of patients were recorded in a Performa, taking into account the age and the number of transfusions given. About 3 ml of patient’s blood sample was collected by venipuncture in disposable vacutainer under strict aseptic condition. The blood was allowed to clot and centrifuged at 3000 rpm for 5 minutes and serum was separated and stored at −20°C.

Serum Iron and TIBC were determined by Ferrozine/Magnesium carbonate method on XL-640 fully autoanalyzer. Serum %Transferrin saturation was calculated as the ratio of Serum Iron to TIBC multiplied by one hundred. Serum Ferritin levels were assessed by direct ELISA (Enzyme Linked Immunosorbent Assay) along with normal and pathological controls on Tulip ELISA reader. The results were analyzed by student’s t-test and p value was calculated by using graphpad instat software 3.06 version.

**RESULTS**

All the cases included in study were on regular blood transfusions and Serum Iron, % transferrin saturation and Serum Ferritin levels were found to be significantly elevated in all the cases as compared to the controls with significant p value (p<0.001). Serum TIBC was found to be significantly decreased in all the cases as compared to the controls with significant p value (p<0.001) (Table 1).

Iron overload status has been graded into mild, moderate and severe categories based on Serum Ferritin levels as shown in Table 2. Among all cases, 13.3% of cases had Serum Ferritin levels between 300-1000 ng/ml and were in a mild iron overload status, 53.3% had Serum Ferritin levels between 1001-2500 ng/ml and were in a moderate iron overload status and 33.3% had Serum Ferritin levels greater than 2500 ng/ml and were in a severe iron overload status. All the control subjects had normal serum Ferritin levels.

**Table 1: Mean and standard deviation values of Serum Iron, TIBC, % transferrin saturation and Serum Ferritin levels in cases and controls along with the p value**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Normal values</th>
<th>Cases (Mean ± SD)</th>
<th>Controls (Mean ± SD)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Iron</td>
<td>µg/dL</td>
<td>60-160</td>
<td>239.7 ± 111.31</td>
<td>107.46 ± 14.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum TIBC</td>
<td>µg/dL</td>
<td>250-400</td>
<td>280.25 ± 76.35</td>
<td>329.96 ± 23.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>-</td>
<td>33%</td>
<td>85.53 ± 14.47</td>
<td>30.89 ± 6.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum Ferritin</td>
<td>ng/ml</td>
<td>7-140</td>
<td>1968.36 ± 861.05</td>
<td>81.5 ± 13.58</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Iron overload status in β thalassemia major

Table 2: Severity of Iron overload (Number and percentage of cases and number of blood transfusions received in different grades of iron overload status)

<table>
<thead>
<tr>
<th>Iron overload status</th>
<th>Serum Ferritin levels (ng/ml)</th>
<th>Number of cases</th>
<th>Percentage of cases</th>
<th>Number of blood transfusions given</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt; 300</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mild</td>
<td>300 - 1000</td>
<td>8</td>
<td>13.3%</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>Moderate</td>
<td>&gt; 1000 but ≤ 2500</td>
<td>32</td>
<td>53.3%</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Severe</td>
<td>&gt; 2500</td>
<td>20</td>
<td>33.3%</td>
<td>20 ± 4</td>
</tr>
</tbody>
</table>

Table 3: A comparative study of mean serum ferritin levels in thalassemic children

<table>
<thead>
<tr>
<th>Study</th>
<th>Mean Ferritin levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayidno et al (2002)</td>
<td>1931 ng/ml</td>
</tr>
<tr>
<td>Cunningham et al (2004)</td>
<td>1696 ng/ml</td>
</tr>
<tr>
<td>Rehman et al (2004)</td>
<td>2861 ng/ml</td>
</tr>
<tr>
<td>Our study</td>
<td>1968 ng/ml</td>
</tr>
</tbody>
</table>

DISCUSSION
Repeated blood transfusions and ineffective erythropoiesis is the major culprit behind the iron overload condition in thalassemic children. Majority of our cases of β thalassemia major are in moderate to severe iron overload status after about 10 transfusions as estimated by measuring levels of Serum Iron, Serum transferrin saturation % and Serum Ferritin. Results of our present study for the above parameters are also in accordance with previous studies.15,16,17,18

The rise in Serum Ferritin levels as observed in the present study correlates well with other studies19, 20, 21 as shown in Table 3. Serum Ferritin levels reflect the tissue iron deposits and thus the total body iron stores. Measurement of serum Ferritin constitutes a simple and reliable method for the early detection of iron overload status.

Thalassemia International Federation has provided guidelines for iron chelation therapy in β thalassemia major. It recommends that iron chelation therapy should be considered whenever either the patient has received at least 10-20 transfusions or Serum Ferritin levels cross 1000 ng/ml. These guidelines are followed at Civil hospital, Ahmedabad and accordingly, Deferasirox 20 mg/kg/day is given orally in averagely transfused patients.22 Serum Ferritin levels are monitored at every 3 months and iron chelation therapy is modified accordingly.

REFERENCES
12. Nadeem Ikram, Khalid Hassan, Muhammad Younas. Ferritin Levels in...
Iron overload status in β thalassemia major


