Original article:

Role of serum ferritin and troponin-I ultra in detecting cardiac injury in children with thalassemia major

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ABSTRACT

Background: Thalassemia major (TM) is a hereditary disease with synthesis defects in beta chains of haemoglobin. Thalassemia major causes red blood cell destruction, leading to anemic symptoms and need for regular blood transfusions. Ferritin which is a diagnostic marker of iron overload in thalassemia patients increases due to inadequate erythropoiesis, iron overload, transfusion, and increased GI absorption. High serum ferritin levels increase the risk of myocardial infarction in thalassemic patients. Myocardial infarct can be diagnosed by the cardiac biomarker cardiac troponin I (cTnI).

Aims and Objectives: Role of cardiac troponin I in early detection of cardiac injury (microinfarcts) in thalassemic children and to correlate serum ferritin and cardiac troponin I in thalassemic children.

Material and Methods: Study was conducted between January 2015 – December 2016 in a total of 100 subjects (50 cases – Group I and 50 controls- Group II).

Results: Mean age of group I patients was 9.98±3.59 and in group II, it was 9.71±3.61 years. A total of 74% male and 26% were females. Mean height was 122.08±14.28 cm and 132.36±21.10 in group I and II respectively. Mean weight was 21.23±7.12 and 28.2±8.24 in group I and II respectively. Mean body surface area of group I cases was 0.84±0.18 and 1.01±0.22 in group II. In study group, a total of 8 cases were found to be positive and none in control group (p<0.01). No patient in the present study was found to be HIV positive or HBsAg reactive. 15 (30%) cases in group I were found to be HCV reactive and none in group II. Investigations such as SGOT, SGPT, S. protein and alkaline phosphatase were found to be statistically significant between both the groups. Maximum number of patients i.e. 27(54%) received 16-20 blood transfusions yearly. Mean blood transfusion was 18.76±3.00. Mean blood urea in group I patients was 24.04±5.86 and 27.1±8.88 in group II. Mean serum creatinine in group I was 0.63±0.70 and 0.46±0.10 in group II. Negative insignificant relationship between serum ferritin and troponin-I was found. Conclusion: The result of our study concluded that in microinfarct, troponin increases independent of ferritin; therefore, it can be used for early detection of cardiac injury in TM patients.

Keywords: Serum Ferritin, Troponin-I, Cardiac injury, Thalassemia major.
INTRODUCTION
Thalassemia major (TM) is a hereditary disease with synthesis defects in beta chains of haemoglobin. Thalassemia major causes red blood cell destruction, leading to anemic symptoms and need for regular blood transfusions. Hemolysis and regular blood transfusions can lead to iron overload in the liver, endocrine organs and myocardium. Iron overload can be evaluated easily and verified by measuring serum ferritin levels. Cardiac involvement is the most important and prevalent complication of Beta-thalassemia.1-5

Ferritin which is a diagnostic marker of iron overload in thalassemia patients8 increases due to inadequate erythropoiesis, iron overload, transfusion, and increased GI absorption. High serum ferritin levels increase the risk of myocardial infarction in thalassemic patients. Myocardial infarct can be diagnosed by the cardiac biomarker cardiac troponin I (cTnI).6-8

Myocardial siderosis is known as the major cause of death in TM patients since it can lead to iron overload cardiomyopathy which occurs due to ineffective erythropoiesis, chronic anemia and hypoxia. Consequently, these patients are more susceptible to ischemia. Troponin is released during cell damage and due to the loss of myocyte contraction force. Irreversible cardiomyocyte damage causes a release of intact cTnI and its degradation products due to metabolic inhibition of cardiomyocytes.9

Various direct and indirect methods of iron assessment, including serum ferritin level, echocardiogram, nontransferrin bound iron, cardiac magnetic resonance T2, heart rate variability, and liver biopsy and myocardial biopsy, have been proposed for early detection of cardiac iron overload in TM patients. However, controversial evidence and limitations of their use in clinical practice exist.

At present, although bone marrow transplantation has been shown to effectively cure some selected patients, the cornerstone of treatment in thalassemia major is still with blood transfusion and iron chelation therapy. The effectiveness of iron chelation has markedly improved since the introduction of oral chelators, such as deferiprone10 and deferasirox,11 resulting in prolonged and increased quality of life. Iron overload cardiomyopathy could be reversible only if early intensive chelation has been initiated.12,13

Once thalassemia major patients develop clinical symptom such as heart failure or arrhythmia, the prognosis usually becomes poor and death occurs thereafter in spite of intensive chelation.14 These findings indicate the importance of early detection of cardiac iron accumulation prior to the development of cardiac dysfunction, and that the intensive chelation can be given promptly to those patients who are at risk.

In this study, we aimed to evaluate Troponin-I for early detection of cardiac injury in thalassemic patients and its interrelationship with serum ferritin.
MATERIAL AND METHODS
This prospective observational study was conducted in the thalassemia unit of Department of Pediatrics, Pt. B.D. Sharma PGIMS, Rohtak. A total of 100 subjects (50 cases i.e. Group I and 50 controls – Group II) were included with age 2 years to 14 years old and Transfusion burden >12/year blood units. Ethical approval was obtained from the institutional ethical committee and parental consent was also taken. All the children were examined and those who were suffering from infectious diseases, known heart disease, iron deficiency anemia, kidney disease, diabetes, fever, and systemic diseases were excluded. Before breakfast, 5ml blood was drawn from these children in red capped vaccutainer for serum ferritin and serum troponin I and 2 ml in purple capped vaccutainer for complete haemogram. After collecting the samples, serum was separated for evaluation of ferritin and troponin I.

Serum levels of ferritin and troponin-I were evaluated using chemiluminiscence technology. Chemiluminiscence is light produced by chemical reaction. The chemiluminiscent substance is excited by the oxidation and catalysis forming intermediates. When the excited intermediates return back to their stable ground state, a photon is released, which is detected by the luminescent signal instrument. Luminescent reactions are measured in relative light units (RLU) that are typically proportionate to the amount of analyte present in a simple. Normal range of serum ferritin was 18–323 ng/ml and troponin I was 0 to 1.5 ng/ml.

STATISTICAL ANALYSIS
Data was collected and analysed by using Chi-square test and student t-test. Pearson's Correlation of Coefficient was used for correlating various parameters with each other.

RESULTS
Mean age of group I patients was 9.98±3.59 and in group II, it was 9.71±3.61 years (p >0.05). A total of 74% male and 26% were females (p >0.05). Socio economic status of all the patients enrolled in the study by using Kuppuswamy Scale showed that maximum number of patients belonged to upper lower class i.e. 70% in group I and 66% in group II. Only 4(8%) patients in group I and 5(10%) in group B belonged to lower class (p >0.05). Anthropometric parameters of patients of both the groups showed mean height 122.08±14.28 cm and 132.36±21.10 in group I and II respectively with statistically significant difference (p <0.01). Similarly mean weight was 21.23±7.12 and 28.2±8.24 in group I and II respectively with significant difference (p<0.001). Mean body surface area of group I cases was 0.84±0.18 and 1.01±0.22 in group II. Body surface area of study population also showed significant difference (p <0.001).

Various laboratory investigations such as Hb, Serum ferritin and Troponin-I were found to be statistically significant between both the groups except TLC which was insignificant (p>0.05). In study group, a total of 8 cases were found to be positive and none in control group (p <0.01). No patient in the present study was found to be HIV positive or HBsAg reactive. 15 (30%) cases in group I were found to be HCV reactive.
and none in group II. Liver & Renal function tests of children of both the groups are shown in Table I and II.

**Table I**

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Group I (n=50) n(%)</th>
<th>Group II (n=50) n(%)</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT (IU/L)</td>
<td>81.14±63.1 2</td>
<td>31.84±6.04 04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>95.82±66.0 7</td>
<td>22.96±9.52 52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S. bilirubin (mg/dl)</td>
<td>0.58±0.166 0.36±0.1</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>190.34±70.26</td>
<td>47.26±9.02 02</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Group I (n=50) n(%)</th>
<th>Group II (n=50) n(%)</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood urea (mg/dl)</td>
<td>24.04±5.8 6</td>
<td>27.1±8.8 8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S. creatinine (mg/dl)</td>
<td>0.63±0.70 0.46±0.1</td>
<td>0.09</td>
<td></td>
</tr>
</tbody>
</table>

Findings of serum ferritin and troponin-I in group I were correlated by using Pearson's Correlation of Coefficient (r value) with a negative insignificant relationship between serum ferritin and troponin-I. In microinfect troponin I increases independent of ferritin; therefore, it can be used for early detection of cardiac involvement in thalassemic patients to determine the sub clinical effects (Table III).

**Table III**

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Serum ferritin (ng/ml)</th>
<th>Troponin-I (ng/ml)</th>
<th>Pearson's Correlation of Coefficient (r value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ferritin</td>
<td>0.159±0.3 28</td>
<td>0.188, p=0.190</td>
<td></td>
</tr>
<tr>
<td>Troponin-I</td>
<td>8 Positive 42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Insignificant relationship between serum ferritin and troponin-I in control group was found which is evident from Table IV.

**Table IV**

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Serum ferritin (ng/ml)</th>
<th>Troponin-I (ng/ml)</th>
<th>Pearson's Correlation of Coefficient (r value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ferritin</td>
<td>0.003±0.0 04</td>
<td>0.188, p=0.190</td>
<td></td>
</tr>
<tr>
<td>Troponin-I</td>
<td>All negative</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Majority of patients were >10 years of age i.e. 24(48%) in group I and 23(46%) in group II with mean age of group I patients was 9.98±3.59 and in group II, it was 9.71±3.61 years (p >0.05). History of consanguineous marriage was not found in any case and cardiac examination of all the patients was normal. Significant growth retardation was present in TM patients which was also seen in study done by Fahim et al\textsuperscript{15} who compared 100 TM patients with 100 controls and concluded that children with beta thalassemia major have delayed growth that signify the importance of therapeutic interventions.

Study reported by Mirhosseini et al\textsuperscript{16} assessed the nutritional status of thalassemia patients and determined the factors involved. 44.3% of boys and 37.7% of girls were found to be of short stature. This significant difference is due to chronic transfusion therapy leading to iron deposition in the endocrine system. This results in high rate of hypothyroidism, hypogonadotrophic hypogonadism, growth hormone deficiency.

Hb, Serum ferritin and Troponin-I found to be statistically significant among both the groups. In study group, a total of 8 cases were found to be troponin-I positive and none of the case was found to be positive in control group (p <0.01). Study done by Shahramian et al\textsuperscript{17} (2013) found a significant difference between the two groups regarding the mean of the serum levels of troponin (p=0.045) and ferritin (p=0.001). Shodikin et al\textsuperscript{18} showed similar results that is the mean of serum ferritin levels in study group was statistically significant (p=0.0004) compared to control group. The mean of serum cTnI in the thalassemia group was higher than in the control group but statistically not significant (p=0.82).

Findings of serum ferritin and troponin-I in group I cases were correlated and a negative insignificant relationship between serum ferritin and troponin-I was observed. In microinfarct troponin I increases independent of ferritin; therefore, it can be used for early detection of cardiac involvement in thalassemic patients to determine the sub clinical effects. Shahramian\textsuperscript{17} also concluded that in microinfarct, troponin increases independent of ferritin; therefore, it can be used for early detection of cardiac involvement in thalassemia patients to determine the sub-clinical effects. Similarly, Shodikin\textsuperscript{18} study showed correlation between serum ferritin levels and cTnI in TM children. In the TM group, the mean of serum ferritin and cTnI levels were 4292.5 μg/L and 0.20 ng/mL respectively. The mean of serum ferritin levels in the TM group was higher than in the control and statistically significant (p= 0.0004). The mean of serum cTnI in the TM group was higher than in the control, but statistically insignificant (p= 0.82). In the TM group, there was a weak correlation between serum ferritin and cTnI levels (r=0.34).\textsuperscript{11} A single unit of packed red blood cells contains approximately 200 mg of iron. This is the reason for increased serum ferritin levels in group I. There are 8 patients in group I and none in group II whose troponin I was positive. This is due to microinfarcts which
can occur in thalassemic patients due to cardiac hemosiderosis.

Findings of serum ferritin and troponin-I in group II were correlated and showed insignificant relationship between serum ferritin and troponin-I in control group. Fifteen (30%) cases in group I found to be HCV reactive and in group II, none of the patient was found to be HCV reactive (p < 0.001 VHS). Study done by Ataei\textsuperscript{19} revealed that the prevalence of HCV positivity was 8% among thalassemia patients. They concluded that blood transfusion was the main risk factor for HCV infection among beta thalassemia patients.

In our study 30% thalassemic children were HCV positive. This difference may be because mean blood transfusion was 18.96±3.00 per year. In our study, maximum number of patients i.e. 27(54%) received 16-20 blood transfusions yearly. Mean blood transfusion was 18.76±3.00. Transfusion should generally be given at intervals of 3-4 weeks with the goal being to maintain a pre transfusion hemoglobin level of 9.5-10.5 g/dl. Mean blood urea in group I patients was 24.04±5.86 and 27.1±8.88 in group II (p <0.05). Mean serum creatinine in group I was 0.63±0.70 and 0.46±0.10 in group II with insignificant result (>0.05). The results were similar to study done by Smolkin\textsuperscript{20} which included 37 patients with beta thalassemia major and 11 with thalassemia intermedia, Twelve children without iron metabolism disorders or renal diseases served as control group. No difference in blood urea nitrogen, serum creatinine, creatinine clearance electrolytes was found.

**CONCLUSION**

Our study concluded that in microinfarct, troponin increases independent of ferritin; therefore, it can be used for early detection of cardiac injury in TM patients.

**REFERENCES**


