Biochemical Marker of Hepatic Injury in Sickle Cell Disease

Dr. Jayesh Warade¹, Dr. Hemant Dahake², Dr. Yogesh Pawade³, Dr. Aparna Pandey⁴

¹Meenakshi Mission Hospital and Research Centre, Madurai
jdyajdo@gmail.com
² & ³Seth G. S. Medical College and K. E. M. Hospital, Parel, Mumbai
⁴Apollo Specialty Hospital, Madurai

Affiliation Where The Work Was Primarily Carried Out): Indira Gandhi Govt.
Medical College, Nagpur

Abstract:

Background: Sickle cell anemia is an inherited blood disorder characterized primarily by chronic anemia and periodic episodes of pain. Evidence of liver disease in sickle cell disease is obtained either from abnormal biochemical tests or postmortem liver biopsy specimen rarely an antemortem liver specimen. Icterus and increased tendency to gall stones is the most common presentation. Materials and methods: To evaluate biochemically, the patients with sickle cell disease (n=100) from medicine and pediatrics wards/OPD were included in the study. 100 age matched healthy individuals were also selected as controls. An informed written consent was obtained from all the study subjects who were enrolled in the study. Results: The levels of serum AST, ALT, ALP and bilirubin is found to be significantly higher in sickle cell disease patients than in healthy subjects. Discussion: Intrahepatic cholestasis is one of the fatal complications of sickle cell anemia. Sickling of red blood cells in hepatic sinusoids and their stasis may also cause serious damage to hepatocytes and Kupffer cells. This can lead to frequent enlargement of liver making it sensitive so that a minor stimulus can lead to hepatic dysfunction which can cause frequent rise in liver enzyme levels in blood. Hepatic injury due to transfusional iron overload and staining of hepatocytes and Kupffer cell with iron leading to hepatic iron overload due to repeated blood transfusion may be a possible reason for hepatic dysfunction in those patients who have received multiple blood transfusions. Conclusion: Serum enzyme (ALP, ALT, and AST), bilirubin and uric acid parameters are significantly increased as compared with the controls. The liver function of the patients was significantly compromised as compared to controls. Increased serum bilirubin indicates the likelihood of continuous ongoing hemolysis.

Keywords: Hemolysis, cholestasis, Iron overload, Hepatocytes, Kupffer Cells, Sickli

Sickle cell anemia is an inherited blood disorder characterized primarily by chronic anemia and periodic episodes of pain. The underlying problem involves hemoglobin, a component of red blood cells. Normal red blood cells live about 120 days in the bloodstream, but sickled red cells die after about 10 to 20 days. Because they cannot be replaced fast enough, the blood is chronically short of red blood cells, a condition called anemia. Sickle cell anemia is an autosomal recessive (1) genetic disorder caused by a defect in the HBB gene, which codes for hemoglobin. The presence of two defective genes (SS) is needed for sickle cell anemia. If a person carries one sickle hemoglobin gene (S) and one normal gene (A), the condition is sickle cell trait. Elevation of the different liver enzymes correlates with the different categories; hemolysis raises plasma
aspartate transaminase (AST) while plasma alanine transaminase (ALT) levels more accurately reflect hepatocyte injury. High levels of serum alkaline phosphatase are commonly seen in patients with sickle cell anemia; this may be because of either cholestasis or bone disease. Evidence of liver disease in sickle cell disease is obtained either from abnormal biochemical tests or postmortem liver biopsy specimen rarely an antemortem liver specimen. Icterus and increased tendency to gall stones is the most common presentation. In this study an attempt has been made to to study the extent of liver injury in sickle cell disease by estimation of biochemical parameters.

Materials and methods:
The present study was carried out during the period of December 2007 - September 2009 in the Department of Biochemistry, Indira Gandhi Government Medical College, Nagpur. The study protocol was approved by the Institutional Ethical Committee. To evaluate biochemically, the patients with sickle cell disease (n=100) from medicine and pediatrics wards/OPD were included in the study. 100 age matched healthy individuals were also selected as controls. An informed written consent was obtained from all the study subjects who were enrolled in the study. The estimation of biochemical parameters such as AST, ALT, ALP and bilirubin in serum was done by Transasia Erba XL 300 fully automated analyzer with dedicated reagents.

Statistical Analysis: All values were reported as mean ± SD. The unpaired two tailed Student’s t test was used to assess the significance of the difference in the values in the sickle cell disease subjects and in healthy controls. The differences were considered as statistically significant at a probability value, P < 0.05. All statistical analyses were performed by using statistical software Graph Pad Prism.

Results:
The results obtained after estimating liver parameters in healthy subject and disease subjects are tabulated as in table no.

Table No. 1: Showing Mean, Standard Deviation of Serum Liver Enzyme and serum bilirubin level in Cases and Control.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (mean ± S.D.) (n=100)</th>
<th>Control (mean ± S.D.) (n=100)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr.ALP (IU/L)</td>
<td>104.8±11.03</td>
<td>62.63±13.58</td>
<td>&lt;0.001(HS)</td>
</tr>
<tr>
<td>Sr.ALT (IU/L)</td>
<td>44.73±11.07</td>
<td>29.54±4.84</td>
<td>&lt;0.001(HS)</td>
</tr>
<tr>
<td>Sr.AST (IU/L)</td>
<td>44.11±10.18</td>
<td>29.02±6.77</td>
<td>&lt;0.001(HS)</td>
</tr>
<tr>
<td>Sr. Bilirubin (mg %)</td>
<td>1.53±0.50</td>
<td>0.80±0.22</td>
<td>&lt;0.001(HS)</td>
</tr>
</tbody>
</table>
Graph No. 1: Bar Diagram Showing Mean & S. D. of ALT level in study group.

Graph No. 2: Bar Diagram showing Mean & S. D. of ALP level in study group.
Graph No. 3: Bar Diagram Showing Mean & SD of AST level in study group.

Graph No. 4: Bar Diagram showing Mean & SD of Bilirubin level in study groups.
Table No. 2: Showing ALP, ALT, AST, bilirubin level in cases and controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Range</th>
<th>Cases(n=100)</th>
<th>Controls(n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP(IU/L)</td>
<td>34-90</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>&gt;90</td>
<td>90</td>
<td>00</td>
</tr>
<tr>
<td>ALT(IU/L)</td>
<td>18-39</td>
<td>32</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>&gt;39</td>
<td>68</td>
<td>00</td>
</tr>
<tr>
<td>AST(IU/L)</td>
<td>12-42</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>&gt;42</td>
<td>60</td>
<td>00</td>
</tr>
<tr>
<td>Sr. Bilirubin</td>
<td>0.4-1.3</td>
<td>23</td>
<td>100</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>&gt;1.3</td>
<td>77</td>
<td>00</td>
</tr>
</tbody>
</table>

Discussion:
In present study, out of 100 patients 90% of the patients have increased alkaline phosphatase level as compared to control group. Taiwo Kotila et al (2005) also reported 74% of the patients in the study group having alkaline phosphatase level above normal while Emel Gürkan et al (2005) reported the increased in alkaline phosphatase level in 27% of the patients. Engin Alþntafl et al (2003) in their case report of Sickle cell anemia with chronic intrahepatic cholestasis found that alkaline phosphatase level was 326 IU/L. Ojuawo et al (1995) found that alkaline phosphatase levels were significantly higher during crisis than at recovery, (p < 0.005) especially in the young patient. In present study, out of 100 patients 68 were having increased alanine transaminase level as compared to levels with control group. Ahmad M et al (2006) studied three cases of sickle cell disease and reported all of them having increased alanine transaminase level. Taiwo Kotila et al (2005) reported 19% of the patients having increased alanine transaminase level. Emel Gürkan et al (2005) also reported 15% of the patient having increased alanine transaminase level. Engin Alþntafl et al (2003) in their case report of Sickle cell anemia with chronic intrahepatic cholestasis found that ALT level was 40U/L. Ojuawo et al (1995) found that alanine transferase levels were significantly higher during crisis than at recovery, (p < 0.005) especially in the young patients. The value for serum aspartate transaminase in control group is 42 IU/L. Out of the100 cases 60% of the patients have aspartate transaminase level above normal value and only 40% patients have serum AST value within normal range. Taiwo Kotila et al (2005) reported that 50% of the patients have aspartate transaminase level above normal and 50% were having values within normal range. Ahmad M et al (2006) in their case report study of 3 cases reported increase in aspartate transaminase value in all the 3 cases. Engin Alþntafl et al (2003) in their case report of Sickle cell anemia with chronic intrahepatic cholestasis found that AST level was 153 U/L. Acute intrahepatic cholestasis may be sequelae of widespread sickling within the sinusoid or extreme hemolysis, which is often accompanied by elevated alkaline phosphatase and variable levels of transaminases. The reason for deranged liver function appears to be the consequence of repeated vaso-occlusive episodes. There are other studies suggesting that the main causes of liver injury in SCD patients are due to factors other than intrahepatic sickling, such as viral hepatitis or transfusional iron overload. Bleeding disorder and increased risk of encephalopathy are other possible causes. Intrahepatic cholestasis is one of the fatal complications of sickle cell
anemia. Massive accumulation of sickled cells in hepatic sinusoids and stasis cause serious damage to hepatocytes and Kupffer cells. The liver frequently enlarges and becomes sensitive. In hepatic sequestration crisis there is an acute increase in the levels of enzymes. (7) The derangement in liver function can also be explained on the basis that the causes of liver injury in SCD patients are due to factors other than intrahepatic sickling, such as viral hepatitis or transfusional iron overload which was considered to be reversible. (8,9) Intrahepatic cholestasis is one of the fatal complications of sickle cell anemia. Sickling of red blood cells in hepatic sinusoids and their stasis may also cause serious damage to hepatocytes and Kupffer cells. This can lead to frequent enlargement of liver making it sensitive so that a minor stimulus can lead to hepatic dysfunction which can cause frequent rise in liver enzyme levels in blood. Hepatic injury due to transfusional iron overload and staining of hepatocytes and Kupffer cell with iron leading to hepatic iron overload due to repeated blood transfusion may be a possible reason for hepatic dysfunction in those patients who have received multiple blood transfusions. In our study, possible reason for elevated alkaline phosphatase and transaminases (ALT, AST) levels may be acute intrahepatic cholestasis, either due to widespread sickling within the sinusoid or due to extreme hemolysis. (7) Patients with sickle cell disease have increased risk of cholelithiasis which could also be a possible reason for hepatic dysfunction in these patients. Thus, the hepatic dysfunction in sickle cell disease patients could be of multi-factorial origin.

Out of 100 cases 77 of the patient were having bilirubin values above control range while only 23% patients were having serum bilirubin concentration in control range. The case report study done on 3 cases by Ahmad M et al (2006) also reported hyperbilirubinemia in all the 3 cases (6). Emel Gürkan (2005) et al also reported hyperbilirubinemia in 13% of the patient (3). Engin Altıntafl et al (2003) in their case report of Sickle cell anemia with chronic intrahepatic cholestasis found that serum bilirubin level was 20.3 mg% (4).

A variety of conditions in SCD result in acute and marked elevations of bilirubin, and acute hepatic enlargement, including viral hepatitis, which clinically presents with nausea, malaise, jaundice, low grade fever, a tender enlarged liver and high bilirubin. Derangement in concentration of bilirubin metabolizing enzymes in liver causes defective bilirubin metabolism that interns result in increased serum bilirubin level. Hemolysis occurring in sickle cell disease is another reason for increased bilirubin level. In fact, some undiagnosed patients may present for the first time with acute hyperbilirubinemia. Intrahepatic cholestasis may contribute to increase in serum bilirubin level due to obstruction to the flow of bile. Both rapid hemolysis and defective bilirubin metabolism result in an increase in the values of direct and indirect bilirubin.

**Conclusion:**

Serum enzyme (ALP, ALT, and AST), bilirubin and uric acid parameters are significantly increased as compared with the controls. The liver function of the patients was significantly compromised as compared to controls. Increased serum bilirubin indicates the likelihood of continuous ongoing hemolysis. **References:**


5) Ojuawo A ; Adedoyin M A ; Fagbule D; The Central African journal of medicine1994-Dec; vol 40 (issue 12) : pp 342-5


7) Banerjee S, Owen C, Chopra S. Sickle cell
8) **Rosenblate HJ, Eisenstein R, Holmes AW.**

9) **Omata M, Johnson CS, Tong M, et al.**