Correlation of Bilirubin with Liver Enzymes in Patients of Falciparum Malaria

Muhammad Waseem Kausar, Khalida Moeed, Naghmi Asif, Farwa Rizwi and Sajid Raza

Introduction: Falciparum malaria is responsible for 1-3 million deaths annually worldwide. Liver involvement is common and may manifest as raised serum bilirubin, hepatomegaly and elevated liver enzymes. Unconjugated hyperbilirubinemia is usually seen leading to increased mortality. Alanine aminotransferase (SGPT) is a marker of liver damage. The present study was conducted on Plasmodium falciparum malarial patients to observe the correlation between liver enzymes and bilirubin.

Objective: To observe the correlation coefficient of bilirubin with liver enzymes (SGPT, SGOT and Alkaline Phosphatase) in patients of falciparum malaria

Design: A Descriptive study

Place and duration of study: Department of Biochemistry, Basic Medical Sciences Institute, JPMC, Karachi from August 2005 to July 2006.

Material and method: Total 81 patients of different ages and both sexes suffering from acute malaria, were selected by convenient sampling. Nine patients, infected by Hepatitis B and C infections were excluded from the study. Among remaining 72 cases, 48 (70%) were suffering from infection by Plasmodium falciparum and 24 (30%) from infection by Plasmodium vivax infection. The Falciparum infected patients were equally divided into two groups on the basis of duration of illness. Group I had duration of 1-7 days illness and Group II had duration of 8-20 days. Patients suffering from plasmodium vivax infection had illness of 1-20 days duration were placed in Group III.

Results: In the group I, SGPT and Alkaline phosphatase showed a statistically significant positive correlation (r=0.50 and r=0.054, respectively with bilirubin (P<0.05). In group II, the SGPT showed a statistically excellent positive correlation (r=0.88; P<0.01), while the SGOT and Alkaline phosphatase also showed a statistically significant positive correlation. In group III both aminotransferases and Alkaline phosphatase showed a statistically significant positive correlation r=0.82, 0.63 and 0.69 respectively.

Conclusion: Positive correlation of liver enzymes and bilirubin shows that liver function tests should be performed along with early diagnosis of Plasmodium falciparum malarial infections in order to prevent complications and to reduce mortality.

Key words: Malaria, liver enzymes, bilirubin, falciparum malaria
Liver involvement in malaria is common in patients of severe malaria and may manifest as jaundice, hepatomegaly and elevated liver enzymes like aspartate and alanine transaminases. The factors leading to severe anemia in malaria are multiple. It may be due to hemolysis, bone marrow dysfunction etc and is proportional to the level of parasitaemia. Hyperbilirubinemia, mainly unconjugated, is a common feature of falciparum malaria and is attributed to hemolysis of both parasitized and non-parasitized erythrocytes and partly due to liver damage. Although hyperbilirubinemia has been linked with increased malaria related mortality, it is often seen in association with other complications such as acute renal failure or cerebral malaria. Alanine aminotransferase catalyzes reactions in which the building blocks of protein (amino acid) are transferred from a donor molecule to a recipient molecule. It is found largely in the liver. Hence, it serves as a marker of liver damage while Aspartate aminotransferase is found in a diversity of tissues including liver, muscle, heart, kidney, and brain. It is increased when any of these tissues is injured. Therefore it is not highly specific indicator of liver damage.

The increased serum alkaline phosphatase activity among the patients indicates that the liver stage of falciparum malaria infection is accompanied by a perturbation of the host hepatocytes drainage pathways and damage to hepatocytes membrane leading to leakage of this enzyme out of the liver cells.

The present study was conducted on Plasmodium falciparum malarial patients to observe the correlation between the liver enzymes and bilirubin.

**Patients and Methods**

This study was carried out in the Department of Biochemistry, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre (JPMC) Karachi. Total 81 uncomplicated, symptomatic patients (confirmed by thick and thin slide method) were selected through convenient sampling technique from Malaria Control Program counter at Accident and Emergency Department of Medical Units I, II and III, JPMC Karachi. Nine patients were positive for hepatitis B and hepatitis C and therefore excluded from study. Remaining 72 patients were enrolled for the study.

**Inclusion Criteria**
All the symptomatic patients belonging to all ages and both sexes whose diagnosis was confirmed by thick and thin film were included in the study. Detail history was taken and complete physical examination was performed.

**Exclusion Criteria**
1. Those patients who were having fever with or without rigors but were negative for malarial parasite.
2. Those having jaundice of other causes than malaria.
3. Those who were taking hepatotoxic drugs.
4. Those patients having a mixed malarial infection.
5. Pregnant women.
6. On serology, if any patient was found positive for hepatitis.

About 5 ml of venous blood was drawn from an antecubital vein through disposable syringe and fractionated as:

One ml blood was transferred into a tube containing citrate for prothrombin time, while another 1 ml blood was transferred into other tube containing EDTA for hemoglobin and hematocrit estimation. A small drop of blood was also placed on the strip of glucometer (Optium, Abbott) to check the random blood glucose level.

The present study was conducted on Plasmodium falciparum positive and having illness of 1 to 7 days.

Group I: Plasmodium falciparum positive and having illness of 1 to 7 days.

Group II: Plasmodium falciparum positive and having illness of 8 to 20 days.

Group III: Plasmodium vivax positive and having illness of 1 to 20 days.

Blood hemoglobin level was estimated by Cynamet Hemoglobin method, Haematocrit values were estimated by Microhaematocrit method, Serum Bilirubin (Total, Direct and Indirect) by Jendrassik Groff Method, Serum glutamate pyruvate transaminase, Serum glutamate oxaloacetate transaminase and alkaline phosphatase were estimated by enzymatic method. The biochemical parameters were compared between all the three groups. The correlation co-efficient between bilirubin and liver enzymes was observed by applying regression analysis using SPSS version 10, taking P value significant when less than 0.05.

**Results**

Total number of patients included in the study was 72. Patients of all age groups and both genders were included in the study. They were divided into three groups.

**Group I** (Plasmodium falciparum +ve, having illness of 1 to 7 days):

Total number of patients in this group was 24. Their age range was from 3-56 years with the mean age 25.2±3.33, out of which 14 (58.3%) were males and 10 (41.7%) were female. On peripheral blood smear examination gametocytes were observed in 2 (8.3%) cases and rings or trophozoites were present in 22 (91.7%) cases. The liver was palpable in 7 (29.2%) patients, while spleen was palpable in) in 3 (12.5%) patients.
Group II: (Including 24 Plasmodium falciparum positive cases with duration of illness of 8-20 days. Their age ranges from 5-50 years. The mean age was 24.7 ±2.71 SD. 16 (66.7%) were males and 8 (33.3%) were females. On peripheral blood film examination; 4 (16.7%) cases showed gametocyte while rings or trophozoites were observed in 20 (83.3%) patients. Liver was palpable in 19 (79.2%), subcostally and spleen was palpable in 20 (83.3%) cases.

Group III: (24 Plasmodium vivax +ve cases, having illness of 1 to 20 days). Age ranges from 3.5 years to 50 years with the mean age of 23.3±2.75SD years. 15 (62.5%) were males and 9 (37.5%) cases were females. Peripheral blood film examination showed presence of gametocytes in 6 (25%) cases and rings or trophozoite forms in 18 (75%) cases. Liver was palpable in 2 (8.3%) cases and spleen was palpable in 4 (16.7%) cases. Statistically highly significant difference in liver and spleen size with a P <0.001 was noted. Table 1 shows the difference in the results of mean values of Bilirubin – Total, Direct, Indirect, SGPT, SGOT and Alkaline Phosphatase.

Table 1: Comparison of Biochemical Parameters between Groups of Plasmodium Falciparum and Plasmodium Vivax Malaria

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>P. Falciparum</th>
<th>P. vivax</th>
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<tbody>
<tr>
<td></td>
<td>Group I (n=24)</td>
<td>Group II (n=24)</td>
</tr>
<tr>
<td>Range(Mean ± s.e.m)</td>
<td>Range(Mean ± s.e.m)</td>
<td>Range(Mean ± s.e.m)</td>
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<tr>
<td>Bilirubin – Total (mg/dl)</td>
<td>0.63 - 2.89 (1.4±0.13)</td>
<td>3.34 – 19.12 (7.1 ± 0.83)</td>
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<tr>
<td>Direct (mg/dl)</td>
<td>0.20 - 1.60(0.6 ±0.07)</td>
<td>1.20 – 10.56 (3.1 ± 0.49)</td>
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<tr>
<td>Indirect (mg/dl)</td>
<td>0.41 - 1.87(0.8± 0.08)</td>
<td>0.32 – 8.56(4.0 ± 0.43)</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>15 - 46 (27.5 ± 1.59)</td>
<td>34 – 210(67.9 ± 7.72)</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>20 - 38 (27.2 ± 1.19)</td>
<td>30 – 100(52.1 ± 4.21)</td>
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<tr>
<td>Alkaline Phosphatase (U/L)</td>
<td>98 - 340 (248 ± 11.6)</td>
<td>260 – 480 (352 ± 10.7)</td>
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</tbody>
</table>

*** P <0.001.

Table 2: Correlation Coefficient (r) among bilirubin and biochemical parameters

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group I P. falciparum &lt;7 days</th>
<th>Group II P. falciparum &gt;7 days</th>
<th>Group III P. vivax 1 to 20 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>- 0.13</td>
<td>- 0.59*</td>
<td>- 0.46</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>0.50*</td>
<td>0.88**</td>
<td>0.82**</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>0.24</td>
<td>0.75*</td>
<td>0.63*</td>
</tr>
<tr>
<td>Alkaline Phosphatase (U/L)</td>
<td>0.54*</td>
<td>0.62*</td>
<td>0.69*</td>
</tr>
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Statistically significant * P<0.05 ** P<0.01
Discussion

Among four species of malaria, Plasmodium vivax is the most commonly observed species in Pakistan followed by Plasmodium falciparum. Malaria involves the liver where infective sporozoites invade and multiply in the hepatocytes and in erythrocytic stage the merozoites cause the destruction of infected red blood cells. Molyneux et al suggested that Jaundice, which may be deep, is usually accompanied by only moderate elevation of hepatic enzymes and results more from hemolysis than from the hepatic damage. This study was performed to evaluate the acute hepatic damage by falciparum malaria. In addition Plasmodium vivax infected cases were also included for comparative analysis. The history showed that the subjects were acute cases of malaria rather than the chronic cases.

In acute malaria, both hepatic and splenic enlargements are due to hyperplasia of the reticuloendothelial cells. In this study the liver was enlarged in 28 (38.9%) patients ranging from 0.5 cm to 4.0 cm, while spleen was found palpable in 27 (37.5%) of patients ranging from 0.5 cm to 3.0 cm. Clinically pallor reflects low hemoglobin. In malaria, low hemoglobin may result from acute hemolysis or destruction of both infected and uninfected red blood cells, dyserythropoiesis and with nutritional deficiencies. Table 2 shows hemoglobin level with the mean value of 9.2 g/dl and 9.5 g/dl group I and group II respectively showed an anemic picture (<10 g/dl). These findings are in agreement to Bhalli and Samiullah but do not match with the mean hemoglobin level of 13.78 g/dl as reported by Nadeem et al. The mean hemoglobin value has excellent positive correlation with haematocrit in both groups. Anemia and hyperbilirubinemia (mainly unconjugated) are common features of falciparum malaria and it is attributed to hemolysis of both parasitized and nonparasitized erythrocytes. In this study hyperbilirubinemia was present in 64.3% of cases when hemoglobin level was correlated with bilirubin, it showed a significant negative correlation reflecting mild to moderate anemia along with unconjugated type of hyperbilirubinemia. Coagulation abnormalities are not uncommon in falciparum infection and impairment of coagulation system is related to severity of disease. The increase serum level of hepatic enzymes, transaminases (SGOT and SGPT) and alkaline phosphatase are the markers of liver damage. SGPT (ALT) is a specific enzyme of liver. In this study SGPT and SGOT were elevated in the group II. These enzymes were not elevated in Plasmodium vivax positive group III patients, showing difference in mean values statistically highly significant (P<0.001). These results match with the results of Premaratna. Similarly, SGPT and SGOT, both showed a negative good correlation coefficient r= -0.63 and r= -0.53, respectively (P<0.01) versus hemoglobin, while SGPT showed an excellent positive correlation coefficient (r=0.88 and r=0.82) in group II and group III respectively (P<0.01) related to bilirubin. The rise in serum alkaline phosphatase shows leakage of this enzyme on the membranes of hepatic drainage system makes it a potentially important biomarker for the assessment of integrity of this system during malaria infection. The results of difference in mean value of this enzyme are highly significant when group I and group II of Plasmodium falciparum infected patients were compared. This finding correlates with the results of Garba and Ubom.

Conclusion and Recommendation
The results of our study provide valuable information and association between hepatic biochemical derangements in Falciparum malarial patients. This study was performed on a small sample size and provides baseline information in these subjects, so we recommend that same type of study should be carried out on large sample size and liver function tests should be performed along with early diagnosis of Plasmodium falciparum malarial infections in order to prevent complications and to reduce mortality.

References