ORIGINAL RESEARCH ARTICLE

Serum enzyme levels as biomarkers in malaria

Dr T. Anil Kumar1, Dr B. Lakshmi Keerthana2

1,2Senior Resident, Department of Biochemistry, Rangaraya Medical College, Kakinada, Andhra Pradesh, India

*Corresponding author
Dr T. Anil Kumar
Email: anil.mbbs@yahoo.co.in

Abstract: Malaria is one of the most serious diseases especially of the tropical world. Pathophysiological process involves both centrilobular liver damage and red blood cells destruction. As a result, enzymes present in these cells are released into circulation. The present study was undertaken to determine the diagnostic value of serum Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine transaminase (ALT) - Liver function biomarkers and LDH, Acid Phosphatase (ACP) - RBC function biomarkers, in patients with acute uncomplicated malarial infection. The study group consisted of 130 subjects, of age groups 20-50 years and of both sexes. Of these, 50 had vivax malaria, 30 had falciparum malaria and 50 were healthy individuals as controls. They were confirmed to be positive for Plasmodium Vivax/ Falciparum parasite by microscopical examination of Giemsa stained blood slides. Results showed that the serum levels of all enzymes are elevated in both vivax & falciparum malaria patients when compared to controls. The increase in ACP, ALP, ALT, AST is statistically significant (p<0.05) while increase in LDH is statistically highly significant (p<0.001) in vivax malarial patients. Also, the increase in ALP, ALT, AST is statistically significant (p<0.05) while increase in ACP, LDH is statistically highly significant (p<0.001). Hence, serum enzymes can be utilized extensively for both definitive and supportive diagnosis of Malaria in clinical scenario.

Keywords: Malaria, vivax, falciparum, serum enzymes

INTRODUCTION

Malaria is one of the most serious diseases especially of the tropical world. Pathophysiological process involves both centrilobular liver damage and red blood cells destruction. As a result, enzymes present in these cells are released into circulation.

The present study was conducted on both Plasmodium vivax & falciparum positive malarial patients to determine the diagnostic value of-
(A) Serum LDH, AST, ALT, ALP activity which are liver function biomarkers.
(B) Serum LDH, ACP activity which are RBC function biomarkers.

MATERIALS & METHODS:

Study Centre & Period:
This research was conducted at clinical laboratory, Department of Biochemistry, Andhra Medical College between September 2013 and June 2015.

Subjects Selection:
Patient selection was done by simple random sampling of individuals presenting at King George Hospital, Visakhapatnam & Dolphin Diagnostics with a history of fever with chills & rigor within a period of 1-8 days. They were subsequently confirmed to be positive for Plasmodium vivax/falciparum parasite by microscopical examination of Giemsa stained thin blood slides.

Inclusion criteria:
All the symptomatic patients whose diagnosis was confirmed by Giemsa stained slides were included in the study. Detailed history was taken and physical examination was performed.

Exclusion criteria:
(1) Patients who were having fever with or without rigors but were negative for malaria parasite.
(2) Acquired Immune Deficiency Syndrome
(3) Liver Cirrhosis
(4) Hepatitis
(5) Alcoholism
(6) Kidney Disorders
(7) Patients on self medication with any anti-malarial drugs prior to presentation.
(8) Pregnant Woman
(9) Malignancy.
Study Pattern:
The study group consisted of 130 subjects of age group 20-50 years and of both sexes. Of these, 50 had vivax malaria, 30 had falciparum malaria and 50 were healthy individuals as controls. Consent was sought and obtained from all the 130 subjects.

Specimen Collection:
Venous blood (2ml) was obtained from each of the subjects by vein puncture of the ante cubital vein using a 21 gauge hypodermic sterile needle and syringe. The blood samples were then transferred into clean sterile centrifuge tubes and allowed to clot. Each clotted sample was centrifuged at 3000 rpm for 3min at room temperature to obtain the serum. The serum was removed from the mixture using a micropipette and transferred to appendroff tubes. The biochemical assay was carried out within 24hrs of collection.

Assay of Enzymes:
AST, ALT, LDH, ALP levels were assayed on Autoanalyzer MICRO-LAB 300 and Semiautoanalyzer while ACP was assayed on Semiautoanalyzer by Kinetic Method. All the reagents were supplied as commercial kits.

Statistical Analysis:
The data obtained were analyzed using Student’s t-test and level of significance was set at p<0.05. p<0.001 was considered as highly significant. All results were expressed as Mean ± SD.

RESULTS & OBSERVATIONS:
The results obtained from the investigation into the changes in liver and RBC biomarkers in malaria patients and controls are shown in Table 1 (vivax malaria patients vs. controls) and Table 2 (falciparum malaria patients vs. controls).

Table 1: Vivax malaria patients vs. Controls

<table>
<thead>
<tr>
<th>S.NO</th>
<th>ENZYME ACTIVITY</th>
<th>CONTROLS(IU/L)</th>
<th>CASES(IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acid Phosphatase</td>
<td>2.2 ± 0.6</td>
<td>6.6 ± 0.3*</td>
</tr>
<tr>
<td>2</td>
<td>Alkaline Phosphatase</td>
<td>56.0 ± 1.6</td>
<td>119.8 ± 8.8*</td>
</tr>
<tr>
<td>3</td>
<td>Aspartate Transaminase</td>
<td>17.5 ± 0.9</td>
<td>46.0 ± 2.5*</td>
</tr>
<tr>
<td>4</td>
<td>Alanine Transaminase</td>
<td>18.2 ± 1.3</td>
<td>47.3 ± 1.5*</td>
</tr>
<tr>
<td>5</td>
<td>Lactate Dehydrogenase</td>
<td>247.1 ± 19</td>
<td>789.4 ± 35**</td>
</tr>
</tbody>
</table>

All values are expressed as Mean ± S.D. *p value <0.05, ** p value <0.001

From the above data (Table 1) it was observed that enzyme ACP, AST, ALT, ALP activity increases significantly (p<0.05) and increase in LDH is highly significant (p<0.001) in vivax malaria patients as compared to the control subjects.

Table 2: Falciparum malaria patients vs. controls

<table>
<thead>
<tr>
<th>S.NO</th>
<th>ENZYME ACTIVITY</th>
<th>CONTROLS(IU/L)</th>
<th>CASES(IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acid Phosphatase</td>
<td>2.2 ± 0.6</td>
<td>8.8 ± 0.8**</td>
</tr>
<tr>
<td>2</td>
<td>Alkaline Phosphatase</td>
<td>56.0 ± 1.6</td>
<td>146.6 ± 8.4*</td>
</tr>
<tr>
<td>3</td>
<td>Aspartate Transaminase</td>
<td>17.5 ± 0.9</td>
<td>57.2 ± 1.8*</td>
</tr>
<tr>
<td>4</td>
<td>Alanine Transaminase</td>
<td>18.2 ± 1.3</td>
<td>64.4 ± 2.6*</td>
</tr>
<tr>
<td>5</td>
<td>Lactate Dehydrogenase</td>
<td>247.1 ± 19</td>
<td>897.6 ± 36.3**</td>
</tr>
</tbody>
</table>

All values are expressed as Mean ± S.D. * p value< 0.05, ** p value < 0.001

From the above data (Table 2) it was observed that enzyme AST, ALT, ALP activity increases significantly (p<0.05) and increase in ACP, LDH is highly significant (p<0.001) in falciparum malaria patients as compared to control subjects.

DISCUSSION:
In the present study, it was observed that the enzyme levels were elevated in both vivax and falciparum malaria patients when compared with controls. The observed increase in serum liver enzymes (AST, ALT, and ALP) could be due to leakage from hepatic cells that were injured by the auto-immune progress and/or by abnormal cell activation induced by the parasites. The findings of present study correlate well with findings of previous studies of Guthrow et al.; [8], MA Pir et al.; in vivax malaria [9], Sudha Jha et al. [10]. Hence, there is a rationale in using these biochemical markers in malaria.

Maegraith postulated that hepatic dysfunction in malaria involve a synergy between local circulatory failure and centrilobular cellular damage. Since LDH is found in clinically significant amounts in both liver and RBC’s, the observed increase in serum LDH activity can be accounted by this synergy i.e. hepatic activity of invading sporozoites leading to centrilobular liver damage and the destruction of host...
RBC consequent to erythrocytic merogony [11]. This finding has important implication because it highlights the potential of using serum LDH activity as an index in monitoring plasmodium malaria infection, particularly when all other possible causes of increased serum LDH levels have been eliminated. The findings of present study correlate well with findings of previous studies of I H Garba et al.; [12], M A Pir et al.;[9].

Red blood cells contain an excess quantity of ACP. The cell membrane plays a central role in the growth and propagation of the malaria parasite. The invasion of the human erythrocytes by the malaria parasite is during the phase of erythrocytic schizogony. The RBC’s are attacked by the pre erythrocytic cryptomerozoites or the later exo erythrocytic micro-meta crypto merozoites. After sometime the cell membrane of the totally exhausted corpuscle bursts and the merozoites, toxic products and the enzymes like ACP are released into the blood plasma in the blood [7]. Also, a number of reactive oxygen species are generated during this host parasite interaction. Increase in reactive oxygen species and decrease in antioxidants are reported in malaria patients [13, 14]. Alteration in major antioxidants and peroxide lysis of erythrocytes may result in release of enzymes like ACP. The findings of present study correlate well with findings of previous studies of Garba et al.; [15], Benedicita D’Souza et al.; [16], Gulab kanwar et al.; [17]. Thus increase in serum ACP levels in malaria patients could serve as a marker for hemolysis indicating the active stage of the disease.

CONCLUSION:

The results of the present research provide valuable information and association between hepatic and RBC biochemical derangements in vivax/falciparum malaria patients. Although the diagnosis of malaria rest on the demonstration of asexual forms of parasite in stained peripheral blood smear, sometimes, no parasite can be found, even in severe infection. This may be explained by partial antimalarial treatment or by sequestration of parasitized cells in deep vascular beds [18]. Similarly, interpretation of blood smear requires some experience because artifacts are common. In such cases indirect evidences such as serum enzyme levels have a definitive role and can be used specifically for the diagnosis of malaria, especially when all other causes of raised enzyme levels are eliminated. Early antimalarial treatment can be started based on these findings in order to prevent complications and to reduce mortality.

These enzyme markers have also a supportive role in that; these can be used along with other routine tests for the diagnosis of malaria, to know the extent of hemolysis and liver damage and thus the severity of the disease. Finally, we conclude that the enzymes can be used as biomarkers in the diagnosis of malaria and has both definitive and supportive role, thus can be used extensively in the clinical scenario.

REFERENCES:

16. Benedicita D’Souza, Rajeevalochana Farthasarathy, Sriekantha, Vivian D’Souza;
