STUDY OF LACTATE DEHYDROGENASE (LDH) AND OTHER BIOCHEMICAL PARAMETERS IN PLASMODIUM FALCIPARUM MALARIA IN TERTIARY CARE CENTER ODISHA

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Abstract
Background: The World Health Organization estimates that plasmodium falciparum malaria causes 500 million clinical episodes yearly, over one million of which result in death. The intracellular enzyme lactate dehydrogenase (LDH) is found in a wide range of organisms. Hepatic parenchyma damage and LDH release are caused by Plasmodium falciparum infection. Aim: To study the role of Lactate dehydrogenase as a potential biochemical marker in Plasmodium falciparum malaria and also to assess its role and other biochemical parameters with severity of malaria. Materials and Methods: From September 2012 to October 2015, the case control study was carried out in the Biochemistry Department at SCB Medical College & Hospital, Cuttack, on 80 patients with complicated malaria, 80 patients with simple malaria, and 80 participants in the control group. Data were gathered using a thorough history and laboratory results. Result: The mean serum LDH level was significantly high in complicated malaria group (1778 ± 221 U/L) as compared to uncomplicated malaria (777 ± 68.3 U/L) & control group (399 ± 71.1 U/L). In both the uncomplicated & complicated malaria group serum LDH level was significantly higher than the control group. (p-value<0.001). Other biochemical parameters like serum AST, ALT, ALP, Urea & Creatinine level was significantly high in complicated malaria group as compared to uncomplicated and control malaria group. Conclusion: A potentially useful enzyme marker of falciparum malaria infection is serum LDH activity.

INTRODUCTION
A variety of clinical syndromes, including malaria, are brought on by protozoan parasites where mosquitoes transmit parasites from one human host to another. These eukaryotic parasitic organisms are single-celled members of the Plasmodium genus. Humans are infected by five species of plasmodium, which significantly increase morbidity. Most severe infections and deaths are caused by Plasmodium falciparum. This parasite’s life cycle in the human host occurs in red blood cells and also in the parenchyma of liver cells, both of which cause centriflobular liver damage and the death of the host’s red blood cells as a result of erythrocytic merogony. At a hematocrit of 1.5% and parasitemia >0.4%, lactate dehydrogenase activity is visible, and it increases with parasitemia. Trophozoites and schizonts predominate between 36 to 48 hours and that is when lactate dehydrogenase activity is typically at its peak. The red blood cells, liver, heart, skeletal muscles, and kidneys all have large levels of LDH, showing that it is an actual intracellular enzyme. Elevated total serum LDH activity is frequently used as a diagnostic marker for diseases such as nephroblastoma, neuroblastoma, small cell lung cancer, and liver, heart, and red blood cell malfunction. The LDH enzyme is the last enzyme in the anaerobic glycolysis pathway, which converts glucose from the host RBC into lactate. Severe falciparum malaria also causes an increase in serum LDH levels. LDH is released into the bloodstream as a result of red blood cell destruction and hepatic parenchymal cell injury, and serum LDH activity is used as an index in the monitoring of acute P. falciparum malaria infection. There is a correlation between parasitemia levels and serum LDH activity.
MATERIALS AND METHODS

The case control research was carried out between 2012 to 2015 at SCB Medical College & Hospital, Cuttack (Odisha), in department of Biochemistry along with the department of medicine, after taking institutional ethical committee approval and patient’s informed consent.

Selection of Cases

The study group comprised of 240 subjects. The group was divided into three subgroups viz., normal controls without malaria (80 subjects), uncomplicated plasmodium falciparum malaria (80 cases) & complicated plasmodium falciparum malaria (80 cases). Complicated plasmodium falciparum malaria patients were identified as per WHO 2000 guidelines. All of the patients were interviewed using a pre-designed proforma after receiving informed consent.

Inclusion Criteria

All diagnosed plasmodium falciparum malaria patients within age 15-65 years.

Exclusion Criteria

1. Age of patients <15 & >65 years.
2. All pregnant women with P. falciparum malaria.
3. Known cases of malignancy, Renal & Alcoholic liver disease.
4. Prior to presentation, the patient was self-medicating with any anti-malarial medications.
5. Haemolytic Anaemia due to other than plasmodium falciparum malaria.

Sample Collection and Laboratory Analysis

The diagnosis of plasmodium falciparum malaria was made by Giemsa stained peripheral blood smear and verified by OptiMAL (pLDH) quick diagnostic kits after receiving written informed consent from the study patients. After confirming plasmodium falciparum malaria cases, five milliliter of venous blood was collected from subjects. 2ml in vials containing 0.05 ml of EDTA as anticoagulant for the study patient's hematological cell counter (Mindray BD-300 plus) & 3 ml in plain vial for LDH & other biochemical parameters like Hb, RBC, TLC & RBC indices were measured by using Automated hematology cell counter (Mindray BD-300 plus) & 3 ml in plain vial for LDH & other biochemical parameters like total bilirubin, Aspartateaminotransferase(ALT), Alanineaminotransferase(ALT), alkalinephosphatase(ALP), urea, creatinine, random blood glucose, electrolytes was estimated by Fully-Automated Clinical chemistry Analyzer model TBA-120FR (Agappe).

Following tests, investigation reports were examined by the biochemistry department.

Statistical Analysis

The data were shown as mean and standard deviation (SD). The data that were observed were examined using an unpaired Student’s t-test. A p value of less than 0.005 was deemed statistically significant, while a p value of less than 0.001 was deemed extremely significant. SPSS version 13 was used to analyse the data. Graphs and tables were created with Microsoft Word.

RESULTS

This study included 80 patients of uncomplicated malaria, 80 patients of complicated malaria taken as cases and 80 were controls. The mean age for control was 30.1 years, uncomplicated malaria group was 44.5 years and complicated malaria group was 36.3 years. Age groups of 25-35 years were most affected from complicated as well as uncomplicated malaria. [Table 1]

Males were more affected in comparison to female patients in both complicated and uncomplicated malaria groups. Out of 240 subjects, 55% (132) were males and 45% (108) females. 42% females suffered from complicated malaria whereas 49% females were affected with uncomplicated malaria. In males 58% had complicated malaria whereas 51% were suffering from uncomplicated malaria [Table 2].

[Table 3] shows the distribution of hemoglobin & biochemical parameters in control, uncomplicated & complicated malaria group. Mean Hemoglobin was significantly low in complicated malaria (5.6±0.50), compared to uncomplicated malaria (10.9±0.60) and control groups (13.2±1.01).

In uncomplicated malaria the mean serum LDH value was 777.0 ± 68.30, serum LDH levels in complicated malaria was 1778.0 ± 221.01 which was significantly higher than controls(399 ±71.1).

Mean serum creatinine, AST, ALP were significantly elevated in uncomplicated and complicated malaria in comparison to control group (p value <0.001). Mean serum total bilirubin, urea and ALT were significantly increased in cases in comparison to control group (p value <0.005).

Random blood glucose was significantly decreased in complicated (61±7.70) and uncomplicated malaria(97.20 ± 24.10) cases compared to control group (118±17.1). No significant difference in total leukocyte count, serum sodium and serum potassium was seen between malaria cases as compared to control group.

Table 1: Distribution of Age in control, uncomplicated & complicated malaria group (n = 240).

<table>
<thead>
<tr>
<th>Age (yrs.)</th>
<th>Control(n=80)</th>
<th>Uncomplicated Malaria Group (n=80)</th>
<th>Complicated Malaria Group (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-25</td>
<td>22 (27.50%)</td>
<td>08 (10%)</td>
<td>17 (21.25%)</td>
</tr>
<tr>
<td>25-35</td>
<td>23 (33.75%)</td>
<td>23 (31.25%)</td>
<td>27 (33.75%)</td>
</tr>
<tr>
<td>36-45</td>
<td>19 (23.75%)</td>
<td>21 (26.25%)</td>
<td>16 (20%)</td>
</tr>
<tr>
<td>46-55</td>
<td>04 (5%)</td>
<td>15 (18.75%)</td>
<td>14 (17.5%)</td>
</tr>
<tr>
<td>56-65</td>
<td>08 (10%)</td>
<td>11 (13.75%)</td>
<td>06 (7.5%)</td>
</tr>
</tbody>
</table>
Table 2: Distribution of Gender in control, uncomplicated & complicated malaria group (n = 240).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Male</th>
<th>Female</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>44 (55%)</td>
<td>36 (45%)</td>
<td>80</td>
</tr>
<tr>
<td>Uncomplicated malaria group</td>
<td>41 (51%)</td>
<td>39 (49%)</td>
<td>80</td>
</tr>
<tr>
<td>Complicated malaria group</td>
<td>47 (58%)</td>
<td>33 (42%)</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>132 (55%)</td>
<td>108 (45%)</td>
<td>240</td>
</tr>
</tbody>
</table>

Table 3: Distribution of hemoglobin & biochemical parameters in control, uncomplicated & complicated malaria group (n = 240).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control(n=240)</th>
<th>Uncomplicated Malaria (n=80)</th>
<th>Complicated Malaria (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (gm %)</td>
<td>13.2±1.01</td>
<td>10.9±0.60*</td>
<td>5.6±0.50*</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dl)</td>
<td>0.74±0.16</td>
<td>1.25±0.40**</td>
<td>5.04±1.51**</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>32.7±7.4</td>
<td>41.03±7.75*</td>
<td>325±22.0*</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>38±7.8</td>
<td>54.28±8.46**</td>
<td>337±20.1**</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>64.8±7.34</td>
<td>118.8±16.41*</td>
<td>359±35.5*</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>23±6.7</td>
<td>43.6±12.7**</td>
<td>218±24.6**</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.81±0.27</td>
<td>1.29±0.42**</td>
<td>4.59±1.4*</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>399±71.1</td>
<td>777±68.3*</td>
<td>1778±221*</td>
</tr>
<tr>
<td>Random blood glucose (mg/dl)</td>
<td>118 ±17.1</td>
<td>97.2 ±24.1*</td>
<td>61 ±7.7*</td>
</tr>
</tbody>
</table>

*Statistically significant (p<0.001); ** Statistically significant (p<0.005)

DISCUSSION

A total of 80 patients of uncomplicated malaria and 80 patients of complicated malaria were enrolled. Age and sex matched 80 persons were taken as control. Mean age for controls was 30.1 years, uncomplicated malaria group was 44.5 years and complicated malaria group was 36.3 years. Among all, 25 to 35 years age groups were more affected. In this study 55% were males and female were 45%. However, because the study was only conducted in a tertiary care hospital, these statistics do not accurately reflect the incidence and gender distribution of disease in the population. The WHO Malaria Report 2009 indicates that men are more susceptible to malaria than women since they are exposed to more mosquito bites due to their occupation.

In comparison to groups with uncomplicated malaria and controls, complicated malaria patients had considerably lower haemoglobin levels. This is because the malarial parasites in human go through a developmental stage in the parenchyma cells of the liver and in red blood cells, and are transported to all the organs by the circulating blood. Later on, there is a rise in the intravascular destruction of red blood cells as well as their sequestration in the spleen, other circulations, and loss of deformability, which results in hemolysis. So anaemia is a common symptom of severe malaria. In addition, it was noted by Clark IA et al (1988), and Wenish C et al (1995) that dyserythropoiesis played a significant role in anaemia of malarial origin.

When comparing complicated malaria to controls and the uncomplicated group, the observed levels of random blood glucose were considerably lower in the complicated group. In complicated malaria cases, lower blood glucose levels are typically caused by the parasites’ excessive use of glucose. The parasite consumes glucose from the host to develop and multiply quickly throughout the intra erythrocytic phases of its life cycle. In the erythrocytic stage, P. falciparum primarily uses anaerobic glycolysis to produce energy, with the regeneration of NAD+ occurring through the conversion of pyruvate to lactate. When compared to the uncomplicated malaria and control groups, it was found that biochemical parameters like serum urea, creatinine, total bilirubin, AST, ALT, and ALP were significantly higher in the complicated malaria group. The same kind of outcome was previously noted by Kocher DK et al in 2003. Additionally, Mishra SK et al (1999) demonstrated that jaundice caused by falciparum infection was associated with a considerable elevation in serum liver enzyme levels. Our findings are consistent with those reported in other investigations.

In comparison to control group, it was seen that LDH was significantly greater in both complicated and uncomplicated malaria. Red blood cells are hemolysed in both complicated and uncomplicated malaria, thereby increasing serum LDH levels. In one study LDH was strongly associated with markers normally elevated in either hemolysis or liver disease. A potentially useful enzyme marker of p. falciparum malaria infection is serum LDH activity. Therefore, LDH seems to have a lot of potential as a biomarker of a hemolytic mechanism of vascular pathophysiology in patients with p. falciparum malaria and an excellent prognostic indicator.

CONCLUSION

The immediate liver damage and red blood cell death, which are both abundant sources of LDH, will be followed by the release of LDH into the circulation. The potential for employing serum LDH activity as an index in the surveillance of acute P. falciparum malaria infection is highlighted by this study, which has significant ramifications, especially after all other probable reasons of elevated serum LDH levels have been ruled out.
Ethical approval: The Institutional Ethics Committee of SCB Medical College & Hospital, Cuttack (Odisha), India gave its approval for the study.

REFERENCES


