

Levels of Parasitaemia and Changes in Some Liver Enzymes among Malarial Infected Patients in Edo-Delta Region of Nigeria

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Abstract: Hepatic dysfunction is a common complication in malaria and this could be characterized by increase in liver enzyme activities. The activities of some liver enzymes: Serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were assayed in 60 individuals comprising both males and females, age between 10-60 years. Malarial parasite status and densities were also determined and correlated with the enzyme values Thirty (30) individuals presenting with acute uncomplicated falciparum malarial infection were selected as test subjects and 30 sex and age-matched apparently healthy individuals without the infection were included as control group. Malarial parasite count was by the Giemsa stain and the liver enzymes were assayed by standard methods. Results show that there was a positive relationship between the enzyme activities and the level of parasitaemia ($p < 0.05$). Changes in the AST (33.4 ± 16.4 IU/L), ALT (21.3 ± 14.4 IU/L) and ALP (87.4 ± 25.6 IU/L) activities for the infected subjects were higher compared with their non-infected counterparts (AST: 22.3 ± 7.4 IU/L, ALT: 15.2 ± 5.8 IU/L, ALP: 56.4 ± 16.7 IU/L). Evidence indicates a measure of compromise in hepatic integrity and this bears a positive correlation with the levels of parasitaemia. Post-treatment values should be obtained and compared in order to provide a detailed understanding for improved treatment.

Key words: ALT, AST, hepatic dysfunction, liver enzymes, Malaria, *P. falciparum*

INTRODUCTION

Malaria is one of the most devastating diseases of the world, particularly in topical countries (Shiff, 2002). The disease is caused by protozoan parasite of the genus; Plasmodium. Five species of the parasites can infect humans. *P. falciparum* is the most common cause (about 80%) of all malaria cases. It is also responsible for about 90% of deaths arising from malaria (Meddis *et al.*, 2001). *P. falciparum* has infected humans for over 50,000 years and may have been a human pathogen for the entire history of the species (Joy *et al.*, 2003).

Each year, there are 350-500 million cases of malaria (90% in Africa), killing between 1.5-2.7 million people, the majority of whom are young children in sub-Saharan Africa (Snow *et al.*, 2005). Malaria causes catastrophies such as maternal/infant death and abortion. Pregnant women are also especially vulnerable. Susceptible groups are children and adults who have lost or never acquired immunity (Eze and Mazeli, 2001).

Despite significant progress in the treatment of malaria, this disease has staged a huge comeback in large areas of the world, due to development of drug-resistant parasites (Najera, 2001). It precipitates such terribly mutilating afflictions (in children) as cancurum and has numerous complication such as anaemia, pulmonary

oedema, renal failure and coma, which may be fatal (Eze and Mazeli, 2001).

Epidemiological factors that make malaria endemic in the tropics include; climatic conditions (Relative humidity, altitudes, rainfall level, mean temperature between 18-29°C) and socio-economic factors. All these have effects on the availability of vectors which maintain the transmission of malaria (Burtler, 1996). Malaria can be transmitted by three known ways; vector transmission (Anderson *et al.*, 1981), blood transfusion (Strickland, 1999) and congenital transmission (Ezechukwu *et al.*, 2004).

The vector for malaria parasite is the female Anopheles mosquito (Cheesbrough, 1998). The malaria parasite interferes with 3 major organs in the body, namely: the brain, kidney and liver (Edington, 1967).

The invasion of the liver cells by malarial parasites can cause organ congestion, sinusoidal blockage and cellular inflammation (Jarique *et al.*, 2002). When these happen, it causes the parenchymal (transminases) and membraneous (alkaline phosphatase) enzymes of the liver to leak into the circulatory system leading to increased enzyme activity (Burtis *et al.*, 2001).

This study is to assess the correlation between the levels of parasitaemia in blood and changes in some liver enzymes: AST, ALT and ALP obtained from newly diagnosed cases of malarial infection yet to be treated.

MATERIALS AND METHODS

Study laboratory and period: The study was performed in the Malarial Research Laboratory, Department of Medical Biochemistry, Delta State University, Abraka, Nigeria, between May and November, 2009.

Subjects: Sixty (60) human subjects comprising of 30 *P. falciparum* malarial infected and 30 non-infected (control) subjects between 10-60 years were selected for the study. Selection and pre-qualification was done by simple random sampling of individuals presenting at the Faith Mediplex, Benin City, Edo State, Nigeria, University of Benin Teaching Hospital (UBTH), Ugbowo, Benin City and Delta State University Health Centre, Abraka, Nigeria with a history of fever, (temperature $\geq 37^{\circ}\text{C}$), headaches, loss of appetite, joint pains, vomiting and malaise for a period of 2-5 days. They were confirmed to be infected with the *falciparum* malarial parasite by microscopic examination of Giemsa stained thin blood slides. The 30 non-infected asymptomatic subjects were apparently healthy adults within the same Edo-Delta region, and were negative for malarial parasite.

Patients selection criteria: Patients whose case history showed a concomitant presentation with the following conditions; pregnancy, renal diseases, liver diseases including cirrhosis, hepatitis, obstructive jaundice, alcoholism, cancer, metabolic bone diseases, gastrointestinal tract infection, protein energy malnutrition, diabetes, heart failure, infectious mononucleosis and magnesium /vitamin D deficiencies, were excluded from the study. This is because these conditions are associated with significant changes in serum alkaline phosphatase, alanine and aspartate transaminases activities.

Similarly, patients on self-medication with any anti-malarial drug prior to presentation were also excluded from the study.

Sample collection and handling: Two specimen bottles were used for each subject; anticoagulant bottle containing K_2EDTA for malarial parasite count and plain, sterile bottle for serum enzymes assays.

Blood samples were collected by clean venepuncture from the antecubital fossa into already labelled clean, sterile bottles with undue pressure on either the arm or the plunger of the syringe. Samples in the K_2EDTA anticoagulant bottles were tested immediately for malarial parasite after staining the thick film with Giemsa stain, while those samples in the plain tubes were allowed to clot and then centrifuged at $3000 \times g$ for ten minutes at room temperature to obtain the sera. The sera samples were collected by aspiration using a pasture pipette into sterile bijoux bottles and stored frozen until required for analysis, which was done within 72 h.

Table 1: The liver enzyme activities at different levels of parasite density

| Level of parasitaemia | 1+(n = 25) | 2+(n = 5) | Nil (n = 30) |
|-----------------------|-------------------|-------------------|-------------------|
| ALT (IU/L) | 18.77 \pm 12.30 | 33.76 \pm 16.35 | 15.24 \pm 7.07 |
| AST (IU/L) | 32.30 \pm 16.40 | 39.74 \pm 17.54 | 22.33 \pm 6.78 |
| ALP (IU/L) | 86.61 \pm 43.50 | 91.36 \pm 43.89 | 55.90 \pm 16.19 |

Tabulated values are expressed as Mean \pm SD (Standard Deviation)

Malarial parasite density determination: The malarial parasite density was determined by examining a thick blood film stained with Giemsa stain (Cheesbrough, 1998) and the density was graded as follows: 1 parasite/field: Low density (+), 2-9 parasites / field: medium density (++), >20 parasites/field: High density (+++) (Cheesbrough, 1998)

Liver enzymes assays: Aspartate and alanine transaminases were assayed by colorimetric method (Reitman and Frankel, 1957) using Roche model Diagnostic Reflotron. Serum total alkaline phosphatase activity was assayed using the thymolphthalein monophosphate method (Bessey *et al.*, 1946).

Statistical analysis: The results were expressed as Mean \pm Standard Deviation. Data were analysed by the paired Student's t-test. $p < 0.05$ was considered significant.

RESULTS

The results obtained are shown in Table 1-3. Table 1 is the relationship between parasite density and liver enzymes' activities in serum.

Level of parasitaemia correlates positively with liver enzyme activities. Specifically 2+ degree of parasitaemia shows higher values of liver enzyme activities when compared with patients having 1+.

Overall, there were significant increases ($p < 0.05$) in the mean activity levels of ALT, AST and ALP for malarial parasite positive subjects when compared with the non-infected (control) subjects.

Table 2 shows the relationship between age and liver enzymes' activities in infected and non-infected subjects.

Among the non-infected (control) subjects, the liver enzyme activity values for age groups 10-29 years and 50-69 years were similar and lower than the values for those between 30-49 years. But for the malarial infected subjects, the liver enzyme activities correlate positively with age. As age group increased, enzyme activities also increased. Advancing age may worsen the effect of malarial infection on hepatic integrity.

Table 3 presents the gender difference in liver enzymes' activities for malarial infected and non-infected individuals.

Table 3 shows the enzyme levels of the male and female subjects with or without malarial infection. When the liver enzyme activity values for the male infected patients were compared with those for the female infected subjects, there were statistically significant increase in the

Table 2: Some Liver Enzyme activities of malarial infected and non-malarial infected subjects at different age groups

| Age (year) | Malarial infected | | | Non-malarial infected | | |
|------------|-------------------|-------------|--------------|-----------------------|------------|-------------|
| | ALT(IU/L) | AST (IU/L) | ALP(IU/L) | ALT(IU/L) | AST(IU/L) | ALP(IU/L) |
| 10-29 | 15.75±9.95 | 27.14±5.75 | 84.36±5.75 | 13.69±6.49 | 19.52±5.16 | 50.98±11.7 |
| 30-49 | 26.97±16.50 | 41.16±22.42 | 90.50±20.84 | 17.77±6.02 | 24.39±9.27 | 62.32±21.7 |
| 50-69 | 31.40±16.83 | 48.95±10.33 | 108.00±23.55 | 14.31±5.03 | 20.59±1.75 | 49.33±14.56 |

Tabulated values are expressed as Mean ± Standard Deviation (±S.D)

Table 3: The liver enzyme activities of malarial infected and non-malarial infected male and female subjects

| Level of parasitaemia | Malarial infected patients | | Non-malarial infected subjects | |
|-----------------------|----------------------------|-----------------|--------------------------------|-----------------|
| | Male (n = 15) | Female (n = 15) | Male (n = 15) | Female (n = 15) |
| ALT (IU/L) | 22.42±13.53 | 20.14±15.22 | 17.19±7.78 | 13.30±3.78 |
| AST (IU/L) | 38.93±17.43 | 28.14±14.03 | 23.66±7.65 | 21.01±7.22 |
| ALP (IU/L) | 108.20±29.37 | 66.60±24.77 | 60.65±19.63 | 51.23±14.90 |

Tabulated values are expressed as Mean ± Standard Deviation (±S.D)

AST and ALP mean values. For the non-malarial infected (control) subjects, there were no statistically significant gender different ($p>0.05$) in the liver enzymes (AST, ALT and ALP) activities.

DISCUSSION

Acute *P. falciparum* malarial infection is the leading cause of disability, particularly in our environment - the Niger Delta region of Nigeria. The pathogenesis of this parasite infection involves the liver stage where infected sporozoites invade and multiply in the hepatocytes and an erythrocyte stage where merozoites cause the destruction of the infected red blood cells prior to their differentiations into male and female gametocytes leading to significant alterations in host hepatocyte physiology and morphology.

From the present study, evidence shows that malarial infection can induce changes in liver enzyme activities. The result clearly indicates that there was a significant difference ($p<0.05$) in the activities of the liver enzymes (ALT, AST and ALP) in malarial parasite infected patients when compared with the non-malarial parasite infected (control) subjects. The normal healthy values for serum ALT, AST and ALP as estimated by the International Federation of Clinical Chemistry (IFCC) are in the reference ranges of 10-40, 8-20 and 38-94IU/L, respectively. The various enzyme activities increased with increase in malarial parasite density (Table 1). This observation demonstrates that the hepatic stage of the parasite's life cycle in its human host is accompanied by significant perturbation in the hepatocyte membrane leading to leakage of the liver enzymes into a general circulation. This finding correlates particularly with earlier reports by Maegraith (1981). Centribular damage is one of the factors involved in hepatic dysfunction in acute *falciparum* malarial infection, leading to hyperbilirubinaemia, which is a direct consequence of the impaired drainage capacity of the liver.

There was also a significant ($p<0.05$) higher enzyme activities (AST and ALP) in the infected male subjects

when compared with their female counterparts (Table 3). A previous study by Uzoegwu and Onourah (2003) reported a percentage malarial parasite infection rate of 51.1% in males and 41.4% in females, and this higher susceptibility of males to malarial parasite infection than females could be responsible for the higher enzyme activities observed in the infected male subjects.

Malarial infection confers a measure of hepatic compromise especially among the older male subjects, study suggests. However, anti malarial treatment and post treatment enzyme (ALT, AST and ALP) activities in serum need to be further investigated in order to provide more understanding of the changes in these hepatic enzyme biomarkers.

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