Liver Function Status of Malaria Patients in Benin-City, Nigeria

EC Onyeneke, EI Oikeh, A Obhakhan

ABSTRACT

This study was carried out to evaluate the liver function status of patients with malaria in different stages of parasitaemia. The study group consisted of patients attending the General Practice Clinic of the University of Benin Teaching Hospital, Benin City, with varying degrees of malaria parasitaemia. Blood samples were obtained from volunteers and assays of liver function parameters were carried out. The results showed significant elevation in the activities of alkaline phosphatase and aspartate aminotransferase ($p < 0.05$*) with increase in the degree of parasitaemia and was positively correlated (*$r = 0.268$ *and 0.228, respectively). An inverse relationship was observed for the activity of alanine aminotransferase and bilirubin (*$r = -0.114$ *and -0.358, respectively). Total protein levels reduced significantly from mild-to-moderate (*$p < 0.05$*) and then increased significantly (*$p < 0.05$*) in severe parasitaemia. A positive correlation (*$r = 0.032$*) between total protein level and malaria parasitaemia was observed. The results obtained at different stages of parasitaemia suggest some degree of liver dysfunction during malaria infection.*

Keywords: Aminotransferases, malaria, parasitaemia, total protein

Estado de la Función Hepática de los Pacientes con Malaria en la Ciudad de Benín, Nigeria

EC Onyeneke, EI Oikeh, A Obhakhan

RESUMEN

Este estudio se llevó a cabo para evaluar el estado de la función hepática de pacientes con malaria en diferentes etapas de la parasitemia. El grupo de estudio consistió en pacientes que asisten a la Clínica de Práctica General de la Universidad del Hospital Docente de Benín, Ciudad de Benín, con diversos grados de parasitemia por paludismo. Se obtuvieron muestras de sangre de voluntarios y se realizaron análisis de los parámetros de la función hepática. Los resultados mostraron una elevación significativa en las actividades de la fosfatasa alcalina y el aspartato aminotransferasa ($p < 0.05$*) con un aumento en el grado de parasitemia y fue correlacionada positivamente (*$r = 0.268$ *y 0.228, respectivamente). Se observó una relación inversa en la actividad de la alanina aminotransferasa y la bilirrubina (*$r = -0.114$ *y-0.358, respectivamente). Los niveles de proteína total se redujeron considerablemente de leve a moderado (*$p < 0.05$*) y luego aumentaron significativamente (*$p < 0.05$*) con una parasitemia severa. Se observó una correlación positiva (*$r = 0.032$*) entre el nivel de proteína total y la parasitemia por paludismo. Los resultados obtenidos en las diferentes etapas de parasitemia sugieren algún grado de disfunción hepática durante la infección de la malaria.*

Palabras claves: Aminotransferasas, parasitemia por paludismo, proteína total

West Indian Med J 2016; 65 (3): 492

INTRODUCTION

Malaria has been a perennial problem around the world. In 2008, an estimated 243 million incidence cases with 863 000 deaths were recorded world over. About 85% of these cases and 89% of deaths were recorded in Africa (1). Malaria occurs

From: Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin-City, Edo State, Nigeria.

Correspondence: Dr EI Oikeh, Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin-City, Edo State, Nigeria. E-mail: ehigbai.oikeh@uniben.edu

DOI: 10.7727/wimj.2014.127

mainly in tropical and subtropical areas of the world, particularly in Sub-saharan African and Southeast Asia (2, 3). In Nigeria, malaria is holoendemic, hence clinical cases of the disease are seen throughout the year. It is the commonest cause of outpatient hospital attendance in all age-groups in Nigeria (4).

Malaria is caused by the infection of red blood cells by the plasmodium parasite. *Plasmodium falciparum* (*P falciparum*) has been implicated in most malarial deaths (5). The parasites are usually inoculated into a human host during feeding by a female anopheles mosquito. Malaria may also be transmitted congenitally, that is, from mother-to-child during pregnancy (6) and during blood transfusion (7).

Three major organs affected by the malaria parasite are the brain, kidney and liver (8). The liver plays a major role in the pathology of malaria infection as it is the organ for the development and replication of the plasmodium sporozoites into merozoites that infect red blood cells. In the liver stage, infected sporozoites invade and multiply in the hepatocytes. In the erythrocyte stage, 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 (9).

Malaria alters the levels of blood biochemical indices. We have, in previous studies on patients with malaria, observed alterations in the plasma lipid levels and some plasma electrolyte levels (10–12). This study therefore intends to evaluate the liver function status of patients with malaria in different stages of parasitaemia infection to ascertain the severity of liver damage as the disease progresses.

MATERIALS AND METHODS

Chemicals

All chemicals used were of high analytical grade. Reagent kits used in the analysis were products of Randox Laboratories (United Kingdom). Other chemicals used were obtained from British Drug House, United Kingdom.

Study group

The study group consisted of patients attending the General Practice Clinic of the University of Benin Teaching Hospital, Benin City, Nigeria. Ethical approval was obtained from the Ethics Committee in the University of Benin Teaching Hospital and verbal informed consent was obtained from the patients before blood samples were collected.

Sample collection and preparation

5 mL of venous blood was collected by venipuncture from each volunteer into heparinized sample bottles. Serum was obtained by allowing blood samples to stand for an hour and then centrifuged at 3000 revolutions per minute for ten minutes. The supernatant serum was collected into plain sample bottles and used for assay of the required parameters. Samples not immediately assayed were stored in a refrigerator at 2 – 8 °C.

Classification of degree of parasitaemia

Plasmodium falciparum parasitaemia level was determined in blood films by Giemsa stain. Parasitaemia density was classified as described by Cheesbrough (13).

Biochemical analysis

Alkaline phosphatase (ALP) was determined according to the principle of Kochner and Moss (14) while alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the method of Reitman and Frankel (15). Total and direct bilirubin levels were determined using the method described by Jendrassik and Grof (16). Total protein was determined using the Biuret method.

Statistical analysis

The group mean ± SEM was calculated for each group and significant difference between means evaluated by analysis of variance (ANOVA). Post-test analysis was done using the Duncan multiple comparison tests. Values of $p < 0.05$ were considered as statistically significant. Regression graphs were also generated for correlation and regression analysis.

RESULTS

The results show general increase in some liver enzymes with increase in the level of parasitaemia. Mean values obtained for alkaline phosphatase activity increased significantly ($p < 0.05$) with increase in the degree of parasitaemia (mild: 92.60 ± 6.11, moderate: 98.39 ± 5.31 and severe: 103.96 ± 9.43 IU/L). Aspartate aminotransferase activity also increased steadily as parasitaemia increased (11.92 ± 2.23, 13.84 ± 1.99 and 15.50 ± 3.57, respectively). Alanine amino transferase activity increased significantly ($p < 0.05$) from mild-to-moderate parasitaemia (7.02 ± 1.20, 8.33 ± 0.87, respectively) and subsequently decreased in severe parasitaemia [6.18 ± 1.53, $p < 0.05$] (Table 1).

Serum total protein reduced significantly ($p < 0.05$) from mild-to-moderate parasitaemia (7.58 ± 0.33 to 6.86 ± 0.23) with significant increase from moderate-to-severe parasitaemia [6.68 ± 0.23 to 7.66 ± 0.26; $p < 0.05$] (Table 1).

Serum total bilirubin level decreased as parasitaemia increased [0.40 ± 0.05, 0.33 ± 0.02 and 0.32 ± 0.04 µmol/L, respectively] (Table 2). Reduction in bilirubin level from mild-to-moderate parasitaemia was significant ($p < 0.05$) while the reduction from moderate-to-severe parasitaemia was not significant ($p > 0.05$). However, there was steady reduction in conjugated bilirubin as parasitaemia increased [0.18 ± 0.02, 0.16 ± 0.02 and 0.12 ± 0.02 µmol/L; $p < 0.05$] (Table 2). The level of unconjugated bilirubin reduced significantly ($p < 0.05$) from mild-to-moderate parasitaemia (0.22 ± 0.05 and 0.17 ± 0.03) then increased significantly in severe parasitaemia (0.20 ± 0.02, $p < 0.05$).

Table 1: Serum levels of some liver enzymes and proteins in malaria patients

Degree of Parasitaemia	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	Total protein (g/L)
Control	90.82 ± 8.22^{a}	11.66 ± 1.52^{a}	6.94 ± 0.05^{a}	7.42 ± 0.14^{a}
+	92.60 ± 6.11^{a}	11.92 ± 2.23^{a}	7.02 ± 1.20^{a}	7.58 ± 0.33^{a}
++	98.39 ± 5.31^{b}	13.84 ± 1.99^{b}	8.33 ± 0.87^{b}	6.86 ± 0.23^{b}
+++	$103.96 \pm 9.43^{b,c}$	$15.50 \pm 3.57^{b,c}$	$6.18 \pm 1.53^{a,c}$	$7.66 \pm 0.26^{a,c}$

Values are expressed as Mean ± SEM.

Values in the same column with different alphabets differ significantly ($p < 0.05$)

+: Mild parasitaemia
++: Moderate parasitaemia
+++: Severe parasitaemia

Table 2: Serum levels of bilirubin in malaria patients

Degree of Parasitaemia	Total bilirubin (µmol/L)	Conjugated bilirubin (µmol/L)	Unconjugated bilirubin (µmol/L)
Control	0.43 ± 0.08^{a}	0.19 ± 0.01^{a}	0.20 ± 0.02^{a}
+	0.40 ± 0.05^{a}	0.18 ± 0.02^{a}	0.22 ± 0.05^{a}
++	0.33 ± 0.02^{b}	0.16 ± 0.02^{b}	$0.17 \pm 0.03^{b,c}$
+++	$0.32 \pm 0.04^{b,c}$	0.12 ± 0.02^{c}	0.20 ± 0.02^{a}

Values are expressed as Mean ± SEM.
Values in the same column with different alphabets differ significantly ($p < 0.05$)
+: Mild parasitaemia
++: Moderate parasitaemia
+++: Severe parasitaemia

DISCUSSION

In recent times, hepatocyte dysfunction has been recorded in some patients with malaria (17, 18). Malaria parasites avoid the immune system when they move from the liver to the red blood cells. These parasites head to the liver after arriving in a human body, changes into a new form that can infect red blood cells and begin to reproduce. The parasites kill the liver cell they occupy and make it detach from its neighbour (19).

Serum levels of transaminases as well as total and conjugated bilirubin give insights into the integrity and functional status of hepatocytes (20). Malaria-induced hepatocyte dysfunction may manifest in the form of elevated serum bilirubin, liver enzymes and hepatomegaly; serum albumin level may also drop (21).

Significant elevation in liver transaminases was observed in this study. Liver transaminases levels were observed to be higher in patients with malaria than in the controls. This agrees with the findings of Maduka *et al* (22) and Uzuegbu and Emeka (19). Liver transaminase levels also increased significantly as malaria severity progressed from mild to severe. According to Kochar *et al* (17), liver enzymes are elevated 2–3 times the normal and may be much beyond this level. This sharp elevation in liver transaminase activity has also been reported in several studies (22–24). We however, did not observe elevations in liver transaminases in the magnitude of 2–3 times normal values as reported by these authors. Our results agree with those of Ogbadoyi and Tsado (25) who also did not report the up to three times increase in liver transaminase activities. The increase in liver enzyme activity can be attributed to destruction of liver parenchyma by the malaria parasite leading to leakage of liver enzymes into general circulation (22).

Elevated levels of ALP are usually indicative of liver dysfunction. Our results agree in part with earlier works of Jarike *et al* (24) and Maduka *et al* (22), who reported increase in ALP activity as parasitaemia increased. Increased serum alkaline phosphatase activity indicates that the liver stage of *P falciparum* malaria infection is accompanied by a perturbation of the host hepatocytes drainage pathways and damage to hepatocytes membrane leading to leakage of the enzymes out of the liver cells. This may make ALP a potentially important biomarker for accessing the integrity of the hepatic drainage system during malaria infection (26).

Several researchers have evaluated protein levels in patients with malaria differing conclusions. Alumanah *et al* (11), observed no significant change in serum protein levels between patients with malaria and control. Adebisi *et al* (27) and Ikekpeazu *et al* (28), however, report significant hypoproteinaemia in malarial infection. Abdagalil and El Bagir (29) and Ogbodo *et al* (30), in comparing protein levels in different degrees of parasitaemia observed a significant increase in protein levels in mild parasitaemia compared to the control and subsequently, significant decrease in protein levels as severity of the malarial infection increased. Our results follow the same trend observed by Abdagalil and El Bagir (29) and Ogbodo *et al* (30). Significant reduction in total protein as parasitaemia increased could be due to impairment of the liver's normal synthetic function (31). Albumin is the major constituent of liver proteins (31, 32). Ogbodo *et al* (30), observed that the increase in albumin at low parasitaemia may have accounted for the non-severity of the fever and other symptoms of malaria at that level. Thus, the decrease in albumin level subsequently gave way to high fever and other complications of malaria as observed clinically. Hence, the trend observed in total proteins. Decrease in protein level as malaria severity increased may be attributed to breakdown of already formed proteins in response to high fever (30, 31).

Bilirubin levels observed in this study decreased as parasitaemia increased. The results corroborate previous studies by Adeoshun *et al* (33), who observed decreasing levels of total and conjugated bilirubin as malaria parasitaemia increased. The reason for the very low levels of bilirubin observed is not well understood but may be attributable to physiological and biochemical processes associated with the burden of detoxification with increasing parasitaemia.

CONCLUSION

We conclude from our findings that hepatocyte function is increasingly compromised as malaria severity increases.

REFERENCES

1. WHO. World malaria report 2009.WHO Press, Geneva 2009.
2. Alumanah EO, Onyeneke EC, Garuba HI, Onoagbe IO. Plasma electrolyte levels in human malaria. J Innovative Life Sci 1994; **1:** 14–19.
3. Okonko IO, Soleye FA, Amusan TA, Ogun AA, Udeze AO, Nkang AO et al. Prevalence of malaria plasmodium in Abeokuta. Malay J Microbiol 2009; **5:** 113–8.
4. Salako LA, Aderounmu AF. In vitro chloroquine and mefloquine resistant Plasmodium falciparum in Nigeria. Lancet 1987; **2:** 572–3.
5. Phillips RS. Current status of malaria and potential for control. Clin Microbiol Rev 2001; **14:** 208–26.
6. Ezechukwu CC, Ekejindu IM, Ugochukwu EF, Egbuonu, Oguatu M, Chukwuka JO. Congenitally acquired malaria in a hyperendemic area: A cohort study. Trop J Med Res 2004; **8:** 44–8.
7. Kitchen AD, Chiodini PL. Malaria and blood transfusion. Vox Sanguinis 2006; **90:** 77–84.
8. Edington GM. Pathology of malaria in West Africa. Br Med J 1967; **1:** 715–8.
9. Ocanar-Morgner C, Mota MM, Rodriguez A. Malaria blood stage suppression of liver stage immunity by dendritic cells. J Exp Med 2003; **197:** 143–51.
10. Ononogbu IC, Onyeneke EC. Plasma lipid changes in human malaria. Trop Med Parasitol 1983; **34:** 193–6.
11. Alumanah EO, Onyeneke EC, Okpogba NA, Okonkwo CJ. Serum protein index in human malaria. Cytobios 2000; **103:** 61–4.
12. Anionye JC, Onyeneke EC, Onovughakpo-Sakpa OE, Anekwe AI. Plasma calcium (Ca^{2+}) and potassium (K^{+}) levels in Nigerian human malaria patients: effect of neuromusculoskeletal symptoms. Nig J Appl Sci 2012; **30:** 166–75.
13. Cheesbrough M. Laboratory diagnosis of malaria parasite In: District Laboratory Practice in Tropical Countries. Cambridge University Press. 1998.
14. Kochmar JF, Moss DW. Fundamentals of clinical chemistry, NW Tietz (ed). WB Saunders and Company, Philadelphia, United States of America 1976.
15. Reitman S, Frankel S. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; **28:** 56–63.
16. Jendrassik L, Grof P. In-vitro determination of total and direct bilirubin in serum. J Biochem 1938; **299:** 81–8.
17. Kochar DK, Agarwal P, Kochar SK, Jain R, Rawat N, Pokharna RK et al. Hepatocyte dysfunction and hepatic encephalopathy in Plasmodium falciparum malaria. QJM 2003; **96:** 505–12.
18. Singh R, Kaur M, Arora D. A prospective study of hepatic involvement in Plasmodium falciparum malaria. J Clin Diag Res 2010; **4:** 2190–7.
19. Uzuegbu UE, Emeka CB. Changes in liver function biomarkers among malaria infected patients in Ikeja Lagos State, Nigeria. Curr Res J Biol Sci 2011; **3:** 172–4.
20. Chikezie PC, Okpara RT. Serum lipid profile and hepatic dysfunction in moderate plasmodium falciparum infection. Global J Med Res Dis 2013; **13:** 15–20.
21. Baheti R, Laddha P, Gehlot RS. Liver involvement in falciparum malaria-A histo-pathological analysis. J Indian Acad Clin Med 2003; 4**:** 34–8.
22. Maduka CI, Neboh EE, Eyisi NB. Effects of malaria parasitaemia on liver enzyme test. Int J Trop Med 2008; **3:** 49–52.
23. Anand AC, Ranji C, Narula AS Singh W. Histopathological changes of liver in malaria: a heterogenous syndrome? Natl Med J India 1992; **5:** 59–62.
24. Jarike AE, Emuveyon EE, Idogun SF. Pitfalls in the interpretation of liver parenchymal and membraneous enzyme in preclinical P. falciparum and malaria in Nigerian environment. Nig J Clin Med 2002; **10:** 21–7.
25. Ogbadoyi EO, Tsado RD. Renal and hepatic dysfunction in malaria patients in Minna, North Central Nigeria. Online J Health Allied Sci 2009; **8:** 8–13.
26. Kausar MW, Moeed K, Mohmand AQK. Correlation of hemoglobin versus liver function test in patients of falciparum malaria. Isra Med J 2011; **3:** 10–5.
27. Adebisi SA, Soladoye OA, Adekoya D, Odunkanmi OA. Serum protein fractions of Nigerians with Plasmodium infections: Ilorin experience. Afr J Clin Exp Microbiol 2002; **3:** 82–4.
28. Ikekpeazu EJ, Neboh EE, Maduka IC, Mamah E, Ejezie FE, Ufelle S et al. Serum protein level and hepatitis – B surface antigen in malaria Infection. Eur J Sci Res 2010; **39:** 542–7.
29. Abdagalil MA, El Bagir NM. Effect of falciparum malaria on some plasma proteins in males: With special reference to the levels of testosterone and cortisol. Afr J Biochem Res 2009; **3:** 349–55.
30. Ogbodo SO, Okeke AC, Obu HA, Shu EN, Chukwurah EF. Nutritional status of parasitemic children from malaria endemic rural communities in eastern Nigeria. Curr Ped Res 2010; **14:** 131–5.
31. Adekunle AS, Adekunle OC, Egbewale BE. Serum status of selected biochemical parameters animal model. Biomed Res 2007; **18:** 109–13.
32. Korubo OT, Reid HL. Serum and plasma protein changes in Nigeria diabetes. Nig J Physiol Sci 1991; **7:** 1–7.
33. Adeosun OG, Oduola T, Akanji BO, Sunday AM, Udoh SJ, Bello IS. Biochemical alterations in Nigerian children with acute falciparum malaria. Afr J Biotechnol 2007; **6:** 881–5.