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Original Research Article

Implication of Malaria on Liver Health

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Abstract

Malaria is the commonest disease in Africa and some Asian countries with a global prevalence of 0.3-2.2%. Malarial infection has multi-organ complications and the liver is not an exception. It has been reported that the plasma concentrations of liver enzymes, bilirubin, protein and albumin are altered during malarial infection in children. In this study, 1000 children within the age bracket of 1 to 10 years attending Palmars Hospital, Omega Children Hospital, Braithewait Memorial Specialist Hospital, and some primary and secondary schools, all in Port Harcourt, Rivers State, were randomly selected and screened for malaria and hepatitis infections, 694 subjects with malaria infection but free of hepatitis were taken as test group while 306 free of both malarial and hepatitis infections were grouped as control. Venous blood samples were obtained from all the subjects for the determination of plasma liver enzymes, protein and albumin. Statistical analysis using ANOVA showed significantly higher concentrations of liver enzymes and bilirubin ($p < 0.05$) of the test group when compared with those of the control group while the concentrations of protein and albumin of the control group were significantly higher than those of the test group. This study has shown that people with malaria may also present with liver damage. It is therefore necessary to assess liver function especially in people with severe malaria.

Keywords: Malaria, Liver function parameters

INTRODUCTION

Malaria is the commonest disease in Africa as well as some Asian countries with a global prevalent range of 0.3-2.2%, however, in cases of its severe form as seen in regions of tropical climate, the range is as high as 11-30% (White *et al.,* 2014). The causative agent of malaria is a protozoon of the *Plasmodium species* group comprising *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi* (White *et al.,* 2014; Walker *et al.,* 2017). Malaria has remained a public health concern due to its increasing morbidity and mortality rates in developing countries as well as the challenge of antimalarial resistance (Jain *et al*., 2016). In fact, in 2015, there were total estimates of about 212 million cases of malaria and 429,000 related deaths globally (WHO, 2018). Malarial infection has multi-organ complications and the liver is not an exception (Mehta, 2018). Hepatic abnormalities of malarial origin have been described with the concept of "malarial hepatopathy", defined as "a bilirubin level >2.5 of the upper limit of normal with aminotransferase elevation of > 3 times the upper limit of normal, taking alanine aminotransferase as the more liver-specific enzyme (Saya *et al*., 2012, Fazil *et al.,* 2013; Jain *et al.,* 2016). However, irrespective of the levels of the hepatic parameters observed due to malarial infection, it has been generally reported that malaria has the potential to compromise hepatic functions through its effects on some biochemical parameters of the liver, also causing histopathological findings like Kupffer cell hyperplasia, hemozoin loading and monocytic infiltration are commonly reported (Kachawaha *et al.,* 2003, McCarthy *et al.,* 2016). Although abnormalities in liver

functions have been investigated, only a little work has been done in relation to other areas of clinical malaria, therefore, it is important to continue to study the effects of malaria infection on liver parameters as this will help to differentiate the characteristics of its pattern of complications from that of viral hepatitis (Kochar *et al*., 2006, Tangpukdee *et al.,* 2006).

MATERIALS AND METHODS

Study Area

The investigations for the study were conducted among children attending Rivers State University Teaching Hospital (RSUTH), Omega Children Hospital, and Palmers Hospital and Schools (Early Breed Group of Schools, St Francis Nursery and Primary school, and Staff Nursery and Primary school) all in Port Harcourt, Rivers State. Port Harcourt is situated at latitude 4º 47´ 21´´ N and longitude 6º 59´ 54´´. Five hundred and Eighty-two (582) children were involved in this study. Three hundred and ninety-six (396) children had malaria and were regarded as the test group while One hundred and eighty-six (186) children who were not infected with malaria were regarded as the control group.

Experimental Design

The study was a cross-sectional study where the subjects were randomly selected. The sample
size was derived using the following formula:

$$N = \frac{Z^2 (PQ)}{D^2}$$ (Araoye, 2003).

Eligibility Criteria

Inclusion criteria

The children included in the study include those within the age range of 1-5 years who had malaria with no history of hepatic disorders and were not on any anti-malarial drug. The control group was children who were not infected by malaria parasite and who had no history of any liver disease after laboratory trials by subjecting them to hepatitis B and C screening.

Exclusion criteria

The subjects excluded include those whose parents did not give consent, children above ten (10) years of age, had any other underlining health issues, and children on anti-malaria treatments/drugs.

Informal consent was obtained from the parents of the children and the institutional authorities.

Sampling Method

Subjects were selected by simple random technique as described by Biambo *et al*. (2021), Catherine *et al*. (2021) and Diorgu *et al*. (2021). In this technique, the subjects who picked “1” from a 0-1 number system were selected while that picked “0” were not selected.

Sample Collection

About 10 ml of venous blood samples were collected aseptically with a disposable hypodermic syringe. About 4ml of which was dispensed into an ethylene diethyl tetra acetic acid (EDTA) sample container for malaria parasite analysis while the remaining 6ml dispensed into heparin bottle and was used for liver function tests; total bilirubin, conjugated bilirubin, and unconjugated bilirubin, total protein, albumin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transferase as parameters.

Sample Analysis Methods

Blood film preparation (Cheesbrough, 2000)

Thin and thick blood film preparation

The blood samples were gently and thoroughly mixed. A smear was made with a drop of blood placed in the middle of a grease-free microscopic slide and another drop of blood on the right side of the same slide with the aid of a pasteur pipette. With the use of a smooth-edged spreader, the blood placed was spread to make a thin film. An applicator was used to spread the drop of blood to make an even thick smear. This was then air-dried before staining.

Staining Thin and Thick films using Giemsa’s stain

The dried thin film was fixed in methanol for 2 minutes. This was then stained with the thick film using Giemsa’s stain. 3% solution of Giemsa stain was made and the films were stained for 30 minutes. The back of each slide was blotted with cotton wool and placed in the draining rack for drying. The films were examined using an Oil immersion (x100) Objective lens. The presence of malaria parasite was done using the thick blood films while the species were identified using the thin blood films. A slide was scored as parasite seen when 100 per

high power fields had been examined without seeing any malaria parasite.

Estimation of Parasite Density using Quantitative Method

Relative malaria parasite count in each blood sample was determined as described by Cheesbrough (2000).

Counting of Percentage (%) Parasitized red cell using thin blood film

The number of parasitized red cells was counted in about 4 fields (250 red blood cells per high power field), to get approximately 1000 cells. This was then divided by 10. This gave the percentage of parasitized red blood cells.

Counting Parasite numbers per microlitre of blood

The number of asexual parasites present in each thick blood film was counted against 100 white blood cells. This was multiplied by the standard total white blood cells count (8000).

One hundred (100) white blood cells were counted while estimating the number of parasites (asexual) in each thick field covered.

Calculations

$$\text{Number of parasites/ µl of blood} = \frac{\text{Total WBC} \times \text{Number of asexual parasites}}{100 \text{ (Number of WBC)}}$$

Aspartate aminotransferase (AST) (Enzymatic method by Reitman and Frankel, 1957).

Procedure

One hundred microlitres (10the 0µl) of the sample was dispensed into a test tube. Five hundred microlitres (500µl) of buffer (phosphate buffer, L-aspartate, and alpha-oxoglutarate were added to the test tube as well as reagent blank test tube and mixed. One hundred microlitres (100µl) of distilled water was added to the reagent blank test tube, and they were mixed and incubated for 30minutes at 37^{o}C. Five hundred microlitres (500µl) of 2, 4-dinitrophenylhydrazine was added to both test tubes, mixed, and was allowed to stand for 20minutes at 20 to 25^{o}C. Five thousand microlitres (5000µl) of sodium hydroxide was added to both test tubes and mixed. The absorbance of the solution was read photometrically against the reagent blank after 5 minutes at 546nm.

Calculation of result

The activity of AST in the serum was obtained from the AST activity table.

Alanine aminotransferase [ALT] (Enzymatic method Reitman and Frankel, 1957)

Procedure

Pipette One hundred microlitres (100µl) of the given sample to a test tube. Then five hundred microlitres (500µl) of buffer (phosphate buffer, L-alanine, and alpha-oxoglutarate) was added to the test tube as well as reagent blank test tube and mixed. One hundred microlitres (100µl) of distilled water was added to the reagent blank test tube, and there were mixed and incubated for 30minutes at 37^{o}C. Five hundred microlitres (500µl) of 2, 4-dinitrophenylhydrazine was added to both test tubes, mixed, and was allowed to stand for 20minutes at 20 to 25^{o}C. Five thousand microlitres (5000µl) of sodium hydroxide was added to both test tubes and mixed. The absorbance of the solution was read photometrically against the reagent blank after 5minutes at 546nm.

Calculation of result

The activity of ALT in the serum was obtained from the ALT activity table.

Alkaline phosphatase (Phenolphthalein Monophosphate Substrate method by Englehardt *et al.*, 1970)

Procedure

In two test tubes, one hundred microlitres (100 µl) of water was dispensed. Pipette fifty microlitres (50µl) of phenophatein monophosphate into the tubes. They were mixed and incubated for 5 minutes at 37°C. The test tubes were filled with 100 microlitres (100 µl) of sample and standard and incubated at 37°C for 20 minutes. Five thousand microlitres (5000µl) of the color developer was added to the test tubes. The absorbance of the solution was read photometrically at 550nm against water as blank.

Calculation of result

Alkaline phosphatase (u/l) =

Absorbance of test x standard concentration
Absorbance of standard

Gamma-glutamyl transferase (5-amino-2-nitro-benzoate method by Szasz and Bergmeyer, 1974)

Procedure

One hundred microlitres (100µl) of the sample was dispensed into a cuvette. One thousand microlitres (1000µl) of buffer/glycerine (Tris buffer, Glycerine, and L-gamma-glutamyl-3-carboxy-4-nitroanilide were added and mixed. Initial absorbance was read and the timer started simultaneously. The solution was read again after 1, 2, and 3 minutes at 405 nm.

Calculation of result

Gamma-glutamyl transferase (u/l) = Absorbance of test x 1158.
1158 (Multiplication factor) =
Activity of GGT standard
Absorbance of GGT standard

Total Protein (Biuret method by Burtis and Ashwood, 1999)

Procedure

Twenty microlitres (20µl) of standard, sample, and Cuvettes were filled with distilled water.. One thousand microlitres (1000µl) of biuret reagent was added to the cuvettes and mixed. This was mixed and incubated for 30 minutes at 20 – 25^{o}c and absorbance read at 546nm against the reagent blank.

Calculation of result

Total protein =
Absorbance of sample × standard concentration
Absorbance of standard

Albumin (Bromocresol Green Method by Grant *& Kachmer*, 1987)

Procedure

Three test tubes were filled with ten microlitres (10 µl) of standard, sample, and distilled water. 3000µl of bromocresol green. concentrate (Succinate buffer, bromocresolgreen, Brij 35) was correctly mixed and incubated at 20-25°C for 20 minutes. At 578nm, the solution was read against the reagent blank.

Calculation of result

Absorbance of sample x standard concentration
Absorbance of standard

Statistical analysis

The data were expressed as mean±SD. The results were analyzed statistically using *t*-test. This analysis was carried out using computer statistics Prism 3.0 Package (Graph and Software, Inc., San Diego, USA). The minimum level of statistical significance was set at $p < 0.05$.

RESULT

Table 1 below is on the Comparative Means (±SEM) of Liver Function Test Parameters of the Test and Control. It was observed that malaria infected subjects had significantly higher levels ($P < 0.05$) of aspartate amino-notransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma glutamy-ltransferase (GGT), than the control group.

Table 1. Comparative Means (±SEM) of Liver Function Test Parameters of the Test and Control Groups

Parameters	Control (n=306)	Test (n=694)	P-value
AST (IU/L)	5.64 ± 0.11	18.20 ± 0.32	P < 0.05
ALT (IU/L)	4.64 ± 0.10	8.65 ± 0.07	P < 0.05
ALP (IU/L)	17.01 ± 0.41	70.03 ± 0.59	P < 0.05
GGT (IU/L)	16.10 ± 0.19	21.66 ± 0.20	P < 0.05
Protein (g/l)	64.59 ± 0.64	42.61 ±0 .32	P < 0.05
Albumin (g/l)	54.03 ± 0.60	31.03 ± 0.26	P < 0.05

Age Range 1-10 years

DISCUSSION

In this study, the comparative means (+SEM) of the liver function test parameters involving 694 subjects serving as the test group and 306 subjects constituting the control group was carried out with all subjects falling between the ages of 1 to 10. It was observed that the concentration of aspartate aminotransferase (AST) was significantly higher in the test group ($p<0.05$) than the control. Also, the concentrations of alanine aminotransferase (ALT) in the test group were signifi-cantly higher ($p<0.05$) when compared with those in the control group. Similarly, the concentrations of alkaline phosphatase (APT) and gamma glutamyltransferase

(GGT) were significantly higher in the test group ($p<0.05$) when compared with those of the control group. However, the concentrations of protein and albumin were significantly lower in the test group ($p<0.05$) when compared with those of the control group. Increase in liver enzymes are suggestive of liver damage while decrease in protein and albumin are suggestive decline in the protein and albumin synthesis in the liver, there it implies impaired synthetic function of the kidney. This may have resulted due to the damage of hepatocytes shown from the increase in the liver enzymes As already established by Onyesom (2011), this study confirmed that malarial infection compromises hepatic functions as seen in the observed increased liver enzymes and reduced protein and albumin concentrations.

Moreover, considering the age bracket of the subjects of this study, our results agreed with the findings of Abro *et al.*,, who in 2011, observed a positive correlation between the concentrations of liver enzymes in the plasma and malarial parasite infection in some Pakistani children below the age of five, a similar observation has also been recorded by Kachar *et al.,* (2006).

More so, our findings of lower plasma levels of protein and albumin in the test group in comparison with those of the control, agreed with Mohammad *et al.,* and Shwetha, who recorded similar results in 2010 and 2014 respectively.

The observed significantly higher increase in the levels of liver enzymes of the subjects in the test group in comparison with those of the control group could be due to the disruption of the membranes of liver cells as the body responds to the presence of the parasite leading to the release of these enzymes into circulation and the degree of this disruption will depend on the burden of the parasite so that the higher the burden, the higher the disruption of the liver cell membranes, thus, the release of higher concentrations of the enzymes.

The observed decrease in the concentrations of protein and albumin in the test group in comparison with the control group could be due to a decreased liver function of protein synthesis since the liver is the site of malarial parasite multiplication.

CONCLUSION

Malaria infection in children disrupts liver cells thereby increasing the plasma concentrations of its enzymes while also reducing hepatic synthesis of proteins and albumin.

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