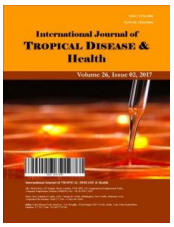

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Effects of Malaria on Selected Liver Function Profiles of Children in Port-Harcourt, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author GNW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EN and OA managed the analyses of the study. Author AO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A cross sectional investigation of the effects of malaria infection on selected liver function profiles of children in Port Harcourt, Rivers State, Nigeria, was conducted. Exactly 1000 randomly selected children aged 1-10 years were involved after institutional ethical clearance and informed consent from their parents were obtained. Exactly 694 of the children, who were malaria positive represented the test group while 306 children apparently healthy, formed the control group. About 10 ml of blood was collected from each child through venopuncture with hypodermic syringe; 4 ml was dispensed into EDTA bottle for *Plasmodium* identification while 6ml each, was dispensed into lithium bottle for liver function tests. Thick and thin blood films were giemsa stained. Total bilirubin, conjugated bilirubin, unconjugated bilirubin, aspartate aminotransferase and albumin were assayed using standard biochemical techniques. Malaria prevalence was 69.4%; age group 1-5 years was

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significantly (P<0.05) more infected (39.7%) than age group 6-10 years (29.7%).Total bilirubin, conjugated bilirubin, unconjugated bilirubin and aspartate aminotransferase were significantly elevated (P<0.05) in malaria subjects.There was a statistically significant (P<0.5) reduction of albumin levels in tests than in the controls. Àmong high malaria parasitaemic subjects, total bilirubin, conjugated bilirubin, unconjugated bilirubin, and aspartate aminotransferase were more elevated significantly (P<0.05) than those of low parasitaemic subjects. These significant changes in these parameters suggest that malaria parasitaemia has significant negative effects on the integrity and functions of the liver which may lead to mortality if ignored.

Keywords: Malaria; liver-function-profiles; effects; children; Port-Harcourt; Nigeria.

1. INTRODUCTION

Malaria is the most important parasitic infection in the world; it is very common in the tropical regions but can also occur rarely in temperate regions. Its transmission, morbidity and mortality are greatest in Africa where most deaths from malaria are among young children and pregnant women [1,2]. It accounts for 1in 5 of all childhood death in Africa. Anaemia, low birth weight (LBW), abortions, stillbirths, growth/mental retardation and neurologic problems, are frequent complications of malaria and are known to have devastating consequences on the general population and these compromise the health and development of millions of children throughout the tropics [3,4,5].

The *Plasmodium* species commonly involved in human malaria are *Plasmodium falciparum, P. vivax, P. ovale,* and *P. malariae*. Some human cases of malaria have also been reported with *P. knowlesi*, monkey - malaria that occurs in certain forested areas of South East Asia [6,7]. Many species of female *Anopheles* mosquitoes transmit malaria parasite in different parts of the world and different species have varied vectorial potentials. Among the most competent malaria vectors are female *Anopheles gambiae* and *Anopheles funestus* which are dominant in Africa while female *Anopheles darlingi* is the major vector in the Amazon basin [8,9]. Children in endemic areas experience frequent episodes of malaria. Initial episodes are commonly severe and the majority of deaths from malaria occur in young children from 6 months of age in endemic areas. The prevalence of malaria among children has been reported in many parts of Nigeria and this is of great importance. Some others, [10,11], reported prevalence of 67.2% and 65.5% among nursery and primary school pupils in Ataba and Port Harcourt, Rivers State, Nigeria respectively while [12] recorded a prevalence rate of 80.5% in Ota, Ogun State. [13] also reported a prevalence rate of 80.4% in Aba, Abia State with Enugu State having the least malaria infection rate of 35.8%. However, Asia, Latin America and to a lesser extent Middle East and parts of Europe are also affected; about 91countries of the world with 216 million cases are infected by this debilitating protozoan infection [14,15].

Plasmodium parasite interferes with the organs of the body namely brain, kidney, liver, lungs, central nervous system and spleen etcetera. Hepatic dysfunction was reported to be 7.15% by [16] at Minna, Niger State, Nigeria. This gives much concern about the need for timely diagnosis, early treatment and the application of effective preventive measures to avert the severe consequences, hence the need for a deeper insight into the problem [17]. According to [18], liver involvement in malaria seemed common in patients with severe malaria and may manifest as jaundice, that is raised serum bilirubin, hepatomegaly, elevated liver enzymes like aspatate aminotransaminase/ alanine transaminase; there may be a redued level of serum albumin and prothrombin time may also be prolonged. Specific histopathological changes and cases with altered liver function tests, (fulminant hepatic failure and hepatic encephalopathy) have been reported [18]. Some researchers [19] also reported that malaria hepatitis is characterized by increased levels of transaminase levels to more than three times the upper limits of normal and alkaline phosphate. When bilirubin is then excreted from the body through the faeces and the elevated levels may indicate certain diseases. Bilirubin circulates in the blood stream in two forms; unconjugated (indirect) bilirubin which does not dissolve in water due to intra molecular hydrogen bonding. It is then bound to albumin and sent through the blood stream to the liver where it is changed into a soluble form (direct or conjugated). Conjugated (direct) dissolves in water because bilirubin is conjugated with glucuronic acid and is then made by the liver from indirect bilirubin much of which goes into the bile and out of the small intestine [20].

Studies on the interactions between malaria and liver enzymes have been carried out in other places like in Minna, Niger State and Edo State [16,21] but much work has not been done among children in study area. Also, in adults there could be many factors that can cause liver disease such as alcoholism [22,23] but children in the study area are not implicated to such; therefore, any derangement in the liver function tests may be attributed to malaria. This work is aimed at investigating the effect of malaria infection on liver enzyme function profiles in children.

2. MATERIALS AND METHODS

The study was carried out among children attending Palmars, Omega Children Hospital, Braithewait Memorial Specialist Hospital (BMSH) and Schools (Early Breed Group of Schools, St Francis Nursery and Primary School and Staff Nursery and Primary School) in Port Harcourt, Rivers State. Port Harcourt is situated at latitude 4º47´21´´N and longitude 6º59´54´´. One thousand (1000) children were included in this study. Six hundred and ninety four (694) children had malaria and were regarded as test group while 306 children who had no malaria were regarded as the control group.

2.1 Experimental Design

This is a cross sectional study where the subjects were randomly selected. The sample size was derived using the following formula [24]:

$$N = \frac{Z^2\ (PQ)}{D^2}$$

Minimum sample size required for this study was calculated using the a reported prevalence of 65.6% from the same town [11]. The test population was 694 with 306 control, making up the number to 1000 subjects. The research work was started in April, 2011 and finished May, 2015; this is just a section of the work.

2.2 Blood Collection and Laboratory Assays

About 10 ml of blood samples were collected through the vein with disposable hypodermic syringe. About 4 ml of which was dispensed into ethylene diethyl tetracetic acid (EDTA) bottle for malaria parasite test while 6ml was used for Liver function tests using total bilirubin, conjugated bilirubin, unconjugated bilirubin, albumin and aspartate aminotransferase, as parameters.

The parameters assayed were total bilirubin using Jendrassik Groff by Mally, et al., [25]. It was used for the quantitative determination of total bilirubin in serum or plasma. Conjugated bilirubin was done using Groff technique by Martinek [26]; unconjugated bilirubin was determined by subtracting the direct bilirubin from the total bilirubin result. Aspartate aminotransferase estimation was done using enzymatic method by Reitman and Frankel [27] for its quantitative determination in serum while Bromocresol Greeen Method by Grant and Kackmser [28] technique was used for albumin qualitative estimation. Blood films preparation and *Plasmodium* identification were made according to the researcher [29]. Estimation of parasite density was done using quantitative method as described by Cheesbrough [29] where 1+ denotes low parasite density (1-10 parasites per 100 thick film fields), 2+ denotes moderate parasite density (11-100 parasites per 100 thick film fields) and 3+ denotes high parasite density (1-10 parasites per thick film fields).

2.3 Statistical Analysis

The data generated were statistically analyzed using statistical package for Social Science (SSS) version 21 and Mega Stat, one factor ANOVA. The results were expressed as mean, standard deviation, per cent, variance and P-value used equals to or less 0.05 as statistically significant. Independent Chi-square test for comparison of proportions was also used.

3. RESULTS

Table 1 shows that overall malaria prevalence was 69.4% (test) while age group 1 – 5years had 39.7%; age group 6- 10 years was 29.7%. There were statistically higher significant differences ($P \leq 0.05$).

Table 2 shows the Mean Parasite Density per microlitre of blood and the corresponding liver function parameters. It was observed that parasite count of <1000 per microlitre of blood had lower values of total bilirubin (TB), conjugated bilirubin (CB), unconjugated bilirubin (UNB) and aspartate aminotransferase (AST), when compared with the parasite count of >1000 ≤ 9999 per microlitre of blood and >10000 per microlitre of blood. Parasite count of >1000 ≤ 9999 per microlitre of blood had lower values of total bilirubin (TB), conjugated bilirubin (CB), unconjugated bilirubin (UNB) and aspartate aminotransferase (AST) when compared with the parasite count of >10000 per microlitre of blood.

Table 1. Age related prevalence of malaria among the studied population

Age group (%) (Years)	Number examined	Number infected (Test group)	Uninfected (%) (Control)	X2(df)	P-value
1-5	584	397 (39.7%)	187 (18.7%)		< 0.05
6-10	416	297 (29.7%)	119 (11.9%)	964.311(1)	
Total	1000	694 (69.4%)	306 (30.6%)		

Legend: Mean = Control (30.6%) = % uninfected of 1000 subjects examined

Protein and albumin levels of parasite count of >10000 per microlitre of blood was lower than the levels in both <1000 and >1000≤9999 parasite count of per microlitre of blood.

Table 3 shows 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 total bilirubin (TB), conjugated bilirubin (CB) and unconjugated bilirubin (UNB) and aspartate aminonotransferase (AST) than the control group. Also there were significant lower levels (P ≤ 0.05) of albumin in malaria infected subjects than the control group.

Table 4 shows that there was a significant higher levels (P ≤ 0.05) of total bilirubin (TB), conjugated bilirubin (CB) and unconjugated bilirubin (UNB) and aspartate aminotransferase (AST), in malaria infected group than the control but there was no significant difference in levels (P ≥ 0.05) of albumin between the two groups. The Comparison between the Parameters in Low and High Densities showed a significant higher level (P ≤ 0.05) of total bilirubin (TB), conjugated bilirubin (CB) and unconjugated bilirubin (UNB) and aspartate aminotransferase (AST).There was no significant difference in level (P > 0.05) of albumin between the two groups.

4. DISCUSSION

The prevalence of malaria infection in the study area was 69.4%. The value is slightly higher 65.5% than [11] in Port Harcourt, Rivers State but lower when compared with 80.5% prevalence rate obtained by [12] at Ota, Ogun State, and 80.4% obtained by [13] at Aba, Abia State all in Nigeria. The result confirmed that children under the age of 5 years are mostly at risk which agrees with the results obtained by earlier researchers [11].This may be probably due to immature immune system, low level of protection against mosquito bites, high exposure rates as well as nutritional factors [13].

In this study, there was an increase in bilirubin level in malaria infected subjects when compared with the control with a statistically significant difference (P ≤ 0.05). This agrees with the findings of [30,31). Who suggested that hyperbilirubinaemia in malaria could be due to a number of causes like intravascular haemolysis of parasitized red blood cells and micro angiopathic haemolysis associated with disseminated intravascular coagulation. Unconjugated hyperbilirubinaemia is due to massive intravascular haemolysis whereas conjugated hyperbilirubinaemia is due to hepatocyte dysfunction and this is associated with raised transaminases.

Table 2. Mean parasite density/µl blood and the corresponding liver function parameters

Parameters	Parasite density <1000/µl (873.8 ± 30.44)	Parasite density >1000 ≤ 9999/µl (3248 ± 109.31)	Parasite density >10000/µl (24813.8 ± 877.22)
TB (µmol/l)	12.43 ± 0.16	15.40 ± 0.25	20.63 ± 0.63
CB (µmol/l)	4.54 ± 0.11	5.52 ± 0.15	7.03 ± 0.21
UNB (µmol/l)	8.06 ± 0.14	9.85 ± 0.22	13.06 ± 0.48
AST (iu/l)	14.73 ± 0.32	17.11 ± 0.11	24.03 ± 0.63
Albumin (g/l)	31.72 ± 0.41	30.61 ± 0.47	30.46 ± 0.46

Statistical significance: P < 0.05.

Legend: Low parasitaemia denotes parasite density of <1000/µl of blood

Moderate parasitaemia denotes parasite density of >1000 ≤ 9999/µl of blood

High parasitaemia denotes parasite density of >10000/µl

TB =Total bilirubin, CB = Conjugated bilirubin, UNB = Unconjugated bilirubin, AST = Aspartate aminotransferase

Table 3. Comparative means (±SEM) of liver function test parameters of the test and control groups from age 1 to 10 years

Parameters	Test	Control	P-value
	n=694	n=306	
TB (µmol/l)	15.61 ± 0.24	4.84 ± 0.11	P < 0.05 = 5.48E- 137
CB (µmol/l)	5.51 ± 0.09	1.84 ± 0.05	P < 0.05 = 3.57E- 109
UNB (µmol/l)	10.16 ± 0.18	2.99 ± 0.83	P < 0.05 = 1.09E- 113
AST (iu/l)	18.20 ± 0.32	5.64 ± 0.11	P < 0.05 = 3.25E - 112
Albumin (g/l)	31.03 ± 0.26	54.03 ± 0.60	P < 0.05 = 2.41E - 218

Age Range 1-10 years
Legend: TB =Total bilirubin, CB = Conjugated bilirubin, UNB = Unconjugated bilirubin, AST = Aspartate aminotransferase, SEM = Standard error of the mean

Table 4. Comparison between the parameters in low, moderate and high densities

Parameters	1+	2+	3+	P-value
	n=298	n=199	n=197	
TB (µmol/l)	12.43 ± 0.16	15.40 ± 0.25	20.63 ± 0.63	P < 0.05 = 2.32E-42
CB (µmol/l)	4.54 ± 0.11	5.52 ± 0.15	7.03 ± 0.21	P < 0.05 = 1.19E-27
UNB (µmol/l)	8.00 ± 0.14	9.88 ± 0.22	13.60 ± 0.48	P < 0.05 = 1.60E-34
AST (iu/l)	14.73 ± 0.32	17.11 ± 0.49	24.03 ±0.63	P < 0.05 = 0.266
Albumin (g/l)	31.72 ± 0.41	30.65 ± 0.47	30.46 ± 0.46	P > 0.05 = 0.0915

Legend: 1+ denotes low parasite density (1-10 parasites/100 thick film fields)
2+ denotes moderate parasite density (11-100 parasites/100 thick film field
3+ denotes high parasite density (1-10 parasites/thick film field)
TB =Total bilirubin, CB = Conjugated bilirubin, UNB = Unconjugated bilirubin, AST = Aspartate aminotransferase.

In this study, most infected subjects showed unconjugated hyperbilirubinaemia which seemed to be due to haemolysis of peripheral parasitized red cells and impairment in bilirubin transport because of reticuloendothelial blockade and disturbance of hepatocyte microvilli which is a feature of falciparum malaria in agreement with [32].There were also statistically significant (P ≤ 0.05) higher values of total bilirubin and unconjugated bilirubin in moderate parasitaemia compared with low parasiteamia. Higher values of total bilirubin and unconjugated bilirubin were noticed in high parasitaemia when compared with both low and moderate parasitaemia. The differences were statistically significant (P ≤ 0.05). This agrees with the results obtained by [33] who reported that hyperparasitaemia is associated with higher serum bilirubin level along with increased incidence of complications such as anaemia, haemoglobinuria leading to black water fever and acute renal failure. The role of liver injury or hepatocellular damage in patients has been proposed by many workers [34,35]. However in this study about 38 (5.5%) children with severe malaria (percentage parasitized red cells >5%) had conjugated hyperbilirubinaemia and this probably suggests hepatopathy. In an earlier work reported by [36], it was shown that the incidence of malaria hepatopathy in children with severe malaria was 8%, while [37] reported 32%. This study suggests that there was an element of hepatic dysfunction characterized by rise in serum conjugated bilirubin especially in the absence of hepatotoxic drugs exposure. Centribular liver damage is one of the factors suggested to have been involved in hepatic dysfunction in acute malaria infection leading to hyperbilirubinaemia which seemed to be a direct consequence of the impaired drainage capacity of the liver, [38] because of suppression of bilirubin excretion due to the effect of parasitaemia on the hepatocye or endotoxemia or metabolic acidosis.

It was observed that aspartate aminotransaminase (AST) was elevated in the malaria infected subjects when compared with the control group and the difference was statistically significant (P ≤ 0.05). This result agrees with the earlier findings of [39,21]. Similarly, there was higher level of AST in high parasitaemia more than in both low and moderate parasitaemia. The differences were statistically significant (P < 0.05). This result agrees with earlier findings of [39].

Lower levels of albumin were observed in the malaria infected children when compared with

the non infected children. This observation agrees with the work of [40,41,42], who reported that subjects infected with malaria, had their serum albumin levels dropped by 15%. However, Asian [42] suggested that the decrease in albumin level may reflect the acute phase reaction and may help in determining the prognosis on admission. It was not then a suprice to observe higher levels in albumin at lower parasiteamic subjects as against those with moderate parasitaemia which was statistically significant (P≥0.05). The higher albumin levels may have accounted for the non severity of the fever and other symptoms of malaria at that level. This result agrees with the report of [41]; which stated that at high parasitaemia, there was a decrease in albumin level when compared with low and moderate parasitaemia but their's was not statistically significant (P ≥ 0.05). The effects of albumin on these complications may be one of the mechanisms by which albumin infusion achieves faster recovery from hypovolemia due to malaria than synthetic colloidal infusions.. Therefore, it is suggested that malaria patients, should be administered albumin infusion specially children with severe malaria because when resuscitated with albumin infusion results in a lower mortality than when with other synthethic colloidial infusions. This may be attributed to the colloidal properties of albumin; possible specific neuroprotective effect, its improved microvascular perfusion in malaria through its rheological effects, volume expansion and influencing fluid shifts across the endothelium.

5. CONCLUSION

The significant reduction in levels of serum albumin and increase in levels of TB, UNB, CB and AST which are liver function parameters indicate that high malaria parasitaemia has some significant adverse effect on the integrity and functions of the liver among children which may lead to mortality if neglected.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

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COMPETING INTERESTS

The authors declare that there were no conflict of interest or bias at the course of this research. I certify that all authors have participated sufficiently in the conception and design of this work. While it was funded by the researchers, the work was done in Rivers State University, Port Harcourt, Nigeria.

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