

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

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 10ml of blood was collected from each child through venopuncture with hypodermic syringe; 4ml 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 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. Among 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.

33 **INTRODUCTION**

34 Malaria is the most important parasitic infection in the world; it is very common in the
 35 tropical regions but can also occur rarely in temperate regions. Its transmission, morbidity
 36 and mortality are greatest in Africa where most deaths from malaria are among young
 37 children and pregnant women (Samba, 1997; Rosenthal, 2007). It accounts for 1 in 5 of all
 38 childhood death in Africa. Anaemia, low birth weight (LBW), abortions, stillbirths,
 39 growth/mental retardation and neurologic problems, are frequent complications of malaria
 40 and are known to have devastating consequences on the general population and these
 41 compromise the health and development of millions of children throughout the tropics
 42 (Ogum, 2006; Hartman *et al.*, 2010; WHO, 2010).

43 The *Plasmodium* species commonly involved in human malaria are *Plasmodium falciparum*,
 44 *P. vivax*, *P. ovale*, and *P. malariae*. Some human cases of malaria have also been reported
 45 with *P. knowlesi*, monkey - malaria that occurs in certain forested areas of South East Asia
 46 (Midega *et al.*, 2007; Cox-Singh *et al.*, 2008). Many species of female *Anopheles* mosquitoes
 47 transmit malaria parasite in different parts of the world and different species have varied
 48 vectorial potentials. Among the most competent malaria vectors are female *Anopheles*
 49 *gambiae* and *Anopheles funestus* which are dominant in Africa while female *Anopheles*
 50 *darlingi* is the major vector in the Amazon basin (Annan *et al.*, 2007; Angella *et al.*, 2014).
 51 Children in endemic areas experience frequent episodes of malaria. Initial episodes are
 52 commonly severe and the majority of deaths from malaria occur in young children from 6
 53 months of age in endemic areas. The prevalence of malaria among children has been reported
 54 in many parts of Nigeria and this is of great importance. Wokem *et al.* (2006, 2008), reported
 55 prevalence of 67.2% and 65.5% among nursery and primary school pupils in Ataba and Port
 56 Harcourt, Rivers State, Nigeria respectively while Olasehinde *et al.* (2010) recorded a
 57 prevalence rate of 80.5% in Ota, Ogun State. Kalu *et al.* (2012) also reported a prevalence
 58 rate of 80.4% in Aba, Abia State with Ughasoro *et al.* (2013) having the least malaria
 59 infection rate of 35.8%. However, Asia, Latin America and to a lesser extent Middle East and
 60 parts of Europe are also affected; about 91 countries of the world with 216 million cases are
 61 infected by this debilitating protozoan infection (WHO, 2014 and 2017).

62 *Plasmodium* parasite interferes with the organs of the body namely brain, kidney, liver, lungs,
 63 central nervous system and spleen etcetera. Hepatic dysfunction was reported to be 7.15% by
 64 Ogbadoyi and Tsado (2009) at Minna, Niger State, Nigeria. This gives much concern about
 65 the need for timely diagnosis, early treatment and the application of effective preventive
 66 measures to avert the severe consequences, hence the need for a deeper insight into the
 67 problem (Rijam and Tor-Agbiyes, 2011). According to Rajesh *et al.*, (2003), liver
 68 involvement in malaria seemed common in patients with severe malaria and may manifest as
 69 jaundice, that is raised serum bilirubin, hepatomegaly, elevated liver enzymes like aspartate
 70 aminotransaminase/ alanine transaminase; there may be a reduced level of serum albumin and
 71 prothrombin time may also be prolonged. Specific histopathological changes and cases with
 72 altered liver function tests, (fulminant hepatic failure and hepatic encephalopathy) have been
 73 reported (Rajesh *et al.*, 2003). Bhalla *et al.* (2006) also reported that malaria hepatitis is
 74 characterized by increased levels of transaminase levels to more than three times the upper
 75 limits of normal and alkaline phosphate. When bilirubin is then excreted from the body
 76 through the faeces and the elevated levels may indicate certain diseases. Bilirubin circulates
 77 in the blood stream in two forms; unconjugated (indirect) bilirubin which does not dissolve in
 78 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 (Pironi *et al.*, 2009).

Studies on the interactions between malaria and liver enzymes have been carried out in other places like in Minna, Niger State and Edo State (Ogbadoyi and Tsado, 2009; Onyesom and Onyemakonor, 2011) 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 (Maduka *et al.*, 2008; Burtis *et al.*, 2001) 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.

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 4° 47' 21'' N and longitude 6° 59' 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.

Experimental Design:

This is a cross sectional study where the subjects were randomly selected. The sample size was derived using the following formula (Araoye, 2003):

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

Minimum sample size required for this study was calculated using the a reported prevalence of 65.6% from the same town (Wokem, *at al.*, 2008). 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.

Blood Collection and Laboratory Assays

About 10 ml of blood samples were collected through the vein with disposable hypodermic syringe. About 4ml 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 Mallor, *et al.*, 1937. It was used for the quantitative determination of total bilirubin in serum or plasma. Conjugated bilirubin was done using Groff technique by Martinek, 1966; 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,

1957 for its quantitative determination in serum while Bromocresol Green Method by Grant and Kachmer, 1987 technique was used for albumin qualitative estimation. Blood films preparation and *Plasmodium* identification were made according to Cheesbrough, (2006). Estimation of parasite density was done using quantitative method as described by Cheesbrough, (2006) 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).

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.

RESULTS

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	306 (30.6%)		

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

Table 1 below 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 $1000 \leq 9999$ per microlitre of blood and >10000 per microlitre of blood. Parasite count of $1000 \leq 9999$ 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. 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: below is 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 ($P \leq 0.05$) of total bilirubin (TB), conjugated bilirubin (CB) and unconjugated bilirubin (UNB) and aspartate aminotransferase (AST) than the control group. Also there were significant lower levels ($P \leq 0.05$) of albumin in malaria infected subjects than the control group.

Table 4: below shows that there was a significant higher levels ($P \leq 0.05$) 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 ($P \geq 0.05$) of albumin between the two groups. The Comparison between the Parameters in Low and High Densities showed a significant higher level ($P \leq 0.05$) 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.

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 $>1000 \leq 9999/\mu\text{l}$ of blood

High parasitaemia denotes parasite density of $>10000/\mu\text{l}$

TB =Total bilirubin, CB = Conjugated bilirubin, UNB = Unconjugated bilirubin,

AST = Aspartate aminotransferase

Table 3: Comparative Means ($\pm$ SEM) of Liver Function Test Parameters of the Test and Control Groups from age 1 to 10 years

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

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+ n=298	2+ n=199	3+ n=197	P-value
TB ($\mu\text{mol/l}$)	12.43 ± 0.16	15.40 ± 0.25	20.63 ± 0.63	$P < 0.05 = 2.32\text{E-}42$
CB ($\mu\text{mol/l}$)	4.54 ± 0.11	5.52 ± 0.15	7.03 ± 0.21	$P < 0.05 = 1.19\text{E-}27$
UNB ($\mu\text{mol/l}$)	8.00 ± 0.14	9.88 ± 0.22	13.60 ± 0.48	$P < 0.05 = 1.60\text{E-}34$
AST (iu/l)	14.73 ± 0.32	17.11 ± 0.49	24.03 ± 0.63	$P < 0.05 = 0.266$

223	Albumin (g/l)	31.72 ± 0.41	30.65 ± 0.47	30.46 ± 0.46	P > 0.05 = 0.0915
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224 **Legend:** 1+ denotes low parasite density (1-10 parasites/100 thick film fields)

225 2+ denotes moderate parasite density (11-100 parasites/100 thick film field)

226 3+ denotes high parasite density (1-10 parasites/thick film field)

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

229 DISCUSSION

230 The prevalence of malaria infection in the study area was 69.4%. The value is slightly higher
231 65.5% than Wokem *et al.*, (2008) in Port Harcourt, Rivers State but lower when compared
232 with 80.5% prevalence rate obtained by Olaseshinde *et al.*, (2010) at Ota, Ogun State, and
233 80.4% obtained by Kalu *et al.*, (2012) at Aba, Abia State all in Nigeria. The result confirmed
234 that children under the age of 5 years are mostly at risk which agrees with the results
235 obtained by earlier researchers (Wokem *et al.*, 2006, 2008). This may be probably due to
236 immature immune system, low level of protection against mosquito bites, high exposure rates
237 as well as nutritional factors (Kalu, *at al.*, 2012).

238 In this study, there was an increase in bilirubin level in malaria infected subjects when
239 compared with the control with a statistically significant difference ($P \leq 0.05$). This agrees
240 with the findings of White (1992) and Kausar *et al.* (2010). Who suggested that
241 hyperbilirubinaemia in malaria could be due to a number of causes like intravascular
242 haemolysis of parasitized red blood cells and micro angiopathic haemolysis associated with
243 disseminated intravascular coagulation. Unconjugated hyperbilirubinaemia is due to massive
244 intravascular haemolysis whereas conjugated hyperbilirubinaemia is due to hepatocyte
245 dysfunction and this is associated with raised transaminases.

246 In this study, most infected subjects showed unconjugated hyperbilirubinaemia which
247 seemed to be due to haemolysis of peripheral parasitized red cells and impairment in bilirubin
248 transport because of reticuloendothelial blockade and disturbance of hepatocyte microvilli
249 which is a feature of falciparum malaria in agreement with Mehta *et al.* (1989). There were
250 also statistically significant ($P \leq 0.05$) higher values of total bilirubin and unconjugated
251 bilirubin in moderate parasitaemia compared with low parasitaemia. Higher values of total
252 bilirubin and unconjugated bilirubin were noticed in high parasitaemia when compared with
253 both low and moderate parasitaemia. The differences were statistically significant ($P \leq 0.05$).
254 This agrees with the results obtained by Kochar *et al.* (2006) who reported that
255 hyperparasitaemia is associated with higher serum bilirubin level along with increased
256 incidence of complications such as anaemia, haemoglobinuria leading to black water fever
257 and acute renal failure. The role of liver injury or hepatocellular damage in patients has been
258 proposed by many workers (Chawla *et al.*, 1989; Kochar *et al.*, 2003). However in this study
259 about 38(5.5%) children with severe malaria (percentage parasitized red cells >5%) had
260 conjugated hyperbilirubinaemia and this probably suggests hepatopathy. In an earlier work
261 reported by Bag *et al.* (1994), it was shown that the incidence of malaria hepatopathy in
262 children with severe malaria was 8%, while Satpathy *et al.* (2004) reported 32%. This study
263 suggests that there was an element of hepatic dysfunction characterized by rise in serum
264 conjugated bilirubin especially in the absence of hepatotoxic drugs exposure. Centribular
265 liver damage is one of the factors suggested to have been involved in hepatic dysfunction in
266 acute malaria infection leading to hyperbilirubinaemia which seemed to be a direct

consequence of the impaired drainage capacity of the liver, (Adekunle *et al.*, 2007) because of suppression of bilirubin excretion due to the effect of parasitaemia on the hepatocyte 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 ($P \leq 0.05$). This result agrees with the earlier findings of Bhavé *et al.* (2005); Onyesom and Onyekonor (2011). 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 Bhavé *et al.* (2005).

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 Golden (1982); Ogbodo *et al.* (2010) and Ahsan *et al.* (2008), who reported that subjects infected with malaria, had their serum albumin levels dropped by 15%. However, Ahsan *et al.* (2008) 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 surprise to observe higher levels in albumin at lower parasitaemic subjects as against those with moderate parasitaemia which was statistically significant ($P \geq 0.05$). 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 Ogbodo *et al.* (2010); 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 ($P \geq 0.05$). 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 (Akech *et al.*, 2006). 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 synthetic colloidal infusions (Akech *et al.*, 2006). 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.

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.

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CONFLICT OF INTEREST

The authors declare that there were no conflict of interest or bias at the course of this research. I certify that both 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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