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Research Article

Hyperbilirubinaemia in Malaria Infected Children in Port-Harcourt

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Abstract

Background: Malaria is an endemic infection in southern region of Nigeria. This study was carried out among 1000 randomly selected school children aged 1 - 10 years to assess the effects of malaria on bilirubin metabolism.

Methods: Six hundred and ninety four (694) of the children (the test group) were of some private and government hospitals in Port Harcourt, while three hundred and six (306) apparently healthy children attending some private Nursery and Primary schools in Port Harcourt were the control group. 5ml of blood was collected from each child and dispensed into ethylene diethyl tetraacetic acid bottle and plain tubes. Whole blood in ethylene diethyl tetraacetic acid bottle was used for malaria parasite identification using thin and thick films stained with Giemsa Romanowsky stain, while the serum in plain tubes was used for bilirubin estimation using Jendrassik Groff method. The subjects were grouped into four; control group (non-infected), high malaria parasitaemic group, moderate malaria parasitaemic group and low malaria parasitaemic group.

Results: Generally, it was observed from the result that total bilirubin, conjugated bilirubin, unconjugated bilirubin, were significantly elevated ($P < 0.05$) in malaria infected individuals when compared to the non-infected subjects. In high malaria parasitaemic subjects, total, conjugated and unconjugated bilirubin, were elevated significantly ($P < 0.05$) more than that of low parasitaemic subjects, and likewise, they were significantly higher than those of moderate parasitaemic subjects. In all age groups (1 - 5, 6 - 10 and 1 - 10 years), total bilirubin, conjugated bilirubin, unconjugated were significantly ($P < 0.05$) elevated in malaria infected subjects when compared with non-infected subjects.

Conclusion: These significant changes in these parameters suggested that malaria parasitemia significantly causes hyperbiribinaemia among children below the age of 10 years and severity of the hyper bilirubinemia creases as the degree of parasitaemia increases. Left unchecked, this could result in bilirubin encephalopathy or child mortality.

Keywords: Children; Malaria; Bilirubin

Introduction

Plasmodium is the genus of protozoa parasite that causes malaria. It is caused by any of four single-celled parasites of the *Plasmodium* species which are carried by mosquito infected from biting someone who had the disease. Female Anopheles mosquitoes when infected transmit malaria to humans mainly night period. Malaria is commonly caused by these plasmodium species, namely, *Plasmodium falciparium, Plasmodium vivax, Plasmodium malariae*, and *Plasmodium ovale*. Among these *plasmodium* species, *Plasmodium falciparium* is regarded as most deadly and it majorly accounts for severe malaria cases. In Africa, it is the common species that is endemic [1]. Another species that is as common as *Plasmodium falciparum* is *Plasmodium vivax* except in Africa but seldom cause severe disease. *Plasmodium malariae* and *Plasmodium ovale* are much less common cause of the disease. Generally they do not cause severe illness. Recently, there have been cases of malaria in humans with *Plasmodium knowlesi* known as monkey malaria found in South East Asian forest [2]. It can rarely cause severe human disease. Globally, different species of *Anopheles* are responsible for transmitting malaria with different capabilities. The most implicated vectors in Africa are *Anopheles gambiae* and *Anopheles funestus* and in Amason Basin *Anopheles darlingi* [3]. Malaria can

also be transmitted from mother to infant (congenital malaria), through blood transfusion or sharing intravenous needles and in endemic areas by mosquito infected after biting infected immigrants or travelers [4]. The highest risk occurs in children from the age of 6 months. 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 in endemic area. With advancing age, increasing protection against malaria is seen with a continuum from frequent infection including severe disease in young children to frequent asymptomatic infection in older children, to uncommon infection in adults. The specific ages most affected by malaria will vary depending on endemicity. In the most endemic areas, malaria is mostly found in children below the age of 5years and in low endemic areas, it may be common later in children, but rare in adults [4].

Each year there is approximately 300 - 500 million malaria infections leading to over 1 million deaths, of which 75% occur mostly in children less than 5years [5]. The rapid spread of resistance to antimalarial drugs; coupled with widespread poverty, weak health infrastructure and in some countries, civil unrest, means that mortality from malaria in Africa continues to rise. In malaria endemic countries, the economy of such areas are affected drastically [6]. Migrants from non malarious areas are mostly affected by the disease when infected because they are non immuned. Malaria is a leading cause of death among Africans especially children less than 5 years of age [7]. Malaria caused nearly 1 million deaths mostly among African children in 2008 [8]. Ogum [9] reported that malaria accounts for 60% of out-patients seen in Nigerian medical centers. AbioduandIyabo [10] reported that the disease kills about 300,000 children annually causing significant morbidity and mortality in the population. At least 1.7 million to 2.5million deaths due to malaria are recorded each year worldwide [11]. Mortality rate in children under five years due to malaria in Nigeria is 30% while that of pregnant women is about 11% [9,12]. In fact, over 90% of Nigeria' population is at risk [10].

The severe symptoms of malaria caused by the parasite *P. falciparum* appear within days and bring death to about 15 to 25% of those stricken when great quantities of infected red cells are destroyed in a single burst; therefore it is the most prevalent parasitic disease against which effective control measures should be implemented [13]. Almost 50% of the world's population is prone to suffer malaria. Morbidity and mortality due to malaria occur in sub-Saharan Africa. This devastating effect of malaria does affect other areas such as Asia, Latin America, Middle East and some parts of Europe [14]. Children who have not had malaria attacks in stable transmission areas, (who have not yet developed protective immunity against the most severe form of the disease), none immune antenatal women, semi immune antenatal women in areas of high transmission, semi immune HIV infected antenatal women in stable transmission areas, HIV/AIDS infected people, international travelers from non-endemic areas because they lack immunity, and immigrants from endemic areas and their children living in non-endemic areas and returning to their home countries to visit friends/ relatives because of waning or absent immunity are at high risk of malaria attack.

Malaria parasite affects some important organs of the body namely brain, kidney, liver, lungs, central nervous system and spleen. Co infections are bound to occur with other infectious diseases as well as non-communicable disorders. This raises concerns about the need for timely diagnosis, early treatment and the application of effective preventive measures to avert the severe consequences arising there-from, hence the need for a deeper insight into the problem [15]. Bilirubin formerly known as haematopinid [16] is a yellowish pigment found in bile and is produced when the liver break down old red blood cells (haeme catabolism). Bilirubin is then removed (excreted) from the body through the faeces and 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 [17].

Malaria affects some organs of the body such as the kidneys, liver, brain and spleen. Hyperbilirubinemia in malaria is mostly called "malarial hepatitis", hyperbilirubinemia involves multiple factors ranging from hepatocellular dysfunction, intravascular hemolysis and disseminated intravascular coagulation [5,18]. The direct causes of hyperbilirubinemia due to malaria include malaria hepatitis, intravascular haemolysis of parasite red blood cells, septicemic hepatitis and microangiopathic hemolysis associated with disseminated intravascular coagulation. The indirect cause includes G6PD-related hemolysis and it can be anti-malaria drug induced.

Hepatic dysfunction ranging from conjugated hyperbilirubinemia to fulminant hepatic failure has been described in falciparum malaria. Liver involvement in malaria is common in patients with severe malaria and may manifest as jaundice that is raised serum bilirubin and hepatomegaly. The exact pathogenesis of jaundice is not clearly understood although the term malarial hepatitis has been used to describe the occurrence of jaundice in patients with falciparum malaria. Organ change in falciparum malaria is said to be related to cyto adherence of parasite and red blood cells to the vascular and sinusoidal endothelium leading to stagnant anoxemia.

Reversible reductions in portal venous flow have been described during the acute phase of falciparum malaria presumably as a consequence of micro occlusion of portal venous branches by parasitized erythrocytes. Jaundice in falciparum malaria may be because of suppression of bilirubin excretion due to either effect of parasitaemia on the hepatocytes or endotoxemia or metabolic acidosis or a combination of these abnormalities. Electron microscopy of liver tissue in severe falciparum malaria has shown hypertrophy of Kupffer cells and sinusoidal macrophages [18].

Hepatocyte swelling, changes in endoplasmic reticulum and mitochondria and loss of microvilli at the sinusoidal pole were reported. The damage to the canalicular membrane (on electron microscopic examination) may be responsible for passive back diffuse of conjugated Bilirubin. Other causes of jaundice in severe malaria include intravascular hemolysis of parasitized red blood cells. Massive intravascular haemolysis has been recognized as the pathogenic mechanism of jaundice in falciparum malaria. This accounts for the common occurrence of unconjugated hyperbilirubinemia [19].

Malaria infection is an endemic disease in the city of Port Harcourt (urban city in Rivers State, Nigeria) as a result of poor environmental state that encourages breeding of Anopheles mosquitoes. Children that have not developed enough immunity against malaria attack easily get infected. On this premise, this study was carried out to evaluate the effect of malaria on bilirubin metabolism.

Materials and Methods

Inclusion criteria

The study was carried out on some children attending some private and government hospitals and schools in Port Harcourt. A total of 1000 children between the ages of 1-10years were used for this study. Six hundred and ninety six (694) of them who had no hepatitis B and C diseases but had *P. falciparum* in their blood were not on malaria treatment during the study. Three hundred and six (306) of the children (were free from hepatitis B and C diseases) who had no malaria were recruited as the control group.

Exclusion criteria

Children who had malaria parasite and were on malaria treatment were excluded in this study. Febrile children whose blood samples were negative for malaria parasite were equally excluded from the study.

Sample collection and analysis

About 5ml of blood sample was collected through the vein with disposal hypodermic syringe; 2ml of which was dispensed into ethylene diethyl tetrameric acid (EDTA) container for malaria parasite identification, while 3ml was dispensed into plain tests for bilirubin estimation using Jandrassik and Groff method. Thick and thin blood film preparations were done using the method described by Cheesebrough [20] for malaria parasite identification.

Estimation of parasite density

Relative malaria parasite count in each blood sample was determined as described by Cheesbrough [20]. The number of asexual parasites present in each thick blood film was counted against 100 white blood cells. This was multiplied by the standard white blood cells count (8000).

Parasite/µl = Total WBC (8000) ×Number of asexual parasites/100 WBC The number of parasitized red blood cells was counted in about 4 fields [250 red blood cells per high power field], to get approximately 1000 cells divided by 10 to get the percentage parasitized red blood cells. Total bilirubin and direct (conjugated) bilirubin were estimated following Jendrassik Groff method described by Mally., et al.[21] and Martinek, [22], respectively. Unconjugated Bilirubin was calculated as the difference between Total and Conjugated bilirubin.

Statistical analysis

All data generated were analyzed statistically using Normal central tendency, Chi square and Test of hypothesis.

Results

From table 1. it was observed that as the density of the parasitaemia increased among individuals, the level of every type of bilirubin (Total bilirubin, Conjugated bilirubin, Unconjugated bilirubin) increased consequently. Low parasite density (873.8 ± 30.44 /ul), moderate parasite density (3,248 ± 109.31 /ul) and high parasite density (24,813.8 ± 877.22/ul) groups had total bilirubin levels (12.43 ± 0.16umol/l, 15.40 ± 0.25umol/l, and 20.63 ± 0.63umol/l respectively), conjugated bilirubin (4.54 ± 0.11umol/l, 5.52 ± 0.15umol/l and 7.03 ± 0.21umol/l respectively), and unconjugated bilirubin (8.06 ± 0.14umol/l, 9.85 ± 0.22umol/l, and 13.06 ± 0.48umol/l respectively). The higher levels of mean total bilirubin, conjugated bilirubin and unconjugated bilirubin of individuals of moderate parasite density were significantly higher ($p < 0.05$) than those of the low parasite density. Likewise, the levels in high parasite density subjects were significantly above ($p < 0.05$) those in moderate parasite density group.

From table 2, the levels of total bilirubin, conjugated bilirubin and unconjugated bilirubin of the malaria infected subjects in age group 1 - 5 years (16.04 ± 0.38µmol/l, 5.52 ± 0.14µmol/l, and 10.56 ± 0.27µmol/l respectively) were significantly higher ($p < 0.05$) than those of the control group in the same age groups (4.7 ± 0.14µmol/l, 1.81 ± 0.07µmol/l and 2.97 ± 0.11µmol/l respectively.

Parameters	*Low Parasite Density (mean parasite count) 873.8 ± 30.44 /ul	** Moderate Parasite Density (mean parasite count) 3,248 ± 109.31 /ul	*** High Parasite Density (mean parasite count) 24,813.8 ± 877.22/ul
Total bilirubin (µmol/l)	12.43 ± 0.16	15.40 ± 0.25	20.63 ± 0.63
Conjugated bilirubin (µmol/l)	4.54 ± 0.11	5.52 ± 0.15	7.03 ± 0.21
Unconjugated bilirubin (µmol/l)	8.06 ± 0.14	9.85 ± 0.22	13.06 ± 0.48
P-value	P<0.05	P<0.05	P<0.05

Table 1: Mean concentrations of the estimated parameters (Total Bilirubin, Conjugated Bilirubin, and Unconjugated Bilirubin) among individuals of different malaria parasite density.

*Low parasite density denotes parasite count less than 1000/µl of blood (< 1000/ul)

**Moderate parasite density denotes parasite count between 1000 and 9999/µl of blood (1000-9999)

***High parasite density denotes parasite count more than 10,000µl of blood (>10,000/ul) [23].

Parameters	Age range (Years)	Malaria infected subjects and sample size (n)	Non-infected subjects and sample size (n)	P-value
Total Bilirubin (µmol/l)	1-5	16.04 ± 0.38 (n=396)	4.78 ± 0.14 (n=186)	P< 0.05
	6-10	15.05 ± 0.24 (n=298)	4.93 ± 0.16 (n=120)	P< 0.05
	1-10	15.61 ± 0.24 (n= 694)	4.84 ± 0.11 (n= 306)	P<0.05
Conjugated Bilirubin (µmol/l)	1-5	5.52 ± 0.14 (n=396)	1.81 ± 0.07 (n=186)	P<0.05
	6-10	5.52 ± 0.12 (n=298)	1.90 ± 0.09 (n=120)	P<0.05
	1-10	5.51 ± 0.09 (n= 694)	1.84 ± 0.05 (n= 306)	P<0.05
Unconjugated Bilirubin (µmol/l)	1-5	10.56 ± 0.27 (n=396)	2.97 ± 0.11 (n=186)	P<0.05
	6-10	9.64 ± 0.21 (n=298)	3.02 ± 0.13 (n=120)	P<0.05
	1-10	10.16 ±0.18 (n= 694)	2.99 ± 0.83 (n= 306)	P<0.05

Table 2: The comparative means (±sem) in umol/l of the estimated parameters (Total, Conjugated and unconjugated bilirubin) of malaria infected subjects and non-infected subjects in different age groups (1-5 years, 6-10years and 1-10 years).

The levels of total bilirubin, conjugated bilirubin and unconjugated bilirubin of the malaria infected subjects in age group 6 - 10 years (15.05 ± 0.24µmol/l, 5.52 ± 0.12µmol/l and 9.64 ± 0.21µmol/l respectively were significantly higher ($p < 0.05$) than those of the control group in the same age range (4.93±0.16µmol/l, 1.90 ± 0.09µmol/l and 3.02 ± 0.13µmol/l respectively).

The levels of total bilirubin, conjugated bilirubin and unconjugated bilirubin of the malaria infected subjects in age group 1 - 10 years (15.61 ± 0.24µmol/l, 5.51 ± 0.09µmol/l and 10.1 6± 0.18µmol/l respectively were significantly higher ($p < 0.05$) than those of the control group in the same age range (4.84±0.11µmol/l, 1.84 ± 0.05µmol/l and 2.99 ± 0.83µmol/l respectively).

Discussion

Liver involvement in malaria infection may manifest as raised bilirubin, elevated liver enzymes and hepatomegaly. Hyper bilirubin, mainly unconjugated bilirubin, is a common feature of falciparum malaria and is attributed to haemolysis of both parasitized and non-parasitized erythrocytes. In this study, there was an increase in bilirubin level in malaria patients when compared with the control subjects and the difference was statistically significant ($P < 0.05$).This agrees with the findings ofMishra., *et al.*[24], White [25] and Kausar., *et al* [26]. Malaria induced hyper bilirubinemia is as a result of intravascular haemolysis of parasitized red blood cells and micro angiopathichaemolysis associated with disseminated intravascular coagulation (DIC). Unconjugated hyperbilirubinaemia-mainly results from generalized intravascular haemolysis while conjugated hyperbilirubinaemia is caused by hepatic cells derangement and this occurs in conjunction with raised transaminases.

Most cases in this study showed unconjugatedhyperbilirubinaemia. This hyperbilirubinemia could be attributed to hyper haemolysis of peripheral parasitized erythrocytes. Other factors like impairment in bilirubin transport resulting from reticuloendothelial blockade and distortion of liver cells microvilli (which is a feature of falciparum malaria) could be possible causes of unconjugated hyperbilirubinaemia [27].

Age of the children infected by malaria seems to have no significant influence on haemolytic effect of malaria on red blood cells and also bilirubin transport mechanism in the liver. In this study, irrespective of the children age, there was still hyperbilirubinaemia as indicated by the three estimated bilirubin parameters.

As regards the degree of parasite density among these malaria infected children, it was observed in this study that as the degree of parasiteamia increased (from low via moderate to high parasiteamia), the level of both conjugated and unconjugated bilirubin increased progressively. This indicates that increase in percentage of vascular malaria parasite is tantamount to accelerated haemolysis of parasitized and unparasitized red blood cells and consequent increase in bilirubin conjugation with glucuronide in the liver [28]. The mechanism of haemolysis of erythrocytes in malaria infection is largely immune-mediated lysis and this rupture of erythrocytes takes place mainly during plasmodium cycle [29].

The higher values of total bilirubin and unconjugated bilirubin as the parasiteamia increases indicates that haemolysis of parasitized and non-parasitized red blood cells increased as parasitaemia increases and probably due to impairment in bilirubin transport because of reticuloendothelial blockade and disturbance of hepatocyte micro villi which actually is a feature of falciparum malaria. This agrees with the results obtained by Kochar., *et al*. [30] who reported that hypercarotenemia 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 [30,31]. Very high serum bilirubin levels with predominant conjugated hyper bilirubinemia along with increase in the liver enzymes are important denominator of liver injury in these patients [30]. Some studies have observed predominant conjugated hyperbilirubinemia patients with malaria hepatopathy especially in adults [31,32]. However in this study about 38 children (5.5%) with severe malaria (percentage parasitized red cells > 5%) had conjugated hyperbilirubinaemia and this probably suggests hepatopathy. In an earlier work reported by Bag., *et al.* [33], it was shown that the incidence of malaria hepatopathy in children with severe malaria was 8%, while Satpathy., *et al.* [34] reported 32%. This study suggests that there was an element of hepatic dysfunction characterized by rise in serum conjugated bilirubin and alanine aminotransferase especially in the absence of hepatotoxic drug exposure, or viral hepatitis (since they tested negative for hepatitis Band C), [35]. Cen tribular liver damage is one of the factors involved in hepatic dysfunction in acute malaria infection leading to hyperbilirubinemia which is a direct consequence of the impaired drainage capacity of the liver [36] because of suppression of bilirubin excretion due to the effect of parasitemia on the hepatocyte or endotoxemia or metabolic acidosis. Hepatic dysfunction has been well documented in cases of falciparum malaria [37]. Majority of this pathological event is due to sequestration of erythrocyte containing highly metabolically active parasites in vascular bed of internal organs leading to capillary blockade and ischemia. Most findings were based on adults, and this study, therefore suggests that much work should be focused on children in order to reduce the disease burden as well as mortality especially in children under 5years of age. The presence of falciparum malaria hepatopathy indicates a more severe illness with a higher incidence of complications and a poor prognosis. Hepatic dysfunction is reversible in the entire patient developing malaria hepatopathy who respond favorably to anti malaria therapy and no residual effects have been documented in survivors [38,39]. Therefore prompt diagnosis and treatment of malaria is required since any treatment delay gives rise to high malaria parasiteamia that causes hyperbilirubinemia and eventual mortality among children.

Conclusion

Therefore this calls for adoption of certain strategies to create better awareness on malaria vector control measures and prevention which is the gold standard in roll back malaria should be intensified in the area.

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