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Malaria parasite status and cholesterol level of malaria patients in parts of the IMO River Basin of Nigeria

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ABSTRACT

Objective: To investigate the relationship between malaria parasite status and cholesterol level of 110 consenting subjects (55 patients and 55 controls) in parts of the Imo River Basin of Nigeria. **Methods:** Giemsa staining was used for malaria parasite examination while Randox cholesterol kit was used for cholesterol level estimation. **Results:** About 49 persons (90%) with malaria had low cholesterol (<180 mg/dL). Highest mean cholesterol levels were 274 mg/dL for study patients and 220 mg/dL for controls respectively; Lowest mean cholesterol levels were 168 mg/dL (patients) and 138 mg/dL (controls) respectively. Low cholesterol levels (<180 mg/dL) were found in patients (84%) and controls (6%) respectively. However, 16.4% of controls and 6% of patients had borderline cholesterol level (200-239 mg/dL). This study establishes a significant correlation (12.9%, $P<0.01$) between malaria parasite status and cholesterol level. **Conclusions:** These findings imply that cholesterol level estimation may be a potential concurrent and valuable diagnosis for malaria status.

1. Introduction

Malaria is an acute protozoa disease characterized by alternating attacks of high temperature, excessive perspiring and exhaustion[1]. It is a parasitic disease that occurs basically in the tropical and subtropical regions of the world.

Though malaria can be caused by other *Plasmodium* species, *Plasmodium falciparum* (*P. falciparum*) is the most feared and most common one in Nigeria and Africa[2]. The life cycle of this parasite in the human host includes the developmental cycle in the red blood cells (RBC), and then the transformation cycle in the liver cell parenchyma. *P. falciparum* has been found to establish successful infection in human host by interacting with a variety of human proteins on the surface of the different cell types, as well as those in the cells of the host[3, 4]. Other biochemical events that have been reported to occur in malaria include cellular changes in energy metabolism, hemo metabolism, membrane lipid peroxidation (LPO) and stress enzymes[5]. Very little is known about the cholesterol changes in malaria patients. However, hypercholesterolemia and hypertriglyceridemia have been observed in the setting of both complicated and uncomplicated malaria and other acute infections, the magnitude of these changes seems to be related to the severity of malaria, but in a study conducted in Africa where malaria is endemic, it was found that between the severity of malaria attack and its effects on high density lipoproteins (HDL) and decrease in cholesterol level the result was not too significant, though it was observed that cholesterol levels of most Africans were lower than that of people living in other continents[6]. There are hypothesis which indicates that low-level *P. falciparum* infection actually induces a significant change in common lipid parameters[5] Most of these studies were carried out in hypoendemic areas, therefore there is need for similar studies in areas with hyperendemic *P. falciparum* infection. The changes observed in lipid composition and cholesterol exchange in malaria patients could have a marked effect on the function of the red cell membrane and may therefore be partly responsible for the increase in fluidity and permeability of *P. falciparum* infected erythrocyte[7].It has also been observed lately that parasites (*P. falciparum*)

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metabolize cholesterol in severe infection and with some enzymes allows the parasites to break up the cell and consume lipids and cholesterol[8].

Cholesterol in the body either originates from diet or is synthesized by the body. It has beneficial effects to the body, but still it is linked positively with coronary heart disease[9]. Cholesterol is a waxy and fat like compound in nature and is found throughout the body. It is an important component in cell membrane. The body can use cholesterol in making sex hormones, adrenal hormones, and vitamin D. Chemically, cholesterol is a complex alcohol of a type known as sterols. It is also a lipid, meaning it does not dissolve in water[10].

Recently malaria has been thought to have significant effect on the level of blood cholesterol and their relationships have raised some concern. This present study, investigates the possible effect of *P. falciparum* infection on the cholesterol level of malaria patients and possibly, abducting it can be a possible parameter for chemical diagnosis of malaria in the absence of a positive blood film.

Ethical clearance for this study was given by the research committee and institutional review board of the the School of Health Technology, Federal University of Technology, Owerri, Nigeria.

2. Materials and methods

2.1. Subjects and sampling methods

A total of 110 subjects attending Federal Medical Centre, Owerri, Nigeria who presented with clinical signs and were diagnosed as malaria in the laboratory were recruited for this study. Both patients and control were related in age and sex. Out of the 110 subjects; 55 were study patients and 55 were control groups. Venous blood samples (7 mL) were collected from each subject in vacutainer tubes from the antecubital vein using sterile venipuncture procedures and preserved in EDTA anticoagulant tubes (K3 EDTA).

2.2. Malaria parasite test

For each blood sample collected, a thick film was made on a clean, grease free slide. After about 1-2 hours of air drying, the thick blood film was stained using the Giemsa staining techniques for 30 minutes, and fixed with methanol (methyl alcohol) for 2-3 minutes. The smear was examined under ×100 oil immersion microscope lens.

2.3. Estimation of cholesterol level

The blood samples were transferred into an anticoagulant bottle containing 75 μ/mL litmus heparin and centrifuged at 3 000 rpm for 5 minutes to separate the serum from the whole blood. A mixture of 10 μL to 1 000 μL of cholesterol reagent (Randox, U.S.A.) was prepared for the standard while the blank was prepared using 10ul distilled water with 1 000 μL of the reagent. The samples were prepared using 10 μL of serum added to 1ml of reagent. This was allowed to stand for 10 minutes at 37 ℃ and was read within 60 minutes. The colorimeter was calibrated to 0.00 using fresh distilled water in a cuvette. The standard was then measured and their values recorded. All the readings were taken with the colorimeter at wavelength of 520 nm.

2.4. Classification of study subjects and controls

The control subjects were designated as + (those who had malaria parasite load of 1-10 per 100 high power fields), while the study patients were designated as ++ and +++ respectively (those with malaria parasite loads of 11 – 1 000 per 1 000 high power field and 1-10 in every high power field respectively)[11]. The controls were chosen to be those designated as + (parasite load of 1-10 per 100 high power field) because as malaria endemic area it was difficult to find patients with no trace of malaria parasite in their blood. Therefore those with the lowest parasite load were chosen as controls.

2.5. Data analysis

Analysis of variance (ANOVA) method was used for the regression and the coefficient analysis of data. This was done by imputing the data in a computer with statistical programme for social sciences software (SPSS) version 10.

3. Results

This study involved a total number of one hundred and ten (110) consenting individuals among whom 55 were the actual study patients and 55 the control patients. Among the control subjects, 30 were males and 25 females, while for the study group (patients), 28 were males (52.7%) and 27 were females (47.3%).

Table 1 showed the age distribution of subjects involved in this study. These subjects were confirmed to be malaria positive. Those between 21-30 years of age had 17 (51%) as controls and 16 (49%) as study patients. Among those of age 41-50 years, 11 (50%) were controls, and 11 (50%) study patients. Those that were 50 years and above had 10 (40%) as controls and 15 (60%) study patients.

Out of 58 males in consenting population subjects, 26 (44.8%) had a +, 16 (27.6%) were ++ and 13 (22.4%) were +++. For the females, out of the 52 subjects, 25 (48.1%) were +, 18 (34.6%) were ++, and 12 (23.1%) were +++.

The cholesterol level distribution among age group is depicted in table 2. The distribution of cholesterol level among the gender shows that out of the 58 males, 22 (40%) had low cholesterol level, 8 (14%) had desirable cholesterol level, 20 (35%) had borderline blood cholesterol level and 5 (9%) had high blood cholesterol level. For the female, 31 (60%) out of the 52 female subjects had low cholesterol level, 2 (4%) had desirable blood cholesterol level, 13 (25%) had borderline blood cholesterol and 9 (17.3%) had high

Table 1
Age distribution table in relation to malaria parasite load (n=10).

Age (yrs)	Population frequency (n,%)	Frequency of malaria parasite load		
		+(control)	++	+++
21–30	33(30.0)	17	7	9
31–40	30 (27.3)	12	10	8
41–50	22(20.0)	11	8	3
⩾50	25(22.7)	10	10	5

Table 2
Distribution of cholesterol level among the age groups (n=10).

Age	Population frequency (n,%)	Cholesterol level (yrs)			
		LC	DC	BBC	HBC
21–30	49(44.5)	13(27.0)	5(10.2)	11(22.4)	4(8.2)
31–40	15(13.6)	10(67.0)	2(13.3)	1(7.0)	2(13.3)
41–50	34(30.9)	8(24.0)	2(16.0)	3(9.0)	21(62.0)
⩾50	12(10.9)	4(33.3)	1(8.3)	6(50.0)	1(8.3)

LC– Low cholesterol(<180 mg/dL), DC–Desirable cholesterol(<200 mg/dL), BBC–Borderline blood cholesterol(200–239 mg/dL), HBC–High blood cholesterol(240 mg/dL and above).

blood cholesterol level

Figure 1 shows the frequency difference of cholesterol levels among control and study patients. Analysis on relationship between malaria parasite status and cholesterol level. that more people with + malaria load(control) had borderline and high cholesterol, more people with ++ had low cholesterol than control group while those with +++ had more of low cholesterol level than other two groups. Also none of those with ++ and +++had high cholesterol level at all.

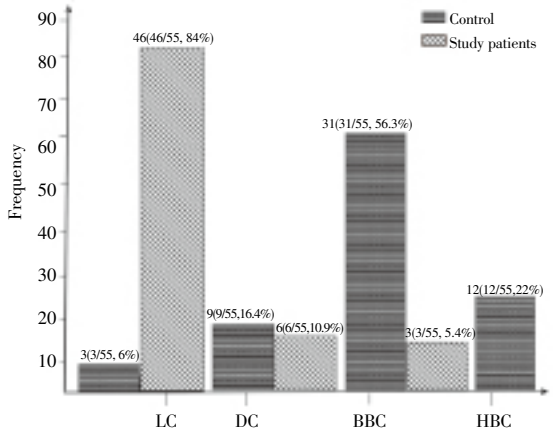

Figure 1. Percentage frequency of cholesterol level among controls and patients.

4. Discussion

This study establishes a significant relationship (12.9%, P<0.01) between malaria parasite status and cholesterol level of malaria patients. These results lend credence to the findings of previous studies in Saudi Arabia on the effect of falciparum malaria infection on blood cholesterol and platelets[12] and Nigeria on biochemical indices of severity in human malaria respectively[12,7]. Both studies showed significant relationship between malaria parasite load and cholesterol level. Similar results have also been obtained in investigations carried out in Sao Tome, an endemic malaria region where it was shown that plasma levels of cholesterol, triglycerides, HDLc and LDLc were significantly low in children infected with *P. falciparum*[13–25].This also agrees with previous review on population studies on common lipid parameters in most endemic malaria countries in Africa[7]. Even though these conflict with findings that showed no relationship between malaria infection and low level of blood cholesterol[27], earlier findings reported that high density lipoprotein (HDL) can be toxic to the parasites at high concentration[26].This relationship found in this study which agrees with previous studies may become a baseline for further investigation on whether blood stage malaria parasites uses blood cholesterol for their metabolic activities. The cholesterol level distribution among age groups in this study indicates that those in the age bracket 21–30 years had the lowest cholesterol levels. This is close to the earlier findings where adolescents had significantly low lipid parameters in endemic malaria areas[28]. This may be attributed to the fact that these groups are very active and therefore have high metabolic reactions which burns out the fats that could have been stored as cholesterol in the body. Those in the 41–50 years age group had highest levels of blood cholesterol and this may be due to the fact that they are more sedentary in their lifestyles, less active and therefore have low metabolic reactions to burn off the fats which are stored as cholesterol, and it could be seen that these group suffer more from stroke, cardiovascular diseases and high blood pressure. Relating Gender with cholesterol level in the study showed that females had lower levels of cholesterol, whereas the males had higher borderline blood cholesterol levels; this may be explained by the fact that women in this region are more active in their farming and other strenuous activities than the males, thereby leading to faster fat metabolism. The study patients

had significantly lower cholesterol levels compared to the controls and this is in agreement with recent reports of a highly significant (P<0.001) correlation between cholesterol and parasite load in study patients group[12].A significant relationship was established between malaria status and cholesterol level in this study. This phenomenon avails an advantage in the concurrent diagnosis of cholesterol level and malaria parasite status. Although these findings may have other clinical implications, this is particularly important in poor endemic areas where it may be difficult for people to run different diagnostic tests to establish their health conditions. The findings of this study may make it possible for cholesterol level and malaria parasite status to be established concurrently with a single test. The study therefore, suggests that while this is not a major diagnostic test, it can still be valuable for malaria diagnosis.

Conflict of interest statement

We declare that we have no conflict of interest.

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