

# Lipid and lipoprotein levels in children with malaria parasitaemia

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## Abstract

It is a common advice in the eastern region of Nigeria that anyone with high fever suspected to be malaria should not take much fatty meal. There is not yet definite biochemical reason for this. We assessed lipid profiles of twenty (20) children, aged between one and twelve years, suffering from malaria. Another fifteen (15) age-matched, uninfected children, were used as controls. Our parameters included total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglyceride. Patients were later classified according to the severity of the infection represented by the density of malaria parasites seen per high field of thick film. Our results showed that these lipoproteins significantly decreased ( $p < 0.05$ ) in the patients than in the control group. However there was no correlation between the malaria density and the levels of these lipoproteins. We suggest that mild fatty nutrients should be recommended to this class of patients for effective stability and prevention of weight loss during the treatment, bearing in mind that they are not even at risk of any condition caused by hyperlipidaemia.

**Key words:** Lipids, Lipoproteins, Children, Malaria parasitaemia.

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## Introduction

Malaria is an internationally recognized devastating disease, producing nearly 600 million new infections and 3 million deaths each year. Today, approximately 40% of the world population suffer from malaria. Mostly, those living in the world's poorest countries are at risk and about 90% of death in Africa are mainly due to malaria, especially children under the age of 5 years [1]. Many children who survive episodes of severe malaria attack suffer from learning impairment or brain damage. Another group of people severely affected by malaria is pregnant women, probably due to reduced immunity [2]. There is observed preferential susceptibility of pregnant women to malaria infection and this may be related to some evidence that immunosuppression is associated with pregnancy and that it is more in the first than the subsequent pregnancies [3, 4], hence prevalence of malaria in pregnancy decreases with increasing gravidity number. Thus, there could be in-utero transfer of parasites across the placenta. Malaria can cause uncountable perinatal and maternal illnesses and death, abortion, stillbirth and low birth weight [2,6,7].

Of the four species of malaria parasite, *Plasmodium falciparum* accounts for the preponderance of malaria morbidity and mortality globally [1]. Variety of potentially fatal symptoms, including liver failure, renal failure, cerebral disease and anaemia are associated with untreated *Plasmodium falciparum* infection [8]. Severe malaria has also been associated with coma, hypoglycaemia, lactic acidosis and apnea. Recurrent episode of malaria in children is dangerous, and mostly lead to cerebral malaria with severe opisthotonic posturing. Statistics shows that 10 – 20% of

children with cerebral malaria die, while approximately 7% are left with neurological sequelae even when treated with absolute care [8].

Lipid deposits are major energy store for many organisms including man. Lipoproteins are particles consisting of core lipids and apoproteins, hence they are responsible for transportation of some specific particles to the membrane, including triglycerides and cholesterol. These lipoproteins include high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). Abnormalities in metabolism of lipids and lipoproteins have been associated with many disease conditions including hypertension, diabetes, liver disease, atherosclerosis etc. All these have been associated with increased total cholesterol and, particularly, low density lipoprotein. In malaria, lipoproteins are oxidatively modified and the degree of modification is related to the severity of the malaria infection [9]. This modification, which ultimately is alteration in the lipoprotein metabolism may have resulted from acute phase responses. Thus, cytoadherence of the infected erythrocytes is also enhanced through oxidation of lipoprotein. It therefore appears that the para-sites take advantage of the oxidative stress to increase its pathogenicity. For instance, oxidized LDL from malaria patient increases the endothelial expression of adhesive molecules and this suggests the role of oxidized lipoprotein in the pathogenicity of the disease [9].

The overwhelming episode of malaria infection in infants is gradually spreading over the world. Malaria infection during pregnancy contributes significantly not only to anaemia in pregnancy but also to low birth weight and high infant mortality rate [7, 10]. It is possible that such infants who survive utero-placental infection usually have reduced immunity to face subsequent infections early in life. A good knowledge of the stabilizing factors for these disadvantaged infants will go a long way in the management of this "wide spreading fire". Ethical clearance was obtained from the Ethical Committee of Ebonyi State University Teaching Hospital, Abakaliki, while additional consent was sought from the infants' mothers.

## **Materials and Methods**

### **1. Subjects**

A total of 35 infants aged between 1 and 12 years were recruited for the study. This number was made up of 20 febrile patients on first visit to the Pediatric Clinic of Ebonyi State University Teaching Hospital Abakaliki, Nigeria and who were later diagnosed of having malaria infection. The remaining 15 were apparently healthy nursery and primary school pupils whose parents' and teachers' consents were sought. They were also screened for malaria parasites. Five out of the initial 20 samples collected were found to be positive for malaria and were therefore excluded.

### **2. Sample collection**

A total of 3.0 ml of whole blood was collected from each subject, either directly with syringe or "tap method", depending on the prominence of the vein. From each sample, a thick film was made directly on a clean grease-free microscope slide and air dried. The remaining was put into a clean plain test tube, allowed to clot and retract and then centrifuged at 5000 rpm for 5 minutes. The serum was then stored frozen until needed for analysis.

### **3. Methods**

(a) Malaria parasite identification: The thick blood films were stained by Field's stains A and B, allowed to air dry and examined under the microscope using x100 objective lens (oil immersion lens). The average number of malaria parasites in a high power field were counted and denoted as follows, according to Dacombe [11].

1-10 parasites per 100 high power fields	= +
11-100 parasites per 100 high power fields	= ++
1-10 parasites per high power field	= +++
>10 parasites per high power field	= ++++

(b) Lipids: Triglycerids, total cholesterol and HDL were estimated using kits prepared by Biosystem (SA) according to Sharma et al [12]. They were all based on enzyme reactions. LDL and VLDL were calculated using Friede-wald equations as follows,

$$\text{LDL} = \text{Total cholesterol} - (\text{HDL} + \text{VLDL}) \text{ and}$$

$$\text{VLDL} = \text{TG}/5$$

## Results

Table 1 shows the mean  $\pm$ SD of total cholesterol, triglyc-eride and the lipoproteins in both infected and uninfected infants. There were significant decreases ( $p < 0.05$  each) in total cholesterol, HDL and VLDL in infected infants. However, though there were relative decreases in LDL and triglyceride levels in infected infants, the decreases were not statistically significant ( $p > 0.05$  each). When infected infants were grouped according to their density of parasitaemia, there was no statistically significant correlation ( $p > 0.05$ ) between the parasite density and the levels of lipids and lipoproteins (Table 2). However, there were relative decreases in these parameters as the malaria density increased. But there were significant differences ( $p < 0.05$ ) when the lowest density (+) was compared with the highest density (++++).

**Table 1:** Mean ( $\pm$ SD) values of lipids and lipoproteins in infected and uninfected infants.

Parameter (mg/100ml)	Infected (n=20)	Uninfected (n=15)	P-value
Total Chol	87.8(16.7)	111.2(22.5)	0.0027
HDL	24.2(7.9)	36.5(10.4)	0.0013
LDL	46.0(16.3)	53.2(14.4)	0.2493
VLDL	18.1(4.3)	22.6(6.8)	0.0332
Triglyceride	88.9(22.8)	108(32.9)	0.0694

**Table 2:** Mean ( $\pm$ SD) values of lipids and lipoproteins in different densities of parasitaemia.

Parameter (mg/100ml)	Low (+) (n=6)	Moderate (++) (n=8)	High (+++) (n=6)	P-value
Total Chol	91.3 (18.7)	88.8 (15.1)	84.17.1 (17.1) f=0.2849	0.7556
HDL	25.2 (11.5)	23.8 (6.3)	23.8 (6.8) f=0.0611	0.9409
LDL	41.4 (15.9)	46.6 (14.2)	43.0 (18.3) f=0.2688	0.7680
VLDL	18.1 (4.2)	17.9(5.4)	17.3 (4.6) f=0.0489	0.9524
Triglyceride	90.2 (20.6)	89.8 (26.7)	86.3 (23.2) f=0.0503	0.9511

## Discussion

Lipids and lipoproteins are affected physiologically by many factors including age, sex, physical activity, weight, eating habit and heredity. For instance, cholesterol generally rises slightly with increasing age [13] while physical activity helps to lower LDL [14]. Excess weight tends to increase LDL while weight loss lowers it, hence it is accepted that even small increase in weight may increase cholesterol and the general risk of cardiovascular disease [15]. It is also believed that smoking increases the level of LDL in the blood [16]. This in turn leads to increase in some clotting factors such as fibrinogen, leading to atherosclerosis and then stroke [17]. Excess alcohol consumption does not only induce fatty liver but also cause damage to the heart muscle, leading to high blood pressure and raised triglyceride; this encourages high level of LDL [18]. Other food materials rich in saturated fats, mostly from animal sources, cause increase in LDL.

Our subjects in this study – infants, are not within the categories prone to these physiological conditions mentioned above. Hence any variation in the values will be attributed to the effect of the disease – malaria. Our results indicated that there were significant decreases ( $p < 0.05$  each) in total cholesterol, HDL and VLDL when compared with the values from control group. Though LDL and triglyceride showed relative decrease, none was significant ( $p > 0.05$ ). The reduction in total cholesterol may be as a result of significant reduction in HDL, probably due to oxidative modification. The increased oxidative stress in malaria, which accounts for the degradation of the lipoproteins, may originate from several sources including intracellular of parasitized erythrocytes and extracellular of haemolysed erythrocytes or host immune responses. Consequently, the products from oxidative stress-reactive species like reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive chloride species (RCS), will interact with the mediators, involved in the pathogenesis of this disease, such as nitric oxide. Also, observed significant decrease in HDL with non-significant decrease in LDL suggests that the acute phase response involves a decrease in the activity of lecithin cholesterol acetyltransferase (LCAT). Once HDL is oxidized, LDL is produced which further inhibits this enzyme and thus impair further HDL metabolism and reverse cholesterol transport [19]. In addition, malaria parasites may release toxins into the system, which stimulate macrophages to release cytokines; these cytokines, though not harmful, can induce additional and uncontrollable production of nitric oxide that would bind to LDL. Furthermore, HDL is believed to be derived from cell membranes and therefore plays a role in removing excess cholesterol from the cell surface while transporting them to the liver as cholesterol esters in the interior of lipoprotein molecules. Moreover, HDL in conjunction with LDL is thought to have regulatory role with regards to the level of cholesterol and its esters in the blood [20]. In this part of the world, rapid weight loss is one of the commonest signs of malaria parasitaemia in infants especially those who could not obtain urgent treatment. This may probably account for the rapid lowering of the lipids, since it is known that weight loss help in lowering of lipoproteins, particularly, LDL and HDL [15]. The progressive reduction of these lipoproteins may also account for the general weakness in the patients and can be attributed to increased replication of the parasites and/or incubation period for the next phase of the infection.

From the foregoing, it is pertinent to institute any measure to forestall weight loss as part of treatment plan for in-fants suffering from malaria. In addition, antioxidants like vitamins C and E should be part of the treatment plan since these antioxidants are known to reduce lipid peroxidation and prevent cerebral malaria [21], as well as prevent drug-induced haemolysis of G6PD-deficient erythrocytes [22]. This is necessary since the G6PD status of most of these infants are not yet known and some anti-malarial drugs are the principal causes of haemolysis in these patients. It is also advisable that intermittent preventive treatment (IPT) must, unfailingly, be part of antenatal care, since this lowers frequency of low birth weight and reduces infant mortality rate [10, 23]. This IPT in which full therapeutic doses of anti-malaria are given at defined intervals during parturition has the potential to provide some of the benefits of sustained chemoprophylaxis in pregnant women and infants. It is a promising new approach to malaria control [24], which has been adopted by many malaria endemic countries, and this usually starts from the “quickening stage” i.e the 16th week of pregnancy. This measure prevents vulnerability of both the mother and the fetus/infant to malaria by keeping their immunity intact.

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