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Full Length Research Article

EFFECT OF MALARIA INFECTION ON BIOMARKER OF LIPID PEROXIDATION (MALONDIALDEHYDE) AND LIPID PROFILE IN PREGNANT WOMEN

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ABSTRACT
Malaria infection has been found to be associated with lipid peroxidation accompanying reduction in antioxidant capacity of infected patients. This study determined the effects of malaria infection on malondialdehyde (MDA) and lipid profile of pregnant women in Dutse, Jigawa State. A total of 103 pregnant women (15-40 years) were enrolled. Blood samples were collected from each consented pregnant women during ante-natal clinic for malaria test, MDA and lipid profile. The mean MDA was significantly higher ($p<0.05$) in malaria positive primigravidae and secundigravidae than in multigravidae. The mean total cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL) and triglyceride levels were higher in malaria positive than in malaria negative primigravidae and secundigravidae ($p>0.05$). The mean total cholesterol level was significantly ($p<0.05$) higher in primigravidae than in malaria positive secundigravidae and multigravidae. Albumin and Total protein were significantly ($p<0.05$) lowered in the severe than in mild and control groups. The result of this study shows that primigravidae and secungravidae are susceptible to malaria infection and the tendency of having atherosclerosis is higher in malaria positive primigravidae due to increased LDL and total cholesterol. Supplementation of diet with antioxidants along anti-malaria drugs during treatment of malaria pregnant women is recommended.

Keywords: Malaria, Pregnancy, Malondialdehyde and Lipid profiles, Jigawa.

INTRODUCTION

Malaria is a parasitic vector borne infectious disease caused by obligate intracellular Protozoa of the genus *Plasmodium*, which is responsible for substantial portion of global disease burden causing nearly 1.4 million deaths annually (Campbell *et al.,* 2005) The zoological family *Plasmodidae* contains protozoan parasites found in the blood of birds, reptiles and mammals (Akinleye, 2009). Malaria is one of the most important causes of morbidity and mortality in the world. The disease is transmitted by female *Anopheles* mosquitoes which carry infective sporozoite stage of *Plasmodium* parasite in their salivary glands, which is transmitted from person to person through the bite of the mosquito (Bryce *et al.,* 2005). Some species which are of medical importance to humans are: *Plasmodium ovale, Plasmodium falciparum, Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium knowlensi* (Kirby, 1996). Pregnant women and children under five years are particularly vulnerable to the disease due to their weaker immune systems (WHO, 2000).

An estimated 25 million women become pregnant in malaria-endemic areas of sub-Saharan Africa, with over 10,000 maternal and about 200,000 infant deaths per year as a result of *P. falciparum* infection (Broen *et al.,* 2007 and Rogerson *et al.,* 2007). Pregnant women experience lowered immunity to malaria (Mockenhaupt *et al.,* 2002). Malaria suppresses responses to immunogens, and placental malaria impairs materno-foetal antibody transfer, which potentially reduces the benefits of maternal immunization strategies (Steketee *et al.,* 2001; Duffy 2003; Metenou *et al.,* 2007). This is particularly frequent and severe in primigravidae (Steketee *et al.,* 2001; Metenou *et al.,* 2007). Also, parasites-infected red blood cells (IRBCs) sequestration in the placenta is a key feature of infection by *P. falciparum* during pregnancy and is frequently associated with severe adverse outcomes for both mother and baby such as spontaneous abortion, preterm delivery, low birth weight and infant death, as well as severe anaemia for both mother and infant (Van de Broek and Letsky, 2000; Guyatt and Snow, 2005; Tuteja, 2007). In Africa, 5-10% of pregnant women may develop severe anaemia (defined as haemoglobin < 70g/l or < 80g/l) (Van de Broek *et al.,* 2000 and Megna *et al.,* 2007). One of the major reasons for development of malarial anaemia seems to be oxidative stress (Das *et al.* 1999 and Kulkami *et al.,* 2003). Malaria parasites utilize cholesterol and phospholipids for survival in their human host (Labaied *et al.,* 2011). For these parasites, circulating high-density lipoprotein (HDL) particles and erythrocytic membrane are the potential sources of cholesterol, whereas the source of phospholipids is erythrocytic membrane (Njoku *et al.,* 1995). Lipid peroxidation is the main manifestation of oxidative stress. MDA is the by-product of non-enzymatic degradation of polyunsaturated fatty acids, and it is mostly found in cell membrane (Yang *et al.,* 2020).

MATERIALS AND METHOD

Study area

General Hospital Dutse, in Jigawa State was used as the study area.

Sample size determination

The sample size (n) was estimated using the formula, $n=Z^2Pq/d^2$ (Cochran, 1976).
Where:
Z = 1.96 (at 95% CI level) q= (1-p)

p = 0.51 (prevalence of malaria of 51% the authors used only 100 blood samples (Dogara *et al.,* 2016)
d = 0.05 (margin of error)
n= $(1.96)^2$ x 0.51 x 0.05/ $(0.05)^2$ = 744 (This study used only 103 samples, because those were the number of participants that consented)

Ethical approval
Ethical clearance with reference MOH/Sec/1.S/528/vol.1 was obtained for this study from the Ministry of Health Jigawa State, while informed consent of patients was sought and obtained before any data is being collected.

Collection of Obstetric Data
With the aid of a structured questionnaire, team of health workers obtained socio-demographic characteristics and obstetric history (age, parity, gravidity and gestational age) from one hundred and three (103) women, who came for antenatal clinic.

Inclusion Criteria
1. Subjects (participants) who were not on antimalarial drug and without other infections.
2. Those from whom informed consent was obtained.

Exclusion Criteria
1. Patients on drugs (e.g. antipsychotics, anticonvulsants and hormones) that affect lipid metabolism were excluded.
2. Patients who refused consent were excluded.

Sample Collection and Processing
With the help of qualified medical personnel, 5 mL of blood was collected by venipuncture from each participating pregnant woman. A total of 3 mL blood was transferred into a plain bottle that was allowed to clot and serum was later obtained. The level of various lipids were determined from the serum. Malaria was diagnosed with the blood smear staining method of Warhurst and Williams (1996). The stages of malaria were confirmed by microscopic examination. Parasitaemia was determined in peripheral blood smears stained by Giemsa stain. The parasitaemia was graded as: + = mild (1-999 μL^{-1}), ++ = moderate (1000-9999 μL^{-1}) and +++ = severe (>10,000 μL^{-1}).
Blood samples collection and analysis for MDA estimation: Five milliliter of venous blood sample was collected from the antecubital vein under aseptic precaution from each subject into EDTA anticoagulant bottles. The blood was then centrifuged at 2500 rpm for 5 min and the plasma removed and stored at 4°C pending assay of product of lipid peroxidation-Malondialdehyde (MDA) estimation (Buege and Aust, 1978).

RESULTS

Effect of Gravidity on Lipid Profiles in Malaria Positive Pregnant Women
The mean total cholesterol level was significantly ($p<0.05$) higher in primigravidae than in both malaria positive secundigravidae and multigravidae. Mean HDL level was slightly higher in malaria positive multigravidae than in any other group. The mean LDL level was significantly ($p<0.05$) higher in malaria positive primigravidae than in malaria positive secundigravidae. Mean triglyceride was significantly higher in malaria positive multigravidae than in malaria positive secundigravidae (Fig. 1)

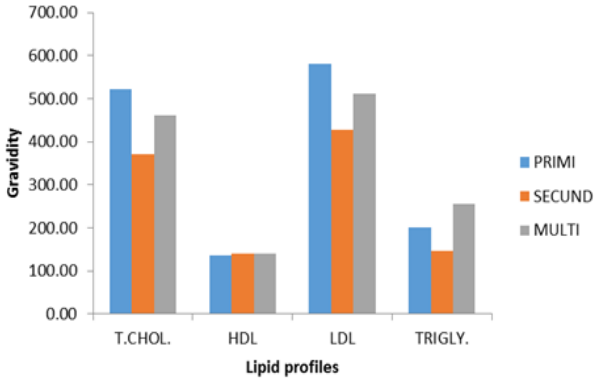

Fig.1: Effect of gravidity on lipid profiles in malaria positive pregnant women

Effect of Malaria Infection on Malondialdehyde (MDA) and Lipid Profile of Pregnant Women
Figure 2 (A to E) compares the levels of malondialdehyde (MDA) and lipid profile in malaria infected pregnant women with their non-malarious counterparts (controls). The result showed that levels of MDA and high-density lipoprotein (HDL) were slightly lower in malarious pregnant women compared to controls (2.4 vs 2.8 nmol/ml and 80.2 vs 82.0 mg/dl respectively), while for cholesterol, triglycerides and low-density lipoprotein, higher levels were noted in malaria infected pregnant women compared to controls (249.4 vs 180.7 mg/dl; 273.2 vs 235.9 mg/dl and 115.2 vs 57.4 mg/dl respectively). However, the differences in levels of MDA and lipid profile between malaria infected pregnant women and controls were not statistically significant ($P > 0.05$).

On the degree of parasitaemia as shown in table 1, the empirical data showed that MDA and lipid profile (with the exception of HDL) levels increased as the degree of parasitaemia increased from mild (+) to severe (+++). However, the difference across the groups (control, mild, moderate and severe) did not attain statistical significance ($P > 0.05$).

Table 1: Levels of Malondialdehyde (MDA) and Lipid Profile in relation to the degree of parasitaemia

		Degree of Parasitaemia		
Parameter	**Control**	**+**	**++**	**+++**
MDA (nmol/ml)	2.8 (0.77)	2.3 (0.68)	2.2 (0.53)	2.9 (0.99)
TC (mg/dl)	180.7 (93.7)	230.6 (118.2)	235.7 (104.5)	290.9 (154.3)
HDL (mg/dl)	81.9 (29.0)	83.6 (26.7)	80.9 (17.9)	74.9 (24.9)
TG (mg/dl)	235.9 (105.2)	217.3 (96.3)	281.9 (203.5)	338.8 (205.1)
LDL (mg/dl)	57.4 (66.6)	104.0 (95.6)	96.8 (66.2)	152.0 (117.2)

Values are mean (standard deviation)
Key: += Mild parasitaemia ++ = Moderate parasitaemia +++= Severe parasitaemia
MDA: Malondialdehyde, TC: Cholesterol, HDL: High density lipoprotein, TG: Triglyceride,
Significant difference was assessed using One-Way ANOVA at P< 0.05
P-values: MDA = 0.06; TC = 0.17; HDL = 0.73; TG = 0.13; LDL = 0.08

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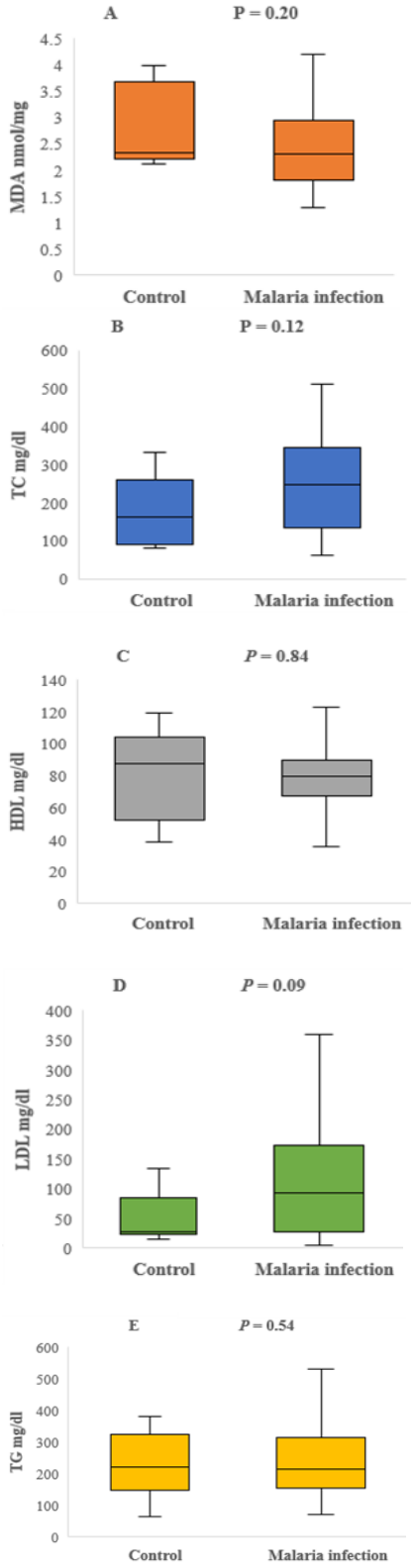

Figure 2: Levels of MDA and lipid profile between malaria infected and non-infected (controls) pregnant women. *Box represents median with 25 and 75 percentiles*

Effect of Malaria on Lipid Profiles in Pregnant Women

The mean total cholesterol (MTC) and HDL levels were higher in malaria negative pregnant women than in malaria positive pregnant women but the difference is not significant. However mean LDL level was significantly ($p<0.05$) higher in malaria positive pregnant women than in malaria negative pregnant women. Mean triglyceride level was significantly ($p<0.05$) higher in malaria positive pregnant women than in malaria negative pregnant women (Fig. 3)

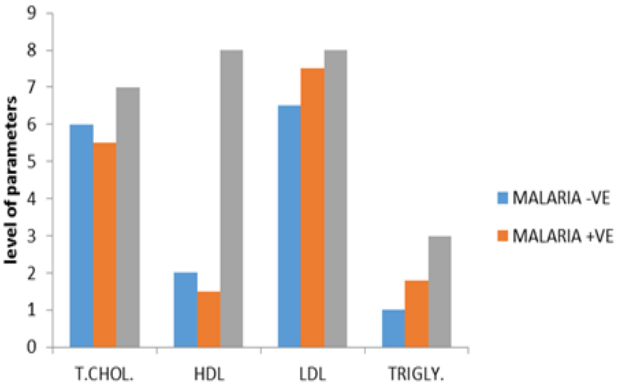

Fig. 3: Effect of malaria infection on lipid profiles in pregnant

Effect of Gravidity on MDA Levels in malaria positive Pregnant Women

There was no significant difference between the mean MDA levels of malaria positive primigravidae and malaria positive secundigravidae while it was significantly lower in malaria positive multigravidae (Table 2)

Table 2: Effect of Gravidity on MDA levels in Malaria Positive Pregnant Women

Gravidity	Malaria positive MDA levels (nmol/mL)	Malaria negative
Primigravidae	2.79±0.44	4.12±0.34
Secundigravidae	2.59±0.14	4.02±0.39
Multigravidae	1.58±0.12	3.59±0.45

Values are mean + S.D

DISCUSSION

It has been reported that pregnant women and children under five years are particularly vulnerable to malaria due to their weaker immune systems (WHO, 2000). Pregnancy is a condition that favors oxidative stress likely due to the mitochondria rich placenta (Casanueva and Viteri, 2003). The present study shows oxidative stress status to be serious with malaria infection, which agrees with the finding of Erel *et al.* (1997) who reported that oxidative mechanism was more dominated in patients with malaria as compared to those without malaria infection. Similarly, the study of Rath *et al.* (1991) reported an increased level of serum lipid peroxidation in *P. falciparum* infected patients, which is in tandem with what was obtained in this study. Furthermore, this study revealed significantly lowered mean MDA level in malaria positive multigravidae than in both malaria positive primigravidae and

secundigravidae. This increase in MDA level in primigravidae and secundigravidae could be that there was an oxidative stress in primigravidae and secundigravidae as reactive oxygen species (ROS) production increases during pregnancy as explained by Akanbi *et al.* (2009) that lipid peroxidation products such as thiobarbituric acid-reactive substances increase in pregnant women. The increase in MDA levels shows that there was increase in the lipid peroxidation in malaria positive primigravidae as earlier explained by Upadhyay *et al*. (2011). Malaria in pregnancy is associated with a range of deleterious effects in women and their babies (Schantz-Dunn and Nour, 2009). Changes in lipid profiles during malaria infection have been reported to contribute to pathological effect of malaria in pregnant women (Akanbi *et al*., 2013). Increase in cholesterol, LDL and triglyceride levels during malaria infection have been reported to contribute to the pathogenesis of malaria and this could be dangerous to human health as it is capable of causing atherosclerosis if necessary treatment is not adopted (Weerapan *et al.,* 2004). Lipoprotein has been reported to represent a major component of serum needed for the growth of the malaria parasite (Akanbi *et al*., 2013). The increase in LDL, cholesterol and triglycerides levels has been reported to be common in malaria positive patients (Weerapan *et al.,* 2004). In this study, the mean LDL and triglyceride were significantly higher in malaria positive pregnant women than in malaria negative pregnant women, while HDL level was higher in malaria negative than malaria positive pregnant women. This agrees with the previous study (Krishna *et al*., 2009).

The mean LDL and cholesterol levels were higher in primigravidae than in secundigravidae and multigravidae. This shows that primigravidae are at the high risk of having atherosclerosis than other pregnant women.

It is possible that this increase could be as a result of the increase in the parasitaemia in the primigravidae than in the secundigravidae and multigravidae which is similar to the report of Akanbi *et al.* (2012) that the level of parasitaemia could determine the extent of changes in lipid profiles in malaria positive patients.

Conclusion

It was concluded that in Dutse, Jigawa State, especially among pregnant women attending antenatal care clinic at Dutse General Hospital that the primigravidae and secundigravidae are susceptible to malaria infection and the tendency of having atherosclerosis is higher in malaria positive primigravidae as suggested by increased levels of LDL and total cholesterol.

We recommend that further study be carried out with larger sample size in case control and cross sectional studies, and especially during raining season, when mosquitoes are breeding in their numbers, to confirm if these findings are consistent. Also Supplementation of diet with antioxidants along anti-malaria drugs during treatment of malaria pregnant women is recommended.

Acknowledgments

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Conflict of Interest

Authors declare that there is no conflict of interest as far as this study is concerned.

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