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Evaluation of Nitric Oxide and Antioxidant Status of *Plasmodium falciparum* Infected Pregnant Nigerian Women with Malaria

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ABSTRACT

Women are highly susceptible to malaria during pregnancy. *Plasmodium falciparum*, which sequesters in the placenta, causes this greatest disease, contributing significantly to maternal and infant mortality. Oxidative stress is thought to be involved in the pathophysiology of malaria, especially in pregnancy where natural resistance is markedly reduced. In this study, the levels of lipid peroxidation products (MDA) and the activity of antioxidant enzymes (GPx, SOD and CAT) and molecular antioxidants (GSH, Vitamins C and D) were evaluated in 112 pregnant women. Levels of lipid peroxidation products (MDA) were significantly higher ($p < 0.05$) in pregnant women with malaria when compared to the control subjects (10.20 ± 0.40 µmol/ml and 5.30 ± 0.35µmol/ml respectively). In contrast, GPx(136.43 ± 5.98 U/mg protein) and SOD (41.79 ± 1.92 U/mgprotein) activities and the levels of GSH (22.99 ± 2.18 mg/ml), vitamins C (0.77 ± 0.04 mg/dl) and E levels (0.78 ± 0.05 mg/dl) of pregnant women with malaria were found to be significantly reduced ($p < 0.05$) when compared to that of controls (136.43 ± 5.98 U/mgprotein; 41.79 ± 1.92 U/mgprotein; 22.99 ± 2.18 mg/ml; 0.77 ± 0.04 mg/dl and 0.78 ± 0.05 mg/dl respectively) while CAT activity was significantly elevated($p < 0.05$) in malaria infected pregnant women (54.80 ± 2.80 U/mgprotein) when compared to that of controls (48.38 ± 1.72 U/mgprotein).The infected non – pregnant women also demonstrated elevated ($p < 0.05$) MDA levels (6.57 ± 0.38 µmol/ml), reduction ($p < 0.05$) in GPx(152.64 ± 7.70 U/mgprotein) and SOD (40.07 ± 1.91 U/mgprotein) activities and vitamins C (0.96 ± 0.04mg/dl) and E (0.78 ± 0.03mg/dl) and increase ($p < 0.05$) in catalase activity. Also, the levels of nitric oxide significantly increased in the malaria infected pregnant women (38.62 ± 1.40µmol/ml) when compared to the control (33.38 ± 1.68µmol/ml). However, the degree of manifestation in these indices, were higher in malaria infected pregnant women than in infected non pregnant women. Our results suggest an imbalance between oxidants and antioxidants in pregnant women suffering from malaria, a situation which could lead to severe consequences in either the mother or the foetus.

Key words: *Plasmodium falciparum*, pregnancy, antioxidants, nitric oxide.

INTRODUCTION

Malaria is a major cause of morbidity and mortality in developing countries, accounting for 2 to 3 million deaths per year worldwide [1]. More than 90% of these deaths occur in sub - Saharan Africa and most of them are due to *Plasmodium falciparum* [2]. As in many other malaria-endemic regions of sub-Saharan Africa, the most affected are pregnant women [3]. Malaria during pregnancy is a major public health concern and an important contributor to maternal and infant morbidity and mortality in malaria-endemic countries [4]. The hallmark of *falciparum* malaria in pregnancy is parasites sequestered in the placenta. Sequestered parasites evade host defense mechanisms: splenic processing and filtration. *P. falciparum* causes greater morbidity (maternal and fetal, principally low birth weight and anaemia) and mortality than non-*falciparum* infections [5][6] but there is mounting evidence that *P. Vivax*is not as benign as had been previously thought [7] [8].

An estimated vulnerable 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 [9]. Pregnant women experience lowered immunity to malaria. Malaria suppresses responses to immunogens, and placental malaria impairs materno-fetal antibody transfer, which potentially reduces the benefits of maternal immunization strategies [10][11]. This is particularly frequent and severe in primigravidae [12]. 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 [13]. Important to note in this context is the fact that ROS are toxic molecules that the body antioxidant regulation system can neutralize at the cellular level through enzymes or scavengers such as vitamins A, C and E [14]. A decreased antioxidant levels have been reported in malaria infection by some authors [15][16][17][18].Oxidative stress occurs when levels of reactive oxygen species (ROS) overwhelm the body's antioxidant defense system that regulates their production. This is a condition in which the elevated levels of ROS damage cells, tissues or organs [19]. The aim of this study is to evaluatenitric oxide and antioxidant status of *Plasmodium falciparum*malaria infected Nigerian pregnant women.

MATERIALS AND METHODS

Study location: This study was conducted at the Central Hospital, Benin - City, Edo State, Nigeria.The study subjects are pregnant women suffering from malaria infection. Infected non – pregnant women were also recruited for the study. Healthy non – malaria (non – pregnant) subjects were used as the controls. The study was approved by the Edo State Ministry of Health Ethical Committee (HA.577/VOL.11/80)before its commencement.

Study group: A total of one hundred and twelve (112) pregnant women were recruited and screened for malaria parasite; 44 (39.29%) pregnant women were positive for malaria parasite and 68 (60.71%) pregnant women were negative for malaria parasite. A total of fifty – four (54) non pregnant women were used as the control subjects.These pregnant women visited the ante - natal clinic at Central Hospital, Benin City, Edo State, Nigeria,forroutine medical check-up. All the patients in this study were infected with the specie*P.falciparum.* Other clinical history such as gestational period, parity, age were obtained from their clinical records.

Blood collection and preparation: 10ml of venous blood was drawn by venipuncture from the women and 8ml was immediately transferred into a plain sterile sample bottle and were allowed to clot before centrifuging for 10 minutes at 3000 r.p.m. The serum was used to determine the activities of glutathione peroxidase, superoxide dismutase, catalase and the levels of malondialdehyde, reduced glutathione, vitamin C, vitamin E and nitric oxide.

Determination of parasitaemia: The remaining 2ml of bloodwas transferred into EDTA tubes, swirled gently, shielded from bright light and was used to determine parasitaemiaby the use of thick and thin blood films using Geimsa stain. The thick blood film was used to attain a qualitative diagnose for malarial infection and the thin blood film was used to identify the *Plasmodium* specie present. The thick and thin films were analyzed for the number of parasites per 200 blood cells. Patients were labelled as malaria negative only if three consecutive smears were negative. The degree of parasitaemia was expressed as mild (+), moderate (++) and severe (+++).

Biochemical assays: Serum lipid peroxidation was quantified by the method of Buege and Aust, [20]. Serum glutathione peroxidase was estimated by the method of Nyman [21]. Serum superoxide dismutase activity was measured by the method of Misra and Fridovich [22]. Serum catalase activity was

assayed by the method of Cohen *et al.,* [23]. Serum reduced glutathione was estimated by the method of Ellman [24]. Serum vitamin C by the method of Omaye*et al.,* [25], serum vitamin E by the method of Desai [26] and nitric oxide by the method of [2].

STATISTICAL ANALYSIS

Results obtained were subjected to statistical analysis using automated package SPSS (version 21.0) and were expressed as mean ±SEM. One way ANOVA and Duncan multiple range comparison tests were used to compare the means and to test for levels of significance at 95% confidence level.

RESULTS

The antioxidant indices of pregnant Nigerian women infected with *Plasmodium falciparum*are represented in Table 1. The results obtained revealed significant increase ($p < 0.05$) in the levels of MDA in the infected pregnant women (10.20 ± 0.40µmol/ml) when compared to the control subjects (5.30 ± 0.35µmol/ml). Glutathione peroxidise (136.43 ± 5.98 U/mg protein) and SOD (41.79 ± 1.92U/mgprotein) activities were significantly ($p < 0.05$) decreasedwhile CAT (54.80 ± 2.80U/mgprotein) activity was significantly ($p < 0.05$) elevated in the infected pregnant women when compared to the control subjects (169.57 ± 5.53U/mgprotein; 52.39 ± 1.74U/mgprotein and 48. 38 ±1.71U/mgprotein respectively). There were significant ($p < 0.05$) decreases in the levels of GSH, vitamins C and E in the infected pregnant women when compared with the control subjects. There was a significant ($p < 0.05$) increase in the levels of nitric oxide in the infected non – pregnant women and the infected pregnant women (36.92±2.32µmol/mland38.62±1.40µmol/ml) when compared to the control (33.38±1.68µmol/ml) (Table 1).

There were no significant differences($p > 0.05$) in the activities ofGPx and SOD as well as in GSH levels in the various degree ofparasitaemia in infected non pregnant and infected pregnant women (Table 2). However, there was a significant ($p < 0.05$) decrease in MDA levels in severe parasitaemia in infected non pregnant women(5.05 ± 2.90µmol/ml) when compared to mild (6.58 ± 0.55µmol/ml) and moderate (8.56 ± 0.97µmol/ml) parasitaemias. Also, there was a significant ($p < 0.05$) reduction in MDA levels during mild parasitaemia in infected pregnant women (5.92 ± 0.41µmol/ml) when compared to moderate (6.82 ± 0.76µmol/ml) and severe(8.25 ± 1.00µmol/ml) parasitaemias. Catalase activity reduced ($p < 0.05$) in moderate (28.31 ± 3.74U/mg)

and severe (27.83 ± 1.62U/mgprotein) parasitaemias in infected non pregnant women when compared to mild (38.68 ± 2.44U/mgprotein) parasitaemia, but no significant differences were observed in mild (43.45 ± 2.34U/mgprotein) and moderate(41.91 ± 4.30U/mgprotein) parasitaemias in infected pregnant women when compared to severe parasitaemia (47.61 ± 6.89U/mgprotein).There was also no significant change ($p > 0.05$) in Vitamin C levels in various parasitaemias in infected pregnant women, but in infected non - pregnant women, there were significant decreases ($p < 0.05$) in moderate(0.75 ± 0.11mg/dl) when compared to mild (0.98 ± 0.06mg/dl) and severe (1.11 ± 0.44mg/dl) parasitaemias. Vitamin E levels were elevated ($p < 0.05$) in severe parasitaemia both in infected non pregnant women and infected pregnant women. Also, there were no significant differences ($p > 0.05$) in the nitric oxide levels in the infected non pregnant women and the infected pregnant women in mild, moderate and severe parasitaemia except in the moderateparasitaemia of the infected pregnant women where there was a significant ($p < 0.05$) increase when compared to the control (49.89 ± 4.24µmol/ml and 33.38 ± 1.68µmol/ml) (Table 2).

Gestational age did not exhibit any significant difference in the levels of GSH and Vit E in both uninfected pregnant women and infected pregnant women (Table 3).However, there were graded significant ($p < 0.05$) increasesin MDA levels (9.87 ± 0.50 µmol/ml; 11.61 ± 0.50µmol/ml and 8.43 ± 0.80µmol/ml respectively) and CAT (50.16 ± 0.10 U/mgprotein; 54.66 ± 4.10U/mgprotein and 57.35 ± 3.60U/mgprotein respectively) activity in the various trimesters in the infected pregnant women while there were no significant changes in that of non - infected pregnant women. Glutathione peroxidase (150.88 ± 8.00U/mgprotein) and SOD(48.17 ± 4.20U/mgprotein) activities as well as vitamin C (0.91 ± 0.10 mg/dl) levels were significantly elevated ($p < 0.05$) in the third trimesterin the infected pregnant women. There were no significant differences ($p > 0.05$) in the nitric oxide levels of the non – infected pregnant women and the infected pregnant women when compared to the control. However, there was a significant decrease ($p < 0.05$) in the level of nitric oxide in the first trimester of the infected pregnant women when compared to the control (23.13 ± 3.90µmol/mland33.38 ± 1.68µmol/ml) (Table 3).

Glutathione levels reduced ($p < 0.05$) with multigravidaein the infected pregnant women while MDA levels increased with multigravidae in infected pregnant women (Table 4). Glutathione peroxidase (106.99 ± 10.00U/mgprotein), SOD (26.28

± 5.70U/mgprotein) and CAT (47.65 ± 3.40U/mgprotein) activities were significantly decreased ($p < 0.05$) with increasing gravidae in the infected pregnant women. Vitamins C (0.71 ± 0.10 mg/dl) and E (0.94 ± 0.70 mg/dl) levels were also reduced ($p < 0.05$) with increasing gravidae in the infected pregnant women when compared to the non - infected pregnant women(1.02 ± 0.07 mg/dl and 0.89 ± 0.05 mg/dl respectively). There were no significant difference ($p > 0.05$) in the nitric oxide levels of the infected non - pregnant women and the infected pregnant women in primigravidae, secondigravidae and multigravidaeexcept inprimigravidae of the infected pregnant women where there was a significant increase ($p < 0.05$) when compared to the control(52.79 ± 4.88µmol/ml and 33.38 ± 1.68µmol/ml respectively) (Table 4).

RESULTS

Table 1: **Antioxidant status of *Plasmodium falciparum* infected pregnant women.**

	CONTROL	INPW	UIPW	IPW
MDA(µmol/ml)	5.30 ± 0.35^{a}	6.57 ± 0.38^{a}	5.18 ± 0.23^{a}	10.20 ± 0.4^{b}
GPx (U/mg protein)	169.57 ± 5.53^{b}	152.64 ± 7.70^{ab}	169.47 ± 6.17^{b}	136.43 ± 5.98^{a}
SOD (U/mg protein)	$52.39 \pm s1.74^{b}$	40.07 ± 1.91^{a}	53.80 ± 2.09^{b}	41.79 ± 1.92^{a}
CAT (U/mg protein)	48.38 ± 1.72^{b}	33.81 ± 1.43^{a}	35.54 ± 0.97^{a}	54.80 ± 28.0^{c}
GSH ((mg/ml)	30.00 ± 1.84^{b}	22.41 ± 1.55^{a}	27.23 ± 1.68^{ab}	22.99 ± 2.18^{a}
VITC (mg/dl)	1.11 ± 0.03^{c}	0.96 ± 0.04^{b}	1.02 ± 0.04^{c}	0.77 ± 0.04^{a}
VITE (mg/dl)	0.96 ± 0.03^{b}	0.78 ± 0.03^{a}	0.89 ± 0.03^{b}	0.78 ± 0.05^{a}
NO (µmol/ml)	33.38 ± 1.68^{a}	36.92 ± 2.32^{ab}	30.02 ± 1.45^{a}	38.62 ± 1.40^{b}

Data are represented as mean ± SEM. Values in the same row with different alphabets differ significantly ($p < 0.05$).

Key: UINPW: Uninfected non pregnant women; INPW: Infected non pregnant women; IPW: Infected pregnant women;MDA: Malondialdehyde; GPx: Glutathione peroxidase; SOD: Superoxide dismutase: CAT: Catalase; GSH: Reduced glutathione; Vit C: Vitamin C; Vit E: Vitamin E; NO: Nitric oxide.

Table 2: Effect of degree ofparasitaemia on antioxidant status of *Plasmodium falciparum* infected pregnant women

	INFECTED NON PREGNANT WOMEN			INFECTED PREGNANT WOMEN		
	DEGREE OF PARASITAEMIA			DEGREE OF PARASITAEMIA		
	MILD (+)	MODERATE (++)	SEVERE (+++)	MILD (+)	MODERATE (++)	SEVERE (+++)
MDA (µmol/ml)	6.58 ± 0.55^{a}	8.56 ± 0.97^{b}	5.03 ± 2.90^{a}	5.92 ± 0.41^{a}	6.82 ± 0.76^{a}	8.25 ± 1.00^{b}
GPx (U/mg protein)	136.39 ± 5.53^{c}	107.05 ± 4.4^{a}	134.32 ± 9.20^{b}	115.67 ± 6.82^{ab}	106.34 ± 7.20^{a}	129.65 ± 4.30^{b}
SOD(U/mg protein)	39.72 ± 2.53^{ab}	39.10 ± 4.20^{ab}	40.63 ± 8.37^{b}	37.36 ± 1.56^{a}	38.68 ± 2.20^{a}	40.53 ± 4.89^{b}
CAT(U/mg protein)	38.68 ± 2.44^{ab}	28.31 ± 3.74^{a}	27.83 ± 1.62^{a}	43.45 ± 2.34^{b}	41.91 ± 4.3^{b}	47.61 ± 6.89^{c}
GSH(mg/ml)	18.83 ± 1.73^{a}	21.05 ± 3.16^{a}	15.21 ± 6.32^{a}	23.64 ± 2.03^{ab}	26.65 ± 4.71^{b}	20.46 ± 5.35^{a}
VITC(mg/dl)	0.98 ± 0.06^{b}	0.75 ± 0.06^{ab}	0.54 ± 0.12^{a}	0.89 ± 0.04^{b}	0.90 ± 0.05^{b}	0.89 ± 0.05^{b}
VITE(mg/dl)	0.82 ± 0.05^{a}	0.79 ± 0.11^{a}	1.11 ± 0.44^{b}	0.82 ± 0.04^{a}	0.80 ± 0.06^{a}	0.90 ± 0.04^{ab}
NO (µmol/ml)	32.18 ± 1.95^{ab}	28.51 ± 3.91^{a}	32.56 ± 14.81^{ab}	32.24 ± 2.89^{ab}	49.89 ± 4.24^{b}	26.11 ± 5.20^{a}

All values are represented as mean ± SEM. Values in the same row with different alphabets differ significantly ($p < 0.05$).

Key: MDA: Malondialdehyde; GPx: Glutathione peroxidase; SOD: Superoxide dismutase: CAT: Catalase: GSH: Reduced glutathione: Vit C: Vitamin C; Vit E: Vitamin E;NO: Nitric oxide; (+): Mild, (++): Moderate, (+++): Severe parasitaemia

Table 3: Effect of gestation on antioxidant status of *Plasmodium falciparum* infected pregnant women.

	UIPW			IPW		
	FIRST TRIMESTER	SECOND TRIMESTER	THIRD TRIMESTER	FIRST TRIMESTER	SECOND TRIMESTER	THIRD TRIMESTER
MDA (µmol/ml)	5.18 ± 0.38^{a}	5.49 ± 0.33^{a}	4.40 ± 0.60^{a}	9.87 ± 0.50^{b}	11.61 ±0.50^{c}	8.43± 0.80^{b}
GPx (U/mg protein)	172.78 ± 9.9^{c}	177.88 ± 9.4^{c}	143.29 ± 12.9^{b}	133.85 ± 80^{ab}	128.18 ± 11^{a}	150.88 ± 8^{bc}
SOD (U/mg protein)	65.72 ± 2.91^{b}	48.11 ± 3.01^{ab}	45.40 ± 2.98^{ab}	38.85 ± 3.0^{a}	39.33 ± 2.50^{a}	48.17 ± 4.20^{ab}
CAT (U/mg protein)	34.49 ± 1.42^{a}	36.86 ± 1.71^{ab}	34.17 ± 1.46^{a}	50.16 ± 0.10^{b}	54.66 ± 4.10^{c}	57.35 ± 3.60^{c}
GSH (mg/ml)	30.14 ± 2.98^{c}	22.12 ± 2.41^{a}	21.12 ± 3.47^{a}	21.68 ± 3.50^{a}	24.04 ± 3.80^{b}	22.65 ± 3.90^{a}
VITC (mg/dl)	1.02 ± 0.73^{b}	1.00 ± 0.44^{b}	1.04 ± 0.9^{b}	0.64 ± 0.50^{a}	0.79 ± 0.10^{a}	0.91 ± 0.10^{b}
VITE (mg/dl)	0.89 ± 0.54^{a}	0.86 ± 0.53^{a}	0.94 ± 0.72^{a}	0.91 ± 0.10^{a}	0.70 ± 0.10^{a}	0.77 ± 0.50^{a}
NO (µmol/ml)	31.23 ± 2.32^{b}	30.63 ± 2.41^{b}	26.33 ± 2.53^{ab}	23.13 ± 3.90^{a}	30.74±3.20^{b}	29.81±1.90^{b}

All values are represented as mean ± SEM. Values in the same row with different alphabets differ significantly ($p < 0.05$).

Key:MDA:Malondialdehyde;GPx: Glutathione peroxidase; SOD: Superoxide dismutase; CAT: Catalase; GSH: Reduced glutathione; Vit C: Vitamin C; Vit E: Vitamin E; NO: Nitric oxide.

Table 4: Effect of parity on antioxidant status of *Plasmodium falciparum* infected pregnant women.

	UIPW			IPW		
	PARITY			PARITY		
	PRIMI.	SECONDI.	MULTI.	PRIMI.	SECONDI.	MULTI.
MDA (µmol/ml)	5.27±0.38^{a}	5.42±0.33^{a}	4.44±0.69^{a}	4.40±0.52^{a}	7.81±1.10^{b}	7.99±0.36^{b}
GPx(U/mg protein)	173.46±9.59^{c}	177.49± 9.7^{c}	166.35±9^{bc}	129.65±1.0^{b}	115.12±5.80^{ab}	106.99± 0.10.^{a}
SODU/mg protein)	66.27±2.85^{c}	55.87±2.04^{bc}	56.43±1.46^{bc}	40.39±3.0^{b}	36.36±4.30^{ab}	26.28±5.70^{a}
CATU/mg protein)	33.85±1.55^{a}	37.55±1.61^{b}	34.17±1.46^{a}	52.08±3.50^{c}	52.87±2.40^{c}	47.65±3.40^{bc}
GSH (mg/ml)	29.40±2.95^{b}	25.83±2.38^{ab}	30.36±3.29^{b}	25.31±2.90^{ab}	16.96±3.30^{a}	18.91±3.80^{a}
VITC (mg/dl)	1.02±0.07^{b}	0.99±0.05^{a}	0.94±0.07^{a}	1.18±0.70^{b}	0.92±0.10^{a}	0.71±0.10^{a}
VITE (mg/dl)	0.89±0.05^{ab}	0.87±0.05^{ab}	0.94±0.07^{b}	0.80±0.80^{ab}	0.63±0.80^{a}	0.94±0.70^{b}
NO (µmol/ml)	32.82 ± 1.80^{a}	31.96 ± 1.56^{a}	35.77 ± 2.47^{ab}	52.79 ± 4.88^{b}	32.80 ± 3.12^{a}	34.77 ± 0.54^{ab}

All values are represented as mean ± SEM. Values in the same row with different alphabets differ significantly ($p < 0.05$).

Key: UIPW: Uninfected pregnant women; IPW: Infected pregnant women; PRIMI: Primigraviae, SECONDI: Secondigravidae; MULTI: Multigravidae; MDA: Malondialdehyde; GPx: Glutathione peroxidase; SOD: Superoxide dismutase: CAT: Catalase: GSH: Reduced glutathione: Vit C: Vitamin C; Vit E: Vitamin E; NO: Nitric oxide.

DISCUSSION

Pregnancy involves the sequence of events that take place after the fertilization of an ovum, thus enabling the fertilized ovum to eventually develop into a full – term foetus and changes in acquired cellular immunity during pregnancy is also important [27].Pregnant women, especially primigravidaeand secondigravidae are known to have low immunity, hence they are easily prone to malaria than the non – pregnant women [28], [29].The level of susceptibility of pregnant women to malaria infection decreases with increase in number of pregnancies [30].The present study evaluated the antioxidant status of

plasmodium falciparum infected pregnant Nigerian women. The results showed significant($p < 0.05$) increases in lipid peroxidation productsin the pregnant women infected with *Plasmodium falciparum* when compared to control subjects. Malaria infection has been found to be associated with lipid peroxidation accompanying reduction in antioxidant capacity of the infected patients especially in *Plasmodium falciparum* infection [17],[31],[32]. The highly elevated ($p < 0.05$) levels of MDA obtained in this study, in the pregnant women with malaria infection, is an indication of increased production of reactive oxygen species. The increase ($p < 0.05$) in lipid peroxidation is probably due to the production of ROS species by the immune cells and also due to the release of O^{-}_{2} during hemoglobin degradation by the malaria parasite. It has been shown that intact *Plasmodium falciparum* trophozoite infected human red cells produce H_2O_2 and OH^{-} radical about twice as much as the normal erythrocyte [33].

The results of the present study support the role of increased oxidative stress in the pathogenesis of *P.falciparum* malaria. It was observed that patients with *P.falciparum* malaria are characterized by enhanced lipid peroxidation in their sera [14]. These findings support the notion that enhanced oxidative stress is an important characteristic in these patients [34]. While enzymatic and non-enzymatic systems preserve the antioxidant status, these defense systems become overwhelmed during oxidative stress, leading to a metabolic derangement due to an imbalance caused by excessive generation of ROS and diminished antioxidant capacity [35]. There are many sources of ROS in malaria, such as generation by intra erythrocyte parasite and production by host-phagocytes as a defense mechanism against the parasite. ROS are also generated during the breakdown of hemoglobin by the malaria parasite [36]. The antioxidant enzyme activities vary inversely with the severity of malaria[17].Antioxidants are used up to counteract the effects of free radicals generated by the immune system of the host, in the cause of malaria infection [17]. This explains why reduction in antioxidant level is dependent on the severity of malaria [17].In this study, patients with severe malaria had significantly ($p < 0.05$) lower antioxidant activities than those with mild/moderate malaria. Results also revealed significant decrease ($p < 0.05$) in serum antioxidant enzyme (GPx, SOD) activities, as well as GSH, Vitamins C and E levels in the infected pregnant women when compared to the controls, but CAT was significantly increased ($p < 0.05$) in the infected pregnant women when compared to the controls. This may be attributed to either, increased requirement or increased destruction of antioxidants during malaria infection.

This is similar to the work of [37] and [38]who reported reductions in the levels of some vitamins. Vitamin C which is a water soluble vitamin and non-enzyme antioxidant serves directly by scavenging aqueous peroxyl radicals and indirectly regenerate reduced vitamin E [30]. Vitamin C levels are reduced ($p < 0.05$) in malaria patients following its use to regenerate vitamin E from alpha tocopheroxyl radical at water lipid interface (31). It is also an efficient quencher of superoxide and hydroxyl radicals [39].The significant reduction ($p < 0.05$) of vitamin E in malaria patients may probably be due to enhanced lipid peroxidation by the *Plasmodium falciparum.* Vitamin E accounts for most of the chain breaking antioxidant activity in the erythrocyte membrane [16]. To be functionally active, vitamin E has to be maintained in its native state for which ascorbate contributes. Ascorbic acid (vitamin C) converts tocopheroxyl radical to its native state. Therefore, loss of ascorbate may interfere in tocopherol regeneration and may lead to impaired membrane function [39]. The lower ($p <0.05$) values obtained in antioxidant levels in malaria in this study may also be attributed to increased utilization of the host antioxidants by the malaria parasites to counteract oxidative damages. Degradation of antioxidant enzymes as well as haemoglobin by malaria parasite to produce its own protein has been reported [40]. This might thus be contributing to the decline in antioxidant status. The infected non – pregnant women also demonstrated elevated MDA levels, reduction in GPx and SOD activities and vitamins C and E and increase in catalase activity. However, the degree of manifestation in these indices were higher ($p < 0.05$) in the infected pregnant women than in the infected non – pregnant women.It has been established that experimentally induced *Plasmodium falciparum* infection strongly induces NOS in PBMCs in malaria-naive adult human volunteers at low levels of parasitaemia that are detectable by PCR but not by microscopy [41]. It is also possible that other subclinical infections such as intestinal parasitosis [42] contribute to basal NO production in tropical regions via activation of monocyte CD23 receptorsand other mechanisms [43].

CONCLUSION

The increased level of MDA and reduced antioxidant status probably implies that oxidative stress plays a role in the pathogenesis of malaria. The pregnant women were more prone to and affected by these compromised antioxidant imbalance, and vary with the severity of infection, gestational age and inversely with gravidae. Having established that the levels of Vitamins C and E are depleted in malaria, it is recommended that supplementation with these antioxidants

through exogenous intake will protect pregnant women from a high risk of oxidative damage which may be associated with malaria. This also will reduce the frequently associated adverse outcomes for mother and baby. The increased activities of some antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress.

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