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Original article

Anti- MSP-1_{19} antibody (IgG) and reactive oxygen species (ROS) response against malaria infection in pregnancy in South Western Nigeria

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Abstract

Objective: Although immunity to malaria is reduced in pregnancy, the maternal immune system still continues to respond to malaria infection by the production of antibodies. IgG has been reported to play significant role in immune response against *P. falciparum*. Anti-MSP-1_{19} antibody and reactive oxygen species have been shown to be protective against malaria infection in children. This work assessed the response of anti-MSP-1_{19} antibody (a promising blood stage vaccine candidate antigen) and oxidative stress in 250 pregnant women. **Methods**: Blood samples were collected in dry and wet seasons. *Plasmodium falciparum* infection was determined by microscopy, anti-MSP-1_{19} IgG level was investigated using ELISA. Malondiadelhyde (MDA) and reduced glutathione (GSH) were used as indicators of oxidative stress and they were quantified spectrophotometrically. **Results**: Parasitaemia was significantly higher ($P<0.05$) in wet than dry season and its level decreased with gravidity. There was a significant increase ($P<0.05$) in anti-MSP-1_{19} IgG and MDA levels in the dry than wet season. Anti-MSP-1_{19} IgG and MDA levels were significantly higher in *P. falciparum* positive primigravidae than *P. falciparum* negative primigravidae in both wet and dry seasons. In wet season anti-MSP-1_{19} IgG level was significantly increased ($P<0.05$) in *P. falciparum* positive multigravidae than *P. falciparum* negative. The anti-MSP-1_{19} IgG and MDA were significant higher in *P. falciparum* positive multigravidae than primigravidae. Reduced glutathione (GSH) level was significantly reduced ($P<0.05$) among malaria positive than malaria negative patients in both seasons. **Conclusion**: This study suggests that IgG and MDA response were positively associated with the presence of malaria infection.

Keywords: Pregnancy; Malaria; Reactive oxygen species; Anti-MSP-1_{19} antibody (IgG); MDA

INTRODUCTION

Malaria constitutes one of the major health problems in Africa and many other tropical countries around the world[1]. There is an increase in the susceptibility of pregnant women to malaria infection when compared to their non-pregnant counterpart living in the same endemic region[2]. Primigravidae has been reported to develop serious malaria infection than multigravidae[2, 3]. This susceptibility, however, decrease with increasing numbers of pregnancies, suggesting that women acquire pregnancy-associated immunity with successive pregnancies. Acquired immunity involves both cellular and humoral factors. Humoral immune responses have been strongly impli-

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cated in blood-stage protection against malaria infection. Antibody activity against *P. falciparum* has been found in immunoglobulin G (IgG) fraction of immune sera[4]. Studies have shown that adults living in malaria endemic regions develop immunity to malaria infection[5, 6], it has been reported that IgG seems likely to be protective in *P. falciparum* infection in man[7]. Field studies have shown that the mean serum IgG level of adult women was depressed in association with pregnancy[8, 9]. This reduction could be as a result of regulation of immune response against foetus[8]. The progressive fall in IgG is of interest, since antibodies protective against malaria are known to exist in this immunoglobulin. Despite this depression, the maternal immune system continues to respond to the parasite and antibodies against malaria parasite are still produced[10]. Passive transfers of IgG have provided protection against *P. falciparum* blood stage in both American monkey and in human[11].

Anti-MSP-1_{19} antibody has been reported to inhibit efficiently *in-vitro P. falciparum* merozoite proliferation[8, 10] and mediates opsonization of infected erythrocyte[10]. The level of IgG against the MSP-1_{19} C terminal region can serve as a good indicator of protective immunity[12]. Different studies have shown the protective role of anti-MSP-1_{19} IgG against the antigen in children in different parts of tropical region including Nigeria[12, 13]. Though the response and protective role of anti-MSP-1_{19} IgG in children has been established, it is need to study the anti-MSP-1_{19} IgG response and its protective role in pregnancy, as MSP-1_{19} has emerged as the most promising blood stage vaccine candidate antigen under development.

Apart from pregnancy, which creates an environment for oxidative stress[14], malaria infection is also accompanied by increased production of ROS which indicates the environment for oxidative stress. The level of oxidative stress could be assessed by quantifying MDA level in the body[15]. Malaria parasite has been reported to be sensitive to oxidative damage and the level of oxidative stress is influenced by the severity of malaria infection as measured from the plasma parameters[15, 16]. Destruction of malaria parasite by the immune system has been related to the increased production of ROS by macrophages and polymorphonuclear neutrophils[14, 17] which serve as effector cells in the elimination of the parasite. These changes may play a key role in host defense against malaria, but may also render host tissues such as erythrocytes more vulnerable to oxidative damage[18, 19] as macrophages generated ROS are known as non-specific effectors molecules in their defense armoury[20]. Though, the destruction of both parasitized and non-parasitized red blood cells by macrophage generated ROS has been reported[12, 14], no work has studied the relationship between malaria parasite, ROS and anti-MSP-1_{19} IgG production in pregnancy in south western Nigeria. Therefore, this study assesses anti-MSP-1_{19} IgG and ROS response against malaria parasite in pregnancy in Ibadan, south western Nigeria.

MATERIALS AND METHODS

Study group

The study was conducted during the dry season (October-January i. e. immediately after the wet season) and wet season (April-July i. e. the beginning of the wet season) at Ade Oyo Maternity Hospital Ibadan, Nigeria. Ibadan is located in the south-western part of Nigeria. Blood samples were collected from 250 pregnant women who came for antenatal care. The women were grouped according to their gravidity (primigravidae and multigravidae). Informed consent was obtained from them. Information on demographic, clinical history, age, last menstruation date, gravidity, and antimalaria drugs previously used were obtained from them by questionnaire. Patients who have been transfused within the last two months were excluded from the study. Those who were malaria positive were treated according to WHO standard regulation for managing malaria. The study was reviewed and approved by the Joint Ethical Committee of the College of Medicine and University of Ibadan, Ibadan, Nigeria.

Parasitological study

Thick and thin peripheral blood film were prepared from each sample, stained with Giemsa stain and examined for the presence of parasites using routine microscopy to determine the parasiteamia. For the positive slides, the number of parasite counted per 200 white blood cells was recorded and used to calculate parasite density assuming 8 000 leucocytes/

μL of blood.

Determination of GSH and Lipid peroxidation

Lipid peroxidation in serum was assessed by measuring the thiobarbituric acid reactive substances (TBARS) and expressed in term of malondialdehyde (MDA) formed per mg protein according to the procedure described by Rice-Evans *et al*[21]. GSH level in the supernatant of serum was determined using the method described by Jollow *et al*[22].

Determination of anti- MSP-1_{19} antibody

The serum samples from the pregnant women were analyzed by ELISA for anti MSP-1_{19} antibody as described by Aucan *et al*[10].

Statistical analysis

Women were divided into primigravidae and multigravidae. Those with first pregnancy were grouped under primigravidae (G1) while those with two or more pregnancy were grouped under multigravidae (G2 - G5). We did not consider age in this study because we realized that age given to us by the participants were unreliable. The antibody titers were log transformed. Student's t-test was used to compare means and means were separated using Duncan multiple range tests for comparison of the parameters studied. The level of significance was estimated at $P < 0.05$. The log reciprocal antibody titers were expressed at log base 10. The software packages used were SPSS 11.0 and Excel.

RESULTS

The results showed that of 250 pregnant women studied, 46.4% were *P. falciparum* positive in both dry and wet seasons. When season was put into consideration, 30% and 61% were *P. falciparum* positive in dry and wet seasons respectively. The result also showed that 45% and 63% of primigravidae were *P. falciparum* positive in dry and wet seasons respectively, while 32% and 61% of multigravidae were *P. falciparum* positive in dry and wet season respectively (Table 1). The mean parasitaemia was significantly higher ($P < 0.05$) in wet than dry season. The mean anti-MSP-1_{19} antibody titers and MDA levels were significantly higher ($P < 0.05$) in dry season than in wet season, while GSH was significantly higher in wet than dry season (Table 2).

Anti-MSP-1_{19} antibody titers was significantly higher ($P < 0.005$) in *P. falciparum* positive primigravidae than *P. falciparum* negative primigravidae in both wet and dry seasons, likewise the mean MDA level was significantly increased ($P < 0.05$) in *P. falciparum* positive primigravidae than *P. falciparum* negative primigravidae in both wet and dry seasons (Fig 1a). The mean anti-MSP-1_{19} antibody titers was significantly higher ($P < 0.05$) in *P. falciparum* positive multigravidae than in *P. falciparum* negative multigravidae in dry and wet seasons, while the mean MDA level was marginally higher in *P. falciparum* positive multigravidae than in *P. falciparum* negative multigravidae in both wet and dry seasons (Fig 1b).

The mean anti-MSP-1_{19} antibody titers and MDA levels in *P. falciparum* positive increased as gravidity increased; the anti-MSP-1_{19} antibody titer was significantly higher in *P. falciparum* positive G5 than *P. falciparum* positive G1. The mean MDA level was also significantly higher in *P. falciparum* positive G5 than in *P. falciparum* positive G1 (Fig 2). As the gravidity increases from G1 to G5, the mean GSH level was also increased in both *P. falciparum* positive and negative pregnant women (Fig 3). The mean parasitaemia decreased as the gravidity increased from G1 to G5 and the mean parasitaemia was significantly higher in primigravidae than in multigravidae (Fig 4).

DISCUSSION

Some studies have shown the response of anti-MSP-1_{19} IgG in malaria endemic areas including Nigeria[8, 12] but no work has been done on the response of anti-MSP-1_{19} IgG and ROS in pregnancy in south western Nigeria. This study therefore assessed the anti-MSP-1_{19} IgG and ROS response in pregnancy in southwestern Nigeria.

The relationship between frequency of exposure to malaria parasite, functional immunity and disease risk are yet to be assessed in pregnancy in Nigeria. Antibody responses against MSP-1_{19} may either block red blood cell invasion or make the merozoite susceptible to phagocytosis through the phagocyte Fc receptor on IgG[6, 12]. Antibody specific to parasite acts in co-operation with cell mediated immunity and natural killer cell to eliminate the parasite. In many

cases, killing of the parasite requires that the parasite should be precoated with specific antibodies, and such parasite will be destroyed by a process called antibody dependent cell mediated cytotoxicity (ADCC). Thus, ADCC occurs only when the target cell is precoated with antibody. It is clear that antibodies serve two distinct functions; first, they provides cognitive function i. e. parasites that have bound IgG will be preferentially killed compared with those not displaying IgG. Secondly, the occupancy of Fcγ Ⅲ serves to activate the natural killer cell to synthesize and secrete cytokines etc as well as to discharge ROS which probably mediate the lytic function to this cell type[23]. This shows that the level of antibodies may determine the rate of production of ROS and thus the rate at which the parasites are eliminated.

Pregnant women have been reported to produce antibody against MSP-1_{19} and its production has been linked to protection against malaria parasite infection[8]. This study was carried out at Ibadan where malaria infection is perennial, but the prevalence is usually higher in wet season than dry season[12]. This study also confirmed that the prevalence of infection was higher in wet than dry season. Our results showed that all pregnant women studied produced antibody to MSP-1_{19}. It has been previously reported that individuals living in malaria endemic regions tends to acquire immunity against malaria infection[6, 8]. The levels of anti-MSP-1_{19} antibody varied with season, gravidity, and *P. faciparum* infection in this study. The mean anti-MSP-1_{19} IgG was significantly higher in the dry season than the wet season. This could be as a result of anti-MSP-1_{19} IgG accumulating over the prolonged period of previous wet season (i. e. from the beginning of wet season to the end), which actually marked the period of high prevalence of malaria infection. Though, malaria infection in Ibadan is perennial, its prevalence is higher in wet than dry season and therefore there is tendency that the exposure rate to malaria parasite of the subjects studied immediately after the wet season (dry season), was higher than the exposure rate in the beginning of wet season and since the rate at which the body produces antibodies in response to the presence of parasite is determined by rate of exposure to malaria parasite, this might also be one of the reasons why anti-MSP-1_{19} IgG was lower among the subjects studied at the beginning of the wet season when exposure rate to malaria parasite was minimal as compared to early part of dry season.

When the effect of *P. falciparum* infection on production of anti-MSP-1_{19} IgG was put into consideration, it was found that anti-MSP-1_{19} IgG was significantly higher among *P. falciparum* positive primigravidae and multigravidae in both seasons as compared with *P. falciparum* negative. This shows that *P. falciparum* is a strong determining factor for anti-MSP-1_{19} IgG production in individuals living in malaria endemic regions. It has been reported that parasite can only be recognized by macrophages when it is coated by anti-MSP-1_{19} IgG, which have special affinity to bind Fc gamma receptors on monocytes and macrophages[10]. There was an increase in anti-MSP-1_{19} IgG as the gravidity increased from G1 to G5. The increase was significantly higher among *P. falciparum* positive multigravidae than primigravidae. This could be responsible for the low level of *P. falciparum* density among multigravidae as compared to primigravidae.

We also studied the production of MDA and GSH as indices of oxidative stress in pregnant women. This was done to appreciate the importance of ROS in the elimination of malaria parasite in pregnancy as malaria parasite has been reported to be sensitive to ROS and its level of production has also been related to the severity of malaria infection[15]. The level of MDA was significantly higher in dry season than wet season; this could be because of the response of individuals against infection in the previous wet season. The significant increase in GSH level in wet season could be as result of low level of MDA level in the wet season which indicates absence of oxidative stress while low level of GSH could be related to the high MDA level in the dry season[17].

The MDA level was significantly higher in both wet and dry seasons *P. falciparum* positive primigravidae than *P. falciparum* negative primigravidae while it was marginally higher among *P. falciparum* positive multigravidae than *P. falciparum* negative multigravidae. This is in agreement with the belief that membrane lipid peroxidation of tissues is possible in *P. falciparum* infection due to increased production of ROS by immune system[19]. This may play a key role in host defense against malaria infection[19]. The mean MDA and anti-MSP-1_{19} IgG level were significantly increased in *P. falciparum* positive subjects as the gravidity increases from G1 to G5. The low

level of MDA and anti-MSP-1_{19} IgG in primigravidae could be responsible for the higher level of *P. falciparum* density among primigravidae studied as compared with the multigravidae. It has been reported that parasite can only be recognized by macrophages when it is coated by anti-MSP-1_{19} IgG which has special affinity to bind Fc gamma receptors on monocytes and macrophages[10] which initiate the production of ROS that serves as non-specific effector molecule in their defense armoury[20]. This could be probably responsible for the killing of the malaria parasites. The malaria parasite density was found to decrease from G1 to G5 and significantly higher in G1 than G5. This could mean that the production of high level of anti-MSP-1_{19} IgG and MDA in G5 may be responsible for the destruction of the parasites and thereby decreasing the parasitaemia in multigravidae than primigravidae.

Mean GSH level was significantly reduced among *P. falciparum* positive than *P. falciparum* negative subjects. This agrees with previous report by[17]. The reduction might be as a result of the increase in ROS produced in malaria positive subjects which may result in depletion of GSH due to its oxidation to GSSG in response to oxidative stress in the host. GSH is known to serve as scavengers for oxidant agents[17]

This study revealed the higher level of production of MDA and anti-MSP-1_{19} IgG in *P. falciparum* positive individuals as compared to *P. falciparum* negative subjects, and the high level of MDA and anti-MSP-1_{19} IgG correlated with the reduction of parasitaemia in dry season and multigravidae as compared with wet season and primigravidae. Further work needs to be done to confirm the relationship between the anti-MSP-1_{19} IgG and ROS in pregnancy.

Table 1 Prevalence of *P. falciparum* among pregnant women in relation to gravidity in wet and dry season.

	*n*1		Dry season	Wet season
	dry	wet	*n*2 (%)	*n*3 (%)
Pregnant women	115	135	35 (30)	83(61)
Primigravidae	62	74	28 (45)	47 (63)
Multigravidae	53	61	17 (32)	36 (59)

*n*1: total number in dry and wet seasons.
*n*2: number positive in dry season.
*n*3: percentages positive in wet season.

Table 2 Mean parasite density, MDA, Anti-MSP-1_{19} and GSH levels in pregnancy in wet and dry season.

	Dry season	Wet season
Mean parasite density	1 112 ± 45 *	2 746 ± 100
Mean MDA level (μmol/mL)	3.04 ± 0.4 *	2.60 ± 0.2
Mean anti-MSP-1_{19} IgG	2.84 ± 0.3 *	2.25 ± 0.1
Mean GSH level (μmol/mL)	35.1 ± 6.3 *	48.3 ± 7.9

The means were separated for significant differences using Duncan's multiple range tests;
* :It is sigrificanthy different from wet season.

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search and Training, University of Ibadan, Ibadan, Nigeria.

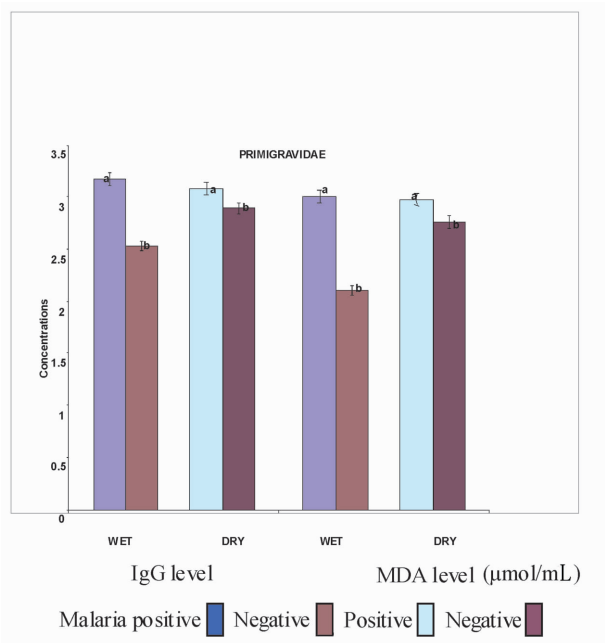

Figure 1a Mean ± SD of MDA (μmol/mL) and IgG levels of malaria positive and negative primigravidae in dry and wet seasons. MDA and IgG levels were significantly higher ($P<0.05$) in all malaria positive than malaria negative, ("a" is significantly different from "b").

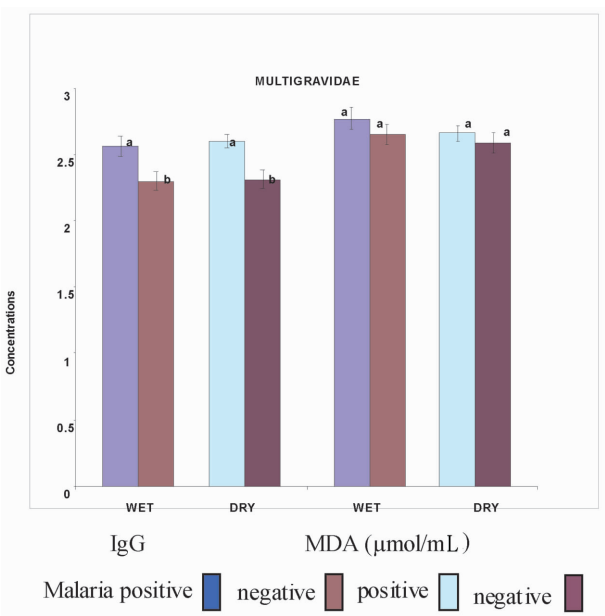

Figure 1b Mean ± SD of MDA (μmol/mL) and IgG of malaria positive and negative multigravidae in dry and wet seasons. IgG levels were significantly higher ($P<0.05$) in malaria positive than malaria negative in wet and dry seasons. ("a" is significantly different from "b").

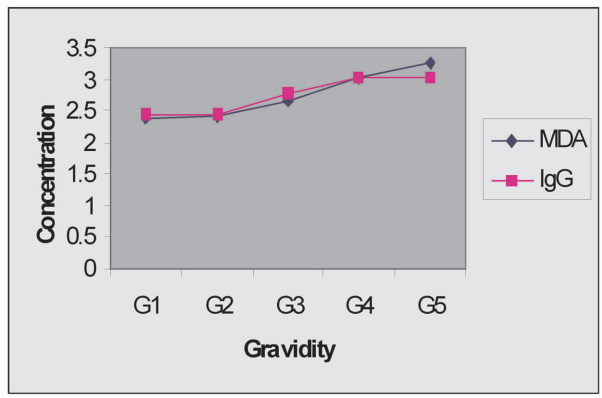

Figure 2 Mean MDA (μmol/mL) and IgG levels in malaria positive primigravidae and multigravidae. MDA and IgG levels were significantly lower ($P<0.05$) in G1 than G5.

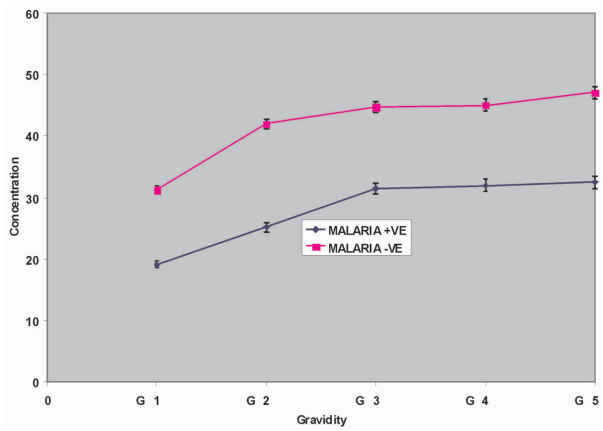

Figure 3 Increasing order of mean GSH as the gravidity increases from G1 to G5 in malaria negative and positive. GSH level was significantly higher in malaria negative than malaria positive ($P<0.05$).

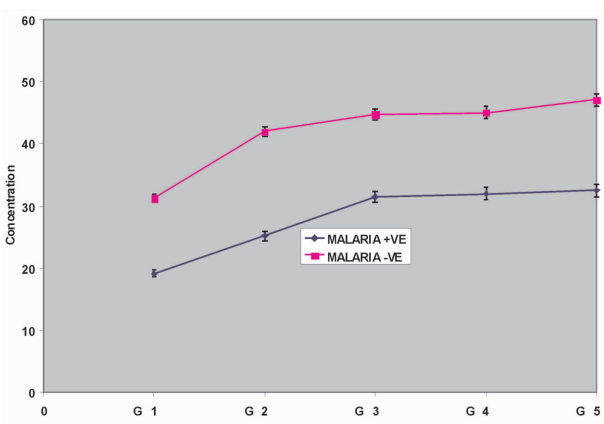

Figure 4 Parasitaemia as the gravidity increases from G1 to G5. The mean parasiteamia was higher in primigravidae (G 1) than in multigravidae (G2- G5).

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