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INFLUENCE OF HAEMOGLOBIN GENOTYPE ON MALARIA PARASITE DENSITY AND ANTI-MSP-1₁₉ IgG (ANTIBODY) RESPONSE IN PREGNANT WOMEN AND BIRTH WEIGHT OF NEONATES

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ABSTRACT

Haemoglobin genotype variance has been reported to be responsible for the high susceptibility of individuals to malaria in the tropics. This study assessed the influence of haemoglobin genotype on the malaria parasite density and anti-MSP-1₁₉ IgG (antibody) during pregnancy and the effect of malaria infection on birth weight of neonates. One hundred and thirty (130) pregnant women and one hundred and fourteen (114) non-pregnant women (Control) were enrolled and grouped according to their haemoglobin genotype. The study assessed only the pregnant women with haemoglobin genotype AA (HbAA) and haemoglobin genotype AS (HbAS). Blood sample from each subject was collected by venipuncture into EDTA, and plain bottles to determine haematological parameters and anti-MSP-1₁₉ IgG (antibody) level, respectively. The parasite density was significantly higher ($P < 0.05$) in malaria positive HbAA women than in malaria positive HbAS women in dry and wet seasons. There was a significant increase ($P < 0.05$) in the mean anti-MSP-1₁₉ IgG (antibody) levels in women with HbAA as compared with women with HbAS in the dry season. The anti-MSP-1₁₉ IgG level was not significantly higher in women with HbAA when compared with women with HbAS. The mean birth weight of neonates born to malaria positive HbAA women was lower than those born to malaria positive HbAS women in the wet season, but there was no significant difference between the mean birth weight of neonates born to malaria positive and malaria negative HbAA and HbAS women in dry season. This study showed that haemoglobin genotype influenced parasite density but did not influence the level of anti-MSP-1₁₉ IgG and the birth weight of neonates.

Keywords: Haemoglobin genotype, malaria parasite, anti-MSP-1₁₉ IgG, pregnant women

INTRODUCTION

Malaria still remains one of the parasitic diseases that are of great concern to World Health Organization. Several steps have been taken to eradicate it, but it continues to spread across the globe, even to the areas where it had been previously eradicated (WHO, 2012). The most challenging aspect of malaria control is the development of drug resistant strains of the parasite, especially *Plasmodium falciparum* to most of the effective and available drugs (Akanbi *et al.*, 2014). The infection is common among the children, and pregnant women are also highly susceptible especially, during their first and second pregnancy (primigravidae and

secungravidae respectively) which may lead to abortion, low birth weight of the baby, neonatal death and even death of the mothers (Idowu *et al.*, 2010; Olusi and Abe, 2014). Several factors have been reported to be responsible for this high prevalence of malaria infection in children and pregnant women, one of which is the low level of immunity (Omosun *et al.*, 2009). In pregnant women, the case of immunosuppression has been reported to be responsible for their high susceptibility to malaria infection (Akanbi *et al.*, 2009). During pregnancy, the T helper cell 1 (Th-1) is suppressed purposely for the implantation of the allograft, allowing some infectious agents take

advantage to establish themselves and cause serious infection in pregnant women (Samak, 2004). Apart from the level of the immunity, red blood cell disorders including variant haemoglobin genotype, blood grouping and thalassaemia have been considered to have impact in the prevalence of malaria infection in endemic areas (Uneke *et al.*, 2007). The protective role which certain haemoglobin genotypes plays against malaria infection has been attributed to the high incidence of haemoglobin genotype variance in the tropical region where malaria incidence is very high (Verra *et al.*, 2007; Edith *et al.*, 2012). The level of susceptibility to malaria infection has been reported to be higher in individuals with HbAA when compared with those with HbAS and HbAC, thus, the high frequency of HbAC and HbAS in malaria endemic areas has been attributed to a decrease in malaria morbidity and mortality in malaria endemic areas (Uneke *et al.*, 2007).

The protective role displayed by HbAC and HbAS in malaria infection is as a result of reduced cytoadhesion of infected red blood cell to microvasculature and impaired rosetting formation as a result of the presence of abnormal PfEMP1 antigen on HbAC and HbCC (Verra *et al.*, 2007). Similarly, the presence of HbAS genotype enhances the recognition of two malaria parasite isolates by the immune system in Gabon (Cabrera, *et al.*, 2005) and this indicates that HbAS genotype is associated with the accelerated acquisition of immunity against malaria infection in an individual (Williams *et al.*, 2005).

Malaria infection and anaemia have been reported to be the two major factors responsible for low birth weight of infants born in tropical region (Akanbi *et al.*, 2009; Smaila *et al.*, 2013). Most of the studies have shown the effect of haemoglobin genotype on the prevalence of malaria infection, but there is a dearth of information on the effect of haemoglobin genotype on the anti-MSP-1₁₉ IgG (antibody) response against malaria infection in pregnant women, and the effect of haemoglobin genotypes on the birth weight of infants in South Western part of Nigeria. This work therefore, studied the role of haemoglobin genotype on parasite density and anti-MSP1-₁₉ IgG

(antibody) response in pregnant women and its effect on the birth weight of neonate in South West Nigeria in dry and wet season.

MATERIALS AND METHODS

Study area

This study was carried out at Ade-Oyo Maternity Hospital, Ibadan, Oyo State, in the South West of Nigeria in November to January and May to August. *Plasmodium falciparum* is the common malaria parasite in this region and the transmission is perennial and usually more prevalent in the rainy season when compared with the dry season.

Study population

Two hundred and forty four (244) women were recruited for this study, one hundred and thirty were pregnant and one hundred and fourteen were non-pregnant (control). The women were grouped according to their haemoglobin genotype. The study assessed only those with HbAA and HbAS while those with HbSS were excluded from the study because they were very few in number. Those who were transfused few weeks before the commencement of the blood collection were also excluded from the study. Those who showed clinical symptom of sickness were referred to medical doctors for treatment. Detailed information including age, parity, number of stillbirth, occupation, gravidity, and episode of malaria infection were obtained from each patient using open/close questionnaire. Information about the neonates were collected from the trained nurse who attended to the patients at delivery and also took the birth weight using the hospital's weighing scale. Informed consents were obtained from the patients and the study was approved by the local Institution Ethical Review Committee.

Blood collection

Blood sample was collected by venipuncture into EDTA and plain bottles from each patient. The blood in EDTA bottle was used to determine the haematological parameters, while serum obtained from the blood in the plain bottle was used to determine the anti-MSP-1₁₉ IgG (antibody) level against malaria parasite.

Determination of parasitaemia

Parasitaemia was determined from the whole blood from each patient by microscopy. Thick film was prepared on the slide for each patient,

and it was flooded with Giemsa stain and was allowed to stay for 20 minutes before it was washed and examined under the light microscope. The parasite density was calculated as described by Akanbi *et al.* (2006).

Determination of haematological parameters

The level of anaemia was determined by quantified haemoglobin concentration level using cyanmethaemoglobin method as described by Dacie (1994) and it was graded according to WHO standard. 0.02ml of whole blood sample was added to 5ml of Drabkin's solution containing 200mg of $K_3Fe(CN)_6$, 500mg of KCN and 1g of $NaHCO_3$ in 1 liter of distilled water, and this was incubated for 15 minutes at room temperature. The haemoglobin was converted to cyanmethaemoglobin (HbCN). The absorbance of the solution was then read against a blank in a spectrophotometer at the wavelength of 540nm. The concentrations of the standard were plotted against optical density on semi-log graph paper. The concentrations of the test and control samples were read from the standard curve.

Determination of haemoglobin genotype

Haemoglobin genotypes were determined using electrophoretic tank with cellulose acetate membrane as described by Schnieder *et al.* (1974). The haemolysate from the whole blood was prepared from each sample and was carefully placed on the cellulose acetate membrane at p^H 8.6. The cellulose membrane was then placed in the Shandon electrophoretic tank which contained 250ml of Tris-EDTA-Borate (TEB) buffer. The electrophoresis was allowed to run with the direct current power pack at 200 volts for 30 minutes at room temperature. The strip was removed and allowed to dry and the haemoglobin genotype was determined using the band of the standard as references

Determination of anti-MSP1₁₉ antibody (IgG) against malaria parasite

The anti-MSP-1₁₉ IgG (antibody) level against malaria parasite was determined from serum using ELISA as described by Aucan *et al.* (2000). Disposable polystyrene microtiter plates with 96 wells were coated with 100 μ l of recombinant *P. falciparum* merozoite surface protein 1, (PfMSP1₁₉) antigen. The plates were incubated overnight at 4°C in the incubator. The plates were aspirated and washed three times

with 0.05% PBS-Tween-20. 200 μ l of blocking buffer was added to each well to block all unspecific binding. The wells were aspirated and washed three times with 0.05% PBS-Tween-20 and then banded dried. 4 μ l of test sera diluted in 1:50 (200 μ l) of blocking buffer was added to column 'A' of well of the plate, while 100 μ l of blocking buffer was added to column 'B-H' wells of the plate. Then serial dilution of 1:50, 1:100, 1:200, 1:400, up to 1:6400 were made from column 'A' well of the plate. Then the plate was incubated for one hour at 37°C in the incubator. The plate was later decanted and washed three times with PBS-Tween-20 and banded dried. 100 μ l of conjugate prepared in 1:3000 μ l of blocking buffer was added to all the wells and incubated for one hour at 37°C, and this was decanted and washed three times with PBS-Tween-20 and banded dried, after which 100 μ l of 2,2'-azino-di(3-ethyl-benzthiazoline sulfonate (ABTS) substrate with peroxide was added to each well and the colour was allowed to develop at 37°C in the incubator for 30 minutes. The absorbance of the mixture was read at 650nm at room temperature with microtiter reader (Molecular Devices Menlo Park, CA, USA).

Statistical analysis

The anti-MSP1₁₉ IgG (antibody) was log transformed and their means were determined. Students't-test was used to compare the means and the level of significance was determined at $P < 0.05$. Inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals. The software package used was SPSS 15.0.

RESULTS

The mean parasite density was significantly higher ($P < 0.05$) in both pregnant and non-pregnant women with HbAA than in women with HbAS in both wet and dry seasons (Table 1), while the mean anti-MSP-1₁₉ IgG (antibody) response against malaria infection and Hb level was not significantly higher ($P > 0.05$) in women with HbAA than in women with HbAS in both dry and wet season (Table 1).

Anti-MSP-1₁₉ IgG (antibody) level was not significantly lower ($P > 0.05$) in both HbAA and HbAS pregnant women than in HbAA and HbAS non-pregnant women in wet and dry

seasons (Table 2), likewise there was no significant difference ($P < 0.05$) between the mean anti-MSP-1₁₉ IgG (antibody) of HbAA pregnant women and HbAS pregnant women in both wet and dry seasons. The Hb level was not significantly higher in HbAA non-pregnant women than in HbAA pregnant women in both dry and wet seasons. Among HbAS women, the Hb level was significantly higher ($P < 0.05$) among non-pregnant women than pregnant women in dry season. There was no significant difference between the Hb levels in HbAA and HbAS pregnant women in both dry and wet seasons (Table 2).

The mean birth weights of the neonates born to both malaria positive and malaria negative HbAA mothers were not significantly different from the birth weight of neonates born to malaria negative and malaria positive HbAS

mothers in the dry season, while the birth weight was significantly higher in neonates born to malaria positive and malaria negative HbAS mothers than malaria positive and malaria negative HbAA mothers in wet season (Table 3). The mean birth weights of the neonates born to malaria positive HbAA and HbAS pregnant women in dry and wet seasons was significantly lower ($P < 0.05$) compared with those born to the malaria negative mothers. Hb level was significantly lower ($P < 0.05$) in HbAA malaria positive when compared with HbAA malaria negative pregnant women, but there was no significant difference between the Hb level in both malaria positive and malaria negative HbAS pregnant women (Table 3).

Table 1. Effect of haemoglobin genotype on mean Anti-MSP1₁₉ antibody ((IgG) response, Hb levels

GENOTYPE (n)	DRY SEASON			WET SEASON		
	Anti-MSP-1 ₁₉ IgG Level	Hb(g/dl) level	Parasite density	Anti-MSP-1 ₁₉ IgG level	Hb(g/dl) level	Parasite density
HbAA (70)	3.43±0.3 ^a	10.0±1.4 ^a	3550±34 ^a	2.9±0.6 ^a	11.0±1.9 ^a	6169±42 ^a
HbAS (39)	2.7±0.4 ^b	9.9±1.9 ^a	635±233 ^b	3.1±0.5 ^a	10.7±1.6 ^a	4799±34 ^b

Key: $P < 0.05$; Mean followed by different letters across the column are significantly different using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals.

(n) represented number of patients

Table 2. Effect of haemoglobin genotype on mean Hb and Anti-MSP-1₁₉ IgG (antibody) levels in pregnant and non-pregnant women in dry and wet seasons

GENOTYPE	(n)	DRY SEASON		WET SEASON	
		Hb (g/dl)	Anti-MSP-1 ₁₉	Hb (g/dl)	Anti-MSP-1 ₁₉
		Level	IgG level	level	IgG level
HbAA					
Pregnant	(68)	10.8±1.2 ^a	2.7±0.6 ^a	10.3±1.6 ^a	2.9±0.7 ^a
Non-pregnant	(62)	13.1±1.0 ^a	3.3±0.3 ^a	12.6±1.0 ^a	3.0±0.8 ^a
HbAS					
Pregnant	(60)	10.2±1.3 ^a	2.7±0.5 ^a	10.1±1.2 ^a	2.9±0.6 ^a
Non-pregnant	(54)	13.1±1.4 ^b	3.2±0.4 ^a	12.4±1.6 ^a	3.2±0.5 ^a

Key: P<0.05; Mean followed by different letters across the column are significantly different using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals.

(n) represented number of patients

Table 3. Effect of haemoglobin genotype on haemoglobin and Anti-MSP₁₉ antibody (IgG) levels in malaria positive and negative pregnant women and birth weight of the neonates in dry and wet seasons

GENOTYPE(n)	DRY SEASON			WET SEASON		
	Hb (g/dl)	Anti-MSP-1 ₁₉ IgG	Birth weight	Hb (g/dl)	Anti-MSP-1 ₁₉ IgG	Birth weight
HbAA						
Positive (43)	10.0±0.4 ^b	3.5±0.1 ^a	3.0±0.1 ^a	11.0±1.9 ^a	2.9±0.1 ^a	2.0±0.1 ^b
HbAS						
Positive (29)	9.9±0.8 ^b	3.4±0.4 ^a	3.0±0.1 ^a	10.7±1.6 ^a	3.1±0.5 ^a	2.8±0.2 ^{bc}
HbAA						
Negative (25)	11.2±0.3 ^a	2.9±0.2 ^b	3.4±0.1 ^a	10.8±1.8 ^a	3.2±0.3 ^a	3.3±0.2 ^a
HbAS						
Negative (31)	10.7±1.8 ^{ab}	2.3±0.4 ^b	3.3±0.1 ^a	11.1±1.9 ^a	3.0±0.2 ^a	3.4±0.2 ^a

Key: P<0.05; Mean followed by different letters across the column are significantly different using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals.

(n) represented number of patients

DISCUSSION

Haemoglobin genotype is known to influence the prevalence of malaria and malaria parasite density in malaria endemic areas. The protective role displayed by HbAS and HbAC against

malaria infection in Africa has been reported to be probably responsible for their high frequency in this region (Rihet *et al.*, 2003). The significant increase in the parasite density in the wet season when compared with dry season in both HbAA

and HbAS indicates that weather is one of the factors that influence the prevalence of malaria infection in the study area. Prevalence of malaria infection is usually higher in the wet season when compared with the dry season, as a result of the increase in the breeding sites for the vector (Uneke *et al.*, 2007).

The effect of haemoglobin genotype on the prevalence of malaria cannot be over emphasized. Individuals with the genotype HbAA have been reported to be more susceptible to malaria infection than those with HbAS genotype (Verra *et al.*, 2007). The significant difference in parasite density in HbAA women than in HbAS women indicated that HbAS individuals develop mild malaria attack compared with those with genotype HbAA. This protective role has been previously reported (Hill *et al.*, 1991; Uneke *et al.*, 2007). This shows that haemoglobin genotype influences parasite density, and women with HbAA genotype are at the risk of serious complications which may arise from malaria infection.

Despite the evidence of protection against malaria infection by HbAS, the mechanisms involved are yet to be understood. The increase in mean anti-MSP-1₁₉ IgG (antibody) levels in both pregnant and non-pregnant HbAA women than in both pregnant and non-pregnant HbAS women in dry season in this study indicated that the protection against malaria infection in HbAS was not conferred by anti-MSP-1₁₉ IgG (antibody) response in this group; otherwise it would be expected that anti-MSP-1₁₉ IgG (antibody) will be higher in HbAS than in HbAA women. The level of anti-MSP-1₁₉ IgG (antibody) responses has been reported to be determined by the level of parasite density in the individual (Omosun *et al.*, 2009). Though the parasite density was significantly higher in HbAA women than in HbAS women in the wet season, but the anti-MSP-1₁₉ antibody level was not significantly different. This shows that the rate of body immune response in HbAA and HbAS individuals in the wet season was not different. This could be because the parasite density was generally higher in the wet season compared with the dry season; therefore the immune response to malaria infection was generally higher in both HbAA and HbAS

women in the wet season compared with the dry season. This study shows that the low level of parasite density in women with HbAS in this study was not a function of the response of anti-MSP-1₁₉ IgG (antibody) to malaria infection but may be a function of the genetic manipulation. It has been reported that when malaria parasite infects individuals with HbAS, the infected erythrocytes are quickly moved into the sinusoid of the liver where they will be destroyed along with the malaria parasites and this will not give the parasite the ability to multiply (Flemin *et al.*, 1984). The haemoglobin level was not significantly different among malaria positive HbAA women and HbAS women in both seasons. This shows that haemoglobin genotype does not have influence on the haemoglobin level.

The role of haemoglobin genotype on the birth weight of the neonates revealed that the mean birth weight of neonates born to malaria positive HbAA women was lower than those born to malaria positive HbAS mother in the wet season. There was no difference in the mean birth weight of neonates born to both malaria positive HbAA and HbAS women in the dry season. The significant increase in the birth weight of the neonates born to malaria positive women with HbAS in the wet season could be as a result of the reduction in the level of parasite density among the women with HbAS compared with the high parasite density in women with HbAA. There was no evidence from this study that haemoglobin genotype could have influence on the birth weight of the neonates. The effect of parasite density on the birth weight of neonates has been previously reported by Akanbi *et al.* (2009) and this was reflected on the lower birth weight recorded for the neonates born to malaria positive HbAA and HbAS pregnant women compared to the birth weight of the neonates born to malaria negative HbAA and HbAS pregnant women in both seasons. This suggests that malaria infection in pregnancy could be the main factor that is responsible for the low birth weight in malaria positive HbAA and HbAS mother, and not the haemoglobin genotype. Though the birth weight of neonate born to both malaria positive and negative HbAA and HbAS women in this study were lower than the WHO standard (WHO, 1977), it was more pronounced

in the neonate born to malaria positive pregnant women.

CONCLUSION

This study concluded that haemoglobin genotypes do not have influence on the anti-MSP-1₁₉ IgG (antibody) response, haemoglobin level and birth weight of the neonate. There is need for more study on the mechanisms involved in the reduction of the parasite density among HbAS individuals.

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