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Host Genetic Factors and Burden of Asymptomatic Malaria Infection in Calabar Pregnant Women, Nigeria

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Abstract

Introduction: Malaria is one of humans' oldest and most frequently occurring infectious diseases. This study was aimed at assessing the distribution of ABO, Rh blood groups, haemoglobin variants, and malaria parasite density of pregnant women with asymptomatic malaria attending the antenatal clinic at the University of Calabar Teaching Hospital (UCTH).

Materials and Methods: This cross-sectional questionnaire study involved fifty pregnant women positive for malaria parasites but without any malaria-related symptoms and attending antenatal clinic at the University of Calabar Teaching Hospital, who constituted the test subjects, and 50 non-pregnant uninfected women, who constituted the control samples. Malaria diagnosis was performed using the standard microscopy method, ABO and Rh blood grouping was done using the standard tube method, while haemoglobin electrophoresis was analyzed using the alkaline cellulose acetate method.

Results: Blood group O and Rh 'D' positivity were the predominant blood group among the pregnant women with asymptomatic malaria (68.0%, 96.0%) and the non-pregnant controls (70.0%, 98.0%), respectively. Haemoglobin type AA was the most prevalent among both the pregnant women (72.0%) and the non-pregnant controls (74.0%). No significant variations were observed in the ABO and Rh blood groups as well as the haemoglobin types of the pregnant women with asymptomatic malaria and the non-pregnant controls ($p > 0.05$). It was also observed that the ABO and Rh blood groups of the pregnant women had no significant bearing on their malaria parasite densities. However, a significant association was observed between the haemoglobin type and the malaria parasite densities of the pregnant women, with those belonging to HbAS having significantly lower malaria parasite densities (689.18±142.38).

Conclusion: The study reported a significant association between haemoglobin types of pregnant women with asymptomatic malaria and their malaria parasite densities. However, no significant association was found between their ABO and Rh blood groups and their malaria parasite densities.

Keywords: Haemogloinopathies, Blood groups, Malaria Parasite infection, ABO Blood group, Rh blood group.

INTRODUCTION

Malaria is one of the oldest and most frequently occurring infectious diseases in humans. It is widespread in tropical and subtropical regions of the world. Malaria is the second leading infectious disease that causes death in Africa, after HIV/AIDS, and it is the first leading cause of death in Nigeria (1,2). Pregnant women are considered to be at higher risk of malaria and adversely affected by the disease (3). According to the WHO, there were 229 million malaria cases in

2019. More than 90% of these cases were located in the WHO African region (4,5).

An individual is said to have asymptomatic malaria when one has PCR (polymerase chain reaction) detectable *P. falciparum* parasites on the day of sampling without associated malaria symptoms such as fever (6). Asymptomatic malaria refers to the presence of malaria parasites in the blood without symptoms. It is also known as subclinical malaria; hence, people with asymptomatic malaria carry the infection but have no idea they do because they do not have any indicators (7). Asymptomatic malaria infections have been recognized for many years and result from partial immunity, which controls but does not completely eliminate the infection. Thus, persistent or repeated asymptomatic infection is sometimes viewed as beneficial to the individual, as it helps to maintain this state of premunition, thereby reducing the risk of severe disease (8,9). Asymptomatic carriage of Plasmodium falciparum parasites is a major challenge to malaria control efforts in all malaria-endemic countries. Some studies have identified factors, including ABO blood type as well as haemoglobinopathies to alter the asymptomatic carriage of malaria parasites (10,11).

In malaria-endemic areas, the ABO blood group has been associated with disease severity, whereas blood group O has been shown to offer protection against severe malaria by minimizing the formation of rosettes; blood group A is linked to severe malaria (12,13). Haemoglobin S (sickle haemoglobin), which is one of the most common haemoglobin variants in sub-Saharan Africa, is formed as a result of a mutation in the beta globin gene that leads to a replacement of glutamic acid at amino acid residue 6 with valine. Individuals who inherit an S gene from each parent are homozygous for HbS and have sickle cell anaemia (HbSS), while the heterogygous AS individuals are referred to as carriers of the sickle cell trait (7,14). Studies have shown that individuals who have the sickle cell trait (HbAS) have 50–90% reduction in parasite density, most likely due to reduced parasite invasion and growth retardation (15,16). More so, children who are AS have been reported to show faster clearance of asymptomatic malaria infections. Haemoglobin C (HbC) results when the glutamic acid residue at position 6 is replaced with a lysine residue. Individuals with HbCC have been found to enhance malaria transmission by harboring highly infectious gametocytes. Children with HbAC have also been found to carry high loads of asexual stage parasites in addition to gametocytes (17,18).

There have been various studies on the associations of ABO antigens with infections since their discovery as the most functional genetic polymorphism in humans. Their association with malaria has been the subject of numerous investigations, since the sickle-cell haemoglobim was discovered to afford protection against falciparum malaria infection (12,19,20). However, very few have been carried out on the inhabitants of the Calabar metropolis. This study therefore set out to assess the distribution of ABO and Rh blood group variants as well as haemoglobin variants, and determine their association with asymptomatic carriage of *P. falciparum* parasites in pregnancy.

Materials And Methods

Study area

This study was conducted at the University of Calabar Teaching Hospital (UCTH). The facility is located at Calabar in Calabar Municipality Local Government Area of Cross River State, South-South, Nigeria, and is affiliated with the University of Calabar, Calabar (5).

Study population

A total of 100 individuals were enrolled in this study. Fifty (50) pregnant women tested positive for malaria parasites but without any malaria-related symptoms and attended antenatal clinic at the University of Calabar Teaching Hospital, and Fifty (50) were apparently healthy individuals.

Sample size:

The sample size was determined using the formula.

$$n = \frac{z^2 \times p(1-p)}{d^2}$$

Where;

n = required sample size

z = confidence level at 95% (standard value of 1.96)

p = expected prevalence or proportion

d = margin of error or precision (standard value of 5%) = (5/100) = 0.05

Prevalence of Haemoglobin AS in Nigeria is 25%, i.e., p = (25/100) = 0.25 (14).

$$n = \frac{1.96^2 \times 0.25(1 - 0.25)}{0.05^2}$$

Therefore, n= 288

However, due to time and financial constraints, a total of 100 subjects were enrolled

Inclusion criteria

Only pregnant women attending the antenatal clinic at UCTH who were positive for malaria parasites upon microscopy, confirmed to be without associated malaria symptoms, and gave informed consent were co-opted into the study.

Exclusion criteria

Those who did not meet the above-stated criteria study were excluded from the study.

Ethical consideration/informed consent

Ethical clearance was obtained from the Research and Ethical Committee of the University of Calabar Teaching Hospital (UCTH/HREC/33/VOL111/150). Participants were given a detailed explanation of the purpose, objectives, risks, and benefits, after which verbal, consent was obtained. The respondent's right to refuse or withdraw from participating in the interview was fully maintained. Data was collected after obtaining informed consent and agreement from the patients under study. Sample collection was performed following ethical steps and procedures.

Administration of questionnaire

After a detailed explanation, the participants were asked for their willingness to enroll in the study. Then, before blood sample collection, participants' responses were taken using structured questionnaire. The questionnaire contained data on socio-demographic parameters. Each respondent's age group, marital status, educational level, occupation, previous malaria infection, time of last malaria episode, and 'use of bed net the previous night' were recorded.

Collection, handling, storage of samples, and Sample analysis

Three milliliters (3ml) of venous blood samples were drawn aseptically from each volunteer participant via venepuncture using a disposable plastic syringe into 5ml plain containers and labeled with the name, gender, and unique identification number of each participant. Thick and thin blood films were made and labeled appropriately. The samples were temporarily stored in a cold flask packed with ice before their transfer to the laboratory for analysis. ABO and Rh cell grouping assay method was Standard tube method. Haemoglobin electrophoresis was done using Cellulose Acetate Electrophoresis at Alkaline pH (8.6).

Malaria diagnosis was performed using the Microscopy method (the gold standard for malaria diagnosis). In the presence of a Romanowsky stain (Giemsa stain), eosin (a component of Giemsa stain) stains the parasite nucleus red, while the methylene blue component stains the cytoplasm blue. This allows for the visualization and identification of malaria parasites in the blood smear.

Assay procedure

Thick and thin films are made. The thick smear was made by placing a larger drop of blood on the slide and spreading it circularly. The thin smear was made by placing a smaller drop of blood on the slide and spreading it in a straight line.

Before staining, the thin film was fixed with

absolute methanol (96% v/v).

The blood smears are stained with 10% Giemsa stain for 15 minutes and allowed to air dry. The stained blood smears are examined under a microscope using a 100x oil immersion objective for the presence of malaria parasites. The number of parasites per microliter of blood is estimated by counting the number of parasites in a specific number of fields under the microscope and using a conversion factor. The microscopy test results are reported as positive or negative for malaria parasites.

Determination of malaria parasite density

Malaria parasite density was determined by counting the number of parasites in the blood sample and then calculating the number of parasites per microliter of blood using the formula [(counted parasites/500WBC) x counted or assumed WBC/µL].

Statistical analysis

Data was entered and analyzed using Statistical Package for Social Sciences (SPSS), version 27.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics like frequency and percentage were used to describe the characteristics of the study population. A chi-square analysis was used to check for association between variables. Results were also expressed as means ± standard deviation, while comparisons were made between different groups using the student's t-test and Analysis of Variance (ANOVA). The level of statistical difference was set at p-value ≤ 0.05 at a 95% confidence interval.

Results

Table 1 shows the socio-demographic characteristics of pregnant women attending antenatal centres at the University of Calabar Teaching Hospital. Out of the 50 respondents, those in the age group of 35 – 39 years had the highest participation, 18(36.0%), closely followed by those in the age group of 30 – 34 years (32.0%). The majority of the respondents were Christians, 42(84.0%), while the remaining 2(4.0%) and 6(12.0%) were Muslims and Traditional worshippers, respectively. Nearly all of the respondents were married, 47(94.0%), with only 3(6.0%) being singles. Participants with tertiary education constituted the majority, 21(42.0%), while those with no formal education, primary education, and secondary education constituted the remaining 5(10.0%), 12(24.0%), and 12(24.0%), respectively. Most of the respondents were business-women, 18(36.0%), while civil servants, hairdressers, and tailors made up the remaining 9(18.0%), 14(28.0%), and 9(18.0%), respectively. All of the respondents reported having previous malaria infections, while the majority reported suffering from malaria infection within the last 6 months.

Table 2 shows the ABO blood group distribution among pregnant women with asymptomatic malaria and non-pregnant controls. Pregnant women with asymptomatic malaria belonged to blood groups O, A, B, and AB in proportions of 34(68.0%), 7(14.0%), 7(14.0%), and 2(4.0%), respectively, while the non-pregnant controls belonged to blood group O, A, B, and AB in proportions of 35(70.0%), 8(16.0%), 6(12.0%), and 1(2.0%) respectively. No significant variations were observed in the ABO blood groups between the pregnant women with asymptomatic malaria and the non-pregnant controls ($p > 0.05$).

Table 3 shows the Rh blood group distribution among pregnant women with asymptomatic malaria and non-pregnant controls. The majority, 48(96.0%) of the pregnant women with asymptomatic malaria, were Rh 'D' positive, while only a few 2(4.0%), were Rh 'D' negative. For the non-pregnant controls, majority 49(98.0%) were Rh 'D' positive, while 1(2.0%) were Rh 'D' negative. No significant variations were observed in the Rh blood groups between the pregnant women with asymptomatic malaria and the non-pregnant controls ($p > 0.05$).

Table 4 shows the haemoglobin electrophoretic patterns of the pregnant women with asymptomatic malaria and non-pregnant controls. Pregnant women with asymptomatic malaria belonged to

haemoglobin type HbAA, HbAS, HbSS, and HbSC in proportions of 36(72.0%), 12(24.0%), 1(2.0%), and 1(2.0%) respectively, while the non-pregnant controls belonged to blood group O, A, B, and AB in proportions of 37(74.0%), 10(20.0%), 2(4.0%), and 1(2.0%) respectively. No significant variations were observed in the ABO blood groups between the pregnant women with asymptomatic malaria and the non-pregnant controls ($p > 0.05$).

Table 5 shows the malaria parasite density (MPD) of pregnant women with asymptomatic malaria and non-pregnant controls. Pregnant women with asymptomatic malaria had mean values of 692.16±151.95parasites/µl, while the controls had mean values of 0.00±0.00parasites/µl. Significant variation was observed in the malaria parasite density (MPD) of the pregnant women and the non-pregnant controls ($p \leq 0.05$).

Table 6 shows the malaria parasite density (MPD) of pregnant women with asymptomatic malaria based on ABO blood group. Pregnant women with asymptomatic malaria belonging to A, B, AB, and O blood groups had mean values of 693.28±142.06 parasites/µl,, 692.36±150.25 parasites/µl, 693.12±152.65 parasites/µl, and 691.20±145.35parasites/µl, respectively. No significant variation was observed in the malaria parasite density (MPD) of the pregnant women based on their ABO blood groups.

Table 7 shows the malaria parasite density (MPD) of pregnant women with asymptomatic malaria based on ABO blood group. Pregnant women with asymptomatic malaria belonging to Rh 'D' positive and Rh 'D' negative blood groups had mean values of 692.16±143.12 parasites/µl and 692.17±149.28parasites/µl, respectively. No significant variation was observed in the pregnant women's malaria parasite density (MPD) based on their Rh blood groups.

Table 8 shows the malaria parasite density (MPD) of pregnant women with asymptomatic malaria based on haemoglobin type. Pregnant women with asymptomatic malaria belonging to haemoglobin types HbAA, HbAS, HbSS, and HbSC had mean values of 693.46±152.81parasites/µl, 689.18±142.38parasites/µl, 693.27±159.37parasites/µl, and 693.10±149.52parasites/µl, respectively. No significant variation was observed in the pregnant women's malaria parasite density (MPD) based on their haemoglobin types.

Table 1: Socio-demographic characteristics of pregnant women attending antenatal centre at the University of Calabar Teaching Hospital

Variables	Frequency(n = 50)	Percentage (%)
Age (Years):		
20 - 24	4	8.0
25 - 29	8	16.0
30 - 34	16	32.0
35 - 39	18	36.0
40 and above	4	8.0
Religion:		
Christianity	42	84.0
Islam	2	4.0
Traditional	6	12.0
Marital status:		
Married	47	94.0
Single	3	6.0
Education:		
No formal education	5	10.0
Primary education	12	24.0
Secondary education	12	24.0
Tertiary education	21	42.0
Occupation:		
Business	18	36.0
Civil servant	9	18.0
Hairdressing	14	28.0
Tailoring	9	18.0
On iron supplementation:		
Yes	47	94.0
No	3	6.0
Previous malaria infection:		
Yes	50	100.0
No	0	0.0
Period of last malaria infection:		
Within the last 6 months	44	88.0
About a year ago	4	8.0
More than a year ago	2	4.0
Use of bed net:		
Yes	48	96.0
No	2	4.0

Table 2: ABO blood group distribution among pregnant women with asymptomatic malaria and non-pregnant controls

ABO blood group	Groups		χ^2	p –value
	Pregnant women with asymptomatic malaria n(%)	Non-pregnant controls n(%)		
O	34(68.0)	35(70.0)	0.011	16.51
A	7(14.0)	8(16.0)	0.075	13.22
B	7(14.0)	6(12.0)	0.471	14.87
AB	2(4.0)	1(2.0)	0.782	15.54
Total	**50(100.0)**	**50(100.0)**		

Table 3: Rh blood group distribution among pregnant women with asymptomatic malaria and non-pregnant controls

ABO blood group	Groups		X^2	p –value
	Pregnant women with asymptomatic malaria n(%)	Non-pregnant controls n(%)		
Rh 'D' positive	48(96.0)	49(98.0)	6.671	4.413
Rh 'D' negative	2(4.0)	1(2.0)	10.388	0.320

Table 4: Haemoglobin distribution among pregnant women with asymptomatic malaria and non-pregnant controls

ABO blood group	Groups		X^2	p –value
	Pregnant women with asymptomatic malaria n(%)	Non-pregnant controls n(%)		
HbAA	36(72.0)	37(74.0)	0.086	2.96
HbAS	12(24.0)	10(20.0)	0.49	2.45
HbSS	1(2.0)	2(4.0)	0.001	15.31
HbSC	1(2.0)	1(2.0)	0.320	10.39

Table 5: MPD of pregnant women with asymptomatic malaria and non-pregnant controls

ABO blood group	Groups		X^2	p –value
	Pregnant women with asymptomatic malaria n(%)	Non-pregnant controls n(%)		
MPD (parasites/µl)	692.16±151.95	0.00±0.00	32.209	0.001*

Values are expressed as Mean±SD; MPD = Malaria Parasite Density;
* = Significant at p≤0.05

Table 6: Malaria parasite density of pregnant women with asymptomatic malaria based on ABO blood group

	ABO blood group					
Parameter	**A**	**B**	**AB**	**O**	**f ratio**	**p-value**
MPD (parasites/µl)	693.28±142.06	692.36±150.25	693.12±152.65	691.20±145.35	0.728	0.425

Values are expressed as Mean±SD; MPD = Malaria Parasite Density; * = Significant at p≤0.05

Table 7: Malaria parasite density of pregnant women with asymptomatic malaria based on Rh blood group

	Rh blood group			
Parameter	**Rh 'D' positive**	**Rh 'D' negative**	**t**	**p –value**
MPD (parasites/µl)	692.16±143.12	692.17±149.28	0.938	0.420

Values are expressed as Mean±SD; MPD = Malaria Parasite Density;
* = Significant at p≤0.05

Table 8: Malaria parasite density of pregnant women with asymptomatic malaria based on haemoglobin type

	Haemoglobin type					
Parameter	**HbAA**	**HbAS**	**HbSS**	**HbSC**	**f ratio**	**p-value**
MPD (parasites/µl)	693.46±152.81	689.18±142.38*	693.27±159.37	693.10±149.52	3.627	0.047*

Values are expressed as Mean±SD; MPD = Malaria Parasite Density; * = Significant at p≤0.05

Discussion

The study investigated the socio-demographic characteristics of pregnant women attending the University of Calabar Teaching Hospital antenatal center. Among the 50 respondents, the age group of 35-39 years showed the highest participation (36.0%), followed by the age group of 30-34 years (32.0%). The majority of participants were Christians (84.0%), with smaller percentages being Muslims (4.0%) and Traditional worshippers (12.0%). Almost all respondents were married (94.0%), while only a small portion were singles (6.0%). In terms of education, those with tertiary education constituted the majority (42.0%), while participants with no formal education, primary education, and secondary education comprised 10.0%, 24.0%, and 24.0%, respectively. The occupational distribution revealed that a significant proportion of respondents were businesswomen (36.0%), followed by civil servants (18.0%), hairdressers (28.0%), and tailors (18.0%). These findings are similar to those obtained in a recent study by Mangusho *et al.* (21).

The study also reported blood group O as the predominant blood group among pregnant women with asymptomatic malaria. This finding is consistent with the reports obtained among the general population in the studied area by Okoroiwu *et al.* (22), who had earlier reported that the blood group O Rhesus D positive was the predominant blood group among blood donors in Calabar. A study by Mukhtar and Abdulkadir, (23) also reported predominance of blood group O among the study participants. This finding could be because the O blood group is highly prevalent in the study's geographical setting, which is the southern part of Nigeria (22).

Similar to the above, Rhesus-positive persons constituted the majority of the study. This finding also agrees with Okoroiwu *et al.* (22) and a previous study by Mukhtar and Abdulkadir (23). This Rh 'D' antigen predominance might be due to its increased prevalence among blacks (23).

The study also reported the predominance of haemoglobin AA among pregnant women with asymptomatic malaria. This trend was also observed by a study by (24), who also noted a predominance of haemoglobin AA among their study participants. This finding is thought to be due to the high prevalence of haemoglobin AA in most geographical settings, including Nigeria.

The study also revealed significantly increased malaria parasite density in pregnant women with asymptomatic malaria than in the non-pregnant uninfected controls. This trend is consistent with those reported by a study in Ghana by Adu-Gyasi *et al.* (25), and reflects the asymptomatic state of the study respondents. The study's finding also agrees with the study conducted by Ogbu *et al.* (26) and Eze *et al.* (27), who found a high malaria prevalence in Nigeria. The high malaria density obtained in this study, despite the high number of pregnant women who are using insecticide-treated mosquito nets (96.0%), shows that the women may not be using the insecticide-treated mosquito nets correctly. Therefore, there is a need for increased education and awareness.

Additionally, no significant differences were observed in the malaria parasite densities of the pregnant women based on their ABO blood groups, implying that the ABO blood group has no significant effect on the malaria parasite density of pregnant women. In contrast, Otajevwo (28) reported a significant association between malaria and ABO blood groups where blood group O individuals are more susceptible than other ABO blood groups. However, blood group AB individuals were more affected by malaria, according to studies by Ilozumba and Uzozie (29) and Oche and Aminu (30).

It was also observed that the Rh blood groups of the pregnant women had no significant bearing on their malaria parasite densities, as reflected by no significant difference in the values obtained for the two Rh blood groups. This is in line with the findings of Tadesse and Tadesse (12), who found no significant association between Rh blood group and malaria. On the contrary, in a study by Ibrahim *et al.* (31), the relationship between parasitemia

and Rhesus factor indicates that 93.18% of malaria-positive patients in their study were rhesus-positive while 6.82% were rhesus-negative, suggesting that rhesus-positive individuals are more prone to severe malaria infection than their rhesus negative counterparts.

The study however, revealed a significant difference in the malaria parasite densities of pregnant women with asymptomatic malaria based on their haemoglobin type, with those belonging to haemoglobin type AS having significantly lower values of the malaria parasite. This implies that there is a significant association between haemoglobin type and malaria parasite density This is in agreement with previous reports. Studies have shown that individuals who have the sickle cell trait (HbAS) have 50–90% reduction in parasite density, most likely due to reduced parasite invasion and growth retardation (15). Commonly proposed mechanisms underlying this resistance include increased sickling, reduced vascular cytoadherence, impaired rosette formation, etc. (17).

Conclusion

The study reported a significant association between haemoglobin type of the pregnant women with asymptomatic malaria and their malaria parasite densities. However, no significant association was found between their ABO and Rh blood groups and their malaria parasite densities. This suggests that carriage of the sickle cell trait (haemoglobin AS) might confer some degree of effect on the malaria parasite density of the pregnant women with the haemoglobin AS variant.

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