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Impact of maternal *Plasmodium falciparum* malaria and haematological parameters on pregnancy and its outcome in southeastern Nigeria

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Malaria during pregnancy is a serious problem in sub-Saharan Africa, affecting an estimated 24 million pregnant women[1]. Pregnancies in women living in malaria endemic regions, particularly in sub-Saharan Africa are associated with a high frequency and density of *Plasmodium falciparum* parasitaemia, with high rates of maternal morbidity including fever and severe anaemia, with abortion and stillbirth, and with high rates of placental malaria and consequently low birth weight in newborns caused by both prematurity and intrauterine growth retardation[1].

The effects of maternal haematological parameters, particularly haemoglobin concentration (HbC) (used to assess anaemia), ABO blood group and haemoglobin genotype on pregnancy outcome have not been adequately evaluated in many malarious areas of the sub-Saharan Africa. It is well established that anaemia is the most common consequence of *P. falciparum* malaria infection and it is generally accepted that in malaria-endemic areas, *P. falciparum* is a major contributor to anaemia in pregnancy. It has been suggested that the ABO system has evolved under a positive selection pressure in both humans and other primates[2]. The implication is that certain ABO groups appear to provide a selective vulnerability to individuals possessing a particular ABO blood group. Whether ABO system influences susceptibility of pregnant women to malaria is yet to be fully ascertained.

Epidemiological and clinical studies have indicated that malaria susceptibility and severity are influenced by haemoglobin genotype with haemoglobin (Hb) AS individuals having a selective advantage in malarial environments[3]. Thus, the high frequency of HbAS in human populations has been attributed to the decreased malarial morbidity and mortality experienced by HbAS heterozygotes[4,5]. However, the extent of the influence of haemoglobin genotype on the susceptibility and severity of malaria in pregnancy is yet to be clearly established. The objective of this study therefore was to evaluate the possible contributory role and impact of maternal malaria and haematological parameters on pregnancy outcome in southeastern Nigeria.

This study was conducted in Abakaliki, the capital of Ebonyi State in southeastern Nigeria from June to December 2006 at the Ebonyi State University Teaching Hospital (EBSUTH), Abakaliki, which is the largest health facility in this region. Malaria transmission in the area is perennial but usually at peak towards the end of the rainy season. The study protocol was ap-

proved by the Department of Medical Microbiology/ Parasitology, Faculty of Clinical Medicine, Ebonyi State University, Abakaliki, Nigeria and Ethical Committee of the EBSUTH, Abakaliki, Nigeria.

Pregnant women who fulfilled the following inclusion criteria were enrolled into the study: (i) attended the antenatal clinic at EBSUTH; (ii) had an uncomplicated singleton pregnancy of 32 weeks' gestation (based on the fundal height estimation); (iii) reside in Abakaliki or neighbouring local government areas; (iv) had no obvious clinical evidence of malaria (asymptomatic); and (v) had no known underlying chronic illness.

Following informed consent, information on the participants' age, occupation and educational level was obtained by interview and shortly before child birth, about 5 ml of the maternal peripheral blood, was obtained from each participant by venepuncture technique into sterile EDTA container for laboratory analysis. Information was obtained on the delivery outcome including; baby's sex and mode of delivery. The birthweight was determined in kilogram (kg) using an electronic weighing machine immediately after childbirth.

Each maternal blood sample was analysed for malaria parasite infection by performing the microscopy of Giemsa-stained thick and thin blood films. The Plus System was used for the determination of parasite density as previously outlined[6]. The HbC was determined to assess maternal anaemia using the cyanmethaemoglobin method described previously[7]. The WHO definition of anaemia in pregnancy (haemoglobin concentration Hb <11g/dl[8]) was adopted in this investigation. The haemoglobin genotype was determined by the haemoglobin electrophoresis technique at alkaline pH using cellulose acetate membrane (CAM) as described previously[9]. The ABO blood grouping test was performed using the slide method as outlined previously[7], with commercially available reagents which produced strong agglutination within 1–2 min (Murex Diagnostics, Inc., Dartford, UK). Percentage prevalence rates were calculated with their respective 95% confidence intervals. Difference between proportions were evaluated using the chi-square tests while differences in means were evaluated using one-way analysis of variance (ANOVA). Statistical significance were achieved at $p < 0.05$.

A total of 300 women at full pregnancy term were studied during childbirth. The average age of the women at childbirth was 28 yr and age ranged from 15–43 yr. According to the criteria of this investigation malaria parasites were found in the peripheral blood of 48 (16%) women and *P. falciparum* was the only species identified. Of the 48 women infected by malaria parasite, 1–10 parasites per 100 thick film fields were recorded in 11of 48, while 11–100 parasites per 100 thick film fields were recorded in the remaining 37.

Individuals of age group 20–24 yr had the highest prevalence of maternal malaria (15/72; 95% CI: 11.4–30.2%) while the least was recorded among those ≥40 yr (1/9; 11.1%; 95% CI: 9.4–31.6%), but there was no significant difference in the trend ($\chi^2 = 2.02$, df = 4, $p > 0.05$) (Table 1). Those whose occupation was farming were significantly more infected with malaria parasite (8/25, 32% ; 95% CI: 13.7–50.3%), than individuals of other occupations ($\chi^2 = 12.9$, df = 6, $p < 0.05$) (Table 1). Women, who had no formal education recorded the highest prevalence of malaria infection (9/32; 28.1%; 95% CI: 12.5–43.7%) though statistically there was no significant difference ($\chi^2 = 2.69$, df = 3, $p > 0.05$). Individuals with HbAA genotype were more infected with malaria parasite than those with HbAS genotype (17.9%; 95% CI: 11.3–24.6% *vs.* 12.5%; 95% CI: 5.3–19.8%) but the difference was not statistically significant ($\chi^2 = 1.11$, df =1, $p > 0.05$). The highest prevalence of malaria infection was recorded among women with blood group 'O' (19.5%; 95% CI: 12.4–26.6%) followed by those of blood group 'A' (8/52,

Table 1. Prevalence of malaria infection in relation to demographic/obstetrics data, and haematological parameters among women at childbirth in Abakaliki, Nigeria

Parameter	No. examined	No. malaria +ve (%)	95% Confidence interval
Age			
≤ 19	11	2 (18.2)	4.6–41.0
20–24	72	15 (20.8)	11.4–30.2
25–29	106	16 (15.1)	8.3–21.9
30–34	78	11 (14.5)	6.6–28.7
35–39	26	8 (11.5)	0.8–23.8
≥40	9	1 (11.1)	9.4–31.6
Total	300	48 (16.0)	11.9–20.1
Occupation			
Students	28	5 (17.9)	3.7–32.1
Civil Servants	72	9 (12.5)	4.9–20.1
Housewives	44	8 (18.2)	6.8–29.6
Business	69	14 (20.3)	10.8–29.8
Farmers	25	8 (32.0)	13.7–50.3
Total	238	44 (18.5)	13.6–23.4
Educational level			
None	32	9 (28.1)	12.5–43.7
Primary	75	12 (16.0)	7.7–24.3
Secondary	73	13 (17.8)	9.0–26.6
Tertiary	58	9 (15.3)	6.2–24.8
Total	238	43 (18.1)	13.2–23.0
HbC (g/dL)			
<7.0	2	2 (100)	–
7.0–8.9	7	3 (42.9)	6.2–79.6
9.0–10.9	22	7 (31.8)	12.3–51.3
≥ 11	149	28 (18.8)	12.5–25.1
Total	180	40 (22.2)	16.1–28.3
Genotype			
AA	128	23 (17.9)	11.3–24.6
AS	80	10 (12.5)	5.3–19.8
Total	208	33 (15.9)	10.9–20.9
Blood group			
A	52	8 (15.4)	5.6–25.2
B	34	5 (14.7)	2.8–26.6
AB	4	0	–
O	118	23 (19.5)	12.4–26.6
Total	208	36 (17.3)	12.2–22.4

15.4%; 95% CI: 5.6–25.2%), there was also no significant difference in the trend (χ^2 = 1.55; df = 3; p >0.05) (Table 1). The prevalence of malaria infection decreased with increase in HbC and the difference was statistically significant (χ^2 = 23.8, df = 3, p <0.05) (Table 1).

The results of the association of neonatal birthweight with maternal malaria infection and haematological parameters among the subjects are summerised in Table 2. A higher proportion of malaria infected women (8/37) had babies with low birthweight compared to women without malaria infection (18.8%). The mean birthweight of babies of malaria infected women was lower (2.37 kg) than the uninfected (2.94 kg). One-way ANOVA showed a significant difference in the trend (F ratio = 15.05, df_1/df_2 = 2/3, p <0.05). Anaemic women (with HbC <11 g/dL) had a higher proportion of low birthweight (LBW) babies (5/25) than the non anaemic women (16.4%) also the mean birthweight was lower among anaemic women, but the difference was not statistically significant (F ratio = 7.34, df_1/df_2 = 2/3, p >0.05). The women with blood group 'O' recorded the highest proportion of LBW (23.4%) followed by those of the blood group A (14%), statistically however, no significant difference was observed in the trend (F ratio = 0.99, df_1/df_2= 2/3, p >0.05). The proportion of LBW was higher among those with HbAA genotype (17.4%) than those with HbAS genotype (12%). The difference in the trend was not statistically significant (F ratio = 6.42, df_1/df_2 = 2/3, p >0.05).

The maternal malaria prevalence of 16% obtained at child birth in this study is comparable to the maternal malaria prevalence rates obtained from recent studies conducted in a number of malarious areas of the sub-Saharan Africa which ranged from 8.6 to 19%[10,11]. Although lower literacy level and younger age appeared to be more predisposing factors to malaria infection in this study, the differences in the trend were however not statistically significant. Moreover, occupation was significantly associated

Table 2. Association of neonatal birthweight with maternal malaria infection and haematological parameters among women at childbirth in Abakaliki, Nigeria

Maternal parameters	Neonatal birthweight in kg (%)			Total	Mean birth-weight (kg)
	<2.5	2.5–3.5	>3.6		
Malaria infection					
Infected	8 (21.6)	26 (70.3)	3 (8.1)	37	2.37
Uninfected	34 (18.8)	115 (63.5)	32 (17.7)	181	2.94
Total	42	141	35	218	
Haemoglobin concentration					
<11g/dL	5 (20)	16 (64.3)	4 (16)	25	3.04
≥11g/dL	19 (16.4)	83 (71.6)	14 (12.1)	116	2.97
Total	24	99	18	141	
Blood group					
A	6 (14)	30 (69.8)	7 (16.3)	43	3.04
B	2 (6.5)	25 (80.6)	4 (12.9)	31	3.09
AB	0	2 (100)	0	2	2.85
O	25 (28)	68 (63.6)	14 (13.1)	107	2.98
Total	33	125	25	183	
Genotype					
AS	9 (12)	59 (78.7)	7 (9.3)	75	2.97
AA	19 (17.4)	72 (66.1)	18 (16.5)	109	2.98
Total	28	131	25	184	

with malaria infection but the reason for this outcome was somewhat obscure. Previous findings from eastern Sudan[12] and Kigali, Rwanda[13] had indicated that age was not significantly associated with malaria during pregnancy and the report of an investigation from Zanzibar, Tanzania[14], showed no apparent relationship between malaria and socio-demographic parameters including occupation.

In this study, anaemic women were more likely to have malaria infection than non-anaemic women. This was consistent with reports from a number of sub-Saharan African countries which indicated that the prevalence of anaemia was consistently higher among pregnant women infected with malaria parasites than those uninfected[12,14].

The prevalence of malaria infection in this study was higher among individuals with HbAA genotype than those with HbAS genotype. Although studies that investigated this relationship between malaria and haemoglobin genotypes in pregnancy are essentially lacking, malaria has been shown to be consistently higher in individuals with HbAA genotype compared to those with HbAS[4]. The greater susceptibility of HbAA individuals to *P. falciparum* malaria and the enhanced severity of an attack in this group may be due to low red cell membrane resistance to the invading parasite and a non-hypoxic environment within the red cell which enhances its development[4,5].

High prevalence of malaria infection in the present investigation was recorded among women with blood group 'O'. Findings from studies evaluating the relationship between malaria and ABO blood group are contradictory[15]. While studies evaluating the

relationship between malaria and ABO blood group during pregnancy are essentially lacking, the only current study related to this was the evaluation of the ABO phenotypes and malaria outcomes in mothers and babies in The Gambia[16]. In that study, blood group 'O' was associated with an increased prevalence of active placental infection in primiparae and with a reduced risk of placental malaria in multiparae. Placental parasitaemia was observed to occur at least twice as frequently in primiparae but only among blood group 'O' women. More systematic studies are urgently needed to further elucidate this.

In this investigation maternal malaria infection was significantly associated with birthweight with infected mothers having a higher proportion of LBW babies than the uninfected mothers. On the contrary, none of the haematological parameters investigated indicated any significant association with birthweight. The proportion of LBW babies was higher among anaemic women, those with blood group 'O' and individuals of the HbAA genotype. The reason for this outcome could not be clearly ascertained; however, it is interesting to note that individuals who belong to these categories had higher prevalence of malaria infection. Therefore, these results suggest that maternal malaria may be the major determining factor to LBW in this study and that the haematological parameters may have played only a secondary role in LBW observed.

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