Asexual and sexual stages of *Plasmodium falciparum* in Nigerian pregnant women attending antenatal booking clinic

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Summary: Susceptibility to infection by *Plasmodium falciparum* is increased in pregnant women. In sub-Saharan Africa, the consequences of maternal malaria include preterm birth, fetal growth restriction and increased infant mortality. Malaria transmission requires the circulation of viable gametocytes that can be ingested by the female mosquito taking a blood meal. This study was conducted to evaluate the presence of asexual and sexual stages of *P. falciparum* in pregnant women attending antenatal booking clinics in south-western Nigeria, an area hyper-endemic for malaria. Gametocyte carriage was about 13%, similar to that documented for children symptomatic for malaria in our area of study.

Keywords: infectious diseases, maternal mortality

INTRODUCTION

Malaria remains a major cause of morbidity and mortality in pregnant women resident in sub-Saharan Africa.^{1,2} Malaria contributes to preterm delivery as well as fetal growth restriction,^{2,3} anaemia and the requirement for blood transfusion in sub-Saharan Africa, adding to the economic burden and the possible transmission of blood-borne diseases including HIV. Women in their first or second pregnancy are known to be relatively more 'at risk' than multiparous women³ and HIV-positive pregnant women have increased susceptibility.^{4,5}

HIV and malaria as well as their treatments interact in complex ways.^{6–9} First, HIV infection has been observed to predispose to malaria infection and probably increase poor malaria treatment outcome. Second, malaria infection causes 'spikes' in viral load in HIV-infected individuals though the importance of this is yet to be fully elucidated. Third, some anti-malarial drugs, notably mefloquine, chloroquine and quinine, possess demonstrable antiretroviral activity while protease inhibitors such as atazanavir, lopinavir possess antimalarial effect at the usual dose for retroviral treatment.^{10,11} In Nigeria, HIV prevalence among pregnant women may be as high as 8% or even higher.^{8,12}

There is an urgent need to control malaria in the general population and especially among pregnant women. Successful transmission and continued circulation of malaria parasite requires ingestion of viable gametocytes by female Anopheles mosquitoes during a blood meal. Profiling of gametocyte carriage will support efforts aimed at malaria control.¹³ Because of increased susceptibility to malaria,^{10,14–16} pregnant

women would be expected to have higher rates of gametocyte carriage in an area hyper-endemic for malaria.

MATERIALS AND METHODS

This cross-sectional observational study was conducted at two public secondary health facilities in Ibadan, Oyo State, south-west Nigeria. Ibadan lies between 7.3°N latitude and 3.4°E longitude in the guinea savannah belt with an average annual rainfall of about 1250 mm. Rainfall occurs from April to October each year, a period which coincides with the highest intensity of malaria transmission, reflecting the breeding pattern of the Anopheles vector.

The study sites, Aremo Maternity and Adeoyo Maternity, belong to the Government of Oyo State, Nigeria, and provide subsidized health care for residents of the city, serving mostly relatively low-income people. The Research and Ethical Committee of Oyo State Ministry of Health, Ibadan provided ethical review for the study. The recruitment lasted from July to September at Aremo Maternity and between August and September 2009 at Adeoyo Maternity Hospital. Pregnant women attending antenatal clinic for the first time during the current pregnancy and who consented were enrolled. Social-biological parameters obtained from the participants included age, parity, gestation age, use of malaria prevention measures, use of antimalarial drugs during the current pregnancy, weight, temperature and other physical characteristics. General physical examination and obstetric examination were carried out by midwives and two of the investigators (FAF, AA). All the participants underwent other tests according to the standard protocol with public hospitals in Nigeria and these included screening for hypertension, serology for ABO and Rhesus blood groups, tests for syphilis as well as HIV status.

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Thick and thin blood smears for quantification and speciation of malarial parasite were prepared from finger prick following strict asepsis procedure. A drop or two drops of blood were spotted on a filter paper for the purpose of polymerase chain reaction determination of parasite species and submicroscopic parasitaemia at a later date. Approximately 100 μ L of blood was collected into a heparinized capillary tube during the preparation of a blood smear for the determination of haematocrit. Haematocrit was determined by using a HawskeyTM Microhaematocrit centrifuge and read on the microhaematocrit reader.

Anaemia was classified as mild, moderate or severe, respectively, with haematocrit of 27–<30, 19–26 and <19%.

Screening and quantification for malaria parasitaemia

The quantification of malaria merozoites and gametocytes used air-dried thick smears stained with 10% freshly prepared Giemsa stain at pH $\sim$ 7.2. Parasite speciation involved studying the morphological characteristics on thin smears that were fixed with methanol prior to staining. In all cases, screening involved at least two microscopists including three of the investigators (STB, FAF and AAA) at different times. No smear was declared negative until at least 100 oil immersion fields had been examined and whenever the results of the observers differed, the opinion of the third became mandatory.

Quantification of asexual and sexual forms of *Plasmodium falciparum*

Parasite density was determined by counting all asexual or sexual stages against a minimum of 500 white blood cells or 500 asexual forms, whichever occurred first. Parasite density was thereafter obtained using the following formula and assuming a white cell count of 8000 per microlitre of blood:¹⁷

$$\text{Parasite density} = \frac{\text{No parasite} \times 8000}{\text{No white blood cell}}$$

Statistical analysis

The primary outcome measure was the presence of asexual forms and/or gametocytes in the peripheral blood films of the participants. Data obtained were fed into SPSS version 15 statistical package comparing categorical parameters like presence or absence of parasite using chi-square test while continuous variables such as age were compared using Student's *t*-test. *P* value of less than 0.05 was considered statistically significant.

RESULTS

Socio-biological characteristics of participants

A total of 361 consenting pregnant women were enrolled between July and September 2009. The mean age for all the participants was 26.93 ± 5.36 years with a range of 15–45 years. The median age was 26 years. Seven women were below 18 years of age, all of whom were nulliparous. The mean age of nulliparous women was 22.9 ± 3.7 years and was statistically lower than multiparous women, 29.2 ± 5.4 years ($F = 6.68$;

$P = 0.01$). The age group that was most commonly represented was 25–29 years accounting for 41.3% of the participants. The mean weight and height, respectively, were 60.79 ± 11.15 kg and 157.18 ± 6.96 cm with 40/361 (11.1%) being below 150 cm and regarded as short and therefore considered obstetrically at risk.

Only 9/361 (2.5%) reported for booking during the first trimester, that is, at <14 weeks, whereas 162 or 44.9% reported for booking during the second trimester, at 14–27 weeks gestation, while the number of women attending booking clinic during the third trimester, at >27 weeks of gestation age, was 190/361 or 52.6% ($\chi^2 = 54.6$; $P < 0.0001$). Mean gestational age at booking was 23.5 ± 5.8 weeks (range 8–36) (see Table 1).

Only one (0.3%) of the participants was HIV positive, representing a very low proportion in comparison to the national average of about 5%.¹⁸

Peripheral asexual and sexual malaria parasitaemia

In all, 67 pregnant women had only asexual forms whereas 10 of 77 or 13% of women had both asexual and sexual forms, while another eight were positive for gametocytes only; thus, giving a total of 85 (23.5%) individuals with one form of malaria parasite. The geometric mean of asexual parasite density was 1887 and the range was 40–30,901, whereas the mean gametocyte density was 39, range 8–264/ μ L. The proportion of nulliparous women with peripheral asexual parasitaemia was significantly higher than that of multiparous women: 40/130 (30.8%) versus 36/231 (16.0%) ($\chi^2 = 10.79$; $P = 0.001$), although no difference was found in the mean asexual parasite density based on parity. Of the nine women who attended the booking clinic during the first trimester, three (33%) were parasitaemic compared with 24% and 18% parasite rates for those booking during the second and third trimesters, respectively ($\chi^2 = 6.08$; $P = 0.047$).

Of the 361 participants, only 27 (7.5%) used insecticide-treated nets (ITN). Of the 77 with peripheral asexual malaria parasites with or without gametocytes, only three (3.9%) used

Table 1 Socio-biological characteristics of 361 pregnant women seen at Antenatal booking clinic at Adeoyo Maternity and Aremo Maternity in Ibadan, Nigeria

Parameters	
Age (years)	
Mean	26.93 ± 5.36
Range	15–45
No age <18 years	7
Weight (kg)	
Mean	60.8 ± 11.15
Range	31–103
Height (cm)	
Mean	157.18 ± 6.96
Range	134–182
No. shorter than 150	40 (11.1%)
Haematocrit (%)	
Mean	32.70 ± 4.11
Range	22–46
Parity	
Mean	2.33 ± 1.30
Range	0–5
Gestational age at booking (weeks)	
Mean	23.46 ± 5.8
Range	8–36

ITN while the remaining 74 did not. Non-use of ITN was clearly associated with the presence of asexual parasitaemia with or without gametocytaemia: 74/334 (22.2%) versus 3/27 (11.1%); $\chi^2 = 4.39$; $P = 0.036$.

Pure gametocytaemia

Eighteen out of 361 (5.0%) pregnant women had gametocytaemia in this study, eight of whom had pure gametocytaemia (Table 2). Five (62.5%) of the participants with pure gametocytaemia were symptomatic and had had a course of antimalarial drugs during the preceding two weeks, namely sulphadoxine-pyrimethamine (three individuals) and chloroquine. Two of the remaining three participants with pure gametocytaemia took no medication while one had taken paracetamol. Pure gametocytaemia did not show any correlation with the use of ITN (Pearson's coefficient = 0.07, $P = 0.34$).

Haematocrit

The mean haematocrit of all participating women was $32.70 \pm 4.11\%$ (range 22–46%) and 21.6% had a haematocrit of less than 30%. In all, 14.7% had mild anaemia haematocrit of 27–<30%, 6.9% presented with moderate anaemia haematocrit of 19–26%. The proportion of participants with anaemia was higher among parasitaemic 32/74 (43.2%) than aparasitaemic 42/272 (15.4%) women; $\chi^2 = 19.04$; $P = 0.0001$. However, mean haematocrit of participants with pure gametocytaemia was $29.8 \pm 4.4\%$ and was not significantly lower than the mean haematocrit of all participants, $32.7 \pm 4.11\%$; $P > 0.05$.

DISCUSSION

It is noteworthy that reports of malaria parasitaemia from parts of Nigeria have been conflicting, perhaps as a result of lack of standardization of technique.^{14–16} Information on sexual forms of malaria parasite is generally unavailable but

gametocytaemia has been reported to be between 10% and 15% among children symptomatic of malaria in Nigeria.^{19–21} Our current small observational study is probably the first attempt at profiling both asexual and sexual forms of malaria parasite in pregnant women in Nigeria. The gametocyte carriage rate of about 13% in symptomatic parasitaemic pregnant women is similar to what has been documented in children in the same environment. Nulliparous women were significantly younger than multiparous women, a finding in keeping with earlier reports.¹⁴ A modal parity of 0 and relative preference of pregnant women to attend antenatal booking clinic during the second and third trimesters were findings similar to those of previous studies in the same area.^{14,15} In the present study, only nine (2.5%) attended booking clinic during the first trimester of pregnancy, the majority of whom probably did so because of perceived discomfort or illness as about two-thirds of them were symptomatic for malaria. It may therefore be inferred from this study that peripheral parasitaemia may be a factor affecting the gestational age at booking in our area of study. A larger study will be needed to confirm this hypothesis.

That only one (0.3%) woman was HIV positive is both interesting and heartening as this is far below the national average of about 5%¹⁸ especially when figures as high as 8.2% have been reported from at least one part of the country in the same population of pregnant women.^{8,12} It is equally noteworthy that this individual was positive and symptomatic for malaria parasitaemia. However, the sample size involved in this study and the extremely low incidence of retroviral positivity precludes any meaningful deductions.^{5,6}

Peripheral asexual parasitaemia among pregnant women has been variously reported as 7.7% and 58.4%, and as high as 87.9% among primigravida in Nigeria.^{14,15,22,23} However, in this study prevalence of asexual malaria parasitaemia was 21.3%, a relatively higher rate than that previously recorded from the same locality, although within the range described for the country. The population of participants in this study should be more representative of the locality considering the fact that the two public health facilities where the study was conducted were located within the heartland of the ancient city of Ibadan. We consider that reasonable deductions that may assist maternal and child health could be drawn from this study while also recognizing the need for community-based studies.

The need to explore gametocyte carriage in pregnant women is further underscored by the introduction of intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine, an antimalarial drug with propensity for gametocyte generation.²⁴ Interestingly, both populations of young children and pregnant women are susceptible to malaria in our area of study. The most commonly used antimalarial drugs have little or no effect on gametocytes, thus pure gametocytaemia found in some of the participants may suggest that the asexual forms have been cleared following the use of such drugs. A period of follow-up of a cohort of pregnant women, coupled with the use of appropriate chemotherapeutic agents, will be needed to fully elucidate the evolution of asexual and sexual stages of malaria parasite in pregnancy.

Although mean haematocrit of the participants with pure gametocytaemia appeared lower than that of all the participants, no statistically significant difference was observed. Anaemia is associated with gametocytaemia in children symptomatic of *Plasmodium falciparum* malaria,²⁵ thus the absence of a significantly lower haematocrit in those with pure gametocytaemia in this study may be due to small sample size.

Table 2 Profile of 85 pregnant women with peripheral asexual and/or sexual parasitaemia

Parameters	Asexual parasitaemia only	Sexual parasitaemia only	Both asexual and sexual parasitaemia	P value
Number	67	8	10	–
Age (years)				
Mean	25.6 $\pm$ 5.2	25.0 $\pm$ 5.3	24.8 $\pm$ 4.6	0.85
Range	(15.0–40.0)	(19.0–37.0)	(17.0–32.0)	
Proportion use ITN				
% in parenthesis	3 (4.5)	0 (0)	0 (0)	–
Haematocrit (%)				
Mean	30.5 $\pm$ 3.6	33.9 $\pm$ 4.4	29.8 $\pm$ 3.1	0.038
Range	(22.0–40.0)	(25.0–39.0)	(26.0–35.0)	
GA at booking (weeks)				
Mean	23.7 $\pm$ 6.4	24.4 $\pm$ 8.1	23.3 $\pm$ 5.5	0.94
Range	(8.0–36.0)	(16.0–36.0)	(16.0–32.0)	
Asexual parasite density (/ μ L)				
Mean	2043	–	837	0.045
Range	(40–30,901)		(40–4928)	
Gametocyte density (/ μ L)				
Mean	–	13	49	0.22
Range		(8–40)	(8–264)	

GA = gestational age

It would appear that pregnant women living in an area that is hyper-endemic for malaria are similarly predisposed to gametocyte carriage, like young children, and therefore serve as a reservoir for continued transmission. ITNs will both protect pregnant women from frequent acute malarial episodes and reduce transmission of the disease. The poor uptake of ITNs by pregnant women in hyper-endemic areas should be addressed with a view to reducing transmission and attacks.

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