Malaria Endemicity Among Pregnant Women Attending Antenatal Clinic in the University of Calabar Teaching Hospital, Calabar, Nigeria.

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ABSTRACT

This study was carried out to ascertain the burden of malaria among pregnant women accessing health care services in the University of Calabar Teaching Hospital, Calabar, Nigeria. A total of 369 pregnant women were tested for malaria infection using the microscopic technique, which is the ''Gold Standard'' for malaria diagnosis. The prevalence of malaria infection in the study was 68.0%. Women in their third trimester had the highest prevalence of malaria infection (74.2%) and intensity (801.7±204.92) of malaria parasites. Malaria infection peaked (84.8%) among younger subjects aged 18-22 years in the study. Single women had a higher malaria prevalence rate (82.9%) than their married counterparts (60.8%). Women with a higher education had a lower malaria infection prevalence rate (60.1%). The occupation of the participants in the study had no effect on the rate of occurrence of malaria infection. Participants who slept under insecticide treated nets (ITNs) had 19% prevalence rate of malaria compared to those who do not (71.4%). Despite efforts geared towards minimizing malaria in pregnancy, the 68% prevalence recorded in this study is still considered high.

Keywords: Malaria, Pregnant women, Microscopy, Calabar, Nigeria, Microscopy.
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INTRODUCTION

Malaria is a vector borne disease caused by the protozoan parasites of the genus *Plasmodium* (1). The predominant species responsible for over 95% of the cases of malaria in Nigeria is *P. falciparum*. It is the most common cause of mortality and morbidity in pregnant women and delivery outcomes; such as premature delivery, low birth weight, neonatal jaundice, miscarriages, still births, neonatal deaths etc (2). It is estimated that 1.5-2.7 million deaths annually results from malaria infection. Ninety percent of deaths due to malaria occur in sub-Saharan Africa, where most of the victims are children and pregnant women (3). Pregnant women are at risk of malaria because of the low immunity associated with pregnancy (4). Malaria infection in pregnancy can be fatal to mother, foetus and new-borns. Maternal mortality is two times likely to occur in pregnant malaria women than among non-pregnant women with severe malaria (5). Complicated malaria, such as cerebral malaria, is common in pregnancy and is responsible for deaths (6).

In Nigeria, malaria is endemic; with more than half of the population experiencing malaria attack at least twice a year (7).The malaria situation in Nigeria, though is improving, but still requires a lot to be done. The most dangerous form of the disease,

falciparum malaria, is endemic in Nigeria and sub-Saharan Africa (8). Calabar, the capital of Cross River State is one of the coastal cities in Nigeria. Malaria is hyper-endemic in the city due to heavy rains (which is almost all year round with peak periods between July and September) coupled with its typical rainforest vegetation and abundance of female *Anopheles* mosquitoes (9).Okafor *et al*., (2012) reported a malaria prevalence rate of 70.1% amongst pregnant women in Calabar (10).

In recent past, there has been aggressive and coordinated effort towards an integrated control of malaria through a combination of early parasite-based diagnosis of malaria, prompt treatment with artemisinin combination therapy (ACT), use of long lasting insecticide treated nets and environmental modification, among others. Women attending antenatal care and children are the focus of malaria control in Nigeria.

This study is an attempt to assess the success of malaria control programme in Nigeria, most especially as the public sector organ charged with the responsibility for the control of malaria has been re-named National Malaria Elimination Programme.

MATERIALS AND METHODS

Study area:

This study was conducted at the University of Calabar Teaching Hospital (UCTH), located in Calabar, Cross River State, Nigeria between August and December 2015. Calabar, the capital of Cross River state, lies on the geographical coordinates of 4º 34′ 27″ N, 6º 58′ 32″ E in the south-south geopolitical zone within the coastal rainforest region of Nigeria with a population of 372,848 people (11). The majority of the population speak Efik and Ejagham dialects. Calabar has a relatively good drainage system with tarred roads and gutters in some parts of the metropolis. The vegetation is typical of the tropical rain forest which makes malaria transmission stable throughout the year but more intense between April and early October (wet season) with a peak period in June/July due to relatively heavy downpour during this period which usually helps to propagate the vector of malaria resulting in malaria hyper-endemicity (9).

Ethical Approval and Consent

The study protocol was reviewed and approved by the Health Research Ethics Committee of UCTH while awritten informed consent was obtained from the participants before the study began.

Study Population: Pregnant women attending the Antenatal Clinic (ANC) at the University of Calabar Teaching Hospital (UCTH) were screened for malaria infection by microscopy.

Inclusion Criteria: All pregnant women attending the ANC at UCTH, who gave their consent were considered for the study.

Exclusion Criteria: Any pregnant woman who objected was excluded from the study.

Sample Size: Three hundred and twenty-two (322) subjects were enrolled in the study. This was derived using the formula described by Daniel (12). A malaria prevalence rate of 70.1% reported for Calabar was used in deriving the sample size (10).

Administration of Questionnaire: Structured questionnaires were administered to the prospective mothers. Information, such as, age, gestational age, marital status, methods of malaria prevention and mosquito control, occupation, source of livelihood and living conditions were obtained.

Collection of Blood Samples

Two millilitres (2 cm^3) of venous blood was collected from each participant aseptically using a syringe and needle, after disinfecting the collection site and applying a tourniquet, onto microscope slides for the study.

Detection of Malaria Infection

Participants were screened for malaria by microscopy. Using an automatic pipette, 6µL and 2µL of blood from same participant was placed at different sites on the same slide for the thick and thin films respectively.The slide was left on a flat surface to air dry, after which the thin smear was fixed for 5 seconds using absolute methanol. The entire slide was then flooded with freshly prepared 3% Giemsa solution for 30 minutes, after which the stain was washed off using buffered water pH 7.2, air dried and examined under the microscope using X100 oil immersion objectives (13). The stained thick smear was used for detection of the presence of malaria parasite and estimation of the malaria parasite density, whereas, the thin film was used for species identification. Using the thick smear, malaria parasites were quantified. After the first white blood cell was seen, malaria parasites were counted and tallied against white blood cells (WBCs) until 200 WBCs were counted with 6,000 used as the total number of WBCs per microliter of blood (13) and the malaria parasite density (MPD) was expressed as:

$$\frac{\text{Parasites counted} \times \text{WBC standard (6000)}}{\text{Total number of WBCs counted (200)}}$$

Data Analysis

The data generated in this study were analysed for level of significance using the Student t-test, Chi square test and Analysis of Variance (ANOVA). Differences were shown to be statistically significant where $p<0.05$.

RESULTS

Figure 1 is based on the prevalence of malaria parasitaemia among pregnant women in Calabar. Of the 322 subjects screened for malaria, 219 (68.0%)were positive for malaria. *Plasmodium falciparum* was responsible for all cases of parasitaemia.

Table 1 shows the frequency of malaria infection by age amongst the study population. Malaria infection was most common amongst study subjects aged 18-22 years (84.0%) while those aged >42 years accounted for the least prevalence (30.0%). The difference in the prevalence of malaria by age of pregnant women was statistically significant ($\chi^2=18.150$; $p<0.05$).

The prevalence of malaria infection among pregnant women by their gestational age is shown on Table 2. Participants who were in their 3rd trimester had the highest prevalence of *P. falciparum* (74.4%). This category of participants also accounted for the highest parasite density with a mean parasite density of 801.7 ± 204.92 parasites/µL, followed by those in their 2nd and 1st trimesters (305.1 ± 148.50 and 188.9 ± 129.44 parasites/µL of blood respectively). There was no statistically significant difference in the prevalence of malaria infection by gestational age ($\chi^2=0.460$; $p>0.05$). The variation of malaria infection intensity was independent of gestational age (F ratio= 0.008, $p>0.05$).

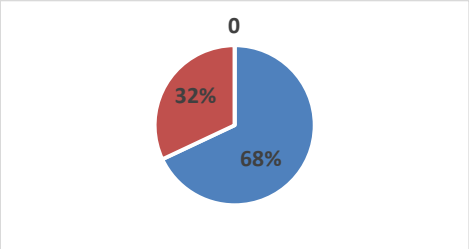

Figure 1: Prevalence of malaria parasitaemia among pregnant women in Calabar
Malaria positive subjects (n=219)
Malaria negative subjects (n=103)

Age group (years)	No. Examined	Number (%) Infected
18-22	25	21 (84.0)
23-27	53	32 (60.3)
28-32	91	56 (61.5)
33-37	96	71 (73.9)
38-42	48	36 (75.0)
>42	9	3 (30.0)
Total	322	219 (68.0

Table 1: Distribution of malaria parasite infection among study subjects by age

Table 2: Prevalence of malaria parasitaemia among pregnant women according to gestational age

Gestational Age	No. of Subjects Examined	No. (%) Infected	Mean Parasite Density±SEM (parasite/µL)
1st Trimester	76	51 (67.1)	188.9±129.44
2nd Trimester	164	106 (64.6)	305.1±148.50
3rd Trimester	82	61 (74.4)	801.7±204.92
Total	322	219 (68.0)	541.2±201.48

Table 3: Prevalence of malaria infection based on marital status, level of education, occupation and living conditions of participants.

Marital Status	**No. of Subjects Examined**	**No. (%) Infected**
Married	217	132 (60.8)
Single	105	87 (82.9)
Total	322	219 (68.0)
Level of Education	**No. of Subjects Examined**	**No. (%) Infected**
None	14	10 (71.4)
Primary School	63	53 (84.1)
Secondary School	77	55 (71.4)
Tertiary School	168	101 (60.1)
Total	322	219 (68.0)
Occupation	**No. of Subjects Examined**	**No. (%) Infected**
Students	90	69 (76.6)
Workers	139	84 (61.8)
Housewives	93	66 (70.9)
Total	322	219 (68.0)
Type of Control Measures Used/Environmental conditions	**No. of Subjects Examined**	**No. (%) Infected**
Use Insecticide treated bed nets	21	4 (19.0)
Did not use Insecticide treated nets	301	215 (71.4)
Good environmental conditions	192	101 (52.6)
Poor environmental conditions	130	118 (90.8)

Good environmental condition- used screens on doors and windows, used insecticide spray, no water-logged channels and clean surroundings.

Poor environmental conditions- did not use screens on doors and windows, did not use insecticide spray, water logged and dirty surroundings.

DISCUSSION

In this study, 68.0% of pregnant women investigated had malaria caused by *P. falciparum*. This is slightly lower than the 70.1% reported by Okafor *et al* (10) amongst pregnant women who attended antenatal clinic in this same locality. Ojiezeh *et al* (14) reported a prevalence of 71.2% of malaria among pregnant women in South West, Nigeria. The high prevalence of malaria infection amongst pregnant women in this study may be attributed to the stable transmission of malaria in the study area coupled with the poor drainage found in some areas of the study locality, rainfall throughout the year, thick vegetation and poor observance and acceptance of malaria control activities (9). Additionally, depressed immunity which is usually associated with pregnancy may also account for the high endemicity of malaria among the pregnant women enlisted in the study (4).

Malaria infection peaked in pregnant women aged 18-22 years (84.0%) and declined thereafter, particularly in subjects aged >42 years (30.0%). This agrees with the findings of Tonga *et al* (15); Njoku *et al*(16) and Wogu *et al*(17). They all reported a higher prevalence rate amongst younger (teenage) pregnant women. The significant (χ^2=18.150; p<0.05) influence of age on the prevalence of malaria amongst the study subjects implied that the younger group of pregnant women carries a higher risk of malaria infection. This may be due to the disposable financial income of participants within this age group, which may affect their ability to assess prompt and early healthcare services, living in better environment with protective measures and good nutrition. The other factor may be the level of acquired immunity against malaria infection which increases with age. This is associated with protection from malaria infection (14). Also, it is likely that, younger women (particularly, teenagers) had a lower level of awareness of their body. They may not have known early enough that they were pregnant in order to assess early antenatal care.

The prevalence (68.0%) of malaria was quite high in the study, particularly amongst participants in the third trimester. The mean parasite density for subjects in the 3rd trimester was 801.7±204.92 parasites/µL of blood, compared to those in the 1st (188.9±129.44 parasites/µL of blood) and 2nd (305.1±148.50 parasites/µL of blood) trimesters. This finding is lower than the intensity of malaria infection reported by Okafor *et al*(10) in a similar locality. A higher mean parasite density of 1913 ± 554.7 parasites/µL of blood, 885.6 ± 364.1 parasites/µL of blood and 577.4 ± 320.6 parasites/µL of blood was found for 3rd, 2nd and 1st trimesters respectively. Also, the frequencies of malaria parasite infection in the 3rd trimester (74.4%) was higher than those in the 2nd (64.6%) and 1st (67.1%) trimesters. A somewhat similar report was made by Okafor *et al*(10) where 60.6%, 67.6% and 80.2% prevalence for 1st, 2nd and 3rd trimesters were recorded. The findings of this study are not in agreement with results obtained by Njoku *et al*(16) in Ebonyi state, Nigeria where the 2nd trimester carried the highest risk of infection (79.3%) with a subsequent decline towards term. The likely reason for the difference in the prevalence rate obtained in this study with that reported

by Njoku *et al*(16) is not known. However, no significant difference existed in the prevalence rate (χ^2=0.460; p>0.05) and intensity (F ratio = 0.008; p>0.05) of malaria infection in the three categories but the prevalence and intensity of malaria infection increased as the gestational age increased.

Participants who were single had a higher prevalence rate (82.9%) of malaria infection than their married counterparts (60.8%). Most likely, the living conditions of the single participants may be responsible for the higher prevalence rate. Proportions of single women that had malaria infection was statistically significant than that of married women (t-test= 8.76; p<0.05).

The prevalence rate of malaria was lower in participants who had acquired a tertiary degree (60.1%). This may be due to their ability to acquire and practice malaria control measures readily. Statistically, there was a significant difference in the prevalence of malaria amongst pregnant women with a tertiary education than those without (χ^2=5.445; p<0.05).

Participants who were students had a higher malaria prevalence rate (76.6%) with workers having the least prevalence rate (61.8%). Statistically, occupation had no effect on the prevalence of malaria infection (χ^2=0.976; p>0.05).

Only 21 out of the 322 participants used insecticide treated nets (ITN). This is very poor and shows that people are either not aware of the importance of ITNs, do not have access to ITNs or do not find it convenient for use. However, participants who used ITNs had a malaria prevalence rate of just 19% compared to those who did not use ITNs (71.4%). Participants who used insecticide spray, mosquito repellents, screens on doors and windows and lived in areas not prone to water log had a lower malaria prevalence rate (52.6%) compared to those who did not use the above and lived in water logged and filthy surroundings (90.8%).

CONCLUSION

The prevalence of malaria infection in pregnancy is still high amongst pregnant women living in the study area despite the increased enlightenment and enforcement of malaria control intervention. The risk of infection was higher in younger than older women. The prevalence and intensity of malaria infection in this study increases with increased gestational age. Marital status, level of education, observance of malaria control measures and living conditions has an effect on the prevalence of malaria infection.

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