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Falciparum Malaria Parasitaemia Among Pregnant Women Attending Antenatal Clinics in a Guinea-Savannah Zone, Southwestern Ebonyi State, Nigeria

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Abstract– A hospital-based, cross-sectional study of *Plasmodium falciparium* infection among pregnant women attending antenatal clinics in three communities of Ebonyi State, Nigeria was conducted between July 2008 and February 2009. Nine hundred and thirty two (932) women, aged ≥ 18 years were offered pretest counseling and screening, 702 were eligible, out of which 660 were selected using random number. Informed consents were collected and their blood screened for malaria parasite. Obstetric and socioeconomic data of participants were colleted using structured questionnaire. Mean age of participants was 24.9 ± 3.1 years; mean number of previous pregnancies 3.2 ± 2.3 per woman. Only *P. falciparum* trophozoites and gametocytes were identified. Altogether, 65.6% of the subjects had *P. falciparum;* parasitaemia was highest (67.1%) in 18 – 25 years aged women and lowest (64.0%) in 26 – 34 years aged women. Prevalence was not affected by age or marital status ($p<0.05$) but was significantly associated with level of education and socioeconomic status ($p>0.05$). Falciparum malaria prevalence decreased with increasing gravidity and was highest in the primigravidae (68.1%): followed by secundigravidae (66.7%) and multiparous (61.6%) women of ≥ 7 pregnancies. Highest falciparum malaria parasitaemia was recorded in second trimester (79.3%) of pregnancy. Mean parasite densities did not show any statistically significant difference between trimesters. Inter-community parasitaemia did not vary significantly ($p>0.05$) between Onicha (71.2%) and Okposi (62.7%). Prevalence of malaria is high in pregnant women in Southwestern Ebonyi State, Nigeria. Primigravidae and secundigravidae were most susceptible to falciparum malaria. It is therefore urgent to design an effective programme of public health education and malaria prophylaxis for this high risk population.

Index Terms– *Plasmodium falciparum*, malaria, parasitaemia, pregnancy, parity, trimester, Nigeria

1 INTRODUCTION

Human malaria, - a protozoan infection caused by *Plasmodium* spp and transmitted by an infected female *Anopheles* mosquito during a blood meal, remains a public health problem in 109 countries worldwide, 45 of them in Africa, where it continues to be the leading cause of maternal and infant morbidity and mortality [1].

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It was estimated that 3.3 billion persons were at risk of acquiring malaria and of these, 247 million were infected (86% in Africa) and nearly one million (mostly African children) died of the infection in 2006 [1].

General estimates of morbidity and mortality associated with malaria suggest that up to 25 – 35% of deaths in African children aged less than five years are caused by malaria [2]. The burden of malaria in Africa appears to have been significantly underestimated and may be increasing largely due to technical and economic constraints in implementing control measures and to the spread of resistance to drugs [3]. In addition, malaria exerts direct financial and indirect costs such as loss of productivity, earnings and school days due to absenteeism which have major impacts on both social and economic development [4].

In Nigeria, malaria infection is endemic with seasonal variations in different geographic and ecological zones. More than 90% of the total population of the country (estimated at over 150 million) is at risk of the infection and about 50% of the population suffer from at least one episode of the disease annually [1, 5]. Nigeria bears a greater burden of malaria than any other country with over 300,000 deaths annually. In particular the disease accounts for 40% of the public health expenditure, 30-50% in-patients admission and up to 50% of outpatients visit [5]. The greatest burden of morbidity and mortality due to malaria falls on children (especially those under 5 years): pregnant women, and non-immune visitors to areas of stable malaria transmission which are mainly concentrated in sub-Saharan Africa [6]. Studies have shown that in areas of high malaria transmission, *Plasmodium falciparum* infection during pregnancy is usually associated with increased susceptibility to peri-natal mortality, increased risk of abortion, low birth weight, and maternal morbidity and anaemia in women of all parities [7 – 11].

It is in the context of the heavy burden on health and the resulting impediment to socio-economic development that malaria control with the use of insecticide - impregnated bed nets during pregnancy has been recommended for adoption as an important component of the antenatal clinic [10, 12]. This survey was undertaken to assess the prevalence of falciparum malaria parasitaemia among pregnant women attending antenatal clinics (ANC) at government-owned general hospitals in three rural communities within the guinea-savannah vegetational zone of southwestern Ebonyi State, Nigeria. It is envisaged that data derived from the study would enhance the development of malaria control strategies in this area of stable malaria transmission.

2 MATERIALS AND METHODS

2.1 Study Area

The study was conducted in three government-operated general hospitals located at Okposi, Onicha and Uburu autonomous communities between July 2008 and February 2009. These communities constitute the southwestern border and integral part of the southern senatorial zone of Ebonyi State. Topographically, Okposi and Uburu constitute 2/3 of current Ohaozara Local Government Area (LGA) with headquarters at Obiozara while Onicha is a major component of Onicha LGA with headquarters at Isu. Ohaozara LGA is situated between latitudes 6˚00', 6˚20'N and longitudes 8˚05', 8˚25'E while Onicha LGA is located between the coordinates 6˚20', 6˚27'N and 8˚05', 8˚45'E. The vegetation of the study area consists of the typical guinea-savannah mosaic pattern characterized by wooded grassland areas punctuated by gently rolling hills and generally undulating terrain [13].

The area experiences two annual climatic conditions of a short, hot, dry season (November to March) and a longer wet season (April to October). The mean annual precipitation is 160mm, relative humidity 71 ± 3.0 and means annual temperature 30˚C. Two major water bodies, the larger Asu and minor Attah Rivers with their feeder streams and rivulets drain the study area and jointly with numerous natural and artificial ponds provide all-season sources of water for the inhabitants. In addition, some of these water sources provide breeding sites for the mosquito vectors and increases the humidity, - conditions which enhance the rapid proliferation and survival of the various mosquito species some of which are vectors of *Plasmodium* malaria.

The population which is basically rural and agrarian is scattered in village-based settlements where large scale subsistence agriculture in the rich alluvial soil, is the dominant occupation.

2.2 Study Design and Population

The hospital-based, cross-sectional investigation was conducted in the obstetric and gynaecology departments of the three hospitals each having antenatal clinics which an average of 90 women attended every month. The choice of three antenatal clinics was to ensure demographic representation for district/zonal-wide evaluation. In the area, majority of pregnant women usually first attend the antenatal clinic in the fourth or fifth month of gestation and thereafter make three or four routine check-up visits before delivery.

Inclusion criteria for participations were: pregnant women aged ≥ 18 years who lived in any of the three study communities and were attending antenatal clinics; gestational age -16 and -35 weeks; absence of general danger signs, for example vomiting, inability to sit or stand; absence of signs of severe and complicated falciparum malaria; absence of recent history of convulsion; absence of hyperpyrexia (i.e. axillary temperature > 39.5˚C) and informed consent of the participating women.

Every participating woman attending antenatal clinics during the study period was offered pretest counseling in the vernacular language by a specially instructed social female worker, confidentially and voluntarily and free of charge. Altogether 932 pregnant women were screened out of which 702 were eligible. A total of 660 pregnant subjects were selected for the study from the study communities using computer-generated random number and consisted of Okposi (225): Onicha (215) and Uburu (220) subjects. At enrolment, the subjects were asked to provide blood for thick and thin blood films once a month. Results of the blood films were explained to the subjects during the next visit. Blood film results were entered in data files in which each subject was identified by her number. In addition, the clinical, obstetric, demographic and socio-economic data for each woman were recorded using a structured questionnaire.

2.3 Ethical Issues

Ethical clearance was sought for and obtained from the State Ministry of Health, Local Government Areas and chief medical officers in-charge of the study hospitals. Drawing blood from the subjects was placed under the responsibility of qualified nurses of each hospital and whose first responsibility was the welfare of the women enrolled in the study. At all times, proper participant management took priority over continuation of the study.

2.4 Blood Examination

Diagnosis of *Plasmodium* malaria was conducted by detecting and identifying the parasites microscopically on blood films. Preparation and staining of the blood films on microscope slides followed standard parasitological protocols [14]. Briefly, the lobe of the finger of each participating woman was cleansed using a swab moistened with 70% v/v alcohol and allowed to dry. For each subject thick and thin blood smears were prepared on the same slide to ensure good staining and species identification, allowed to air-dry for a minimum of 24 hours with slides in horizontal position. Each thin film was fixed in absolute methanol for 1-2 minutes. The dry films were stained in 3% Giemsa stain buffered with physiological saline (pH 7.2): allowed to dry completely overnight. Thick films were examined (100 fields) microscopically for malaria parasitaemia and parasite density using 100 X oil immersion objective and 7 X eye piece [15]. Confirmation of the *Plasmodium* species was by examination of thin blood films. Parasite density was estimated on the assumption that one parasite per high power field equals 500 parasites per microlitre (µl) in accordance with the guidelines of Greenwood & Armstrong [16]. A blood slide was considered negative when examination of 100 thick film fields did not show the presence of asexual forms or gametocytes of *P. falciparum* using the key of Cheesbrough [14]. Categorisation of individuals as infected or uninfected was based on blood film results. For quality control, 10% of the negative samples and 20% of the positive samples were reexamined by different microscopists during the study period.

2.5 Data Analysis

Epi Info statistical software package was used for data analysis. Difference between means was tested using one-way analysis of variance (ANOVA) and differences between proportions were evaluated by Chi-square (χ^2). Socioeconomic risk factors were classified by a scoring system taking into account housing characteristics (type of roof, windows, mud wall or cement block): provision of well or overhead water tank, provision of electric generating set, television/radio, bicycle, motorcycle, motor car. Prevalence ratios were computed at 95% confidence interval to measure the strengths of the associations. For all statistical tests, a p-value > 0.05 was considered significant.

3 RESULTS

Our study showed that the only malaria parasite species detected in the blood films of infected pregnant women was *Plasmodium falciparum* and only the trohozoite and gametocyte stages were identified.

The prevalence of falciparum parasitaemia in relation to some demographic and socioeconomic variables among the study population is shown in Table 1. During the study period 932 pregnant women aged ≥ 18 years who attended antenatal clinics were offered malaria counseling and screened for the study; 702 were eligible while 660(94.0%) consented and tested for malaria infection. The mean age of the tested subjects was 24.9 ± 3.1 years (range 18 – 47 years). The mean number of previous pregnancies was 3.2 ± 2.3 (range 1 – 8) per women. Majority of the subjects 582(88.2%) were married while 11.8% were single during the study period. Overall, 47.0% of the women were housewives, 33.3% were engaged in various aspects of retail trade and 20.0% were corporate employees. Only 8.0% of the study women were unable to read or write even in the ethnic language; 62.0% completed primary education and 30.0% attained secondary school standard.

Falciparum parasitaemia prevalence did not differ with age or marital status of the pregnant women (p>0.05). However, prevalence was associated with low level of education, occupation and low socioeconomic status (p>0.05). Table 2 showed the prevalence of falciparum malaria in relation to age and some obstetric variables among the studied subjects. A total of 433(65.6%) of the studied women (n = 660) aged 18 to ≥ 35 years were infected with *P. falciparum* during the study period. Infection was highest (67.1%) (n = 310) in subjects in the 18 – 25 years age category, lowest (64.0%) in those aged 26 – 34 years, while 65.1% (n=86) of those women aged ≥ 35 years also tested positive for falciparum malaria parasitaemia. However, age did not appear to significantly affect the prevalence of falciparum malaria in pregnancy (p>0.05) (Table 2).

With regards to parity, the results showed that falciparum malaria prevalence decreased with increasing gravidity. Thus, 216(68.1%) of 317 women in their primigravidae (first pregnancy) were *P. falciparum* parasitaemic, compared with 88(66.7%) in secundigravidae (n = 132); 63.4% of those in gravidae 3-6 (n = 112) and 61.6% in their seventh (or more) pregnancies (n = 99).

In relation to gestation age, the highest falciparum parasitaemia (79.3%) was recorded for subjects (n = 311) in second trimester, subsequently parasitaemia decline of 67.1% was recorded towards term. The distribution of malaria parasitaemia by parity and trimester among the subjects is shown in Table 3.

Table 1: Demographic and selected socioeconomic indices associated with malaria in pregnancy in southwestern Ebonyi State, Nigeria by univariate analysis

Characteristics	Number of pregnant women (n = 660; (%)	%	Malaria unadjusted prevalence ratio	P value
Age(years)				
18 – 25	310(47.0)	67.1	1.04(0.89-1.11)[b]	0.25
26 – 34	264(40.0)	64.0	1.00	
≥ 35	86(13.0)	65.1	1.03(0.94-1.14)	0.58
Marital status				
Single	78(11.8)	69.0	1.05(0.96-1.15)	0.35
Married	582(88.2)	65.7	1.00	
Occupation				
Housewife cum farming	310(47.0)	66.9	1.32(1.08-1.63)	< 0.01
Informal business	220(33.3)	66.3	1.31(1.07-1.62)	< 0.01
Employee	130(19.7)	50.5	1.92(1.01-1.64)	
Education				
None	53(8.0)	67.9	1.15(1.04-1.27)	< 0.01
Primary education	409(62.0)	64.4	1.09(0.97-1.22)	0.16
Secondary education	198(30.0)	59.2	1.00	
Socioeconomic status				
Low	386(58.5)	70.0	1.22(1.09-1.38)	< 0.01
Middle	262(39.7)	63.6	1.09(0.97-0.23)	0.14
High	12(1.8)	58.0	1.00	

[b] Figures in parentheses are the 95% confidence interval

Table 2: Obstetric characteristics associated with malaria parasitaemia in pregnancy in southwestern Ebonyi State, Nigeria

Characteristics	Number of women tested (n = 660; (%)	Falciparum Number infected (%)	Parasitaemia (unadjusted prevalence ratio)	P value
Age(years)				
18 – 25	310(47.0)	208(67.1)	1.04(0.89-1.11)[b]	0.25
26 – 34	264(40.0)	169(64.0)	1.00	
≥ 35	86(13.0)	56(65.1)	1.03(0.94-1.14)	0.58
Total		433(65.6)		
Parity				
Primigravidae	317(48.0)	216(68.1)	1.08(0.97-1.19)	0.18
Secundigravidae	132(20.0)	88(66.7)	1.04(0.95-1.14)	0.42
Gravidae 3-6	112(17.0)	71(63.4)	1.03(0.93-1.15)	0.58
Gravidae ≥ 7	99(15.0)	61(61.6)	1.00	
Total		433(65.6)		
Gestation age				
1st trimester	189(28.6)	69(36.5)	1.00	
2nd trimester	392(59.4)	311(79.3)	1.28(1.16-1.42)	< 0.001
3rd trimester	79(12.0)	53(67.1)	1.92(1.21-1.50)	< 0.001
Total		433(65.6)		

[b] =Figures in parentheses are the 95% confidence intervals

Table 3: Distribution and intensity of falciparum malaria parasitaemia by parity and gestation stage among pregnant women attending antenatal clinics in southwestern Ebonyi State, Nigeria

Parity / Mean parasite Intensity	Stage of pregnancy						Total	
	1st trimester		2nd trimester		3rd trimester			
	Number of cases	Number positive (%)	Number of cases	Number positive (%)	Number of cases	Number positive (%)	Number of case	Number positive (%)
Primigravidae	90	23(25.6)	210	182(86.7)	17	11(64.7)	317	216(68.1)
Secundigravidae	50	35(70.0)	63	56(88.9)	19	9(47.4)	132	88(66.7)
Gravidae 3 - 6	34	10(29.4)	62	45(72.6)	16	16(100.0)	112	71(63.4)
Gravidae ≥ 7	15	1(6.7)	57	28(41.1)	27	25(92.6)	99	61(61.6)
Total	189	69(36.5)	392	311(79.3)	79	53(67.1)	660	433(65.6)
Mean parasite Intensity	10120 ± 1835		9828 ± 4140		10444 ± 1002		30393 ± 6977	

Table 4: Community – specific distribution of falciparum malaria among infected pregnant women in relation to parity in southwestern Ebonyi State, Nigeria

Parity	Community parasitaemia			Total (%)
	Okposi (n=225)	Onicha (n=215)	Uburu (n=220)	
Primigravidae	30(21.3)	34(22.2)	26(18.7)	90(20.8)
Secundigravidae	69(48.9)	77(50.3)	65(46.8)	211(48.7)
Gravidae 3-6	23(16.3)	22(14.4)	26(18.7)	71(16.4)
Gravidae ≥ 7	19(13.5)	20(13.1)	22(15.8)	61(14.1)
Total	141(62.7)[a]	153(71.2)	139(63.2)	433(65.6)

[a] = Figures in parentheses are percentages

Out of the 317 women in their primigravidae, 90, 210 and 17 were in their first, second and third trimesters of pregnancy respectively. The highest *P. falciparum* infection was observed for 182 (86.7%) primigravid women in their second trimester while 11(64.7%) other women in their third trimester were also falciparum malaria parasitaemic. Differences in falciparum malaria prevalence among pregnant women in different trimesters were statistically significant ($p>0.05$).

Of the 132 women in their secundigravidae 35, 56 and 9 in their first, second and third trimesters respectively, were infected. While 79.3% of the 433 infected women were in their second trimester, 67.1% were in their third trimester, and only 36.5% were in their first trimester of pregnancy.

Generally, in relation to parity, falciparum malaria parasitaemia appeared to decline in the study population as parity increased being highest in the primigravidae and lowest in the ≥ 7 pregnancies. The results also showed that interparity parasitaemia prevalence was not statistically significant ($p>0.05$). The mean parasite densities were considerably high in the three trimesters of pregnancy. However, the mean parasite densities in the trimesters (all parities combined) did not show any statistically significant difference between trimesters ($p>0.05$).

The community-specific distribution of *P. falciparum* malaria infection among infected pregnant women subjects in relation to parity is shown in Table 4. The results show that inter-community malaria parasitaemia prevalence did not vary significantly although highest in Onicha community (71.2%): followed by Uburu (63.2%) and lowest in Okposi (62.7%). However, the intercommunity prevalence difference was not statistically significant ($\chi^2 = 3.180$, df = 2, $p = 0.16$). In each of the three study communities, women experiencing their second to fourth pregnancies (n = 211) recorded highest *P. falciparum* infection (48.7%) (range, 46.8 – 50.3%) compared with lowest prevalence (14.1%) recorded for multiparous women of ≥ 7 pregnancies (range, 13.1 – 15.8%).

4 DISCUSSION

Our present hospital-based assessment of malaria parasitaemia among pregnant women in southwestern Ebonyi State, Nigeria is of special significance since there is a dearth of published data on the relationship between malaria infection and pregnancy in the State. The study methodology which was aimed at evaluating the burden of malaria parasitaemia during pregnancy in a rural setting also highlighted opportunities for intervention.

The study showed that *P. falciparum* was responsible for all the cases of parasitaemia in pregnant women studied, and that about 2/3 (65.6%) of pregnant women living in the study communities and were attending ANC were falciparum malaria parasitaemic. The level of parasitaemia prevalence recorded in the study is lower than data from some other developing countries endemic for malaria. In the analysis of patterns of malaria infection in a cohort of prenatal women in Ethiopia, falciparum malaria prevalence of 88% was established and in India an overall falciparum prevalence of 97.2% was reported for pregnant women [17, 18]. However, in Gabon, Bouyou-Akotet and co-workers recorded falciparum malaria prevalence of 57.5% among pregnant antenatal women [19]. The level of parasitaemia prevalence recorded in this study is also higher than the *P. falciparum* infection rates recorded for prenatal women elsewhere in Nigeria; 52.6% and 56.0% in Aba and Okigwe, respectively; 20 7.3% in Port Harcourt [20]. The malaria prevalence recorded in our present study is however 4 times higher than the usual estimates from industrialized countries [21]. The high rate of parasitaemia recorded in this survey could be attributed partly to environmental factors inherent in aspects of the ecology of the study area which are favourable to the transmission of the parasite. For example, it has been established that a temperature range of 16˚C – 38˚C and relative humidity of ≥ 60% enhance the transmission of *P. falciparum* [22]. In this study, falciparum malaria parasitaemia prevalence was associated with parity with parasitaemia declining with increasing gravidity. The highest prevalence was observed among the primigravidae (68.1%): followed by secundigravidae (66.7%). Jointly, primigravidae and secundigravidae accounted for 70.2% of total falciparum infection as against 30.5% of gravidae 3 to ≥ 7. These results are consistent with previous reports which found *P. falciparum* parasitized primigravidae at a significantly greater risk of clinical malaria [23 – 25]. Elsewhere in Nigeria, Nnaji and co-workers had also observed statistically significant difference between prevalence rates of malaria parasitaemia in the primigravidae (87.9%) and grand multigravidae (63.6%) among their cohort of malaria-infected pregnant subjects [26]. The reason for the present result of gravidae-associated predisposition to falciparum malaria may be due to the fact that adults who live in malaria-endemic regions generally have some acquired immunity to malaria infection due to immunoglobulin production stimulated by previous malaria infection. This acquired immunity diminishes significantly in pregnancy particularly in primigravidae. It has also been suggested by various authors that the early onset of antibody response in multigravidae and the delayed antibody production in primigravidae may be responsible for the gravidity-dependent and differential prevalence of falciparum malaria among pregnant women [23, 25].

The results of this study indicated that *P. falciparum* infection was highest (79.3%) during the second trimester of pregnancy after which a decline (67.1%) was observed towards term. This reflects the maternal acquisition of immunity resulting from prolonged exposure to the transmission risk factors. The high parasitaemia prevalence recorded during the second and third trimesters could be ascribed to the progressive collapse of weak reserves of iron and folic acid during pregnancy due to increased demand by the foetus.

The high mean parasite intensity in *P. falciparum* infected subjects observed in all trimesters indicates primary infections, which suggest that the pattern of malaria infection may vary in areas of different malarial endemicity. Despite apparently different entomological inoculation rates, falciparum malaria parasitaemia during pregnancy was found to be similar in the three study communities. The high parasitaemia rates recorded in the communities indicate that possible transplacental infection can be substantial. This observation highlights the need for intervention to prevent malaria during pregnancy in a wide range of transmission areas of the country.

5 CONCLUSION

Data from this study showed a high prevalence of falciparum malaria among pregnant women, indicating the need for increased and sustained chemoprophylaxis as a routine policy in malaria-endemic rural settings. It has been estimated that in sub-Saharan Africa, 68% of pregnant women make at least one antenatal visit before term [27]. This group of women at risk deserves serious consideration. Attendance at ANC provides an opportunity to implement malaria prevention strategies during pregnancy. Sustained enlightenment campaigns to educate women of child-bearing age about the dangers of malaria in pregnancy and the potential benefits to expectant mothers could help improve the rate of early attendance at the clinics. In many developing malaria-endemic countries policies to address the problem of malaria are already in place. However, our study has demonstrated that many pregnant women who enlist in ANC are already malaria parasitaemic and some of the preventive measures may be more effective if implemented earlier.

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