

The Demographical Prevalence of Malaria in Pregnant Women in Oshimili South Local Government Area of Delta State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the prevalence of malaria in pregnant women attending antenatal clinics in Delta state. Thick-film microscopy was used to detect parasitaemia while polymerase chain reaction (PCR) was employed to confirm positivity. Statistical method used were Chi square, Paired Sample T test and Pearson Correlation test at 5% level using SPSS version 23.0. Out of three hundred and fifty (350) HIV-negative and asymptomatic pregnant women examined at first antenatal registration, one hundred and twenty-two (122) were found positive for malaria parasite. The percentage prevalence of malaria positive cases recorded using microscopy was 34.86% (122/350) while PCR confirmed the positivity of 84% (21/25) for both positive and negative samples derived from microscopy. Prevalence of malaria in relation to age, gravidity, and educational qualification were statistically significant while prevalence in relation to occupation, the hospital they attended were not statistically significant. Participants within the age group of 15-19(66.6%), primigravidae (42.6%), primary education (100%) and traders/businessmen had the highest prevalence of malaria. It is therefore recommended that malaria test should be one of the routine tests compulsorily done on pregnant women to avoid complications and public awareness campaign programs on preventive measures against malaria should be regularly conducted to make the journey to eradication of malaria from Nigeria an easier one.

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1. BACKGROUND

Malaria has been one of the main causes of death in sub-Saharan Africa, especially in Nigeria. With reference to World Malaria Report, about 229 million cases of malaria and 409,000 deaths occurred across the world in 2020 [1] and Nigeria had 25% of the total global cases. Malaria is caused by eukaryotic Protista of the genus, *Plasmodium* and the five species that infect humans are *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Out of all these species, *P.falciparum* is the most predominant and most fatal in Nigeria. Irrespective of the fact that malaria can be prevented and treated, it is still one of the world's most predominant and medically important tropical disease usually transmitted from one person to another through the bite of its vector, a female *Anopheles* mosquito. Due to the large amount of money and effort spent to prevent malaria, there was an encouraging decline in the number of cases recorded globally between 2000 and 2016 [2].

Economists have depicted that the amount spent on malaria yearly in Africa is about \$12 billion and this huge amount poses a great economic burden to government's productive work [3]. Malaria presents as a critical disease due to its severeness and ability to become fatal and it is one of the major causes of illness and death in tropical regions. In pregnancy, new hormones and a new organ, the placenta are formed and these accord the malarial parasites more binding sites increasing the chances of a pregnant woman of coming down with malaria infection thus, posing a lot of threats to them and the fetuses. Malaria in pregnancy (MiP) is usually severe causing most of the morbidity and death in sub-Saharan African regions as it results in miscarriage, preterm birth, maternal anemia, foetal growth retardation and infant low birth weight (LBW) [4,2]. Due to the severity of malaria infection, it requires to be overcome in malaria endemic regions such as the Sub Saharan Africa [5].

Malaria has been linked to poverty since it is known to affect people and regions in poverty that cannot afford malaria treatment. Malaria has been a critical health issue in Nigeria where its rate of morbidity and mortality is higher than any other country in the world. In the remote areas of Nigeria, malaria is holoendemic (transmission

takes throughout the year) while it is mesoendemic (transmission takes place at particular seasons) in the urban areas. In the southern region of the country, the rate of spread is almost uniform throughout the year [6].

In malaria endemic regions, *P.falciparum* infections during pregnancy is usually asymptomatic and therefore becomes undetected and untreated [7]. World Health Organisation (WHO) recommended the use of insecticide-treated bed nets (ITNs), intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine (SP), and immediate diagnosis and treatment of infection to curtail the severity of illness and death in pregnancy. Although these measures have resulted in decline in the number of cases of malaria, there has been rise in the cost of treating malaria in pregnancy to the individual and the nation.

Pregnant women are more prone to contracting malaria infection because of the changes in their immune system and the development of a new organ, the placenta with new sites for parasite binding.

The severity of Malaria in Pregnancy (MiP) depends on the intensity of malarial transmission in a particular geographical region and an individual's level of acquired immunity. Every year about 25–30 million women become pregnant in malaria-endemic areas in Africa and most maternal morbidity cases and low birth weights of infants are related to malaria caused by *Plasmodium falciparum* infection which occurs predominantly in Africa. Studies have revealed that the following factors are associated with the commencement of malaria in pregnancy: maternal age, parity, gestational age, place of residence, household wealth status, maternal educational level, knowledge of malaria in pregnancy, and non-use of Sulphadoxine-pyrimethamine (IPTp) during pregnancy [8,9].

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Oshimili South Local Government Area of Delta State, Southern Nigeria. The local government area consists of towns and villages which include: Asaba, Okwe, Oko, Illah, Ebu and Eze which are located at the northern end of the state. It has an estimated

population of 149,603(2006 census) with an estimated area of 762 square kilometres. The average annual temperature in the area is 27.2°C and about 1764 mm of precipitation falls annually. The temperatures are highest on average in March at around 29.0°C. August has the lowest average temperature of the year, 25.5°C. The least amount of rainfall occurs in December and most precipitation falls in September.

The area creates a settlement of people engaged in farming, fishing, sports, commerce and industry. The sandy beaches of the river present a beautiful sight and good ground for recreation and tourism. As a result of these activities; the town has attracted millions of migrants from other neighbouring states and regions. Migration is a global issue of concern which if poorly managed can result in various problems including an increase in the transmission of diseases such as malaria.

2.2 Sample Population

The participants were sampled in the study because they offer unique opportunity to monitor the progress of malaria infection. The study was on pregnant women who attended antenatal clinics in Federal Medical Centre (FMC), St. Joseph's Catholic Hospital and Okwe General Hospital.

2.3 Determination of Sample Size

Yamane [10] formula was adopted for sample size determination. The annual 3% growth rate for the female population as at 2020 was 111,861.9. The population of women of reproductive age, which is 24% of all female population (WHO annexes and work sheets), was 26,846.9. Also determined was the population of pregnant women; who constitute 5% of women of reproductive age (1,342.3) in the study area. The figures obtained were substituted resulting to 305.

2.4 Sample Size Calculation

Yamane [10]

$$n = \frac{N}{1+N(e)^2}$$

Where,

n = sample size
N = total population

e = error term at 5% (95% confidence interval)

2.5 Eligibility

The study subjects consisted of pregnant women who met the following criteria:

- Attendance at an antenatal care unit in the hospital.
- Provide informed consent to participate in the study.
- Residence within the state or nearby study states from the period of pregnancy through delivery.
- Willingness to deliver at the hospital.
- Four to six months pregnant mothers within the age of 15 - 49 and had not been placed on IPT.
- Mothers without fever.
- Normal cases (i.e., mothers who do not suffer from terminal illnesses, renal failure, HIV, diabetes and hypertension during present or previous pregnancy).

2.6 Advocacy and ethical Consideration

The study protocol was reviewed and approved by the Ethical Committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Amaku, Awka. Approval was obtained from Delta State Hospitals' Board, the ethical committee of Federal Medical Centre and the Management of St. Joseph's Catholic Hospital with an introduction letter stating the aim of the study. The head of maternity unit as well as the nurses in charge of the unit were informed. The participants were enlightened of the objectives and aim of study.

2.7 Research Design

This cross sectional study was conducted from September 2020 to December 2020. The three hospitals were systematically selected namely; Okwe General Hospital, St. Joseph's Catholic Hospital and Federal Medical Centre, Asaba. Quantitative methods used were questionnaire and biological sample collection and analysis for data collection.

2.8 Administration of Questionnaire

A structural questionnaire was used to capture obstetric and medical history as well as demographic and socio-economic data on the day of recruitment. Each of the participants was

given an identification number which was subsequently used to match all data obtained during the parasitological survey and molecular analyses.

2.9 Blood Sample Collection and Analysis

All HIV negative pregnant women were clinically examined on their first antenatal visit for fever before blood collection with the intent of excluding the ones that have fever. Fever was defined as an axillary temperature ≥ 37.5 °C. Blood samples (finger) were collected for the following analysis:

2.10 Thick-film Microscopy for Determination of Presence of Malaria Parasite and Type

A drop of each of the peripherally drawn blood samples (6 microlitre) was placed at the centre of a clean grease free microscope glass slide. With the aid of a clean spreader, the blood was spread in a circular form of 10mm diameter. The slide was kept to air dry. The blood films were stained with 10% Giemsa for 10 minutes as described by WHO [11] washed off with 7.2 buffered water and allow to air dry.

A drop of immersion oil was placed at the centre of the blood films. The slides were examined under the microscope using x100 objective lens. The number of parasites/microliter of blood was counted by examining the thick smear under oil immersion.

2.11 Molecular Analyses for Extraction of Genomic DNA, Genotyping of Malaria Parasite and Assessment of Polymorphism

Blood samples of the participants spotted in triplicates on Whatmann 3mm filter papers were air dried. The Dried Blood Spot (DBS) of the samples were transported to the Biochemistry laboratory of the Nigerian Institute of Medical Research (NIMR), Lagos. Extraction of genomic DNA was carried out using Qiagen Mini Kit (Qiagen, UK) from the individual dried blood spots. The quality as well as quantity of each DNA sample was determined using nanodrop

(Thermo Fisher Scientific, United States of America). The extracted DNA samples were stored at -20°C until the following analyses were carried out:

2.12 Polymerase Chain Reaction (PCR) for the Genotyping of Malaria Parasite

The DNA of the malaria parasites detected was amplified at merozoite surface protein 2 for genotyping. Parasite positivity was confirmed by the presence of msp-2 DNA fragments of 100-500 bp irrespective of the allelic family on the agarose gel after electrophoresis. The absence of DNA indicated parasite negativity in the dot blood sample.

2.13 Statistical Analysis

Data obtained from the study were summarized using charts and tables. Test of statistical significance was done at 5% level using SPSS version 23.0. Chi square analysis was used to test the relationship between malaria parasite prevalence in hospitals and the diagnostic tool used, prevalence of malaria based on age, gravidity, educational level, and occupation. Paired t-test was used to determine the prevalence among the samples screened by PCR systematically chosen by age. The distribution of participants and prevalence of MiP in relation to occupation and educational qualifications were represented with tables and bar charts.

3. RESULTS

A total of 350 pregnant women were enrolled in this study within the period of September, 2020 to December, 2020 to detect parasitaemia using microscopy and their haemoglobin level was also examined. The number of the pregnant women that was confirmed positive for malaria parasite using microscopy was 122 out of the 350 examined. However, 7% (25) of the total number of samples were systematically selected based on age group and sent as dried blood samples to NIMR for confirmation of parasitaemia and identification of the genes of *Plasmodium falciparum* present.

Table 1. Prevalence of malaria infection amongst pregnant women attending antenatal clinic

Technique	No. Examined	Positive samples	Negative samples	Prevalence (%)
Microscopy	350	122	228	34.86
PCR	25	21	4	84

$X=0.000$ $df=1$

Table 2. Prevalence of MIP in relation to age among the pregnant women

Age	No of Samples	No of positive samples	Percentage (%)
15 – 19	21	14	66.6
20 – 24	63	23	36.7
25 – 29	104	41	39.4
30 – 34	92	30	32.6
35 -39	48	10	20.8
40 -44	22	4	18.2
45 – 49	0	0	0

$X=0.004$ $df=5$

Table 2.1 Relationship between MIP and age of pregnant women

Correlations			
		Age	Positive results
Age	Pearson Correlation	1	.948**
	Sig. (2-tailed)		.001
	N	7	7
Positive results	Pearson Correlation	.948**	1
	Sig. (2-tailed)	.001	
	N	7	7

** Correlation is significant at the 0.01 level (2-tailed)

Table 3. Prevalence of MIP in relation to different gravidity of the pregnant women

Gravidity	No of samples	No of positive samples	Percentage (%)
Primigravidae	129	55	42.6
Secundigravidae	92	39	42.4
Tertigravidae	69	15	21.7
Multigravidae(>3)	60	13	21.7
Total	350	122	100

$X=0.001$ $df=3$

Table 3.1 Relationship between MIP and gravidity

Correlations			
		Gravidity	Positive results
Gravidity	Pearson Correlation	1	.980*
	Sig. (2-tailed)		.020
	N	4	4
Positiveresults	Pearson Correlation	.980*	1
	Sig. (2-tailed)	.020	
	N	4	4

* Correlation is significant at the 0.05 level (2-tailed)

Table 4. Prevalence of MIP in relation to educational qualification of the pregnant women

Highest educational qualification	No of samples	No of positive samples	Percentage (%)
No formal education	15	14	93.3
Primary education	28	28	100
Secondary education	134	53	39.6
Tertiary education	173	27	15.6
Total	350	122	100

$X=0.000$ $df=3$

Table 4.1 Relationship between MIP and educational qualification of the pregnant women

Correlations			
		Edu Qualification	Positive results
EduQualification	Pearson Correlation	1	.225
	Sig. (2-tailed)		.856
	N	3	3
Positiveresults	Pearson Correlation	.225	1
	Sig. (2-tailed)	.856	
	N	3	3

$X=0.856$

Table 5. Prevalence of MIP in relation to participant's occupation

Occupation	No of samples	No of positive samples	Percentage (%)
Civil servants/Private	72	23	31.9
Students	28	8	28.6
Traders/Business	142	57	40.1
Artisans	71	24	33.8
Unemployed	37	10	27.0
Total	350	122	100

$X= 0.472 \text{ df} = 4$

Table 5.1 Relationship between MIP and Participant's Occupation

Correlations			
		Occupation	Positive results
Occupation	Pearson Correlation	1	.996**
	Sig. (2-tailed)		.004
	N	4	4
Positiveresults	Pearson Correlation	.996**	1
	Sig. (2-tailed)	.004	
	N	4	4

**. Correlation is significant at the 0.01 level (2-tailed)

Table 6. Prevalence of MIP among the three hospitals

Hospital	No of samples	No of positive samples	Percentage (%)
FMC	115	29	25.2
St Joseph	92	42	45.7
Okwe General hospital	143	51	35.7
Total	350	122	34.9

$X= 0.729 \text{ df} = 2$

Table 7. Prevalence of MIP among the samples screened by PCR systematically chosen by age

Age	No of samples	No of positives for microscopy	No of positives for PCR
15 – 19	2	2	2
20 – 24	5	3	3
25 – 29	8	4	8
30 – 34	6	3	5
35 – 39	3	1	3
40 – 44	1	0	0
45 – 49	0	0	0
Total	25	13 (52%)	21 (84%)

$\text{sig} = 0.099. \text{SD} = 1.67332$

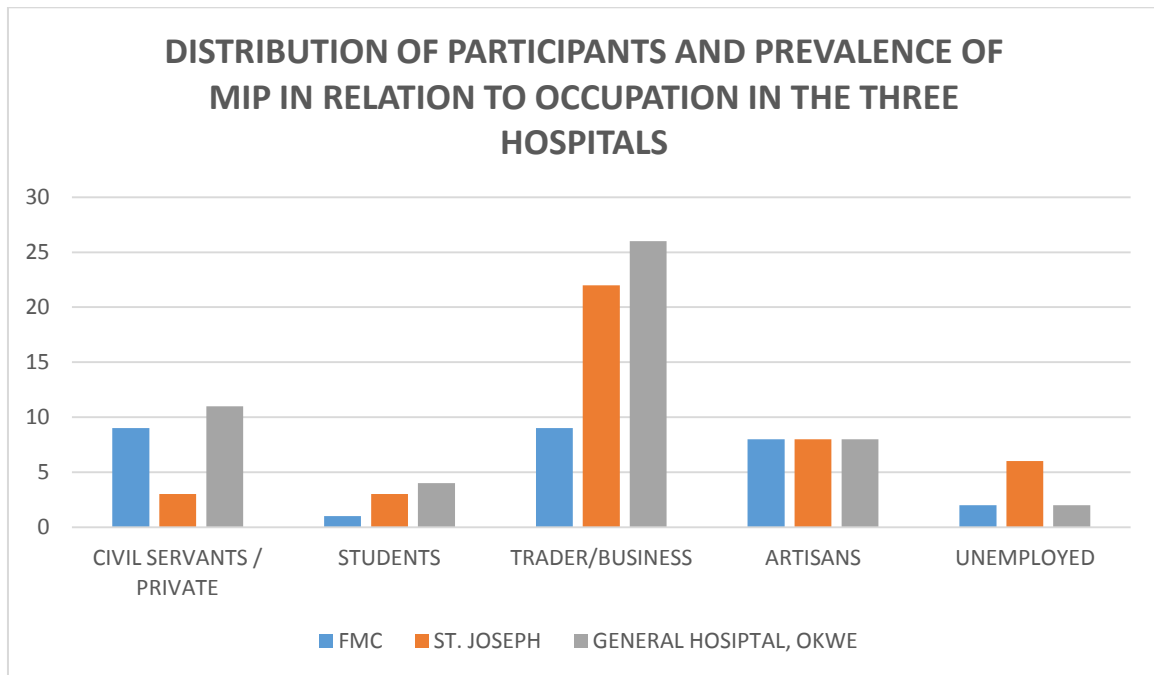


Fig. 1. Distribution of participants and prevalence of MiP in relation to occupation in the three hospitals

Table 8. Distribution of participants and prevalence of MIP in relation to occupation in the three hospitals

	Occupation	No Examined	No Of Positive Samples	Percentage (%)
FMC	Civil Servants/Private	49	9	18.4%
	Students	3	1	33.3%
	Traders/Business	22	9	40.9
	Artisans	35	8	22.9
	Unemployed	6	2	33.3
	Total	115	29	
ST. JOSEPH	Civil Servants/Private	10	3	30%
	Students	16	3	18.8%
	Traders/Business	46	22	47.8%
	Artisans	14	8	57.1%
	Unemployed	6	6	100%
	Total	92	42	
OKWE, GENERAL HOSPITAL	Civil Servants/Private	13	11	84.6%
	Students	9	4	44.4%
	Traders/Business	74	26	35.1%
	Artisans	22	8	36.4%
	Unemployed	25	2	8.0%
	Total	143	51	

Table 9. Distribution of participants in relation to educational qualification

Hospital	Educational Qualification	No Examined	No Of Positive Samples	Percentage (%)
FMC	No Formal Education	0	0	0%
	Primary	0	0	0%
	Secondary	10	9	90%
	Tertiary	105	20	19%
	Total	115	29	25.2%
ST. JOSEPH	No Formal Education	6	5	83.3%
	Primary	9	9	100%
	Secondary	44	23	52.3%
	Tertiary	33	5	15.2%
	Total	92	42	45.7%
GENERAL HOSPITAL, OKWE	No Formal Education	9	9	100%
	Primary	19	19	100%
	Secondary	80	21	26.3%
	Tertiary	35	2	5.7%
	Total	143	51	35.7%

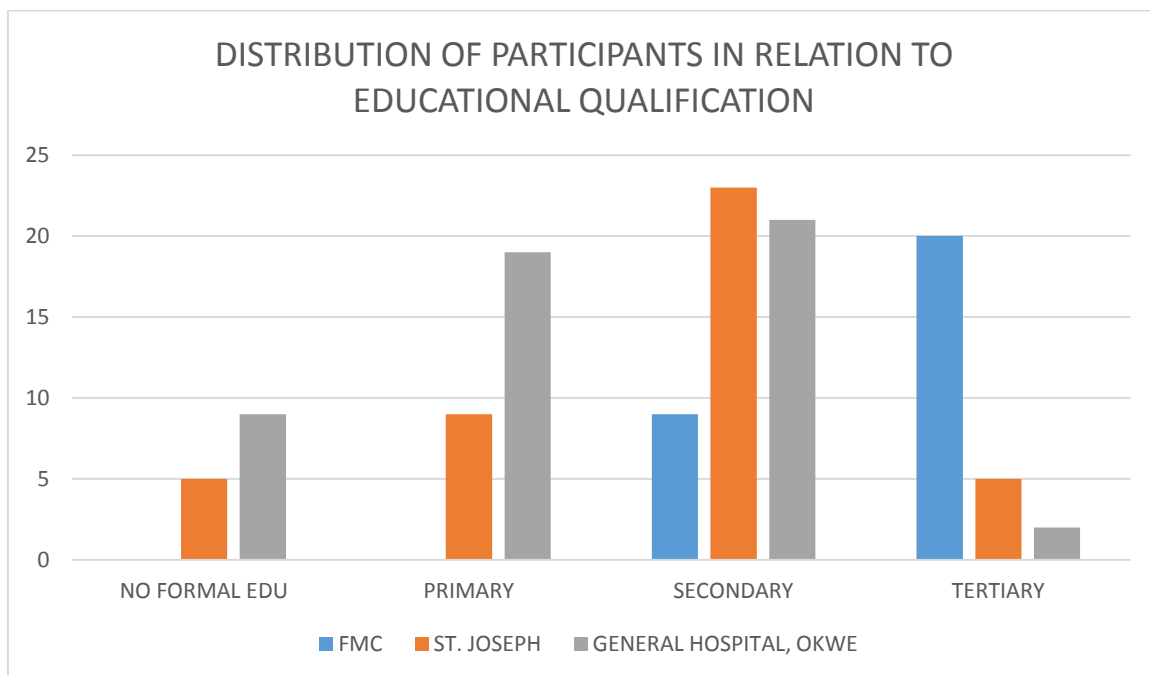


Fig. 2. Distribution of participants in relation to educational qualification among the three hospitals

4. DISCUSSION

The study has shown that there are asymptomatic cases of malaria parasite infection in pregnancy and therefore the essence of administration of IPTp-SP to pregnant women irrespective of whether the women have malaria or not should not be underestimated. Nevertheless, malaria during pregnancy is a problem of major concern due to its effect on the mother and the unborn child [12]. The prevalence of 34.9% (Table 1) was obtained for malaria parasites by microscopy which is higher than the low prevalence of 6.6% observed in Kwale, Delta state [13] among pregnant mothers. The result does not agree with the previous report of Jemikalajah [14] who recorded 63% in Ughelli in the same state. The prevalence of malaria in pregnant women here is an indication of a declined progress in meeting the Delta vision 2020; aimed at providing standard and adequate facilities, infrastructure and human resources to achieve the highest quality of healthcare [15]. In other words, some of the pregnant women in the study area might have been taking measures to prevent malaria parasite infection while others might have declined on their efforts in using the preventive measures. The differences in prevalence within the state could be linked to the season of the year when the study was conducted, the infrastructural development and the level of environmental sanitation in these locations. Furthermore, the prevalence rate obtained in the study is higher than that obtained in Portharcourt, 6.2% by microscopy [16] while 2% was recorded in Lagos [17]. These results are obviously lower than the previous studies in Anambra state which recorded 73.1% [18], 58% and 65 [19,20], 64% 41% in Ebonyi State [21] Portharcourt ,72.5% [22] and Lagos, 52% [23].

False negative (32%) blood samples of microscopy based on PCR can be attributed to human errors in recording *P.falciparum* malaria infection due to other *Plasmodium* species and thick-smear preparation on the slides [24]. Pregnant women found negative and positive of parasite were engaged in the study and no false positive was recorded for microscopy. However, PCR remains a useful diagnostic tool in terms of accuracy though it requires sophisticated machines, uncommon reagents and highly skilled personnel.

Association of malaria prevalence with age showed that the participants between 15-19

years of age had the highest malaria prevalence (66.6%) while the participants between 40-44 years of age had the lowest prevalence (18.2%) (Table 2). Statistically, the association was significant in that malaria parasitaemia in relation to age may be dependent on the maternal age which could be linked to the level of immunity the pregnant woman has acquired with time. This report agrees with that of Agomo and Ukibe [25] where the highest malaria prevalence in younger pregnant women of < 20 years (20.5%) and between 17-22 years, respectively. Bolaji *et al.* also a high prevalence of 84.6% between the age group of 20 to 30 years while those above 40 years had the least. Other studies by Wogu *et al.* [26] and Mbah *et al.* [27] recorded the highest prevalence between 21-30 and 18-28 years, respectively. The prevalence (66.6%) seen in women between 15-19 years could be related to their young age and inexperience.

The highest prevalence rate in association with gravidity (Table 3) was found among the primigravidae (42.6%). The result of this study is in line with many other reports among pregnant women where higher prevalence was consistently reported among the primigravidae [28-30]. Primigravidae do not have an acquired immunity to placental malaria which is usually acquired from exposure to malaria parasites during pregnancy [31]. In other words, exposure to malaria parasite builds up immunity with successive pregnancy [32]. On the contrary, some studies found prevalence rates to increase with increasing gravidity [33]. Statistically, there was a significant difference between prevalence of malaria and gravidity so there may be a relationship between malaria infection and the number of pregnancies a woman has had.

Based on educational level (Table 4), the prevalence was highest in pregnant women with primary education (100%) and varies from tertiary school (15.6%), secondary school (39.6%) and no formal education (93.3%). This observation is in agreement with the previous report by Kiptoo, [34], of which the prevalence was not related to lack of knowledge of the causative agents and preventive measures rather the level of understanding and effective practice of the preventive measures. Pregnant mothers who had tertiary education had a low prevalence and this may be related to their level of education and effective practice of the preventive measures. This does not correlate to a study in Kano, Nigeria where the knowledge of malaria and its preventive measures was high but adherence to preventive measures were

below expected targets [35]. However, educational status of the pregnant women had a significant association; emphasizing on more sensitization of pregnant women and adherence to control measures will go a long way in eradicating malaria.

There was no significant association observed between malaria prevalence and participants' occupation while the highest prevalence (40.1%) was found among traders/business women (Table 5). It is likely that the nature of their jobs exposes them to mosquito bites especially when they have to travel to other states or regions to buy their goods. However, one's occupation determines one's earning and standard of living therefore this could affect their compliance and adherence to the control measures like buying insecticides, insecticides treated nets (ITNs) and antimalarial drugs as well as where they live.

Nevertheless, the level of significance showed above for Table 1, Table 2, Table 3 and Table 4 was determined using an independent correlation (Chi-square) and it showed that malaria infection had a significant association with age, gravidity and educational qualification but not with occupation although Pearson Correlation Statistics which is a dependent test showed correlation between malaria infection and age, gravidity, occupation and not with educational qualification.

The prevalence of malaria in the three hospitals studied showed that St. Joseph Catholic Hospital had the highest prevalence of 45.7% as compared to that of FMC (25.2%) and Okwe, General Hospital (35.7%) (Table 6). Although, statistically, there was no significant association between malaria prevalence and the particular hospital the participants attended. The high prevalence of malaria at St. Joseph hospital may be because it is a missionary hospital which operates at a subsidized rate as concerns bills compared to private hospitals in the area so people from different parts of the local government area and beyond who do not like to use government hospitals prefer to patronize the hospital.

Out of 25 blood samples screened with PCR technique, a total of 21(84%) samples were found to be positive with malaria parasitaemia while only 13(52%) samples were found positive with microscopy, hence 8(32%) were false negatives (Table 7). The prevalence of malaria parasitaemia among the samples screened by PCR was found more among the age grades of

25 -29 (32%) and for the microscopy was also found more among the same age group, 25 – 29 (16%) but was lowest among the age group of 40-44. This report agrees with that of Bolaji *et al.* (2018) where 84.6% between the age group of 20 to 30 years had the highest number of parasitemia while those above 40 years had the least. The statistical analysis showed no significant relationship between the samples screened with microscopy and PCR.

The prevalence of malaria in relation to occupation in the three hospitals studied showed the highest prevalence of malaria among traders/business women in FMC (40.9%), unemployed women in St. Joseph Catholic hospital (100%) and civil servants/private workers in Okwe, General Hospital (84.6%) (Table 8). This shows no correlation between malaria infection and the type of job a person is doing; a person just needs adequate knowledge of malaria and its preventive measures and also adherence to the preventive measures (Michael *et al.*, 2017).

The educational qualification of the participants in relation to malaria prevalence was represented with a bar chart (Fig. 2) and it was seen that a majority of the participants in FMC hospital had a tertiary qualification while St. Joseph Catholic hospital and Okwe General Hospital had more participants with secondary qualification and no formal education respectively (Table 9). FMC being a Federal hospital has more professionals, departments which are distant from one another and does not offer free treatment to pregnant women so some pregnant women who are either uneducated or have primary school qualification may not have enough money to put up with the cost of services in FMC hence will prefer to attend antenatal care at Okwe hospital where they offer them free treatment and delivery.

5. CONCLUSION

The present study has shown that the low prevalence of malaria may have been due to the season of the year when the study was carried out and PCR remains an important diagnostic tool when accurate epidemiological data are needed to monitor the prevalence of malaria.

6. RECOMMENDATIONS

It is recommended that more public awareness and sensitization should be made concerning the

risks of malaria and every pregnant woman should be tested for malaria parasitaemia as one of the compulsory routine tests at the antenatal care unit to know their status during and after their pregnancy term so that they can be properly taken care of.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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