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ORIGINAL ARTICLE

MALARIA PREVALENCE AND PREVENTION METHODS AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINICS IN PRIMARY HEALTH CARE CENTRES IN OSOGBO, NIGERIA

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ABSTRACT

Introduction: Infection caused by *Plasmodium falciparum* in pregnancy is a major public health concern, posing significant risks for both mother and foetus. This study determines the malaria prevalence and prevention practices among pregnant women attending antenatal facilities in Osogbo.

Methods: A total of twelve primary health care centers were selected randomly to ensure representation from the different parts of the study area. Individual demographic data and blood samples were collected from participants attending antenatal clinics in 12 primary health care centres. Microscopy, rapid diagnostic test (RDT) using 2 kits in order to validate the results and also minimize the risk of biased results from a single kit, and polymerase chain reaction (PCR) were used for the detection of *P. falciparum* in the samples. Individual demographic information was collected with a structured questionnaire

Results: A total of 301 pregnant women participated in the study. The mean age was 27.25 ± 0.29 years, mean axillary temperature was 35.5°C ± 1.1 and mean PCV was 32.75± 0.23. Malaria prevalence by microscopy, RDT and PCR were 53%, 70% and 83% respectively. Mean parasite density was 1629.25± 55.69. Based on their gravidity, 97(32.2%) of the women were primigravid while 204 (67.8%) were multigravid, 7 (2.3%), 85 (28.3%), and 209 (69.4%) were in their 1st, 2nd and 3rd trimesters respectively. The use of insecticide treated nets (ITNs), was reported by 212 (70.4%) of the participants. The difference in the use of IPTp SP and *P. falciparum* infection was not statistically significant with p=0.592. The difference in the use of IRS and having *P. falciparum* infection was not statistically significant (p=0.960).

Conclusion: The study revealed that of the malaria prevention methods employed by pregnant women, the most effective was IPTp-SP use which was associated with a reduced prevalence of *P. falciparum* infection.

Keywords: Malaria, pregnant women, *P. falciparum*, IRS, IPTp-SP

INTRODUCTION

Malaria, a vector-borne infectious disease, is caused by protozoan parasites belonging to the genus *Plasmodium*. It is presently endemic in a broad band around the equator,

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in areas of the Americas, many parts of Asia and much of Africa, however, it is in sub-Saharan Africa that 85– 90% of malaria fatalities occur.[1] Four African countries accounted for just over half of all malaria deaths worldwide: Nigeria (31.3%), the Democratic Republic of the Congo (12.6%), United Republic of Tanzania (4.1%) and Niger (3.9%).[2] Of particular concern are pregnant women living in malaria-endemic countries, because of the reduced immunity during pregnancy.[3] Malaria in pregnancy affects more than 25 million pregnant women every year, both in high and low malaria-endemic areas.[4] Pregnancy is a period of increased vulnerability, even for those living in malaria-endemic areas, who develop immunity against malaria. Pregnant women in their first or second pregnancies are particularly susceptible to malaria. This leads to complications ranging from 8.4% to 58% in pregnancies in Nigeria.[5] Therefore, malaria infection (especially with *Plasmodium falciparum*) during pregnancy remains a major public health problem, particularly in Sub-Saharan Africa.[6]

The world Health Organization has advocated for a three-pronged approach to tackling malaria which are: the use of Insecticide Treated Net (ITN), Indoor Residual Spray (IRS) and Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-Pyrimethamine (IPTp-SP).[7] Reports have indicated the gradual decline of malaria in different parts of Africa. These have been made possible by various factors although there are fresh and unfolding challenges for malaria control intervention.[8] One major factor is the use of chemical insecticides that are applied on the walls and roofs of houses. They lead to the reduction of mosquitoes with the parasite, which in turn reduces contact with humans.[9]

Insecticide treated nets (ITNs) work by killing mosquitoes that land on them or repelling them, consequently reducing their contact with humans. Insecticide treated nets benefit pregnant women by reducing low birth weight and maternal anaemia. The World Health Organisation recommends that pregnant women start using insecticide treated nets as soon as possible during their pregnancy.[3] Studies have shown the effectiveness of ITN and it has been recommended as a strategy to reduce adverse effects due to malaria.[10]

Intermittent Preventive Treatment in Pregnancy (IPTp) is a complete medicinal course of malaria drug given to women in pregnancy routinely when they attend antenatal clinics, irrespective of whether they are infected with malaria or not. Low birth weight, neonatal mortality, placental parasitemia, foetal and maternal anaemia, and other adverse outcomes of malaria infection are all reduced by IPTp.[11]

When we look at the effect of malaria on the health of pregnant women, there is definitely a need to examine the factors that influence the application of malaria preventive practices in different locations. The findings from this study, will add to the existing body of knowledge and will be helpful in identifying blueprints for malaria interventions in the region. It is against this background that this study was designed to determine malaria prevalence and prevention methods among pregnant women attending antenatal clinics in primary health care centres in the study area.

METHODS

Study Area

This cross-sectional study was conducted in Osogbo, the capital city of Osun State, Nigeria. It covers a total area of 144 km^2 and is located within latitudes $7^0 43'$ N to $7^0 56'$ N and longitudes $4^0 33'$ E to $4^0 35'$ E, with an elevation beyond 502 m above sea level.[12] The study participants were pregnant women who were attending antenatal clinics at primary health care centres in 12 different locations in the city between September 2015 and March 2016. The primary health care centres were at the following locations: Atelewo, Enikan-oyun, Sabo, Kelebe, Mubinu, Ota-Efun, Ayekale, Olude, Dagbolu, Oba-Oke, Oke-Baale and Idi-Amu. These primary health care centres offer basic primary health care services to the people living in and around these locations.

Inclusion and exclusion Criteria

Pregnant women between the ages of 15 and 49 years who gave their consent to participate in the study and had been residents of Osogbo for at least 12 months prior to the commencement of the study were included. Pregnant women who were ill were excluded from the study.

Ethical Considerations

The study protocol was approved by the Research and Ethics committee of the state's ministry of health with reference number OSHREC/PRS/569^{T}/33 while informed consent was obtained from the pregnant women. Participants' information was kept confidential and individual data was protected and not used for any other purpose apart from the study.

Sample Size Determination

Sample size was determined using the Fisher's formula:[13] $n = t^2 \times p\ (1-p)/\ m^2$

where n= the required sample size, t = confidence level at 95% (standard value of 1.96), p = estimated prevalence of the infection in the project area;[14] and m = margin of error at 5% (standard value of 0.05).

Thus, $n = 1.96^2 \times 0.22\ (1-0.22)/\ (0.05)^2$; = 3.8416 x 0.1716/0.002; = 263

Adding 10% attrition rate to the calculated sample size gave 263 + 26 = 289.

Questionnaire Administration

Structured Questionnaires were administered to the participants. The questionnaire was used to identify the demographic information of the respondents. In addition, the participants' attitude to use of antimalarial drugs and insecticide treated mosquito nets and other means of controlling man-mosquito contact as practiced in the locality and malaria management practices by the study population were also identified. Personal data of participants relating to age, occupation, parity, level of education, marital status, housing, toilet facilities, family size, and gestational age were also obtained through the use of the questionnaire.

Sample Collection

Blood samples were collected from each of the 301 participants by venipuncture. This was done to check the presence of healthy asexual parasites in the peripheral smear of patients. Safety procedures were adopted in the collection of blood samples by swabbing the area to be sampled with 70% alcohol and allowing it to dry before collection. It was also ensured that a new needle and syringe was used for each participant. The bleeding was done in the health care centres by clinicians and medical laboratory scientists. Approximately 2ml of blood was drawn (venipuncture) with a sterile disposable syringe and transferred to sterile EDTA bottles. The blood samples were transported to the laboratory in ice packs at 4^0C.

Rapid Diagnostic Test (RDT)

Two Rapid Diagnostic Test (RDT) kits were purchased and used for rapid diagnostic technique of *P. falciparum* detection. This is to give room for comparative analysis as samples were tested with both kits. The two RDTs were: Bioline SD and Carestart, they were used according to manufacturers' instruction to determine the presence of malaria parasite in blood samples. It was ensured that the test kits were not exposed to high temperatures and they were kept at room temperature as specified by the manufacturers.

Staining techniques for thin and thick blood films

Two glass slides were labelled for each participant. A drop of blood was placed on a clean, grease free glass slide and allowed to dry. The thin smear was spread on the glass slide and this was immediately fixed in absolute methanol for 5 seconds and allowed to air dry completely before staining. Thick blood film was used to determine the presence and quantification of malaria parasites. The thick blood smear was allowed to dry completely under a drier before staining. Giemsa staining technique was used for staining the slides. A staining time of 30 minutes in a 2% volume/volume dilution was used. The air–dried thick blood film was stained in a trough containing the 2% giemsa stain for 30 minutes. The slides were then removed, rinsed in buffer water and the back wiped clean with dry wool. The slides were then placed vertically on the staining rack to air–dry before examination.

Microscopic Examination

Blood films were examined microscopically using 100X (oil immersion) objectives as described by Cheesbrough.[15] The thick films were used to determine the parasite densities. Parasite density per microliter of blood was estimated from the thick film by taking the number of leucocytes per microliter of blood as 8,000 and expressed as follows:

$$\text{Parasite density/µl} = \frac{\text{Parasite count x 8000}}{\text{No of WBC counted}}$$

Polymerase Chain Reaction (PCR)

Plasmodium falciparum in blood samples was detected using a previously described assay.[16] Parasite DNA was extracted from whole blood using the BIOLINE Isolate II Genomic DNA extraction Kit according to manufacturer's instruction. This nested PCR uses Plasmodium genus-specific primers for the initial PCR amplification, followed by specie-specific primers for the second amplification. The primary reaction primers targeted the conserved regions of block 2, block 3, and R2 regions of msp-1, msp-2 and glurp genes, while the nested reaction utilizes a set of primer that target the allelic families of msp-1 (K1, MAD20, and R033), msp-2 (3D7 and FC27) and glurp (R2) region respectively. PCR amplification for each allelic family for the primary and nested reactions were performed separately. Amplified PCR products were analyzed by gel electrophoresis on a 1.5% agarose stained with SYBR Green and visualized by UV trans-illuminator (UVP® DigiDoc-It™, USA). Amplicon sizes were detected relative to the 100 bp molecular DNA ladder (New England Biolab).

Statistical Analysis

Statistical Analysis was performed using GraphPadInstat 3.06 (San Digeo, California, USA). Non-parametric Chi-square test was performed to evaluate the relationship between the presence and absence of malaria in relation to the observed characteristics. Two-sided p-values of <0.05 indicated statistical significance.

RESULTS

General Characteristics of Participants and Prevalence of Malaria

This study showed that the mean age of the participants was 27.2±5.0 years and the mean axillary temperature was 35.5°C ± 1.1. The prevalence of *P. falciparum* infection based on gravidity and duration of their pregnancy is shown in Table 1. Out of the 301 pregnant women who were recruited into the study, the lowest frequency of the participants was observed in the first trimester (2.3%) while the highest was in the third trimester (69.4%). The highest prevalence of *P. falciparum* of 28.97% was recorded among the pregnant women in their first trimester while those in their second and third trimester had 15.3% and 18.2% respectively. The difference between the prevalence of *P. falciparum* among the pregnant women according to trimester was not statistically significant (p=0.225). The overall prevalence of malaria in this study was 17.6% (Microscopy), 23.3% (RDT) and 27.6% (PCR).

Table 2 shows that 97(32.2%) of the study participants were primigravid while 204(67.8%) were multigravid. Overall, the primigravid pregnant women were more infected (21.6%) than their multigravid counterparts (15.7%) however, the difference in their prevalence was not statistically significant (p=0.534).

Socio-demographic characteristics of study participants

Table 3 shows the association between the study participants' socio-demographic characteristics and their malaria status. Based on their age groups, majority of the women were in the age group 26-30 years (34.9%) while the lowest number of participants were in the >35 years age group (6.3%). The young age group (21-25 years) had the highest prevalence of *P. falciparum* infection (18.6%) while the age group 31-35 years had the least prevalence (14%). The difference in the prevalence according to age was not statistically significant (p= 0.477).

Table 1: General Characteristics of Participants and Prevalence of Malaria

Characteristics	Number ±SD
Mean Age	27.25± 0.29
Mean Temperature	35.51± 0.06
Mean PCV	32.75± 0.23
No.positive by Microscopy (%)	53 (17.6)
Mean Parasite Density	1629.25± 55.69
No.positive by RDT (%)	70 (23.3)
No.positive by PCR (%)	83 (27.6)

The influence of educational status on *P. falciparum* parasitemia is shown in Table 3. Majority (55.5%) of the participants had secondary education. Those with primary education had the highest prevalence of malaria which is 28.6%. Those with tertiary education had the least prevalence of 8.2% while those with secondary education had 20.4%. None of the participants who had no education at all had malaria. The difference in the prevalence according to educational status was statistically significant (p=0.012)

Based on their occupation, artisans and traders were in the majority, with 76.7% belonging to this group while the student group had the least number of participants (6.3%). The students' group had the highest occurrence of malaria with a prevalence of 26.3% while the civil servants' group had the least prevalence of 15%. The difference in the prevalence according to occupation was not statistically significant (p= 0.609).

Malaria prevention methods used by study participants

Table 4 shows the malaria prevention methods used by the study participants. The use of insecticide treated nets (ITNs), was reported by 212(70.4%) of the participants, out of which 39(18.4%) were found to be infected with *P. falciparum.* Those who reported not to have used ITN were 89(29.57%) with 14(15.73%) of them having *P. falciparum* infection. Although those who used ITN were more infected than those who did not use, the difference in the use of ITN and *P. falciparum* infection was not statistically significant (p=0.579).

The use of IPTp-SP in the study was reported among 71(23.54%) participants; 11(15.5%) out of these had *P. falciparum* infection while 230(76.41%) were not using IPTp-SP, 42(18%) of whom were found to have *P. falciparum* infection. Those who did not use IPTp-SP had more *P. falciparum* infections than those who used it. The difference in the use of IPTp-SP and *P. falciparum* infection was not statistically significant with p=0.592.

As regards the use of indoor residual spraying (IRS), 107(35.55%) claimed to be using IRS, out of which 19(17.76%) had *P. falciparum* infection while 194(64.45%) were not using and 34(17.53%) out of them were found to be infected with *P. falciparum* infections. Not much difference was found between the prevalence of *P. falciparum* infections in the two groups. The difference in the use of IRS and having *P. falciparum* infection was not statistically significant (p=0.960).

Table 2: Prevalence of *P. falciparum* among pregnant women based on gravidity and trimester

Pregnancy Status		Number Examined (%)	Number Positive (%)	p-value
Gravidity	Primigravid	97 (32.2)	21 (21.6)	0.534
	Multigravid	204 (67.8)	32 (15.67)	
	Total	301	53 (17.61)	
Trimester	1ST	7 (2.3)	2 (28.97)	0.225
	2ND	85 (28.2)	13 (15.29)	
	3RD	209 (69.4)	38 (18.18)	
	Total	301	53 (17.61)	

Table 3: Association between participants' socio-demographic characteristics and malaria status

Factor	No. Examined (%)	No. Positive (%)	P.Value
Age Group			
< 20	30 (10)	6 (20)	
21-25	97 (32.2)	18 (18.6)	0.477
26-30	105 (34.9)	19 (18.1)	
31-35	50 (16.6)	7 (14)	
>35	19 (6.3)	3(15.8)	
Educational level			
None	7 (2.3)	0 (0)	
Primary	42 (14)	12 (28.6)	0.012*
Secondary	167 (55.5)	34 (20.4)	
Tertiary	85 (28.2)	7 (8.2)	
Occupation			
Unemployed	31 (10.3)	7 (22.6)	
Students	19 (6.3)	5 (26.3)	0.609
Civil Servants	20 (6.6)	3 (15)	
Artisans / Traders	231 (76.7)	38 (16.5)	

Table 4: Malaria Prevention methods used by the Study participants

Preventive Method	No. Positive (%)	No. Negative (%)	P. Value
Use of ITN	39/212 (18.4)	173/212 (81.6)	0.579
Not using ITN	14/89 (15.7)	75/89 (84.3)	
Use of IPTp	11/71 (15.5)	60/71 (84.5)	0.592
Not using IPTp	42/230 (18.3)	188/230 (81.7)	
Use of IRS	19/107 (17.8)	88/107 (82.2)	0.960
Not using IRS	34/194 (17.5)	160/194 (82.5)	

DISCUSSION

Malaria in pregnancy is a major public health problem in both tropical and sub-tropical regions of the world. *P. falciparum* is the most prevalent specie in these regions. Many times, pregnant women in these regions are found to be asymptomatic. Despite being asymptomatic, parasites may be present in the placenta and could be responsible for a number of adverse effects on the foetus and newborn, contributing to neonatal mortality. The sequestration of

Plasmodium species in the placenta is associated with low birth weight, pre-term delivery, miscarriages and stillbirths.[17]

P. falciparum which causes the most serious type of malaria especially in Sub–Saharan Africa was studied in this research work among pregnant women in Osogbo, Osun State Nigeria. The percentage of *P. falciparum* by PCR, RDT and microscopy were 27.6%, 23.3%, and 17.6% respectively. This result is in agreement with an earlier observation of prevalence of malaria in southwest Nigeria where malaria prevalence of 19.6% was reported.[18] The results from this study are also closely related to those of Ojurongbe *et al.* in which the PCR test also performed best among the three methods followed by thick film microscopy and RDT in that order.[19] However, in this study the PCR performed best followed by the RDT using the two strips and then microscopy. The reason why microscopy in their study might generate a higher prevalence than the RDT could be due to the fact that the participants in their study were children and they were with higher parasitemia than participants in this study who were pregnant women with mostly low parasitemia.

The world Health Organization has advocated for a three-pronged approach to tackling malaria which are: the use of ITN, IRS and IPTp-SP.[7] In this study, the use of IPT-SP has been shown to reduce malaria prevalence in pregnancy significantly; this agrees with the findings of Stephens *et al*.[20] Also, the use of IPT-SP was not significantly associated with *P. falciparum* infection in pregnancy. Similar finding was also observed in a study in Lagos, Nigeria.[21] Reports indicate that the use of ITN substantially reduces the risk of malaria in pregnancy. However, in this study, the use of ITN did not seem to have effects on the acquisition of *P. falciparum* infection, same goes for the use of IRS. Indeed, this lack of significant association could be explained by the fact that majority of the study participants had very good knowledge of malaria preventive methods. This is attributed to radio and television campaigns on malaria prevention strategies and appropriate treatment options available in the country. Moreover, pregnant women attending antenatal clinics are usually given a health talk on malaria and other conditions affecting them before being attended to.

Malaria prevention methods in Osogbo were similar to those practiced in Badagry Lagos and in other malaria-endemic areas which include the use of insecticide sprays, use of IPTp-SP, and use of IRS.[22]

CONCLUSION

This study has established the incidence of *P. falciparum* infection amongst pregnant women in Osogbo, Osun State, Nigeria. Malaria control must include strong effective health education and behavioural change communication programs as well as efficient vector control measures in order to ensure positive outcomes in terms of scaled up malaria control interventions towards reducing malaria morbidity and mortality. There is also the need to introduce efficient vector control measures as part of the overall malaria control strategy in order to reduce the population heterogeneity of the parasite

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CONFLICT OF INTEREST

There is no conflict of interest.

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