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Original Article

Prevalence and Risk Factors of Asymptomatic Malaria Diagnosed Using Rapid Diagnostic Kits Among Pregnant Women Attending Antenatal Clinic at Benue State University Teaching Hospital, Makurdi

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Abstract

Background: Malaria in pregnancy remains a significant public health concern in sub-Saharan Africa. Pregnant women in areas of stable malaria transmission are particularly vulnerable to its adverse effects. Many infections are asymptomatic and often go undetected and untreated, posing risks to both mother and foetus. We sought to determine the prevalence and associated risk factors for asymptomatic malaria infection among pregnant women attending antenatal care at a tertiary hospital in Nigeria. **Methodology:** A cross-sectional study was conducted among 357 pregnant women between May and December 2021. Malaria infection was assessed using Rapid Diagnostic Tests (RDTs), and sociodemographic and clinical data were collected through structured questionnaires. Statistical analysis included descriptive statistics, chi-square tests, and logistic regression, with a significance level set at $p < 0.05$. **Results:** The prevalence of malaria infection was 11.8%. The level of education, employment status, parity, and gestational age of participants were significantly associated with malaria in pregnancy. Additionally, the use of Sulphadoxine-Pyrimethamine as chemoprophylaxis, indoor spraying with insecticides, and the use of long-lasting insecticide-treated nets were also associated with the likelihood of malaria in pregnancy. Logistic regression analysis revealed that increased literacy, use of intermittent preventive treatment with Sulphadoxine-Pyrimethamine, and indoor insecticide spraying were associated with reduced likelihood of malaria in pregnancy. **Conclusion:** The study highlights the need for effective malaria prevention and control strategies for pregnant women in Nigeria, including the use of IPTp-SP and indoor insecticide spraying. Addressing socioeconomic factors, especially education, may further enhance malaria control efforts among pregnant women in endemic regions.

Keywords: Malaria in pregnancy, prevalence, risk factors, antenatal care, Makurdi, Nigeria

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INTRODUCTION

Malaria is a life-threatening disease caused by the infection of human red blood cells with protozoan parasites of the genus Plasmodium that are transmitted to people through the bites of infected female Anopheles mosquitoes. [1] It affects the lives of almost 40 per cent of the world's population, with the high-risk group being pregnant women and young children (under 5 years of age). [2.] About 10,000 women and 200,000 babies die annually because of malaria in pregnancy. [3] Furthermore, 85 per cent of malaria cases in the world occur in sub-Saharan Africa.[4] Malaria is

endemic in Nigeria, with high prevalence rates in pregnancy ranging from 19.7% to 72.0% and 11% of maternal deaths are attributed to the disease.[5] Previous studies conducted in Benue State have shown that the prevalence of malaria in pregnancy ranges between 33.9% and 68.3% [6,7] using microscopy and 29.7% [8] using Rapid Diagnostic Tests (RDTs).

Pregnant women are at increased risk of contracting malaria compared to their non-pregnant counterparts. This increased susceptibility can be explained by the immunological changes induced by pregnancy, hormonal factors [9,] and the greater attractiveness of pregnant women to mosquitoes. [10] In addition, *Plasmodium falciparum* -infected erythrocytes in pregnant women bind to specific receptors, i.e., chondroitin sulphate A (CSA), and sequester in the placenta. [11] They rarely bind to the other two commonly described receptors in non-pregnant individuals, i.e., CD36 and the intracellular adhesion molecule (ICAM-1). In pregnancy, the parasite antigens expressed on infected erythrocytes are collectively known as variant surface antigen-pregnancy associated malaria (VSA_{PAM}). They differ from those expressed in non-pregnant individuals and, in stable transmission settings, are not recognised by the immune system, thereby explaining the higher risk in primigravidae.[12] The binding of the variant surface antigen (VAR2CSA) with chondroitin sulphate A has been implicated in the pathology of falciparum malaria in pregnancy. [13,14]

During pregnancy, partial maternal immunosuppression occurs in order to accommodate the foetus.[15] This is achieved by suppressing the cell-mediated immune pathway, thereby allowing the humoral immune system (antibody-mediated) to dominate, which is not effective against intracellular pathogens, including Plasmodium. [16] This suppression has been linked with high circulating immunosuppressive hormones such as progesterone and cortisol during pregnancy.[15] This effect makes pregnant women more susceptible to malaria, especially in the first trimester.[17]

In endemic areas, women have developed immunity that generally prevents severe disease; however, the placenta presents a new environment which the parasite specifically targets, thereby leading to an increased risk during pregnancy.[15] Malaria infection is more likely to contribute to maternal anaemia and delivery of a low-birth-weight infant (2500g or <5.5 pounds).[4] It is a particular problem for women in their first and second pregnancies, and for younger women. In low-transmission areas, women generally have developed no immunity to malaria. Malaria infection is more likely to result in severe malaria disease, maternal anaemia, premature delivery, or foetal loss.[4]

Maternal risk factors for malaria in pregnancy (MIP) include maternal age, low parity and low gestational age. The main effects of MIP include maternal anaemia, low birth weight (LBW), and increased infant and maternal mortality. It has been well documented that younger women (primigravidae and multigravidae), especially adolescents, are at a higher risk of malaria infection than older women, and this is independent of parity.[18] Where transmission is stable and relatively high, mainly in sub-Saharan Africa, adults have acquired immunity against malaria, including pregnant women who, despite the immune tolerance occurring during pregnancy, are able to control but not clear malaria infections. Therefore, in this high-risk group, asymptomatic infections are common while clinical malaria is relatively rare.[18] It has been found recently that the reported malaria prevalence was lower, reflecting the decrease in malaria transmission seen in many African countries recently.[19]

Since 2010, WHO has recommended RDTs to enhance diagnosis and management of cases, prevent complications of delayed treatment, prolong survival, and monitor treatment.[20] When compared to microscopy, RDTs have a lower sensitivity, but when the quality of microscopy is low, RDTs may be more reliable, especially in the West African region where there is a dearth of both facilities and personnel. The most widely used RDTs for malaria detection are based on detection of parasite histidine-rich protein II (HRP2), as well as Plasmodium lactate dehydrogenase (pLDH) or p-aldolase, molecules produced by the parasite during the erythrocytic cycle.[21] RDTs have a sensitivity of ~ 100 parasites/µl.[22]

Microscopy remains the "gold standard" for malaria diagnosis; however, the advent of antigen-detecting point-of-care rapid diagnostic tests (RDTs) has transformed the diagnostic landscape. RDTs have attracted interest because they offer accurate diagnosis while circumventing obstacles associated with microscopy in peripheral health care settings, including equipment costs, unstable reagents, and the need for electricity and skilled personnel.[23] RDTs are relatively easy to use and provide a rapid time to result (< 30 min).[22] The major constraint of RDTs is false positives, because HRP2 persists in the blood for several days after infection clearance.[21] This study aimed to determine the prevalence and associated risk factors for asymptomatic malaria using RDT among pregnant women attending antenatal care at Benue State University Teaching Hospital, Makurdi.

MATERIALS AND METHODS

Study Design, Population and Area

This was a descriptive cross-sectional study aimed at determining the prevalence of asymptomatic malaria infection among pregnant women attending the antenatal clinic at Benue State University Teaching Hospital (BSUTH) from May, 2021 to December, 2021. BSUTH is a 360-bed capacity tertiary health care institution which serves as a referral centre for primary and secondary care hospitals within Benue, and

neighbouring states. Makurdi is the state capital and has a population of about 405,500 people projected from the 2006 national population census figures.[24] There are two distinct seasons, the rainy and dry. The former lasts from April to October, while the latter lasts from November to March.[7]

Sampling Technique and Participant Selection

A convenience sampling technique was used. Consecutive pregnant women who presented for antenatal care at Benue State University Teaching Hospital, Makurdi, during the study period were screened for eligibility. Women who met the inclusion criteria and provided written informed consent were recruited consecutively until the calculated sample size was achieved.

Inclusion and Exclusion Criteria

Eligible participants were pregnant women attending the antenatal clinic who were clinically asymptomatic for malaria and consented to participate. Asymptomatic status was determined by measuring axillary temperature using a digital thermometer and conducting symptom screening. Women with a temperature below 37.5°C and no history of fever, chills, rigours, headache, or generalised body weakness within the preceding 48 hours were considered asymptomatic.

Women were excluded if they had a measured fever (≥ 37.5°C), reported recent malaria-related symptoms, were currently on antimalarial treatment, or had taken antimalarial drugs within the previous two weeks. Those with known chronic illnesses such as sickle cell disease, documented HIV infection, or other chronic systemic conditions were also excluded. Women who declined consent were not enrolled.

Data Collection

Data were collected using a pretested structured interviewer-administered questionnaire. Information obtained included age, educational status, occupation, gestational age, parity, history of malaria in the current pregnancy, use of intermittent preventive treatment, insecticide-treated nets, and indoor residual spraying.

Rapid Diagnostic Test (RDT)

A rapid lateral flow immunochromatographic in vitro antigen detection test kit (Care start™ Access Bio Inc., USA) for detecting malaria P. falciparum infection was used to detect malaria HRP2 (Histidine-rich protein 2, Plasmodium falciparum) in patients' blood samples according to the manufacturer's instructions. About 5 µl of blood sample was collected using the provided micro-pipette; the whole blood was added to the "S" well, and 60 µl of assay buffer solution was added to the "A" well. The result was read after 20 minutes. The presence of two lines (one line in the result window adjacent to “C” and another adjacent to “T”) indicated a positive result for Plasmodium falciparum. The presence of only the line adjacent to “C” indicated a negative result. The test was invalid when a line did not appear adjacent to “C”. If this occurred, the test was repeated using a new cassette. Care start™ has a sensitivity of 98% for Plasmodium falciparum. The RDT kits were stored at the manufacturer’s recommended temperature (<40°C) for quality control. The validity of each kit used was certified by laboratory scientists.

Determination of Sample Size

Minimum sample size for this study was determined by Fisher’s formula:[25]

$$N = \frac{Z^2pq}{d^2}$$

N = minimum sample size
Z = standard normal deviate at 95% confidence interval corresponding to 1.96.
p = Prevalence of the disease among the population = 29.7% [8]
q = 1 – p (29.7/100) = 1-0.297=0.703
d = degree of accuracy (5%)

$$N = \frac{1.96^2 \text{ x } 0.297 \text{ x } 0.703}{0.05^2}$$

N = 320.837
Considering a 10% assumption of non-response among study participants, the minimum sample size was adjusted using this formula:

$$N_s = \frac{N}{1-f}$$ [25]

N_s = Selected sample size
N = minimum sample size
f = non-response rate = 10% = 0.1

$$N_s = \frac{N}{1-0.1} = 320.837/\ 0.9$$

N_s = 356.485
N_s ~ 357; therefore, a selected sample size of 357 pregnant women was recruited for the study.

Data Analysis

Data were collated, coded, entered, and analysed using SPSS version 25.0 (IBM® SPSS Statistics Inc., Armonk, New York, USA). Descriptive statistics were generated for all relevant variables. Univariate analysis for categorical variables was performed using Pearson’s chi-squared test, with a p-value < 0.05 considered statistically significant. Variables that were statistically significant at the bivariate level were entered into a multivariate logistic regression model to identify independent predictors of malaria in pregnancy. The model was adjusted for confounders, including maternal age, parity, gestational age, educational level, and use of preventive measures. Adjusted odds ratios (AORs) with corresponding 95%

confidence intervals were computed to estimate the strength of associations. Results were presented in tables and charts.

Ethical Considerations

Verbal/written informed consent was obtained from each participant. Formal approval for the study was obtained from the Health Research Ethics Committee of the Benue State University Teaching Hospital, Makurdi.

RESULTS

The total number of participants in the study was 357. The prevalence of asymptomatic malaria infection in this study was 11.8% (Figure 1). The mean age of the study participants was 30.7 ± 5.0 years, and the majority, 206 (57.7%), were aged 30 years and above. However, of those who tested positive for malaria using the RDT, the majority, 35 (29.6%), were in the age range 25-34 years, and none were below 24 years. Most (64.7%) of these participants were educated up to the tertiary level; however, only 42.6% were employed.

The majority (103; 28.9%) of participants had at least one parous experience, with those in low parity (0-2) constituting the largest proportion (268; 75.1%), and a significant number (37.5%) testing positive for malaria. Fewer than a fifth (17.6%) of the respondents were in their first trimester, 111 (31.1%) in their second trimester, and the majority, 183 (51.3%), in their third trimester. The level of education (X^2 = 7.7, P-value = 0.02), employment status (X^2 = 11.3, P-value = 0.001), parity (X^2 = 16.4, P-value = 0.012), and gestational age (X^2 = 23.2, P-value = 0.001) of participants were significantly associated with malaria in pregnancy. (Table 1)

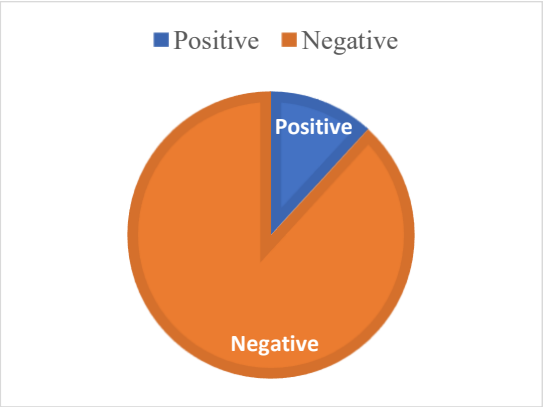

Fig. 1. Malaria in pregnancy

About half (50.1%) of the respondents had taken at least one dose of Sulphadoxine-Pyrimethamine as chemoprophylaxis, and this was also significantly associated with malaria in pregnancy (X^2 = 47.9, P-value = 0.001). About two-thirds (63.3%) of respondents reported always using long-lasting insecticide-treated nets (LLITNs), which was also significantly associated with malaria in pregnancy (χ^2 = 5.5, P-value = 0.001), as was indoor spraying with insecticide (χ^2 = 120.5, P-value = 0.001). (Table 2).

Table 3 presents the multivariate analysis of factors independently associated with malaria among pregnant women. After adjusting for potential confounders, level of education remained a significant predictor of malaria infection (AOR = - 0.08; p = 0.001), indicating that higher educational attainment was associated with reduced odds of malaria infection. Employment status was also significantly associated with malaria (AOR = 0.14; p = 0.002). Parity showed a statistically significant association with malaria infection (AOR = 0.14; p = 0.045), as did gestational age (AOR = 0.10; p = 0.001). The use of intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) was significantly associated with reduced odds of malaria (OR = -0.31; p = 0.001). Use of long-lasting insecticide-treated nets (LLITNs) was not associated with malaria infection (AOR = -0.02; p = 0.734). However, the use of insecticides was significantly associated with lower odds of malaria infection (AOR = -0.36; p = 0.001).

DISCUSSION

The prevalence of asymptomatic malaria among pregnant women in this study was 11.8% using Rapid Diagnostic Tests (RDTs). This is lower than the 29.7% previously reported in Makurdi [8] and the 25.9% documented in Ondo [26], but higher than the 4.8% observed in Sokoto.[27] It is comparable to the 13.1% prevalence reported in Southwestern Nigeria.[28] Variations in prevalence across regions may be explained by differences in ecological conditions, malaria transmission intensity, seasonal patterns, and implementation of preventive strategies. The relatively lower prevalence observed in this study may reflect improved malaria control measures, high literacy levels, and the timing of data collection outside peak transmission periods.

Educational status was significantly associated with reduced likelihood of malaria infection. This finding is consistent with previous Nigerian studies showing higher malaria burden among women with low literacy.[29] Education likely enhances awareness, early antenatal booking, and adherence to preventive measures. Poor literacy has been linked to inadequate understanding and suboptimal implementation of malaria prevention strategies. [30,31] Although employment status did not confer protection in this study, this may be related to occupational exposures or work patterns that limit consistent use of preventive measures such as LLITNs.

The protective association of IPTp-SP with malaria infection aligns with findings from other studies [30,32] and reinforces its established role in malaria prevention during pregnancy. IPTp-SP remains a key component

of the World Health Organization's three-pronged strategy for malaria prevention in pregnancy, alongside insecticide-treated nets and effective case management.[33]

Unexpectedly, LLITN use was not significantly associated with malaria infection, despite evidence from previous studies demonstrating its protective benefit. [30,34] Possible explanations include inconsistent or improper use, insecticide resistance, or concurrent use of other environmental control measures such as

Table 1. Socio-demographic/clinical characteristics of the study participants (n=357)

Variables		Total N (%)	RDT N (%)		X2	P-value
			Positive	Negative		
Age (Years)	<20	7 (2.0)	0 (0.0)	7 (100.0)	9.6	0.06
	20-24	34 (9.5)	0 (0.0)	34 (100.0)		
	25-29	110 (30.8)	14 (12.7)	96 (87.3)		
	30-34	124 (34.7)	21 (16.9)	103 (83.1)		
	>35	82 (23.0)	7 (8.5)	75 (91.5)		
Level of Education	Primary	14 (3.9)	0 (0.0)	14 (100.0)	7.7	0.02
	Secondary	112 (31.4)	7 (6.3)	105 (93.8)		
	Tertiary	231 (64.7)	35 (15.2)	196 (84.8)		
Employment Status	Employed	152 (42.6)	28 (18.4)	124 (81.6)	11.3	0.001
	Unemployed	205 (57.4)	14 (6.8)	191 (93.2)		
Parity	0	75 (21.0)	7 (9.3)	68 (90.7)	16.4	0.012
	1	103 (28.9)	21 (20.4)	82 (79.6)		
	2	90 (25.2)	7 (7.8)	83 (92.2)		
	3	40 (11.2)	0 (0.0)	40 (100.0)		
	4	42 (11.8)	7 (16.7)	35 (83.3)		
	5 and above	7 (2.0)	0 (0.0)	7 (100.0)		
Gestational Age (Trimester)	First	63 (17.6)	14 (22.2)	49 (77.8)	23.2	0.001
	Second	111 (31.1)	21 (18.9)	90 (81.1)		
	Third	183 (51.3)	7 (3.8)	176 (96.2)		

Table 2. Malaria preventive measures (n=357)

Variables		Total N (%)	RDT N (%)		X^2	P-value
			Positive	Negative		
Use of IPTp-SP	Yes	179 (50.1)	0 (0.0)	179 (100.0)	47.9	0.001
	No	178 (49.9)	42 (23.6)	136 (76.4)		
Use of LLITNs	Always	226 (63.3)	42 (18.6)	184 (81.4)	5.5	0.001
	Very often	14 (3.9)	0 (0.0)	14(100.0)		
	Sometimes	70 (19.6)	0 (0.0)	70 (100.0)		
	Rarely	10 (2.8)	0 (0.0)	10 (100.0)		
	Never	37 (10.4)	0 (0.0)	37 (100.0)		
Use of insecticides	Always	48 (13.4)	0 (0.0)	48 (100.0)	120.5	0.001
	Very often	41 (11.5)	0 (0.0)	41 (100.0)		
	Sometimes	227 (63.6)	28 (12.3)	199 (87.9)		
	Rarely	14 (3.9)	14(100.0)	0 (0.0)		
	Never	27 (7.6)	0 (0.0)	27 (100.0)		

window nets and indoor spraying. Further studies are needed to clarify this finding within the local context. Use of insecticides showed a significant protective association, consistent with findings from Uganda where districts implementing outdoor spraying had lower malaria prevalence. [35] Environmental vector control remains an important complementary strategy in malaria-endemic settings.

Table 3. Multivariate comparison of factors associated with malaria in pregnant women

Variables	Adjusted Odds Ratio (AOR)	95% CI Lower	Upper	P-value
Level of Education	-0.08	- 1.00	-0.00	0.001
Employment Status	0.14	0.30	0.15	0.002
Parity	0.14	- 0.13	0.54	0.045
Gestational Age	0.10	0.01	0.10	0.001
Use of IPTp-SP	-0.31	-0.27	0.43	0.001
Use of LLITNs	-0.02	- 0.02	-0.01	0.734
Use of insecticides	-0.36	- 0.38	-0.14	0.001

Younger maternal age has been consistently identified as a risk factor for malaria in pregnancy [36,37], and our findings support this established pattern. Although primigravidae are traditionally considered most vulnerable [36,37], a higher proportion of infection was observed among women with one previous birth in this study. This may reflect differences in behavioural or preventive practices rather than biological susceptibility alone. Malaria prevalence was highest in the first trimester (22.2%) compared with the second and third trimesters, similar to findings from Burkina Faso. [38] Early gestation may represent a period of increased vulnerability before adequate IPTp-SP coverage is achieved.

The limitations of the study include the possibility that the use of RDTs alone may have overestimated malaria prevalence due to antigen persistence after clearance. The cross-sectional design limits our ability to infer causality. In addition, self-reported use of preventive measures may be subject to recall bias.

Based on the study's findings, it is recommended that efforts focus on strengthening early antenatal booking and ensuring optimal uptake of intermittent preventive treatment with sulfadoxine-pyrimethamine, particularly in the first and second trimesters. Health education interventions should be intensified to promote consistent and correct use of long-lasting insecticide-treated nets and indoor insecticide spraying. Targeted strategies aimed at younger and lower-parity women are also necessary to reduce the burden of asymptomatic malaria in pregnancy.

CONCLUSION

The study highlights the moderate prevalence of asymptomatic malaria infection among pregnant women with similar risk factors, including younger maternal age and low parity, particularly among women of low gestational age, and the importance of effective prevention and control strategies. Factors such as literacy, occupational status, increasing parity, use of IPTp-SP, and insecticide spraying were associated with a reduced risk of Plasmodium infection.

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