Comparative evaluation of rapid diagnostic tests and microscopy for placental malaria diagnosis among primigravidae after delivery in health care centres in Kwara State, North Central, Nigeria, 2023-2024

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ABSTRACT

Introduction: Malaria infection in pregnancy can lead to placental malaria, which is linked to negative pregnancy outcomes. This study aimed to evaluate different diagnostic techniques, determine the prevalence, and identify the risk factors associated with placental malaria. **Methods:** This hospital-based cross-sectional study was conducted between January 2023 and September 2024 in healthcare facilities in Ilorin North, Kwara State. A total of 654 consenting women participated, with placental blood samples collected at delivery. Malaria diagnosis was performed using RDTs, and Giemsa-stained blood smears were examined microscopically for malaria parasites. Descriptive statistics, chi-square tests, and calculations of sensitivity and specificity were conducted, with a significance level set at $p<0.05$. **Results:** Of the 654 participants 394 (60.24%) were positive for placental malaria by RDT and 375 (57.34%) by microscopy. Only *P. falciparum* was detected during this study. Blood group O+ had the highest infection at 141 (35.79%) and 135 (36.00%) by RDT and microscopy, while infection was highest among business women, 337/564 and 325/564 by RDT and microscopy. A significant association between placental malaria and blood group was identified using both RDT ($\chi^2 = 30.2$, $p < 0.001$) and microscopy ($\chi^2 = 46.5$, $p < 0.001$). Ethnicity also showed a significant relationship with malaria detection by microscopy ($\chi^2=9.94$, $p=0.019$). Besides, occupation was significantly associated with malaria positivity by microscopy ($\chi^2=18.5$, $p<0.001$). The diagnostic methods demonstrated good performance, with a sensitivity of 0.9976 (95% CI: 0.9930–1.0023) and a specificity of 0.9094 (95% CI: 0.8779–0.9408). Furthermore, BMI was significantly associated with a decreased likelihood of placental malaria, as higher BMI was linked to a lower risk (aOR = 0.0346, 95% CI: 0.175-0.683). **Conclusion**: Placental malaria prevalence in Ilorin North, Kwara State, indicates a significant burden, with younger parturients (ages 26–30) and those in outdoor occupations, such as traders, facing a higher risk. The findings showed the need for continued monitoring and targeted intervention strategies, including improved diagnostic strategies, to address placental malaria in this population.

KEYWORDS: Placental malaria, Diagnostic methods, Prevalence, Performance

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Introduction

Pregnancy increases vulnerability to malaria, primarily due to the sequestration of infected red blood cells in the placenta, which can lead to chronic infection and placental inflammation [1, 2]. This condition, known as placental malaria, is driven by the expression of VAR2CSA [3], a pregnancy-specific surface antigen that allows the infected red blood cells to bind to chondroitin sulphate A in the placenta. Women, especially those in their first and second pregnancies, are more susceptible to infection because they have not yet developed immunity to parasites expressing VAR2CSA. Placental malaria is a leading cause of low birth weight and preterm births in sub-Saharan Africa, contributing to an estimated 900,000 affected deliveries annually [4]. The sequestration of infected erythrocytes containing mature trophozoite and schizont stages in the placenta, particularly within the intervillous spaces, results in higher parasite densities in the placenta compared to the peripheral circulation [5].
In Nigeria, malaria during pregnancy remains a significant public health challenge, with an estimated 25 million pregnant women at risk annually [6]. The country accounts for a substantial proportion of the global burden of malaria in pregnancy, contributing to high rates of maternal anaemia, low birth weight, and neonatal mortality. Studies in Nigeria have reported varying prevalence rates of placental malaria, ranging from 15% to 40%, depending on geographic location, gravidity, and the use of preventive measures [7, 8]. For instance, a study in Ibadan, Southwestern Nigeria, found a placental malaria prevalence of 69.6% among parturient, with primigravidae being the most affected group [9]. Similarly, in Bauchi, Northeastern Nigeria, a prevalence of 38.4% was reported among primigravidae, highlighting the burden of placental malaria in the region [10].

The burden of malaria during pregnancy is not unique to Nigeria but is a widespread issue across sub-Saharan Africa. In Kenya, for example, a study in the Lake Victoria region reported a placental malaria prevalence of 4.7%, with higher rates observed among primigravidae and women with preeclampsia [11]. In Tanzania, placental malaria prevalence was found to be 15.0%, with significant associations between infection and adverse birth outcomes such as preterm delivery [12]. Similarly, in Uganda, where malaria transmission is perennial, placental malaria prevalence was reported at 26.1%, with primigravidae and secundigravidae being disproportionately affected [13].

The risk of placental malaria is influenced by a combination of biological, environmental, and socioeconomic factors. Primigravidae and secundigravidae are at higher risk due to the lack of immunity to pregnancy-specific malaria parasites expressing VAR2CSA [3]. Environmental factors, such as proximity to stagnant water bodies and seasonal variations in malaria transmission, also play a significant role. For instance, in Burkina Faso, placental malaria prevalence was found to be higher during the rainy season, when mosquito breeding sites are more abundant [14]. Socioeconomic factors, including poverty, age, low educational attainment, and limited access to healthcare, further exacerbate the risk of malaria during pregnancy. In Ghana, a study found that parturient from low-income households were twice as likely to have placental malaria compared to those from higher-income households [15].

Nutritional status, as indicated by Body Mass Index (BMI), and hypertensive disorders during pregnancy are critical factors influencing placental malaria susceptibility. In Nigeria and other African regions, undernutrition, reflected by low BMI, has been associated with increased vulnerability to malaria due to compromised immune function. For instance, a study in Nigeria by Emmanuel et al. [16] found that underweight parturient had a higher prevalence of placental malaria, attributed to weakened immune responses and reduced ability to combat *Plasmodium* infections. On the other hand, hypertensive disorders, such as preeclampsia, have also been linked to altered placental function, which may create a favourable environment for malaria parasite survival. Oranuka et al. [10] demonstrated that hypertensive parturient in Nigeria were more likely to experience placental malaria, possibly due to impaired placental blood flow and immune dysregulation. Furthermore, it was highlighted that hypertensive disorders in pregnancy are associated with increased oxidative stress and inflammation, which may facilitate malaria parasite proliferation in the placenta [17]. The interaction between BMI and hypertension in influencing placental malaria is biologically plausible, given their shared effects on immune modulation and placental health. For example, underweight parturient with hypertension had a unique immune profile that increased their susceptibility to malaria [18].

The consequences of placental malaria extend beyond maternal health, significantly impacting neonatal outcomes. Placental malaria is associated with intrauterine growth restriction, low birth weight, and increased neonatal mortality [19]. In Mozambique, a study found that infants born to mothers with placental malaria had a 40% higher risk of low birth weight compared to those born to uninfected mothers [20]. Similarly, in Bangladesh, malaria in pregnancy was linked to a 2.5-fold increase in the risk of preterm birth, highlighting the urgent need for effective prevention and management strategies [21].

Current recommendations for preventing malaria during pregnancy include the use of intermittent preventive treatment with sulfadoxine-pyrimethamine (SP-IPT) after the first trimester, insecticide-treated bed nets (ITNs), and prompt case management [22, 23]. These interventions have been shown to significantly reduce the burden of placental malaria, maternal anaemia, and adverse birth outcomes in malaria-endemic regions. However, the effectiveness of these strategies is often compromised by systemic and operational challenges, including delayed antenatal care (ANC) enrollment, poor adherence to preventive measures, and the emergence of drug resistance [24–26].

Prompt case management of malaria during pregnancy is critical to reducing maternal and foetal morbidity. However, diagnostic and treatment delays are common in many African countries. In Tanzania, for example, a study found that only 50% of parturient with malaria symptoms received prompt treatment, with delays attributed to stockouts of antimalarial drugs and inadequate health worker training on diagnostic methods [27].

Various diagnostic techniques, including placental blood smears, histology, rapid diagnostic test tests, and polymerase chain reaction (PCR), have been employed to assess placental malaria in sub-Saharan Africa [28]. Placental histology is considered the gold standard for diagnosing malaria during pregnancy, as it can detect both active and past infections based on the presence of infected erythrocytes and malaria pigment [29]. However, due to limited expertise and resources in endemic regions, this method is not widely available. Consequently, many studies in sub-Saharan Africa rely on placental blood smears or RDT, which are more accessible but may vary in sensitivity and specificity.

In Nigeria, the use of rapid diagnostic tests (RDTs) for diagnosing placental malaria has gained attention due to their ease of use, rapid results, and suitability for resource-limited settings. RDTs detect malaria antigens, such as histidine-rich protein 2 (HRP-2) and lactate dehydrogenase (LDH), providing a practical alternative to microscopy and histology, which require specialized equipment and expertise. However, studies comparing the performance of RDTs with microscopy or histology for placental malaria diagnosis have yielded mixed results, indicating the need for specific validation.

A study in Yaoundé, Cameroon, reported a sensitivity of 96.15% and specificity of 80.76% for RDTs compared to placental histology, demonstrating their potential as a reliable diagnostic tool in urban settings [30]. In contrast, a study in Eastern Sudan found lower specificity (57.7%) for RDTs in detecting placental malaria, particularly in cases with low parasite density, underscoring the variability in RDT performance across different settings [31]. Similarly, in Uganda, a study reported a sensitivity of 80.9% and specificity of 87.85% for RDTs compared to placental blood smears [32].

The performance of RDTs in diagnosing placental malaria is influenced by several factors, including the type of antigen targeted, the timing of testing, and the presence of residual antigens from cleared infections. HRP-2-based RDTs, which are widely used in sub-Saharan Africa, have been shown to remain positive for weeks after effective treatment, potentially leading to false-positive results [33]. In Papua New Guinean, a study found that HRP-2-based RDTs had a positive predictive value of only 68.4% for placental malaria, largely due to the persistence of HRP-2 antigens in the bloodstream [34].

The emergence of *P. falciparum* strains with HRP-2 gene deletions poses a significant challenge to the reliability of HRP-2-based RDTs. Recently in Southern Ethiopia, a study reported a high prevalence of *pfhrp*2/3 deletions, leading to false-negative RDT results in confirmed malaria cases [35]. Similarly, *pfhrp*2/3 gene deletions were detected in some provinces surveyed in Mozambique, raising concerns about the continued use of HRP-2-based RDTs in these regions [36].

Despite these challenges, RDTs remain a valuable tool for diagnosing placental malaria, particularly in settings where microscopy and histology are unavailable. In Colombia, a study found that RDTs

had a sensitivity of 92.5% and specificity of 100% compared to placental histology, with higher accuracy observed when testing was performed at delivery [37]. In Cameroon, RDTs were shown to be more effective than clinical diagnosis in identifying placental malaria cases, leading to improved case management and reduced adverse outcomes [30]. However, the integration of RDTs into routine antenatal care requires training for healthcare workers, quality assurance programs, and strategies to address supply chain challenges.

The validation of RDTs for placental malaria diagnosis is particularly critical among primigravidae, who are at the highest risk of adverse outcomes due to their lack of immunity to pregnancy-specific malaria parasites. In African countries, studies found a higher accuracy observed in high-transmission areas compared to low-transmission areas [32, 34]. Similarly, in another African nation, RDTs were shown to be more effective in detecting placental malaria in primigravidae than in multigravidae, supporting their use in targeted interventions for high-risk groups [38].

The mixed performance of RDTs across different African settings shows the importance of specific validation and the need for standardized diagnostic criteria. This study aims to contribute to this body of knowledge by evaluating the diagnostic accuracy of placental RDTs compared to microscopy among primigravidae in Kwara State, Nigeria. By focusing solely on placental malaria, this study provided insights into the diagnostic accuracy of RDTs in a high-risk population and contributed to the development of more effective strategies for managing malaria during pregnancy in resource-limited settings.

Methods

Study area and population

The study was conducted in Ilorin North Local Government Area (LGA), Kwara State, from January 2023 to September 2024. Ilorin North, an urban centre in the Kwara North Senatorial Zone, has experienced significant urbanization over the past decade, with its population exceeding 900,000 as of 2022. Data was collected from health facilities at various levels—primary, secondary, tertiary, and private—offering antenatal, preventive, and curative services. The region is predominantly inhabited by Yoruba, Hausa, and Fulani ethnic groups, with Islam and Christianity as the major religions [39]. Annual temperatures range between 22°C and 34°C, with the hottest months being February and March (32–34°C) and the coolest months, December and January (22–25°C) [40]. Relative humidity fluctuates, reaching up to 80% during the wet season and dropping to around 35% during the dry season, influenced by daily weather variations [41].

Study Design and Setting

This study was a cross-sectional comparative evaluation of placental rapid diagnostic tests (RDTs) and microscopy for the diagnosis of placental malaria among primigravidae who had received antenatal care in urban health facilities in Kwara State, North Central Nigeria. Participants who had completed 28 weeks of gestation were recruited [9]. They were followed up and placental blood samples were collected at the point of delivery in the maternity wards of the same facilities.

Sample Size Determination

The sample size (n) was calculated using the Cochran's sample size formula [42] with a precision of 5%, and a z score of 1.96 for a 95% confidence level, error margin (e) of 0.0437 and a p of 0.5 [43]. Based on these parameters, a minimum sample size of 503 participants was required.

$$n = \frac{Z^2 \cdot P \cdot (1–0.5)}{e^2}$$

$$n = \frac{(1.96)^2 \times 0.5 \times (1–0.5)}{(0.0437)^2}$$

n = 502.6 ~503

To account for potential exclusions and non-responses, an additional 30% was added to the minimum required sample size. The final sample size was therefore adjusted to 654 parturients.

Sampling Technique

A multistage sampling technique was employed to select participants. In the first stage, six health facilities were purposively selected based on their patient load and representation of urban settings. In the second stage, random sampling was used to enrol primigravidae who met the inclusion criteria (first-time parturient aged 18–45 years, attending health care facilities). Women with multiple pregnancies, severe anaemia, or chronic illnesses were excluded.

Health Facility Selection

In Nigeria, the health system is structured into primary, secondary, tertiary, and private health institutions, each serving distinct roles. Primary health institutions, such as Primary Healthcare Centers (PHCs), deliver basic preventive, promotive, and curative services to local communities, forming the foundation of healthcare delivery [44]. Secondary health institutions, including general hospitals, provide specialized services and inpatient care, acting as referral centres for primary facilities [45]. Tertiary health institutions, such as teaching hospitals and specialized medical centres, offer advanced medical services, including specialized diagnostics and treatment, and serve as referral hubs for secondary institutions [46]. In addition, private healthcare facilities play a significant role by catering to individuals who can afford their services, often at higher costs compared to public institutions.

For this study, six urban health facilities were included in the study: two primary healthcare centres, one secondary hospital, one tertiary hospital, and two private health centres. The facilities were purposively selected to ensure representation of the diverse healthcare delivery systems within urban settings. The selection criteria included a high volume of delivery cases, availability of laboratory facilities, and willingness to participate in the study. This approach ensured a comprehensive evaluation of placental malaria diagnostic methods in urban healthcare environments.

Participant Selection Procedure

Eligible participants were primigravidae who planned to deliver in the selected facilities. At each facility, a line list of eligible parturient was generated from the antenatal register. From this sampling frame, simple random sampling was employed using random numbers to select the required number of participants per facility (Table 1).

Laboratory Procedures

Placental Blood Collection Procedure

Placental blood was collected immediately after delivery to minimize the risk of maternal blood contamination. Following delivery, the placenta was placed in a sterile container and transported to the laboratory within 30 minutes to maintain sample integrity [47]. A trained laboratory technician collected the blood by making a small incision on the maternal side of the placenta and aspirating blood from the intervillous spaces using a sterile syringe into an ethylenediaminetetraacetic acid (EDTA) bottle. To prevent contamination with maternal blood, the first few drops were discarded, and only blood from the deeper intervillous spaces was collected [47]. The collected blood was then divided into aliquots for rapid diagnostic tests (RDT), microscopy, and blood grouping, ensuring comprehensive analysis for diagnosis.

Rapid Diagnostic Test (RDT) for Placental Malaria

Placental malaria rapid diagnostic tests (RDTs) were conducted using a commercially available HRP-2-based kit, following the manufacturer's instructions. The procedure involved applying 5 μL of placental blood to the test strip, followed by the addition of 2–3 drops of buffer solution to the sample well. Results were interpreted after 15–20 minutes, with a positive result indicated by the presence of both the control and test lines, and a negative result confirmed by the appearance of only the control line. This method provides a rapid and reliable approach for detecting placental malaria, particularly in resource-limited settings [37].

Microscopy

For each participant, both thick smear and thin film were prepared from placental blood on clean glass slides. The smears were air-dried before staining. The thick smears were stained with 10% Giemsa stain for 10 minutes to enhance parasite visibility. Microscopic examination of the thick smears was used to detect the presence of malaria parasites, while thin films were used to determine the *Plasmodium* species in cases where thick films were positive. Two independent microscopists, blinded to the rapid diagnostic test (RDT) results, examined the slides under a light microscope using a 100x oil immersion lens. In cases where the two

microscopists reported discordant results, such as disagreement on the presence or absence of parasites, a third, more experienced microscopist was consulted to review the slides. Parasite density was quantified by counting the number of parasites per 200 white blood cells (WBCs) and extrapolating to parasites per microliter (μL), based on an assumed average WBC count of 8,000/μL [48].

Blood Grouping

Blood grouping was conducted using the slide agglutination method, a common technique for determining ABO and Rh blood types. In this procedure, a drop of placental blood was placed on a clean glass slide, and separate drops were mixed with Anti-A, Anti-B, and Anti-D sera. After a 2-minute incubation period, agglutination was observed to identify the blood group. The presence or absence of agglutination with each antiserum allowed for the determination of the ABO type (A, B, AB, or O) and the Rh status (positive or negative) [49].

Inclusion and Exclusion Criteria

We included primigravidae aged 18–45 years who were attending antenatal care (ANC) and were followed until delivery at the selected urban health facilities. Participants were required to provide written informed consent to be eligible for inclusion. Parturients with multiple pregnancies, such as twins or triplets, were excluded from the study. Besides, those with severe anaemia (haemoglobin < 7 g/dL), chronic illnesses like diabetes or HIV/AIDS, or a history of antimalarial drug use within the past four weeks were not eligible. Women with incomplete medical records or those unwilling to participate were also excluded.

Socio-Demographic and Clinical Data Collection

Socio-demographic data, including age, education, occupation, ethnicity, religion, and marital status, were collected using a structured questionnaire. Trained research assistants administered the questionnaire through face-to-face interviews before delivery to ensure accurate and reliable data collection.

Anthropometric and Clinical Measurements

Body Mass Index (BMI) was calculated using weight and height measurements obtained with a calibrated scale and stadiometer, respectively. BMI was derived as weight in kilograms divided by height in meters squared [50]. Hypertension status was assessed using a digital sphygmomanometer, with hypertension defined as a systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, recorded on two separate occasions [51].

Quality Control Activities

To ensure the quality and reliability of the study, several quality control measures were implemented. All study research team underwent comprehensive training on data collection, placental blood sampling, and laboratory procedures to ensure consistency and accuracy in their work. RDT kits were purchased and delivered by the study team, with storage temperatures maintained through continuous temperature monitoring during storage. All laboratory tests, including rapid diagnostic tests (RDT), microscopy, and blood grouping, were conducted using standardised protocols to minimise variability and enhance reproducibility. In addition, microscopists were blinded to the RDT results to prevent potential bias during the analysis. To further ensure accuracy, a third microscopist independently reviewed the slides, and any discrepancies were resolved.

Regular calibration of laboratory equipment was performed to maintain precision and reliability in measurements. Data validation was also a critical component of the quality control process. Collected data were double-entered and cross-checked to identify and correct any errors, ensuring the integrity of the dataset. These measures collectively contributed to the robustness and credibility of the study findings.

Index Test and Reference Test

The rapid diagnostic test (RDT) was employed as the index test in this study. A commercially available HRP-2-based RDT kit, specifically the First Response (Premier Medical Corporation Private Limited, A1 302 GIDC Sarigam Dist. Valsad, Gujarat, India. MFG/MD/2018/000064), was used following the manufacturer's instructions. This test is designed to detect *P. falciparum* malaria antigens, providing a rapid and accessible diagnostic tool for malaria infection.

The reference standard for this study was placental blood microscopy. Thick blood smears were prepared, stained with Giemsa, and examined under a light microscope by two independent microscopists to confirm the presence of malaria parasites. This

dual-assessment approach ensured the reliability and validity of the diagnostic outcomes, minimising the risk of misdiagnosis.

Data analysis

Data was entered into Microsoft Excel and analyzed using STATA version 14 (Stata Corp, College Station, TX, USA). Proportions were compared using the Chi-squared test. Pearson's Chi-squared (χ^2) test assessed associations between malaria diagnostic methods and variables such as sociodemographic characteristics, BMI, and blood group. Regression analysis examined the relationships between BMI, hypertension status, and placental malaria. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of the RDT were calculated using microscopy as the gold standard, and Cohen's kappa assessed agreement. Statistical significance was defined as $p < 0.05$.

Ethical Considerations

The study was reviewed and approved by the Kwara State Ministry of Health Review Board (ERC/MOH/2022/003/019). Written informed consent was obtained from all participants before enrollment. Participants were informed of the study objectives, procedures, risks, and benefits, and were assured of confidentiality and the right to withdraw at any time without affecting their access to care.

Results

Socio-demographic characteristics of the parturient women

The sociodemographic characteristics of parturient recruited for the study are summarized as follows. The majority of the participants were aged 26–30 years (39.5%), with a mean age of 27.6 ± 4.5 years, while the lowest representation was among parturient aged 41 years and above (0.5%). Most participants were Yoruba (90.9%), followed by Igbo (3.1%), with the least represented ethnic group being Fulani (2.3%). Regarding religion, Islam was the predominant faith (78.3%), while Christianity accounted for 21.7%. Educational attainment was highest at the secondary level (43.0%) and lowest among those with no formal education (7.0%). The most common occupation was business or self-employment (81.0%) (Table 2).

Relationship between sociodemographic characteristics and placental malaria

Only *Plasmodium falciparum* was detected during this study. Of the 654 participants 394 (60.24%) were positive for placental malaria by RDT and 375 (57.34%) by microscopy. The 26-30 age group had the highest number of positive cases for both RDT 174 (44.16%) and microscopy 163 (43.47%), while the ≥41 age group had the lowest number of positive cases. There was a significant association between malaria status and age group by RDT (χ^2=11.3, p = 0.046). The majority were of Yoruba ethnicity (601, 91.96%), with 358 (90.86%) tested positive by RDT. The Igbo group had 17 (4.31%) positive cases, while Fulani and Hausa groups had 7 and 12 positive cases each, respectively by RDT. The association between ethnicity and malaria by RDT was not significant (χ^2 = 6.70, p = 0.082); however, the association between ethnicity and malaria by microscopy was statistically significant (χ^2 = 9.94, p=0.019). By RDT method, 177 (44.92%) parturient with secondary education and 133 (33.76%) women with tertiary education tested positive. The association between educational level and malaria by RDT was not statistically significant (χ^2=5.01, p=0.171).

A total of 530 parturient engaged in business or trading, 337 (85.53%) tested positive while 15 unemployed parturient (3.81%) tested positive by RDT. The association between occupation and malaria positivity by RDT was statistically significant (χ^2=13.5, p=0.004). The association between occupation and malaria positivity by microscopy was also statistically significant (χ^2=18.5, p<0.001). The highest prevalence of placental malaria was observed among parturient practicing Islam for both RDT (78.93%) and microscopy (78.67%). Statistical analysis showed no significant association between religion and placental malaria prevalence, RDT (χ^2=0.234, p=0.62) and microscopy (χ^2=0.074, p=0.79) (Table 3).

Distribution of Placental Malaria Among Parturient by Test Type and Healthcare Institution

Of the total of 654 parturient, based on RDT, the highest prevalence was observed in secondary institutions (33.0%), while the lowest was in private facilities (18.5%). For microscopy, secondary institutions also recorded the highest prevalence (31.47%), whereas private institutions had the lowest (19.47%) (Table 4).

Blood Group-Wise Comparison of Placental Malaria Detection by Microscopy and RDT

Among the patients who tested negative by RDT, blood group O+ had the highest proportion (35.0%), while the lowest proportion was observed in B- (1.54%). Among the patients who tested positive by RDT, blood group O+ again had the highest prevalence (35.79%) while the lowest found in O- (0.76%). A significant association between placental malaria and blood group was observed by RDT ($\chi^2 = 30.2$, $p < 0.001$). For the microscopy results, the highest prevalence was seen in blood group O+ (36.0%), while those with O- showed the lowest prevalence (0.27%). A significant association was also found between placental malaria and blood group by microscopy ($\chi^2 = 46.5$, $p < 0.001$) (Table 5).

Risk Analysis in relation to blood group

The risk of the placental malaria infection analysis among parturient revealed significant variations in the odds of infection across different blood groups (reference group B-). Parturient with blood group AB had the highest odds of placental malaria (OR = 3.13, 95% CI: 1.96–5.01), followed by those with blood group A+ (OR = 1.69, 95% CI: 1.24–2.32), and these associations were statistically significant. In contrast, parturient with blood group O- had the lowest odds (OR = 0.11, 95% CI: 0.03–0.48) and was statistically significant. (Table 6).

Relationship Between Body Mass Index (BMI) and Placental Malaria

The analysis of placental malaria in relation to BMI categories revealed significant associations between both RDT and microscopy results. Among parturients with a normal BMI, 41.88% tested positive for placental malaria by RDT and 42.40% by microscopy. Underweight parturients had the lowest percentage of positive cases, with 8.38% by RDT and 8.53% by microscopy. These associations were statistically significant ($\chi^2 = 16.27$, $p < 0.001$ for RDT, and $\chi^2 = 16.59$, $p < 0.001$ for microscopy) (Table 7).

Agreement Between RDT and Microscopy

The diagnostic performance measures for malaria tests using the RDT and microscopy methods among parturients in Ilorin North is shown in Table 8. Sensitivity was very high at 0.9976 (95% CI: 0.9930-1.0023) as well as the specificity (0.9094 [95% CI: 0.8779-0.9408]). The positive predictive value is 0.9356 (95% CI: 0.9129-0.9582), while the negative predictive value is 0.9966 (95% CI: 0.9899-1.0033). Accuracy, representing the overall correctness of the test, is 0.9596 (95% CI: 0.9454-0.9737), showing that the test performs reliably. The Cohen's kappa analysis showed a high agreement between the RDT and microscopy for placental malaria detection ($\kappa = 0.92$, $p < 0.001$). The observed agreement was 96.09%, substantially higher than the expected agreement by chance alone (51.43%).

Interaction Analysis

A logistic regression analysis was performed to explore the relationship between Body Mass Index (BMI), hypertension status, and their interaction on the likelihood of placental malaria detected by a rapid diagnostic test (RDT) among parturient in Ilorin North, Kwara State. These variables were selected a priori based on their biological plausibility and known associations with both maternal immunity and susceptibility to malaria infection during pregnancy. The analysis revealed that BMI was significantly associated with a decreased likelihood of placental malaria, with higher BMI linked to a lower risk (aOR = 0.346, 95% CI:0.175-0.683). Hypertension status did not show a significant effect on placental malaria (aOR =0.718 , 95% CI:0.381-1.352). Interaction terms indicated that underweight parturient with hypertension had a significantly lower likelihood of placental malaria (aOR =0.045-, 95% CI:0.003-0.788) (Table 9). Similarly, parturients with normal BMI and normal blood pressure, as well as overweight parturient with normal blood pressure, were significantly less likely to test positive for placental malaria (aOR =0.149, 95%CI:0.038-0.578; aOR = 0.408, 95% CI:0.186-0.895, respectively).

Discussion

The results on the prevalence of placental malaria among parturients at different health care facilities in Ilorin North, Kwara State, Nigeria demonstrated consistency between two diagnostic methods: Rapid Diagnostic Test (RDT) and microscopy. The high prevalence of placental malaria among parturients suggests a substantial burden of malaria in parturients in the studied areas, which could have

serious implications for maternal and foetal health. This result is higher than 27.7% recorded in Colombia [52], and 44.6% in Uganda [53]. The observed differences in prevalence may be attributed to several factors, including variations in malaria transmission intensity, healthcare infrastructure, and population characteristics. For instance, the current studied areas are known for higher endemicity of malaria due to environmental factors such as climate, vector density, and limited access to effective malaria control interventions [54, 55].

The highest infection rates in the 26-30 age group suggest that this demographic may be at higher risk of placental malaria, potentially due to factors such as increased exposure to malaria vectors or lower immune responsiveness compared to younger or older parturient. This demographic should be prioritized in public health interventions by health practitioners, such as focused malaria prevention measures and education [56]. The lower infection rates in the 36-40 age group could indicate that older women have a greater acquired immunity against malaria or that they have a more robust immune response [3], which is an important aspect to consider when designing tailored health interventions.

The observation that business/trading occupations had the highest positive rates (85.53% for RDT and 86.67% for microscopy) may reflect the mobility and extended outdoor exposure inherent in these professions. Traders, for instance, may frequently travel to malaria-endemic rural markets or spend extended hours in areas with high mosquito vector densities, increasing their risk of infection [57]. This pattern emphasizes the need for malaria control programs tailored to occupational risks, such as promoting the use of insecticide-treated nets (ITNs) and advocating for protective measures during high-risk activities. Conversely, civil servants (i.e. government workers at local, state, or federal) exhibited the lowest rates of infection. This may indicate better access to healthcare resources, higher socioeconomic status, and a greater likelihood of residing in urban areas with improved malaria control measures [58]. These results support the notion that socioeconomic factors, often linked to occupational categories, significantly affect malaria exposure and prevention outcomes. The strong correlation between occupation and malaria prevalence indicates the potential for occupation-based malaria interventions. Parturient in high-risk occupational groups, such as traders, could benefit from targeted education campaigns and prevention strategies, such as malaria prophylaxis or the distribution of long-lasting insecticide-treated nets.
The distribution of positive cases, with the majority observed among parturient with secondary and tertiary education, aligns with their higher representation in the study population. This result is at variance with similar study [59], where there a commonly held assumptions that higher education levels are inherently linked to improved health outcomes. Parturient with no formal education accounted for the lowest proportion of positive cases, possibly reflecting their lower overall participation rather than a protective effect against malaria.

The observed significant association between ethnicity and placental malaria infection, as indicated by the chi-square tests for RDT and microscopy ($p < 0.001$), suggests that ethnicity may play a role in the risk of infection among parturients in Ilorin North. The Yorubas, representing the largest proportion of the study population, also accounted for the majority of both RDT-positive and microscopy-positive cases. This high prevalence may be influenced by cultural, environmental, or socioeconomic factors [60, 61], specific to this group. For example, their living conditions or proximity to malaria-prone areas may increase exposure to mosquito vectors. The comparatively low rates of infection among the Hausa and Fulani groups may reflect differences in housing types or preventive behaviours.

The significant association between BMI and placental malaria infection showed the role of nutritional and physiological factors in influencing malaria susceptibility during pregnancy [59]. Parturient with normal BMI exhibited the highest infection rates, which could reflect the physiological vulnerability of this group to malaria. A normal BMI might correlate with optimal metabolic and immune functions, which paradoxically may provide a favourable environment for *Plasmodium* parasites to thrive and evade host defences mechanism. In contrast, the lower infection rates observed among obese and overweight parturient could suggest a protective effect linked to higher adiposity [62]. Adipose tissue has been shown to modulate immune responses [63], possibly altering the inflammatory

pathways associated with malaria pathogenesis. Moreover, the physical or hormonal changes associated with higher BMI might influence vector exposure or parasite development. The underweight group, though small, showed a relatively high infection. This aligns with previous evidence that malnutrition can impair immune defences [64], making individuals more susceptible to infections, including malaria.

The significant relationship between blood group and placental malaria infection aligns with previous research suggesting that genetic and immunological factors associated with blood groups influence susceptibility to malaria [65]. The higher proportion of RDT-positive and microscopy-positive results among parturient with blood group A+ may be attributed to differences in erythrocyte membrane characteristics, which could affect the binding efficiency of *Plasmodium* parasites [66]. Blood group O+, which showed the highest frequency of negative results, has been associated with reduced rosetting [67], a key factor in malaria pathogenesis. The substantial positivity observed in blood group AB might reflect an intermediate susceptibility, potentially influenced by the presence of both A and B antigens. Conversely, the lower infection rates in blood groups A- and O- could be due to the protective effects linked to the Rh-negative phenotype, which may modulate immune responses or parasite survival [65].

The interaction term (underweight by hypertensive) revealed a significant inverse relationship between being underweight and hypertensive status, which implies that underweight parturient with hypertension have a markedly lower risk of placental malaria infection. This finding could suggest that hypertensive parturient who are also underweight may experience changes in their immune response or other physiological factors that reduce susceptibility to infection. This result, while somewhat unexpected, warrants further investigation to understand the underlying mechanisms, as it may offer a unique perspective on how hypertension and BMI interact in placental malaria. The interaction between normal BMI and absence of hypertension also demonstrated a significant protective effect for parturientwith a normal BMI and absence of hypertension, indicating that these parturients are less likely to experience placental malaria infection compared to those in other BMI and hypertension categories. This result aligns with existing studies that suggest normal BMI may be associated with better general health and a reduced likelihood of malaria infection due to a potentially stronger immune system [59, 68].

Limitation

One limitation of this study is the non-inclusion of the design effect, which may introduce some level of clustering bias. While this was a necessary trade-off for feasibility, the study's findings remain methodologically sound and provide valuable insights into the research.

Conclusion

The prevalence of placental malaria among parturients in Ilorin North, Kwara State, Nigeria, indicates a significant burden of malaria, with an obvious association between age (26-30 years), occupation (i.e traders), and Yoruba ethnic groups. The data suggest that younger parturient, particularly those in the 26-30 age group, as well as those in occupations (i.e traders) with high outdoor exposure, such as traders, are at greater risk. The findings indicate the importance of targeted malaria interventions tailored to specific risk factors, including occupation, age, and ethnicity, to ensure high-risk groups receive the necessary prevention strategies. The diagnostic methods demonstrated good performance, with a high sensitivity and specificity. The findings showed the need for continued monitoring and targeted intervention strategies, including improved diagnostic strategies, to address placental malaria in this population.

What is already known about the topic

- Women, especially those in their first and second pregnancies, are more susceptible to malaria infection
- Placental malaria is detected by histological methods

What this study adds

- This study showed that there are agreement between placental malaria detection by microscopy and RDT methods
- We found that BMI is associated with placental malaria

Competing Interest

The authors of this work declare no competing interest

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Authors´ contributions

JOS, conceptualized the study. JOS, OKA, and OGO designed the study. OKA participated in fieldwork and data collection. OKA and OGO performed the data analysis; OKA, and OGO interpreted the data. JOS, OKA, and OGO prepared the first draft of the manuscript, reviewed by JOS and OGO. All authors contributed to the development of the final manuscript and approved its submission.

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Table 1: Distribution of study participants across health facility types				
Facility type	**Number of facilities**	**Participants per facility**	**Total participants**	**Percentage (%)**
Tertiary Health Facility	1	189	189	28.9
Secondary Health Facility	1	195	195	29.8
Private Health Facilities	2	81	162	24.8
PHC Facilities	2	54	108	16.5
Total	**6**		**654**	**100**

Table 2: Socio-demographic Characteristics of the parturient in Ilorin North Central Nigeria, N=654		
Variables	**Frequency**	**Percentage**
Age Group		
≤20	20	3.06
21–25	230	35.17
26–30	258	39.45
31–35	106	16.21
36–40	37	5.66
≥41	3	0.46
Ethnicity		
Yoruba	601	91.9
Hausa	18	2.8
Fulani	15	2.3
Igbo	20	3.0
Religion		
Christianity	142	21.7
Islam	512	78.3
Educational Level		
No formal	46	7.0
Primary	104	15.9
Secondary	281	43.0
Tertiary	223	34.1
Occupation		
Farming/Fishing	11	1.68
Business/Self Employed	530	81.04
Civil Servants	82	12.54
Unemployed	31	4.74

Table 3: Placental malaria status by sociodemographic characteristics				
Characteristics	**RDT**		**Microscopy**	
	-ve	**+ve**	**-ve**	**+ve**
Age				
≤20	9 (3.46%)	11 (2.79%)	10 (3.58%)	10 (2.67%)
21–25	96 (36.92%)	134 (34.01%)	101 (36.20%)	129 (34.40%)
26–30	84 (32.31%)	174 (44.16%)	95 (34.05%)	163 (43.47%)
31–35	52 (20.00%)	54 (13.71%)	54 (19.35%)	52 (13.87%)
36–40	18 (6.92%)	19 (4.82%)	18 (6.45%)	19 (5.07%)
>41	1 (0.38%)	2 (0.51%)	1 (0.36%)	2 (0.53%)
χ^2, p-value	(χ^2=11.3, p=0.046)		(χ^2=7.8, p=0.167)	
Ethnicity				
Yoruba	243 (93.46%)	358 (90.86%)	259 (92.83%)	342 (91.20%)
Hausa	6 (2.31%)	12 (3.05%)	7 (2.51%)	11 (2.93%)
Fulani	8 (3.08%)	7 (1.78%)	10 (3.58%)	5 (1.33%)
Igbo	3 (1.15%)	17 (4.31%)	3 (1.08%)	17 (4.53%)
Total	260 (100%)	394 (100%)	279 (100%)	375 (100%)
χ^2, p-value	(χ^2=6.70, p=0.082)		(χ^2=9.94, p=0.019)	
Educational Level				
No Formal Education	25 (9.62%)	21 (5.33%)	26 (9.32%)	20 (5.33%)
Primary	41 (15.77%)	63 (15.99%)	44 (15.77%)	60 (16.00%)
Secondary	104 (40.00%)	177 (44.92%)	111 (39.78%)	170 (45.33%)
Tertiary	90 (34.62%)	133 (33.76%)	98 (35.13%)	125 (33.33%)
χ^2, p-value	(χ^2=5.01, p=0.171)		(χ^2=4.92, p=0.178)	
Occupation				
Farming/Fishing	7 (2.69%)	4 (1.02%)	7 (2.51%)	4 (1.07%)
Business/Trading	193 (74.23%)	337 (85.53%)	205 (73.48%)	325 (86.67%)
Civil Servants	44 (16.92%)	38 (9.64%)	50 (17.92%)	32 (8.53%)
Unemployed	16 (6.15%)	15 (3.81%)	17 (6.09%)	14 (3.73%)
χ^2, p-value	(χ^2=13.5, p=0.004)		(χ^2=18.5, p<0.001)	
Religion				
Christian	59 (22.69%)	83 (21.07%)	62 (22.22%)	80 (21.33%)
Islam	201 (77.31%)	311 (78.93%)	217 (77.78%)	295 (78.67%)
χ^2, p-value	(χ^2=0.234, p=0.62)		(χ^2=0.074, p=0.79)	

Table 4: Comparison of RDT and Microscopy Results for Placental Malaria Across Healthcare Institutions								
Test	**RDT**				**Microscopy**			
	PHC (n=108)	**Secondary (n=195)**	**Tertiary (n=189)**	**Private (n=162)**	**PHC (n=108)**	**Secondary (n=195)**	**Tertiary (n=189)**	**Private (n=162)**
Negative	36 (13.9)	65 (25.0)	70 (26.9)	89 (34.2)	36 (12.90%)	77 (27.60%)	77 (27.60%)	89 (31.90%)
Positive	72 (18.3)	130 (33.0)	119 (30.2)	73 (18.5)	72 (19.20%)	118 (31.47%)	112 (29.87%)	73 (19.47%)
Total	108 (16.5)	195 (29.8)	189 (28.9)	162 (24.8)	108 (16.51%)	195 (29.82%)	189 (28.90%)	162 (24.77%)

Table 5: Placental Malaria Positivity Rates by Blood Group Using Microscopy and RDT				
Blood Group	**RDT**		**Microscopy**	
	-ve	**+ve**	**-ve**	**+ve**
A+	49 (18.85%)	88 (22.34%)	51 (18.28%)	86 (22.93%)
A−	11 (4.23%)	12 (3.05%)	11 (3.94%)	12 (3.20%)
B+	69 (26.54%)	77 (19.54%)	75 (26.88%)	71 (18.93%)
B−	4 (1.54%)	8 (2.03%)	4 (1.43%)	8 (2.13%)
AB+	20 (7.69%)	65 (16.50%)	23 (8.24%)	62 (16.53%)
O+	91 (35.00%)	141 (35.79%)	97 (34.77%)	135 (36.00%)
O−	16 (6.15%)	3 (0.76%)	18 (6.45%)	1 (0.27%)
p-value	($\chi^2 = 30.2$, $p < 0.001$)		($\chi^2 = 46.5$, $p < 0.001$)	

Table 6: Risk of Placental Malaria Infection Across Blood Groups (Reference: B−)				
Blood Group	**Positive**	**Negative**	**Odds**	**95% CI**
A+	105	62	1.69355	1.23727 – 2.31809
A−	14	20	0.7	0.35358 – 1.38584
B+	83	82	1.0122	0.74598 – 1.37340
AB+	72	23	3.13043	1.95763 – 5.00587
O+	138	112	1.23214	0.96029 – 1.58095
O−	2	18	0.11111	0.02578 – 0.47886

Table 7: Microscopy and RDT Results of Placental Malaria Across BMI Categories				
BMI (kg/m²)	**RDT**		**Microscopy**	
	-ve (n=260)	**+ve (n=394)**	**-ve (n=279)**	**+ve (n=375)**
Underweight (< 18.5)	3 (1.15%)	33 (8.38%)	4 (1.43%)	32 (8.53%)
Normal (18.5–24.9)	111 (42.69%)	165 (41.88%)	117 (41.94%)	159 (42.40%)
Overweight (25.0–29.9)	83 (31.92%)	116 (29.44%)	90 (32.26%)	109 (29.07%)
Obese (≥ 30.0)	63 (24.23%)	80 (20.30%)	68 (24.37%)	75 (20.00%)
p-value	($\chi^2 = 16.27$, $p < 0.001$)		($\chi^2 = 16.59$, $p < 0.001$)	

Table 8: Diagnostic Performance of Malaria Tests Using RDT Among Parturient in Ilorin North			
Variables	**Value**	**95% CI**	**SE**
Sensitivity	0.9976	0.9930 – 1.0023	0.0024
Specificity	0.9094	0.8779 – 0.9408	0.0160
Positive Predictive Value	0.9356	0.9129 – 0.9582	0.0116
Negative Predictive Value	0.9966	0.9899 – 1.0033	0.0034
Accuracy	0.9596	0.9454 – 0.9737	0.0072
Positive Likelihood Ratio	11.0086	–	–
Negative Likelihood Ratio	0.0026	–	–

Table 9: Associations Between BMI, Hypertension Status, and Placental Malaria				
Variable	**OR**	**95% CI**	**SE**	**P-value**
BMI	0.0346	0.175 – 0.683	0.3469	0.002
Hypertension status	0.718	0.381 – 1.352	0.3228	0.305
Underweight by Hypertensive	0.045	0.003 – 0.788	1.4617	0.034
Normal BMI by No Hypertension	0.149	0.038 – 0.578	0.6914	0.006
Normal BMI by Hypertensive	0.571	0.101 – 3.226	0.8833	0.526
Overweight by No Hypertension	0.408	0.186 – 0.895	0.4012	0.025
Overweight by Hypertensive	0.375	0.134 – 1.046	0.5232	0.061