Original Article

Malaria Rapid Diagnostic Test Effectiveness In Pregnant Women Attending First Antenatal Checkups At A State Hospital In Southwest Nigeria.

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DOI: 10.47750/pnr.2022.13.S08.516

Abstract

Background: The effects of pregnancy-related malaria on the mother, foetus, and infant continue to be a general health concern. Timely diagnosis and treatment of confirmed malaria infections can facilitate malaria case management in pregnancy. Malaria microscopy takes time and is influenced by several factors. Malaria rapid diagnostic tests (mRDTs) enable parasitological diagnosis and can aid precise and prompt diagnosis in pregnancy.

Methods: A cross-sectional study was conducted at State Hospital Iwo Osun State, Nigeria, between April and August 2017. Pregnant women visiting antenatal care for initial appointment during their current pregnancy were enrolled. Questionnaire that captured socio demographic data such as age, educational level, occupation and marital status were administered. Blood smears were prepared and RDT test was done for each participant. Slides were viewed under microscope to detect, count and different malaria parasite species. RDT outcomes were categorized as either positive or negative.

Results: Two hundred and eighty-four (284) pregnant women with age range of 15 to 46 years old were enrolled. Out of the occupation documented, farming was more, 74 (26.1%). More than half of the pregnant women enrolled, 167 (58.8%) had completed secondary school education. Many of the pregnant women were in their first trimester at enrolment, 271 (95.4%). Of the enrolled pregnant women, 53 (18.7%) of them were positive for malaria microscopy, while 55 (19.4%) were RDT positive. RDT proficiency showed sensitivity and specificity of 94.34% and 97.84%, respectively, with positive predictive values (PPV) of 90.93% and negative predictive value (NPV) of 98.69%. The results of microscopy and RDT results in this study in relation to gestational age was not significant.

Conclusion: The performance of RDT in this study showed it can be a supplementary diagnosis for malaria examination and thereby reduce laborious process in malaria microscopy, particularly for pregnant women attending ANC. Malaria screening using RDT in every ANC can enhance proper case management of malaria in pregnant women.

Keywords: Malaria, Rapid Diagnostic Test, Antenatal Care, Sensitivity, Specificity

INTRODUCTION

Malaria is a common health condition, one of the dangerous infectious diseases and is a principal purpose of death and illness globally especially in the tropical and subtropical areas. In the year 2020, there were 241 million cases of malaria reported globally. Ninety-six (96%) of the cases were reported in twenty-nine countries worldwide and six countries, comprising Nigeria (27%), accounted for about 55% of all cases globally [1]. The 2018 Nigeria Demographic and Health Survey found that the country's current malaria prevalence was 23%. [2].

Each year, *Plasmodium falciparum* infection poses a threat to over 25 million pregnant women in Sub-Saharan Africa [3]. Pregnancy consequences associated with asymptomatic *P. falciparum* infection include maternal anaemia, abortion, premature delivery, low birth weight (LBW), stillbirth, and preterm delivery [4.5]. Due to the presence of malaria parasites in the placenta, low birth weight and maternal anemia have been documented as being the primary effects of malaria illness during pregnancy [6].

Prompt diagnosis, treatment of malaria infections, and regular usage of long-lasting insecticidal nets (LLINs) in conjunction with intermittent preventive treatment of malaria during pregnancy (IPTp) have been recommended as approaches for reducing malaria's detrimental impact in pregnancy [7]. These approaches may control transmission by preventing them from developing into detectable infections [8,9]. Timely diagnosis enables medical professionals to

differentiate between malaria and non-malaria infections, enabling appropriate case management for malaria in pregnancy [8].
The gold standard for detecting malaria is typically considered to be the malaria microscopy technique since it is less expensive, allows species differentiation, permits parasite density quantification, and is sensitive [10]. Light microscopy, on the other hand, takes time and has been shown to miss up to 88% of malaria parasites in low transmission areas [11]. The technician's level of competence, as well as the reagents and equipment quality, can influence the outcome of malaria microscopy result [12].

The rapid diagnostic tests (RDT) in some endemic countries have allowed for timely diagnosis and appropriate case management of malaria. RDT permits parasitological diagnosis even in areas where good microscopy services are unavailable because it is able to detect antigens and this represents an important step forward in the diagnostic strategy [13]. Malaria antigens, not parasites, are detected by RDTs, giving RDT a competitive advantage in diagnosing malaria in patients with low parasitaemia. "Test and Treat" approaches are practice by many Asian–Pacific countries. Regardless of symptoms, pregnant women are examined for malaria parasite during the initial antenatal appointment [14], or investigating methods for periodic malaria testing and treatment at each scheduled antenatal visit for women [9].

Plasmodium falciparum in blood can be identified more accurately by the histidine-rich protein-2 (HRP-2) antigen [15]. HRP-2 is the most selected RDT in Nigeria that is frequently employed because it is only unique to the most prevalent *Plasmodium* species (*P. falciparum* (~98%) [16]. Some factors can influence the performance of HRP-2 RDTs and may lead to false positive result, such as residual antigenicity, which can last for up to 28 days following parasite clearance [10,17]. Pfhrp-2 gene deletions or mutations, as well as the prozone effect, may result in false negatives results [18,19].

RDTs should not be used to replace malaria microscopy, but rather to improve malaria diagnosis and support evidence-based malaria treatment decisions while reducing microscopy limitations [20]. Malaria RDTs can be a valuable diagnostic method, especially in malaria-endemic regions where microscopy expertise is scarce in many health facilities [21]. Considering the difficulties in malaria microscopy, such as reagent quality, equipment, the time-consuming procedure, a scarcity of expertise in malaria microscopy in several malaria-endemic countries, and the need for reliable malaria diagnosis for every pregnant woman attending antenatal care (ANC). Implementation of RDTs for malaria screening and diagnosis in Nigerian health facilities may play a significant role in malaria diagnosis, particularly for pregnant women attending ANC. Therefore, we assessed the effectiveness of malaria RDTs in pregnant women visiting antenatal clinic for initial booking during their current pregnancy in Iwo Osun, Southwest Nigeria.

MATERIAL AND METHODS

STUDY LOCATION

The study location was a state hospital in the Iwo Local Government Area of Osun State, Nigeria. Iwo is in western Nigeria, between the latitudes 7°39′N and 4°11′E. (Figure. 1). Iwo is situated between latitudes 7°39'50" N and 7°39'51" N, and longitudes 4°10'56" E and 4°10'57" with an area of 245 km2 and a population of nearly 191,343. Iwo Local Government is known to be one of the most famous in the state of Osun. From February to November, the rainy season lasted 9.5 months, with an average rainfall of 9.2 inches.

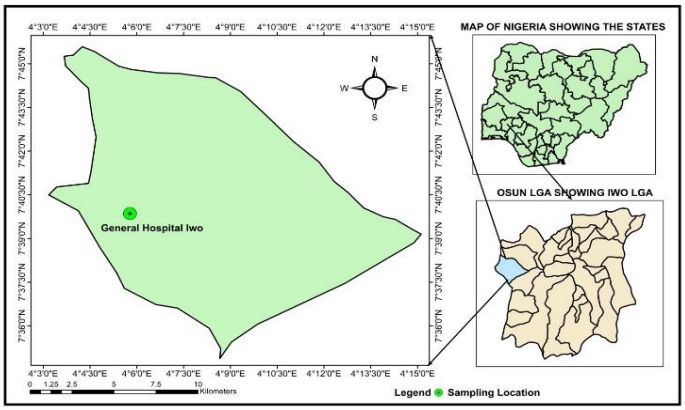

Figure. 1: Map of Location of State Hospital Iwo Osun State, Nigeria

STUDY DESIGN AND POPULATION

A cross-sectional study was conducted by including 284 pregnant women at a healthcare facility in Iwo, Southwest Nigeria. Pregnant women attending their first ANC visit during their current pregnancy at State Hospital Iwo, Osun State, and have agreed to participate were enrolled in this study. The study was conducted between April and August of 2017. Pregnant women coming for their first ANC during their current pregnancy without severe malaria symptoms were included. Those who have made their first visit or are returning for the second or subsequent time, as well as those with severe malaria symptoms, were excluded from the study.

DATA AND SAMPLE COLLECTION

Prior to blood collection for malaria RDT and microscopy, a questionnaire was completed that captured age, occupation, marital status, gender and presenting symptoms. A pre-tested semi-structured questionnaire was used. Interviews were conducted by a trained interviewer to collect demographic data and details of presenting symptoms. Capillary blood was obtained through sterile using sterile lancet. For thin film, one drop of blood (~ 2μL) was collected, and three drops of blood (~ 6μL) for thick film. Blood smears (thick and thin) were made on a slide and stained using Giemsa after drying properly. The RDT test was carried out immediately following slide preparation.

MICROSCOPIC EXAMINATION AND PARASITE COMPUTATION OF STAINED SMEARS

Assuming a white blood cell count of 8000/μL on average, the parasite number on a thick film per 500 WBCs was determined as parasites per microlitre of blood. Each person had their RDT test and microscopy reading done. Two trained microscopists read the slides and they were unaware of each other's results. The RDT result were read and another did a random reading to confirm the RDT results. Under a light microscope with a 100-objective lens, stained slides were examined (immersion oil). If a slide was scanned over 100 times on high power fields (HPF) and no malaria parasites are visible, it was considered negative. Rereading was done by third microscopist for any parasite density slides with more than 20% disagreement between the two readers. The mean parasite count was used to calculate parasitemia for all positive individuals, with parasite counts differing by less than 20% between the first and second readers. Slides were read by trained malaria microscopists.

MALARIA PARASITE DIAGNOSIS BY RDT (HRP 2-PF)

Five microlitres (5 μL) of blood samples were collected following the manufacturer's instruction, using sterile transfer device immediately from the same finger where blood was collected for smear preparation to avoid clotting of blood. The blood was tested using CareStart^{TM} Malaria HRP2 (Pf) {LOT NO: MO15G03}. After 20 minutes, the results were read in accordance with the manufacturer's directions (Access Bio, Inc). If a distinct Pf line appeared in comparison to the control line, the results were recorded as positive, indicating *P. falciparum* infection. If only the control line appeared, the results were recorded as negative.

SAMPLE SIZE

Using data from an initial study that was formerly conducted in Iwo with 23% prevalence [22], the sample size of 272 was calculate. Using the statistical formula [n = Z2p(1−p)/d2] n = Z2p1-p/d2, the sample size was determined, taking into account the level of precision (d) of 5% and the 95% confidence interval (z = 1.96).

ETHICAL APPROVAL AND INFORMED CONSENT

Osun State Hospitals' Management Board ethically approved this study (Reference No: OSSHMB/1144/316). Before the study began, all participants were given consent forms, which they completed and signed. Good clinical laboratory practices were applied for all procedures. Based on the RDT results, all patients with confirmed malaria infection were referred to a physician for appropriate treatment.

DATA ANALYSIS

Data was entered into Excel 2013. SPSS 20.0 version statistical software was used to analyze the data. Two experts validated the questionnaire, which was administered by a qualified field scientist. The results were presented in tables, and the data was presented using frequency and percentages. The percentage of positive blood smears that are true positive malaria as accurately detected by positive RDTs was referred to as sensitivity, while the percentage of negative blood smears that are true negative malaria as accurately identified by negative RDTs was referred to as specificity. The percentage of precise malaria cases among those who had positive RDT results was represented as the positive predictive value. The percentage of precise negative malaria cases among all negative RDT test results was represented as the negative predictive value. The level of significance was set at P 0.05. The Rapid Diagnostic Test's sensitivity, specificity, NPV, and PPV were calculated and compared to those of blood slide microscopy, which was used as the gold standard [23].

RESULTS

In this study, 284 pregnant women visiting the health facility for the first antenatal clinic during their present pregnancy were included. The sociodemographic details and characteristics of the pregnant women who took part in this study showed age range from 15 to 46 years old, with the many of them being between the ages of 26 and 30 years, 104 (36.6%). Occupations reported were: farming 74 (26.1%), trading 63 (22.2%), and fashion designing 18 (6.3%). More than 167 of the enrolled pregnant mothers have completed their secondary education (58.8%). Most participants were married, 248 (87.3%), according to data on marital status and majority of the pregnant women, 271 (95.4%), were still in their first trimester (Table 1).

Out of the 284 pregnant women examined for malaria, 55 were RDT positive and 53 were microscopically positive. Considering the microscopy as the reference method, Table 2 showed the comparative result between RDT and microscopy. We identified 5 false positive results and 3 false negative results in this study. Majority of the *Plasmodium*

species encountered were *P. falciparum*, 52 (98.1%) and only one mixed species of *P. falciparum* and *P. ovale was* reported, 1 (1.9%). RDT's performance in this study showed 94.34% (95% CI: 84.3 - 98.8) sensitivity and 97.84% (95% CI: 95.0 - 99.3) specificity, with corresponding positive predictive values (PPV) of 90.93% (95% CI: 80.8 - 96.0) and negative predictive values (NPV) of 98.69% (95% CI: 96.2 - 99.6). The parasite grouping was not statistically significant (p=0.139). Parasitemia ranges between 100 – 50,000 p/μL in this study and we therefore categorized them into; 100 - 1,000 p/μL (64.2%), 1,001 – 10,000 p/μL (28.3%) and 10,001 – 50,000 p/μL (7.5%).

There was no statistical difference in microscopy and RDT results in the different gestational age of the pregnant women in this study. Microscopy and RDT results in relation to the gestational age showed that pregnant women at first trimester have the highest malaria prevalence of 49 (18.1%) and 52 (19.2%) for microscopy and RDT respectively (Table 3). Tiredness was reported as one of the main presentations, 243 (85.6%) during enrollment (Table 4).

Table 1: Sociodemographic Details and Characteristics of the Study Population

Age Group (Years)	Frequency (n=284)	Percentage (%)
15-20	23	8.1
21-25	72	25.4
26-30	104	36.6
31-35	58	20.4
36-40	21	7.4
41-45	4	1.4
46 and above	2	0.7
Occupation		
Student	29	10.2
Civil Servant	24	8.5
Trader	63	22.2
Fashion Designer	18	6.3
Tailor	29	10.2
Business Woman	41	14.4
Farmer	74	26.1
Others	6	2.1
Education Status		
Primary	33	11.6
Secondary	167	58.8
Tertiary Institution	83	29.2
Marital Status		
Single	25	8.8
Married	248	87.3
Divorced	11	3.9
Gestational Age	271	95.4
First Trimester	10	3.5
Second Trimester	3	1.1
Third Trimester		

Table 2: Malaria Microscopy and RDT Results of Enrolled Pregnant Women

Malaria Test Results		Microscopy Results		Total
		Positive	Negative	
RDT	Positive	50	5	55
	Negative	3	226	229
Total		53	231	284

Table 3: Microscopy and RDT Results in Relation to Gestational Age

Gestational Age	No. Examined (%)	Microscopy		RDT	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)
First Trimester	271 (95.4)	49 (18.1)	222 (81.9)	52 (19.2)	219 (80.8)
Second Trimester	10 (3.5)	2 (20.0)	8 (80.0)	2 (20.0)	8 (80.0)
Third Trimester	3 (1.1)	2 (66.7)	1 (33.3)	1 (33.3)	1 (66.7)
Total	284	p value = 0.093		p value = 0.620	

Table 4: Signs and Symptoms Reported by the Pregnant Women

Symptoms	Frequency (n=284)	Percentage (%)
Fever	34	12.0
Tiredness	243	85.6
Headache	6	2.2
Yellow Urine	1	0.4

DISCUSSION

The effectiveness of rapid diagnostic for malaria test was evaluated in this current study employing the gold standard, malaria microscopy in pregnant women visiting ANC for the first time during their current pregnancy. Peripheral blood smear using microscopy revealed a malaria prevalence of 18.7%. This result was in contrast with was reported earlier in the same state where this study was done. Malaria prevalence of 72% [24] and 29.5% [25] were reported in pregnant women in Osogbo Osun state, Nigeria. Result of studies of malaria prevalence also reported 26.2% in Khartoum state [26], 56.1% in Gambia [27], 40.1% among pregnant women in Abia State, Nigeria [28], 39.7% in Kaduna, Nigeria [29] and 45.38% in pregnant women in Jos [30]. Some studies however, reported a lower malaria prevalence in South Western Nigeria with prevalence of 2% [31] and 7.7% [32], both in Lagos state, and 8.4% [33] in Oyo State.

The variations in malaria prevalence reports observed in pregnant women could be attributed to factors such as diagnostic methods used, geographical location, ongoing malaria interventions, and seasonal variations in malaria endemicity. Other factors may include the level of expertise required to read the blood smear, the staining reagents used, and the tools used, such as a microscope [34]. An evaluation conducted in Nigeria found that peripheral blood smear reading of malaria parasite performed poorly among scientists doing malaria microscopy. It has been suggested that regular training of scientists reading malaria slides, in addition to formal academic laboratory training, will improve malaria parasite diagnosis using a malaria microscope [35].

This study demonstrated the accuracy of the RDT in the detection of malaria in pregnant women. This demonstrated that the RDT is reliable in areas without access to skilled malaria microscopists for accurate malaria diagnosis. RDT performance has overall sensitivity and specificity of 94.38% and 97.84% respectively, with analogous PPV of 90.93% values and NPV negative predictive values of 98.69%. This study is comparable to sensitivities of both First Response® and Care Start TM when compared with microscopy in a study were 97.0% (95% CI: 84.2-99.9) and 97.0% (95% CI: 84.2-99.9) respectively. The specificities were 91.0% (95% CI: 87.6-93.7) and 90.2% (95% CI: 86.7-93.0) respectively [36] (Ameyaw *et al*., 2017). The Performance characteristics of some RDT kits; SD Bioline, First Response, Care Start Pf/ pan and Acon Pf/pan kits were sensitivity - 98.6%, 98.6%, 92.9% and 92.9%; specificity - 90.0%, 90.0%, 93.3% and 93.3%; test accuracy 97%, 97%, 92% and 92%; PPV- 95.8%, 95.8%, 97.0% and 97.0%; NPV - 96.4%, 96.4%, 84.4% and 84.4% [37] (Dozie and Chukwuocha, 2016). Care Start Malaria Pf/Pv combo test showed sensitivity and specificity of 93.2% and 98.6%, respectively in a study in Ethiopia [38] (Dejazmach *et al*., 2021). The overall parasite positivity using light microscopy and Care Start™ RDT was 41 (12.8%) and 43 (13.4%), respectively. The sensitivity and specificity of Care Start™ RDT regardless of species, were found to be 95.4 and 99.3%, respectively in another study done in Togray, Ethiopia [39] (Feleke *et al*., 2017),

In contrast to the value of sensitivity and specificity reported in this study, previous studies that used HRP-2 in pregnant women showed sensitivity and specificity of 88.7% and 89.8%, PPV and NPV of 71.2% and 96.0% respectively [40], and another study with sensitivity of 86.7%, PPV of 93.7%, and NPV of 94.5% [41] and a study that reported sensitivity and specificity of 81.5% and 92.1%, with PPV and NPV of 39.8% and 98.7% [42].

We had 5 (9.1%) false positive results in this study. This false positive result could be due to HRP-2 persistent in the peripheral blood after treatment [43] or a positive RDT and a negative microscopy result could have been caused by the sequestration of parasitized erythrocytes in tissue capillaries and the presence of malaria in placental in the absence of peripheral blood parasitemia. All the false negative results in this study 3 (5.7%) were reported in the parasitemia category of 100 – 1,000 p/μL. Low parasitemia [44] or mutant parasites resistant to the antigenic determinant of the RDT [45] may have resulted in this observation.

The performance of HRP-2 RDT in this study suggests its ability for diagnosis of malaria, especially in pregnant women where malaria infection during pregnancy may have substantial risks for the mother, her foetus, and the neonate [1]. Malaria RDT is not to be replaced by malaria microscopy as the gold standard because microscopy permits species differentiation, parasite density quantification, and is sensitive [10]. Malaria microscopy is known to be tiresome because it requires more timimg and labour intensive [12]. However, the use of RDT for regular screening of malaria during ANC visit may play a key role in reducing the burden of malaria disease in pregnant women especially in remote areas where malaria microscopy is difficult to assist early detection and treatment of the malaria disease.

This study found that malaria infection rates were greater among pregnant women in the 26–30 age group (36.6%) than in other age groups. This may be due to the fact that majority of the enrolled pregnant women were in this group. This is consistent with studies that show a greater rate of malaria infection in people between the ages of 25 and 30 [31,40]. This is however in contrast with studies that reported higher *Plasmodium* infection rates among pregnant women below the age of 25 years [46,47].

The gestational age of the pregnant women in this study in relation to malaria results showed that those in their first trimester had the highest malaria prevalence for both microscopy and RDT. The majority of the pregnant women recruited were in their first trimester and this may be the reason for the highest malaria prevalence reported in this group. This is however, in contrast to studies that reported higher malaria prevalence in the second trimester [48, 49].

Various signs and symptoms were reported by the participant at enrollment. Tiredness was the main presentations reported in this study 243 (85.6%). The reason may be due to sampling criteria that involved those coming for ANC for the first time during their present pregnancy as seen that majority were still in their first trimester. This is however in contrast with a study that reported fever as the main presentation for malaria infected and non-malaria infected pregnant women in Benin [50]. Headache was also reported to be significantly associated with malaria parasitemia in pregnant women followed up in Benin [51]. This present study however showed that majority of the women enrolled were asymptomatic, as tiredness, a non-specific symptom was mainly reported in this study. The limitation in the study is the fact that we did not use molecular techniques to further compare our diagnostic methods.

CONCLUSION

This study shows that malaria RDT could be an alternative diagnostic tool instead of malaria microscopy, especially where expert microscopists are not available and in rural arears where microscopy diagnosis is difficult. The use of RDT in pregnant women for malaria screening during every ANC visit can complement malaria microscopy and reduce the laborious process that could cause delay in microscopy. This will aid proper case management of malaria in pregnant women.

ACKNOWLEDGEMENTS

This study acknowledges the moral support of all nurses in antenatal section attending to pregnant women in State Hospital, Iwo Osun during the period of this project. The study appreciates Mr Olaniyi Sarafa, a Medical Laboratory Scientist in State Hospital Iwo for his helping hand throughout the sample collection. Finally, the study thanks Dr. Idowu, the medical director, for allowing us to conduct the research at State Hospital Iwo in Osun State, Nigeria.

CONFLICT OF INTEREST

No conflict of Interest

Author Contributions

OO and AA designed the study. OO and AA carried out the experimental work. OO wrote the paper. AA did the data entry. All authors participated in data analysis and interpretation, and read and approved the final manuscript.

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