

Assessment of the performance of mRDT versus blood film microscopy in diagnosis of malaria in febrile out-patients in Benue State, North-Central Nigeria

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Abstract

Background: Accurate malaria diagnosis remains a cornerstone in the control of malaria infection in endemic regions. Despite increased availability of malaria rapid diagnostic tests (mRDTs), microscopy remains the gold standard for confirming malaria cases.

Aim/Objective: This study examined the level of diagnostic agreement between mRDT and blood film microscopy in diagnosis of malaria in febrile patients attending the General Outpatient Clinic of Benue State University Teaching Hospital (BSUTH), Makurdi, Nigeria.

Methods: This was a hospital-based cross-sectional analytical study involving 120 consecutive febrile patients aged ≥ 5 years who gave consent/assent, over a period of six months at the GOPC of BSUTH Makurdi, Benue state. Data on socio-demographics, clinical and laboratory information were collected using a pre-tested semi-structured interviewer administered questionnaire. Blood samples were examined using mRDT and microscopy for malaria diagnosis. Data were analyzed with SPSS version 25. Descriptive statistics summarized the data and Cohen's kappa statistic was used to assess agreement between mRDT and microscopy.

Results: 120 participants were recruited, majority of whom were less than 40 years of age with higher number of females 72 (60.0%) than males. The prevalence of malaria was 48.3% by mRDT and 47.5% by microscopy. *Plasmodium falciparum* was the predominant species (45.8%), followed by *P. malariae* (1.7%). There was an excellent agreement between mRDT and microscopy ($\kappa = 0.983, p < 0.001$).

Conclusion: Malaria positivity among febrile patients in Makurdi remains high with preponderance of *Plasmodium falciparum* specie. The strong agreement between mRDT and microscopy supports the reliability of mRDT for malaria diagnosis, especially in resource-limited settings.

Keywords: Malaria, mRDT, microscopy, diagnostic agreement, febrile patients, Benue State

Introduction

Malaria has been identified as a leading cause of morbidity and mortality especially in developing countries of the world. The West African Sub-region and Nigeria are also not left out of this menace. In Nigeria, there was an estimated 68 million cases and 194,000 deaths due to malaria in 2021 [1]. It is a disease that impacts negatively on the economy with substantial costs to both individuals and the society despite various concerted efforts at various levels to curb it.

Malaria is an infectious disease caused by the plasmodium parasite, transmitted mostly by the bite of an infected female Anopheles mosquito. There are five species of the parasite that cause malaria, namely, *plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi*. Malaria in Nigeria is principally due to *P. falciparum* and, to a lesser extent to *P. malariae* and *ovale* [2].

Although the prevalence of malaria declined from 27% in 2015 to 22% in 2021, Nigeria still has the highest burden of malaria globally, accounting for nearly 27% of the global malaria burden [2]. The national malaria prevalence according to RDT and microscopy are 39.6% and 22.3% respectively in 2021 [2].

In 2019, of the six countries accounting for more than half the 229 million cases of malaria worldwide, Nigeria contributed to 23%. During the same year, Nigeria also contributed 24% of the twenty countries that were responsible for 85% of the 409,000 global deaths attributable to malaria [1].

Benue state with a population of 6,627,000 in 2021, is among stable malaria arrears with all year-round transmission of malaria, though there may be seasonal variations. The state contributed an estimated 2.5% of Nigeria's 68 million malaria cases in 2021 [2]. From 2018 to 2021, the estimated malaria cases increased from 1.5 million to 1.7 million, while the estimated incidence increased from 240.3 to 257.2 per 1,000 [3]. However, malaria prevalence by microscopy decreased from 44.5% in 2015 to 17.6% in 2021 [2]. In the same year, the estimated malaria cases were 1,704,000, while the estimated incidence per thousand of the population was 257.2/1000 [2]. Thus, overall, malaria continues to exert a huge burden on the people of the state despite the progress that has been achieved.

The new policy on malaria promotes parasite-based diagnosis. Thus, all individuals presenting with clinical symptoms of malaria must be diagnosed using microscopy

or rapid diagnostic test. This is because the symptoms of malaria are non-specific and can occur as a result of many other diseases. Therefore, confirmation of malaria parasite through microscopy or positive result with RDT is a precondition for making malaria diagnosis in all individuals [3, 4].

Also, despite recent improvement, malaria parasitological diagnosis remains low in Benue state in particular, and Nigeria in general, leading to inappropriate treatment of patients, irrational use of anti-malaria and wastage of resources [5, 6].

Malaria microscopy is considered the gold standard in malaria diagnosis; however, this is subject to the skills and experience of the microscopist as well as availability of electricity. It is done by examining a stained thick and thin film of blood for presence of malaria parasite [7].

mRDT are tests based on colour change to detect plasmodium-specific antigens in a blood sample. The test can be performed in approximately 15 minutes using recommended test kits. It does not require electricity or special equipment and the result is comparable to those of microscopy. The type of mRDT kit approved for use in Nigeria is the Histidine Rich Protein 2 (HRP2) which has been evaluated by WHO/FIND and passed Quality Control. (WHO. Malaria rapid diagnostic test performance. Results of WHO product testing of malaria RDTs Round 8 (2016-2018). Geneva: World Health Organization; 2018). This is justified by the predominance of *P. falciparum* species in Nigeria accounting for 97% of uncomplicated malaria and also the specie most responsible for severe malaria [2, 4, 8].

However, in many areas, suspected malaria patients are treated without parasitological diagnosis in our communities, homes, and even formal health centres/hospitals. The possible reasons could be lack of skills and facilities for microscopy, unavailability/inaccessible RDTs, and most importantly, doubts by individuals and health workers alike as to the reliability/accuracy of RDTs (even when available), compared to microscopy. Therefore, there is need to explore the diagnostic agreement between mRDT and microscopy in this environment.

In Benue state, North-Central Nigeria, where this study was conducted, to the best of the author's knowledge, there is paucity of literature on the performance of mRDT vs blood film Microscopy in febrile out-patients with suspected malaria. This study provided some information to fill this gap, as well as created a platform and data for future studies, especially in Benue state.

Materials and Method

This study was a hospital-based cross-sectional analytical study conducted between May 2022 and November 2022 involving 120 febrile patients aged 5 years and above who attended the General Out-Patient Clinic (GOPC) of the Benue State University Teaching Hospital in Makurdi, the capital of Benue State, North-Central Nigeria. The Benue State University Teaching Hospital Makurdi is a three hundred (300) bed capacity tertiary health institution. It has a Family Medicine Department which runs the GOPC where first contact with most of the undifferentiated patients of all ages and gender seeking medical care at the facility occurs.

The sample size was predetermined. A purposive sampling technique was employed to consecutively recruit febrile patients aged 5 years and above presenting at the GOPC who provided informed consent/assent, until the sample size of 120 was attained. Those who were too ill to participate in the study; serious diseases or medical emergencies, were excluded.

Demographic and clinical information of the participants such as age, sex, body temperature and test results were collected using a pre-tested interviewer-administered questionnaire.

All patients presenting daily (during working days) at the GOPC of BSUTH who met the selection criteria for the study were seen by the researchers. The nursing team at the nurses' station were informed and educated accordingly. All selected patients were sent to the researchers' consulting rooms for consent and recruitment after consultation. Informed consent was obtained as a signature or thumb print in the consent form and assent for minors with parent/legal guardian where applicable, in the presence of a witness. This was done from Monday to Friday until the sample size was reached. The folders of recruited patients were marked behind with "s" at the top right corner to avoid subsequent repeat selection of same patients.

Fever was confirmed using FIT CARE® digital thermometer, and was defined as an axillary temperature $\geq 37.5^{\circ}\text{C}$, consistent with WHO malaria case definition guidelines.⁹ About 2–3 mL of venous blood was collected from each participant aseptically into EDTA tubes by a trained phlebotomist. Rapid Diagnostic Test (RDT) using Bioline Malaria Ag P.f/Pan by Abbott Diagnostics Korea Inc, was performed immediately on-site, according to the manufacturer's instructions, ensuring no degradation of antigens. Positive mRDT was reported as "POS", while negative mRDT was reported as "NEG".

Thick and thin blood films - were prepared and stained with 10% Giemsa stain for 10 minutes. The slides were examined by an experienced medical laboratory scientist who is a WHO level 2 microscopist, using oil immersion at 100x magnification. Malaria positive slide was reported as "Malaria parasite seen" indicating the parasite specie, stage and quantity, while malaria negative slide was reported as "No malaria parasite seen" A 10% subset of slides were re-examined by a second independent microscopist. Discrepancies were resolved by consensus or by a senior reviewer.

Data was analysed using the Statistical Package for Social Sciences version 25.0 software. Descriptive statistics were used to summarise the data. The prevalence of malaria was calculated. Cohen's kappa statistic was used to assess agreement between mRDT and blood film microscopy.

Ethical approval with reference number BSUTH/CMAC/HREC/101/V.111/XX was obtained from the Health Research Ethics Committee of BSUTH. Participants were free to withdraw at any point during the study without any consequences. Data confidentiality was maintained throughout the study, and all patient identifiers were anonymised in analysis and reporting. Participants found positive for malaria were promptly prescribed treatment according to the National Malaria Treatment Guidelines [10].

Results

Socio-Demographic Characteristics of the respondents

Table 1: Socio-Demographic Characteristics of the respondents (n=120)

Socio-demographic characteristics	Frequency	Percentage (%)
Age (in years)		
5–18	28	23.3
19–29	23	19.2
30–39	35	29.1
40–49	26	21.7
50–59	6	5
≥ 60	2	1.7
Gender		
Male	48	40
Female	72	60

Among the 120 participants, 72 (60.0%) were females, while the participants’ ages ranged from 5 to 61 years with majority of them below 40 years of age.

Prevalence of Malaria Positivity among the Respondents Using RDT and Microscopy

Table 2: Prevalence of Malaria Positivity by Diagnostic Method (N = 120)

Test Method	Positive n (%)	Negative n (%)
mRDT	58 (48.3)	62 (51.7)
Microscopy	57 (47.5)	63 (52.5)

Table 2 presents the distribution of malaria test results using malaria Rapid Diagnostic Test (mRDT) and Microscopy. 58 (48.3%) of the respondents tested positive for malaria using mRDT, while 57 (47.5%) were positive by microscopy.

Prevalence and Distribution of Plasmodium Species

Table 3: Prevalence and Distribution of Plasmodium Species

Plasmodium Species	Frequency	Percentage (%)
<i>P. falciparum</i>	55	45.8
<i>P. malariae</i>	2	1.7
None detected	63	52.5
Total	120	100.0

Out of the 120 respondents examined, 55 (45.8%) were positive for *Plasmodium falciparum*, which was the most predominant malaria parasite species detected among the study participants.

Agreement Between mRDT and Microscopy

To assess diagnostic performance, cross-tabulation between mRDT and microscopy results was carried out.

Table 4: Agreement Between mRDT and Microscopy

	Microscopy Positive	Microscopy Negative	Total
mRDT Positive	57	1	58
mRDT Negative	0	62	62
Total	57	63	120

The comparison between mRDT and microscopy results showed excellent diagnostic agreement ($\kappa = 0.983$, $p < 0.001$). The mRDT demonstrated 100% sensitivity and 98.4% specificity, with a positive predictive value of 98.3% and a negative predictive value of 100%.

Discussion

This study revealed a malaria prevalence of 48.3% by mRDT and 47.5% by microscopy, underscoring that malaria remains a significant cause of fever among patients attending BSUTH, Makurdi. This indicates that the prevalence of malaria positivity was 48.3% by mRDT and 47.5% by microscopy, showing a very close similarity between the two diagnostic methods. The minimal difference of 0.8% suggests that both methods produced nearly identical results in identifying malaria cases among the study population. Similar malaria positivity rates have been documented in other Nigerian and African studies [8]. The overall accuracy in this study was high (99.2%), indicating that mRDT results were almost identical to microscopy findings. This high concordance implies that the mRDT is highly reliable for malaria diagnosis and can serve as an effective alternative to microscopy, especially in resource-limited or primary healthcare settings.

This aligns with the findings of Mens, *et al.*, who found 88.6% agreement between the mRDTs and blood film microscopy in Kenya and Tanzania [11]. It also agrees with another study in Makurdi Benue state North-Central Nigeria which reported an overall test accuracy of 93.5% for mRDT with positive and negative predictive values of 83.3% and 94.7% respectively among asymptomatic antenatal women [12]. The discordance observed has been attributed to low density *P. falciparum* infections, non-falciparum infections, or incorrect microscopy [13].

The high sensitivity (100%) and lower specificity (98.4%) found in this study have also been reported in other studies and is in keeping with well-established empirical findings that the HRP2-based mRDTs, the most widely employed diagnostic test for malaria, have a high sensitivity and lower specificity, particularly in a high transmission setting [14-16]. Similarly, a cross-sectional survey conducted in 32 districts in Uganda between November 2021 and March 2023 found a high sensitivity of more than 91% for mRDT, using microscopy as gold standard, and thus was reliable for diagnosis of malaria [13]. In Burkina Faso, a prospective study of febrile children reported a high sensitivity of 98.5% and specificity of 40.5% for mRDT, with microscopy as gold standard [14]. The possible reasons for low specificity of the mRDT across all the studies could be because the HRP-2 antigen persists for about 3-9 weeks in the blood after successful treatment, as well as possible issues with the transportation and storage conditions of the kits.

The positive and negative predictive values of mRDT in this study is comparable to the values from other studies in Nigeria and other African countries. Falade *et al* found a negative and positive predictive value of 86.1% and 65.6% respectively for mRDT in a study across multiple African countries [8]. In Burkina Faso, positive and negative predictive values of 77.7% and 92.7% respectively have been reported for mRDT in a prospective study in the Nanoro district [14]. The slight differences in the values could be explained by the different study designs and populations utilized.

Meanwhile, the predominance of *P. falciparum* in this study aligns with established findings in Nigeria and other West African countries, where this species accounts for the majority of infections and contributes to severe malaria cases [17].

Conclusion

Malaria remains prevalent among febrile patients in Makurdi, with nearly half testing positive by both mRDT and microscopy. The strong agreement between mRDT and

blood film microscopy supports the reliability of mRDTs for malaria diagnosis in routine practice in resource-constrained settings.

Recommendation

mRDT where availability and accessible should be used for the parasitological diagnosis of malaria before treatment especially where microscopy is not feasible since the result is comparable to that of blood film microscopy. More multicentre studies are advocated to verify these findings and enable generalization. Also, considering the high prevalence of malaria, efforts should be made at all levels to ensure proper management and targeted prevention.

Limitations

This was a hospital-based study conducted in one hospital thus, the findings might not be a complete representation of what may be obtainable in other hospitals or the general population. Also, this study made use of microscopy as a gold standard/reference test. However, in recent time, in other climes, researchers have used polymerase chain reaction (PCR)-based techniques to compare the diagnostic performance of mRDTs. This modality has been demonstrated to be one of the most sensitive and specific diagnostic methods. However, it is expensive and not readily available in our environment at the moment.

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