(RESEARCH ARTICLE)

Prevalence of malaria and comparative diagnostic performance of malaria rapid diagnostic test against microscopy in Nasarawa-west senatorial District, Nasarawa, Nigeria

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Abstract

Malaria is a vector-borne parasitic disease caused by *Plasmodium* species, and transmitted by the bite of an infected female anopheles mosquito. It remains a major public health challenge in Sub-Saharan Africa, a major cause of mortality and morbidity, especially in children and pregnant women. This study evaluated the prevalence of malaria and the comparative performance of rapid diagnostic test (RDT) against Microcopy, the gold standard. A total of 385 febrile consenting patients attending randomly selected health facilities in Nasarawa-West Senatorial District were recruited using simple random sampling technique. Malaria parasite tests were conducted using RDT and microscopy technique. Of the 385 samples tested, 115 (29.9%; 95% CI = 25.34 to 34.71%) were positive by microscopy and 103 (26.8%) were positive by RDT. The RDT had a sensitivity of 89.6% (95% CI = 82.48% to 94.49%), specificity of 100.00% (95% CI = 98.64% to 100.00%), Positive Predictive Value (PPV) of 100.00% (95% CI = 96.48% to 100.00%); and Negative Predictive Value (NPV) of 95.8% (95% CI = 92.94% to 97.46%). The accuracy of the RDT was 96.88% (95% CI = 94.62 to 98.38%). Thus, the high diagnostic performance of SD Bioline indicates that it can be effectively used for the diagnosis of malaria. Microscopy however, was able to detect a higher number of positive malaria cases thus implying that microscopy remains a better diagnostic tool for malaria parasite detection than the RDTs.

Keywords: Malaria; Microscopy; RDT; Sensitivity; Parasite density; Nasarawa

1. Introduction

Malaria is a vector-borne obligate intracellular parasitic disease caused by *Plasmodium* species and transmitted by the bite of an infected female anopheles mosquito [1]. It remains a major public health challenge in Sub-Saharan Africa [2,3]. Precise statistics of morbidity and mortality are unknown because many cases occur in rural areas where people do not have access to hospitals or lack the means to afford health care. Consequently, the majority of cases of malaria are undocumented. It is estimated to cause 241 million clinical episodes and 627,000 deaths with an estimated 94% of deaths occurring in the World Health Organization (WHO) African Region [4,5]. According to the 2020 World Malaria Report, Nigeria has the highest number of global malaria cases with 27 % of global malaria cases in 2019 and accounted for the highest number of global malaria deaths which stands at 23% [3,6]. Of the 5 species of malaria parasites (*Plasmodium* species) infecting humans, *Plasmodium falciparum* is the most deadly due to its ability to subvert the physiology of its host during the blood stages of its development [4].

WHO recognizes microscopy as a gold standard, but also recommends RDTs as a good alternative method for malaria diagnosis especially in rural areas where presumptive treatment is still practiced and the availability of constant light for microscopy and PCR methods is difficult to achieve [7]. Microscopy technique remains the gold standard for malarial

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diagnosis in endemic countries. It has a sensitivity of 50-500 parasites/µl [8]. It is inexpensive, allows the identification of species and provides information on the parasite density for monitoring the efficacy of treatment. It is also necessary to observe many fields to detect infection. However, some of the disadvantages of microscopic diagnosis include difficulty in determining the species of the implicating *Plasmodium* parasite, labor-intensive, time-consuming and the need for constant electricity supply, which is erratic in Nigeria [9].

Rapid Diagnostic Tests (RDTs) are immune-chromatographic-based diagnostic tests. In remote parts of sub-Saharan Africa, RDTs have become the primary tool for the parasitological diagnosis or confirmation of malaria. The most widely used RDTs for malaria are based on the detection of parasite histidine-rich protein II (HRP2). They are fast Point of Care (PoC) tests, cost-effective, easy to use, and easy to interpret; results are provided in a few minutes; and no electrical equipment is required neither does it require intense training but few personnel. The major constraint of RDTs is false positive or negative results. False positives occur because HRP2 persists in the blood for several days after infection clearance while RDT-negative but microscopy positive results can occur due to operator error, poor storage conditions, *P. falciparum* histidine-rich protein 2 and 3 gene deletions, poor performance of specific RDT brands [10].

Proper screening and diagnosis of affected persons is an important step in the control of malaria transmission. There is also paucity of literature on the prevalence and comparative investigation on malaria diagnosis using RTDs versus microscopy in the study area. This study is therefore aimed at determining the prevalence of malaria parasite and the comparative diagnostic performance of RDT against Microscopy among febrile adults attending selected health facilities in Nasarawa-West Senatorial District, Nasarawa State, Nigeria

2. Material and methods

2.1. Study Area

Blood samples were collected from febrile consenting adults in the Medical Laboratory units of 5 selected health facilities in Nasarawa-West Senatorial District, Nasarawa State, Nigeria. The samples were analyzed in the Microbiology Laboratory of the Department of Microbiology, Nasarawa State University, Keffi (NSUK), Nasarawa State, Nigeria. Keffi, the headquarters (Collation centre) of the Senatorial District is located in Nasarawa state, North Central Nigeria between latitudes 8°51 and 8°53^1 North of the equator and longitudes 7°50' and 7°51' East of the Greenwich meridian. It is located about 128 km away from Lafia, the Nasarawa State capital, about 57 km away from Abuja, the Federal Capital Territory of Nigeria. Keffi is the smallest Local Government Area in Nasarawa State, with a total land area of approximately 140 km^2 [11, 12].

2.2. Study Population

The Senatorial District has a population of 723, 608 distributed into five local governments namely Karu, Keffi, Kokona, Nasarawa and Toto.

2.3. Sample Size Determination

The sample size was calculated with a 95% Confidence Interval (CI) and precision level of 5% using the standard sample size calculation formula described by [13].

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where:

n = Sample size if the target population is > 10,000
 Z = Z statistic for a level of confidence (For 95% confidence level, Z = 1.96)
P = Expected prevalence or proportion (in proportion of one; if 50%, P = 0.5) and
d = Precision (in proportion of one; if 5%, d = 0.05).

Given that Z = 1.96, P = 0.5, d = 0.05 and substituting the values, sample size was calculated thus:

n = 1.96 x 1.96 x 0.5 (1 – 0.5) ÷ 0.05 x 0.05

n = 3.8416 x 0.5 x 0.5 ÷ 0.0025

$$n = 0.9604 \div 0.0025$$

$$n = 384.2$$

Approximately, 385 samples were collected for the study.

2.4. Ethical Approval

The ethical approval was obtained from the Research and Ethical Committee of the Nasarawa State Ministry of Health, Nasarawa State, Nigeria.

2.5. Subject Selection (Eligibility criteria)

2.5.1. Inclusion criteria

Patients who had clinical symptoms of malaria determined by a headache, body aches and febrile condition of 37.5 °C and above and have not taken antimalarial drug in the preceding week prior to visitation to the health facility were recruited in the study. Similarly, only consenting Patients residing within the senatorial district were enrolled in the study.

2.5.2. Exclusion criteria:

Patients residing outside the senatorial district, without clinical symptoms of malaria determined by a headache, body aches and febrile condition of 37.5 °C and above and have taken antimalarial drug in the preceding week prior to visitation to the health facility were exempted from the study.

2.6. Blood Collection

Blood samples were collected by venipuncture from the study participants at the collection sites namely: General hospitals, Keffi (GHK), Nasarawa (GHN), Garaku (GHG), Medical centre Mararaban Gurku (MCM-G) and SAPETRO Medical Centre (SMC) - Nasarawa State University, Keffi using standard procedure. The collected blood samples were let into labeled EDTA containers at the respective collection sites and conveyed to Microbiology Laboratory, Nasarawa State University, Keffi for analysis.

2.7. Laboratory Analysis

2.7.1. Microscopy

At the Microbiology laboratory at NSUK, thin and thick blood films made on well labeled clean grease free slides and air dried were stained for 10 min using a 1 in 10 dilution of freshly prepared Giemsa stain in buffer water of pH7.2. The stained dry films were read by two independent Microscopists, discrepancies resolved by a third reader.

2.7.2. Rapid Diagnostic Test

Whole blood from the collected blood samples were also tested for malaria parasites using specie-specific RDT kit (SD Bioline®) which is based on Histidine Rich Protein II (HRP II) and test specifically for *Plasmodium falciparum* following Manufacturer's protocol. Briefly, 5μl of whole blood from the test sample was added into the sample well of a labeled, warm SD Bioline malaria antigen *P. falciparum* test device placed on a flat surface. Two drops (100μl) of assay diluent was then added into the diluent well and timed for 15 minutes. Positive samples were indicated by show of 2 colour bands (one in the control line region and the other in the test line region). Results were considered negative when there was colour band only in the control line but none in the test line. Similarly, results were also considered invalid when there was no colour band at all in either the test nor control line or if colour band appeared only in the test line but none in the control line.

2.7.3. Determination of Diagnostic Indices

The diagnostic indices (Sensitivity, Specificity, Positive predictive value, Negative predictive value, and Accuracy) of the RDT were determined using standard protocol as described by [14]. Sensitivity is defined here as the probability that a test result will be positive when the disease is present (true positive rate). It is calculated using the formula: Sensitivity = TP/TP + FN X 100 (where TP = True positive, FN = False negative). Specificity is defined here as the probability that a test result will be negative when the disease is not present (true negative rate). It is calculated using the formula: Specificity = TN/FP + TN X 100 (where TN= True negative, FP = False positive, TN = True negative). Positive Predictive Value (PPV) is defined here as the probability that the disease is present when the test is positive. It is therefore the

percentage of patients with a positive test who actually have the disease. PPV tells us about the number of test positives that are true positives and if these numbers are higher (as close to 100 as possible), then it suggests that this new test is doing as good as 'gold standard'. It is calculated using the formula: PPV = TP/TP + FP X 100 (Where TP = True positive), TP + FP (true positive + false positive). Negative Predictive Value (NPV) is defined here as the probability that the disease is not present when the test is negative. It is therefore the percentage of patients with a negative test who did not have the disease. NPV tells us how many of test negatives are true negatives; and if this number is higher (should be close to 100), then it suggests that this new test is doing as good as 'gold standard'. It is calculated using the formula: NPV = TN/TN + FN X 100 (Where TN= True negative, TP = True positive and FP = False positive. Accuracy (A) is defined here as the overall probability that a patient is correctly classified. It is calculated using the formula: A = Sensitivity × Prevalence + Specificity × (1 − Prevalence).

2.7.4. Determination of Parasite Density

The parasite density was done to provide information on the severity of infection using a formula by [7]. Briefly, an average of 8000 leukocytes per µl was taken as the standard. Two hundred (200) fields under 1000× magnification (10x ocular and a 100x oil immersion objectives) were examined from the thick blood film before the slide was considered negative. For positive slides, parasitaemia (parasite density) was determined by counting only the asexual stages against 200 white blood cells. The asexual parasites and leukocytes were counted separately using two tally counters. If the number of parasites counted was up to 100 or more when 200 leukocytes were counted, the results were recorded, showing parasites per 200 leukocytes. If after 200 leukocytes had been counted and 99 or less asexual parasites had been counted, the counting continued until 500 leukocytes had been counted and the results recorded as parasites per 500 leukocytes. In each case, the parasite count in relation to the leukocyte count was converted to parasites per µl of blood by the simple formula:

$$\text{Parasite density (Parasites per µl)} = \frac{\text{No of asexual parasites counted x 8000}}{\text{No of WBC counted}}$$

This meant that if 200 leukocytes were counted, the parasites were multiplied by 40, and if 500 leukocytes were counted, the parasites were multiplied by 16. The "plus system" was used to record the levels of parasitaemia (parasite density) as follows: 1+ = 1 - 10 parasites per 100 oil-immersion thick smear field; 2+ = 11 - 100 parasites per 100 oil-immersion thick smear field; 3+ = 1 - 10 parasites per thick smear field; 4+ = >10 parasites per thick smear field. The level of parasitaemia was graded as low (< 1000 parasites/µl of blood), moderate (1000–9999 parasites/µl of blood) and severe (≥ 10,000 parasites/µl of blood) [15, 16, 17, 7, 18].

2.8. Statistical Analysis

Distribution of participants' characteristics and malaria parasite prevalence were assessed using contingency tables. Taking microscopy as the gold standard, the performance of the Rapid Diagnostic Test was compared to it by computing the sensitivity, specificity, the negative predictive value, the positive predictive values and the accuracy of the test method and the data obtained was analyzed using MedCalc version 22.009 statistical package (MedCalc Software Ltd. Diagnostic test evaluation calculator).

3. Results

3.1. Prevalence of Malaria in the Study Population

A total of 385 blood samples were collected in this study and 115 were positive by microscopy. The Percentage prevalence of malaria parasite infections in the patients' blood sample in the 5 collection sites is as presented in Table 1. Results from the table indicated a percentage prevalence of 29.9% (115/385) (95% CI = 25.34 to 34.71%). Individual study sites results revealed that GHN had the highest prevalence of 33.8% followed by GHG 32.5%. The other study facilities namely: SMC, MCMG and GHK had a percentage prevalence of 28.6, 28.6 and 25.9 respectively.

Table 1 Prevalence of malaria parasite by Microscopy

Facility	Microscopy		Prevalence value (%)
	No collected	No positive	
GHK	77	20	25.9
GHN	77	26	33.8
GHG	77	25	32.5
MCMG	77	22	28.6
SMC	77	22	28.6
TOTAL	385	115	29.8

GHK = General Hospital, Keffi, GHN = General Hospital, Nasarawa, GHG = General Hospital, Garaku, MCMG = Medical Centre Mararaban Gurku, SMC = SAPETRO Medical centre, % = percentage prevalence

3.2. Comparison of malaria RDT positivity with parasite count by microscopy

The results of RDT positive tests and the parasite count by microscopy was calculated using the formula as described by [7] and the result is presented in Table 2. Parasite count is presented using plus (+) sign method where + = 1 - 10 parasites per 100 oil-immersion thick smear field; ++ = 11 - 100 parasites per 100 oil-immersion thick smear field; +++ = 1 - 10 parasites per thick smear field; ++++ = >10 parasites per thick smear field. Results from Table 4.2 indicated that out of the 115 samples tested positive by microscopy, 60 samples were positive in the one plus (+) category of which 52 samples showed positivity by RDT. Forty (40) samples were microscopically positive in the ++ category with RDT positivity of 38 samples. Similarly, of the 12 samples positive by microscopy in the +++ category, 10 were positive by RDT while the highest parasite density of ++++ had 3 samples microscopically positive and the RDT method was also positive to them all.

Table 2 Comparison between malaria RDT positivity with Microscopy parasite density

RDT Results	Microscopy										Total
	+		++		+++		++++		Nil		
	n	%	n	%	n	%	n	%	n	%	
Negative	08	13.3	02	5	02	16.7	00	-	270	74.4	282
Positive	52	86.7	38	95.5	10	83.3	03	100.0	00	00	103
Total	60	-	40	-	12	-	03	-	270	-	385

+ = 1 - 10 parasites per 100 oil-immersion thick smear field; ++ = 11 - 100 parasites per 100 oil-immersion thick smear field; +++ = 1 - 10 parasites per thick smear field; ++++ = >10 parasites per thick smear field, n = number positive, % = percentage.

Using the formula as described by [7], RDT positivity in comparison with parasite density by microscopy = RDT positive divide by Microscopy positive x 100. The percentage of positive results of RDT and parasite count using microscopic parasite density of +, ++, +++ and ++++ was therefore calculated to be 86.7%, 95.0%, 83.8% and 100.0%, respectively.

3.3. Diagnostic Performance of Rapid Diagnostic Test

The diagnostic performance of the Bioline malaria RDT was compared using microscopy as the gold standard and calculated using the formula on a 2 x 2 contingency table (Table 3) as described by [7]. The sensitivity (TP/TP + FN X 100) of RDT was 89.6% (95% CI = 82.48% to 94.49%) while its specificity (TN/FP + TN X 100) was 100.00% (95% CI = 98.64% to 100.00%). The Positive Predictive Value (PPV) (TP/TP + FP X 100) was 100.00% (95% CI = 96.48% to 100.00%) while the Negative Predictive Value (NPV) (TN/TN + FN X 100) was 95.8% (95% CI = 92.94% to 97.46%). The accuracy (Sensitivity × Prevalence + Specificity × (1 – Prevalence)) of this diagnostic tool was 96.88% (95% CI = 94.62 to 98.38%). This result is presented in table 3.

Table 3 Comparative diagnostic performance of the malaria RDT with microscopy

RDT result (Index test result)	Microscopy (Reference standard)		Total
	Positive	**Negative**	
Positive	103	0	103
	(TP)	(FP)	(TP + FP)
Negative	12	270	282
	(FN)	(TN)	(FN + TN)
Total	115	270	385
	(TP + FN)	(FP + TN)	(TP +FP + FN + TN)

FN = False Negative; FP = False Positive; TN = True Negative; TP, True Positive. RDT = Rapid Diagnostic Test

4. Discussion

Malaria remains a major public health challenge in Sub-Saharan Africa [2,3]. According to the 2021 World Malaria Report, Nigeria had the highest number (26.6%) of global malaria cases and the highest number (32%) of global malaria deaths [19]. The prevalence of 29.9% obtained in this study emphasizes the fact that malaria still remains endemic in the study area. This prevalence is a public health concern considering all known intervention programs aimed at halting malaria parasite transmission. The observed prevalence rate of 29.9% is consistent with a study conducted in Cameroon where malaria prevalence rate of 29% was reported [17]. It is also closely related to the WHO African region's current malaria prevalence report of 26.6% [19]. The observed prevalence is however lower than reports from other parts of Nigeria namely: 53% in Asaba [7]; 64.9% in Kano [20] and 82.7% in Ondo, South West Nigeria [21]. Even though this rate of 29.6% obtained in this study is a bit higher by 3.3% when compared to the 26.6% reported in Nigeria by the WHO African region, the prevalence showed a reduction in the prevalence of the infection in the study area compared to earlier years.

Results from individual study sites showed that GHN had the highest prevalence of 33.8% followed by GHG who had a percentage prevalence of 32.5%. The other study facilities namely: SMC, MCMG and GHK had a percentage prevalence of 28.6, 28.6 and 25.9 respectively. The highest prevalence results obtained from GHN (33.8%) could mean that the rate of adherence to malaria preventive measures is lower compared to other study sites particularly GHK with the lowest prevalence rate of 25.9%. It could also mean that the level of abuse of antimalarial drugs with its attendant antimalarial resistance rate is higher in GHN than in Keffi. Another reason that could be advanced for the high rate of malaria occurrence in GHN could be that there could be multiplicity of malaria in the area compared to other study sites. The decrease in prevalence could also be attributed to the Nigerian government's conscious efforts towards eradicating the disease by implementing relevant preventive and control policies by ensuring that young children and pregnant women, who are most vulnerable to the disease, are promptly diagnosed, treated and protected. Some of the conscious efforts aimed at eradicating the disease include improved adherence to preventive measures like use of insecticide treated mosquito nets, use of indoor residual sprays, clearing of bushy surroundings, oily spray on water surfaces and ensuring that there is less stagnant water for the breeding of the parasite vectors among other preventive measures.

Results from Table 2 indicated that the parasite density reported in plus (+) format in this study has more positive samples in the one plus (+) category. This implies that the parasite density which accounts for severity of malaria was not very high in the participants in the study area. The low severity may also be as a result of early infection of the participants at the time of sample collection. Even though there was low parasite density, the effect of the parasiteamia is still dependent on the immunity of the individual patients in question. Severity of malaria is dependent on several factors such as multiplicity of infection (MOI), resistance of the antimalarial drugs, failure of preventive measures such as ineffective long lasting insecticide nets (LLINs) which will increase exposure of the vector to human population who to them, are sleeping inside treated mosquito nets and should ordinarily be protected not knowing that the treated nets are ineffective. The low yield of positive results with one plus (+) in this study agrees with the fact that the malaria rapid test result positivity is low at low parasite density. Similarly, a report by [22] as quoted by [7] , found that RDT sensitivity was only 45% when the parasite density was below 100 parasite/μl.

Confirmation of malaria positivity using RDT kit alongside the gold standard method revealed that microscopy was able to detect the highest number of positive malaria cases of 115 (29.9%) when compared to RDT with 103 (26.8%). This

finding is similar to other reports across Nigeria [23, 24, 25]. This thus implies that microscopy remains a better diagnostic tool for malaria parasite detection than the RDTs. When comparing microscopy result positivity (29.9%) with that of RDT (26.8%) in this study, the detection of peripheral blood HRP-2 genes by RDT implies that RDT can be used as an alternative diagnostic method for malaria especially in emergency situations or in areas with erratic power supply.

Results from this study showed the sensitivity of 89.6%, specificity of 100.0%, positive predictive value of 100.0% and negative predictive value of 95.8%. By definition, sensitivity in this study is the probability that the test result will be positive when malaria is present and specificity is the probability that the test result will be negative when malaria is not present. Thus, the 100% specificity for SD Bioline together with the high positive predictive value of 100% implies that malaria parasite screening using RTDs can be accurate. The negative predictive value of 95.8% means that the RDTs is strongly reliable in ruling out the chances of malaria infection and can be used to effectively diagnose malaria parasite [26]. This high rate of specificity and sensitivity of RDT is consistent with literature of other researchers [7, 27]. It is also consistent with others reported in FCT and Keffi [27, 28,]., Rivers, Nigeria [26]. Our findings are also in agreement with the findings of a study in Ogun State [29]. The high performance of the RDT could be attributed to good storage conditions because extreme temperatures affect the efficacy of the RDT which may occur during storage and transportation [30].

5. Conclusion

The prevalence of malaria obtained in this study indicates the endemicity of malaria in Nasarawa-West Senatorial District, Nasarawa state, Nigeria. The study observed that Microscopy and RDT techniques can be effectively used for the diagnosis of malaria, however negative RDTs should be microscopically confirmed. The RDT kits showed higher sensitivity, specificity, positive and negative predictive values and this makes it a valuable tool where facilities for microscopy are not available. The endemicity of malaria in the study population could be as a result of failure to adhere to preventive/control measures like use of insecticide treated mosquito nets, use of indoor residual sprays, clearing of bushy surroundings, oily spray on water surfaces and ensuring that there is less stagnant water for the breeding of the parasite vectors among other preventive measures. It could also be as a result of antimalarial drug abuse and misuse whose consequence is drug resistance hence the persistence of the parasite in the study area. I therefore recommend adherence to preventive/control measures as well as correct use of antimalarial drugs only when prescribed by Professionals.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared no conflict of interest.

Statement of ethical approval

The ethical approval was obtained from the Research and Ethical Committee of the Nasarawa State Ministry of Health, Nasarawa State, Nigeria.

Author's Contributions

The study was designed by Igbawua IN and Ngwai YB. Igbawua IN conducted the experiments. Ngwai YB, Ishaleku D supervised the study. Owuna JE prepared the manuscript. All authors proof-read and approved the article.

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