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Comparative laboratory diagnosis of malaria: Microscopy versus rapid diagnostic test kits in a tertiary institution of North-Central Nigeria

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Abstract

Laboratory diagnosis of malaria is a vital key for its effective management. Diagnosis of malaria includes rapid, sensitive, and specific test methods. This study was aimed to assess the diagnostic performance of PfHRP2 rapid malaria test with reference to light microscopy for the diagnosis of malaria at the Bingham University Teaching Hospital (BhUTH) Jos – Plateau State, Nigeria. A total of 150 febrile patients attending BHUTH who were sent to the Hospital Laboratory from the General out patients Department (GOPD) for malaria parasite test (MPT)request had their blood sample collected and tested for malaria parasites (MP) using Field Stain A and B stain microscopy and PfHRP2 rapid malaria test between September and November 2021.Results shows that the sensitivity and specificity of PfHRP2 rapid malaria were 9.23% and 90.0% respectively, with corresponding positive and negative predictive values (PPV) as 90.0%and (NPV) as13. 2%. PfHRP2 rapid malaria test showed good sensitivity and specificity that is in agreement with that of the reference light microscopy. The rapid diagnostic test (RDT) results compared well with the light microscopy (Gold standard) for Laboratory diagnosis for malaria. Sustained use of RDT as an alternative to light microscopy is recommended especially in malaria endemic areas and the rural communities where electricity is out of reach.

Keywords: Malaria; HRP-2; *Plasmodium falciparum*; Sensitivity; Specificity; Comparison

1. Introduction

Malaraia is a disease caused by *Plasmodium* species in the tropics and the most common fatal globally accounting for 3.4 billion people at risk of acquiring malaria with 80% cases and 90% deaths occurring in the African region with highest risk in sub-Saharan African (1). In Nigeria, 68% of the people are reported to live in malaria risk areas with 40 – 60% relative frequencies of *P. vivax* and *P. falciparum* leading to severe and complicated diseases and deaths (2). Clinical diagnosis is the most widely used diagnostic method in rural areas with non-existing laboratory facilities; it is inexpensive to perform and require no special equipment or supplies (3). However, in 2011, according to WHO guidelines on clinical diagnosis of malaria based on signs and symptoms alone is not recommended since it has low

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specificity and increase the chances of the patients being misdiagnosed leading to misused of anti-malaria drugs (4). Laboratory diagnosis is one possibility in the management of a patient presenting with fever (5). In order to improve the quality care of the patient, many diagnostic procedures have been developed with the aim to have accurate diagnosis, reduce the turnaround time and training needed (6). Even though the light microscopy is considered as the gold standard method for malaria diagnosis in many developing countries, its sensitivity and specificity still remain a challenge (7, 8, 9). Whereas, malaria rapid diagnostic test (RDTs) is based on the detection of one of the following antigens, histidine-rich protein-2 (HRP-2), lactate dehydrogenase (LDH) and aldolase showing differences in the sensitivity and specificity in the test kits with majority targeting HRP-2 for *P. falciparum* than any other antigens [10, 11, 12, 13].A study conducted by Ansah*et al*., 2010 reported that clinicians treat febrile presentation with anti-malaria drugs, even when the results of the RDT is negative for malaria parasitic antigen [10].

2. Material and methods

This study was conducted in Bingham University Teaching Hospital (BhUTH) Jos, Plateau State between the months of September and October, 2021.

A minimum sample size of 150 venous blood (about 2 mls) was collected from the patients aseptically into labeled EDTA bottles after administration of questionnaire and consent obtained using a pilot study conducted in Jos by Nanvyyat*et* al., who revealed a 10% prevalence of malaria using the Cochran formula for calculating sample size as stated. Demographic and clinical data were collected with a focus on their age variables and gender who were sent to the Hospital service Laboratory for malaria test from the general out-patients department (GOPD). Malaria parasite present in the blood was determined immediately using mRDT, blood smears prepared on grease-free slides as described byNanvyat and Mharakuwa et al., [15, 16]

2.1. Laboratory diagnostic procedures

2.1.1. Malaria rapid diagnostic testing

The presence of *Plasmodiumspp.* in bloodwas determined using the PfHRP2 malaria rapid diagnostic kit (SD Bioline, Alere, Repulic Korea) and according to the manufacturer's instructions. Aliquot of 5 µl of sample from each was placed in the sample window of the RDT cassette and three drops of diluent added. The results were then read after 15 min, with the presence of two (or three), one or no distinct line indicative of a positive, negative or invalid result respectively.

2.1.2. Light microscopy

Thin and thick blood smears were prepared and allowed to air dry. The thin films were fixed with methanol and both smears stained with field stain A and B for 30 second each as described by Mharakuwa et al.,[16]. Subsequently, the stained slides were then air-dried and viewed under the x100 oil immersion objective of a binocular Olympus microscope. Each slide was examined by two independent microscopists, results were considered to either positive or negative and documented [17].

2.2. Statistical Analysis

The data was entered into SPSS version 20 software for analysis; sensitivity, specificity, positive predictive value, negative predictive value was also determined with Kappa value calculated at 95% confidence interval.

3. Results and discussion

Out of a total of 150 subjects that were diagnosed for malaria, 98 (65.3%) were females and 52 (34.7%) were males. The mean age of the participants 36 years (49.5%) while majority were within the age range of 30–39 years (Table 1).

Table 1 Age range of RDT and LM positive subjects

Age group	No of subjects	RDT positive%	LM positive %
1-9	20	-	27
10-19	18	7.5	25.5
20-29	11	1.5	15
30-39	36	1.5	49.5
40-49	26	7.5	33
50-59	19	-	24
60-69	8	-	7.5
70-79	7	-	9
80-89	5	-	6

Table2 Specificity and sensitivity of light microscope and RDT

Positive/Negative	Sensitivity	Specificity	Total
Positive	12	2	14PPV
Negative	118	18	136NPV
Total	130	20	150

PPV = Positive predictive value, NPV = Negative predictive value; Sensitivity = true positive/(true positive + false negative) × 100%; Specificity = true negative/(true negative + false positive) × 100%

$$PPV = \text{true positive}/(\text{true positive} + \text{false positive}) \times 100\%$$

$$NPV = \text{true negative}/(\text{true negative} + \text{false negative}) \times 100\%$$

The predictive values were determined as described by Moges et al., [26]

- Sensitivity =9.23%
- Specificity =90.0%
- Ppv =85.7%
- NPV =13. 2%s

Using light microscopy as standard test for diagnosing malaria, the sensitivity and specificity of the malaria RDT was 9.23% and 90.0% respectively, with corresponding positive and negative predictive values (PPV and NPV) of90.0% and 13. 2%(Table 2).

The current study revealed a high sensitivity and specificity of PfHRP2 rapid malaria test. The high sensitivity and specificity are in agreement with the reports in Sudan [21]. However, the sensitivity and specificity are lower than the reports in Zimbabwe [23], Ghana [12 23], Sudan [24], and Uganda [25]. These differences might be due to observer variation or host factors.rapid malaria test had high PPV and NPV. Thus, a high NPV indicates that a person does not have the disease with high certainty, meaning that PT is reliable test method in diagnosing of malaria parasites.

From our study, PfHRP2 rapid malaria test 12 patients were positive while light microscopy 130patients were positive, these indicate that 118 were mixed out.In addition, the presence of artifacts such as immature erythrocytes, or bacterial cells might have been misinterpreted as*Plasmodium* DNA [22]. On the other hand, RDT produced 118 false negative results which were positive by light microscopy. This might be due to the fact that unlysed red blood cells may lie on each other or overlap with each other thereby preventing parasites in red blood cells that may be lying beneath other cells.

The overall prevalence of malaria in the study area was very high, as detected by both the LM (86 .7%) and the RDT (13.3%). The district is malarious and endemic for all*Plasmodium* species. But the prevalence of *P. falciparum* is very high.

This result is higher than the report from other regions in Ethiopia [24, 25]. The high prevalence could be partly explained by the fact that the study was conducted in malaria transmission season of the country.

PfHRP2 rapid malaria test showed good sensitivity and specificity with an excellent agreement to the reference light microscopy. RDT has very short turnaround time, requires little training, and is applicable under field conditions. Therefore, RDT can be considered as alternative diagnostic tools in malaria endemic areas. In this study, Light Microscopy showed the highest prevalence of malaria parasites. These confirmed the fact that Light Microscopy is still the golden standard of diagnosing malaria parasite.

4. Conclusion

The rapid diagnostic test (RDT) results compared well with the light microscopy (Gold standard) for Laboratory diagnosis for malaria. Sustained use of RDT as an alternative to light microscopy is recommended especially in malaria endemic areas and the rural communities where electricity is out of reach.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors hereby declare no conflict of interest.

Statement of ethical approval

Ethical approval (Ref - NHREC/201/05/2005 /00842) was obtained from the Health Research Ethics committee of the Bingham University Teaching HospitalJos, Nigeria.

Statement of informed consent

All participants gave written consent for this study.

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