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Evaluation of Rapid Methods in Malaria Diagnosis from Persons attending Primary Health Facilities, Ogun State, Nigeria


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Abstract

Malaria in Nigeria is treated blindly in most cases and where diagnostic measures exist, efficacies of several are still in doubt. This study evaluated performance of microscopy and rapid (RDT) methods in confirming prevalence of malaria parasites (MP), parasite species and percent parasitized red blood cells in 384 persons systematically selected from seven primary healthcare facilities in Ogun State, Nigeria from October to December 2012. Giemsa thin and thick film techniques and RDT (SD Bioline Ag Pf/Pan kit) were employed on the blood samples. Data were analysed using SPSS version 16. Demographic results showed that females were 61% and males 39%, but of varying age groups. Seventy-one percent of the blood samples were positive for MP by microscopic blood films, and only 24% samples were positive by RDT. Paired sample T-test showed a significant difference (P=0.000) between microscopy and RDT methods. Of the 273 positive blood samples, 95.6% were P. falciparum, 3.3% were P. malariae, 0.7% was P. ovale and P. vivax (0.4%). For RDT, P. falciparum was 74% while mixed infections were 26%. Prevalence in risk groups 0–10 and 11–20 age groups was observed to be higher with significant differences (p=0.011, p=0.023) for both microscopy and RDT respectively. RDT sensitivity and specificity were 23.4% and 74.7% compared to microscopy. Positive Predictive Value and Negative Predictive Value were 69.6% and 28.4%. The efficiency of the RDT was 38.3%. The low sensitivity of RDT observed means that malaria diagnosis by microscopy method remains the gold standard.

Keywords: Rapid Diagnostic, microscopy, malaria parasites, health facilities, Ogun State.

Introduction

Malaria is one of the highest killer diseases in most tropical countries affecting millions of people world-wide [1]. Human malaria is caused by the protozoan parasite of the genus, Plasmodium. The species of Plasmodium are P. vivax, P. ovale, P. malariae and P. falciparum, and a fifth species P. knowlesi[2]. In Nigeria, P. falciparum is the most virulent specie and accounts for over 80% of human malaria infections leading to about 200,000 deaths annually [3, 4, 5]. Microscopy had long been...
the method of choice for the diagnosis of malaria, which requires skills and a power supply [6]. The major drawback of routine microscopy in malaria studies is the expertise in the parasite identification. As a result of this, the use of rapid diagnostic test (RDT) to ensure prompt and early diagnosis as a first step to the control of malaria was advocated as a necessity for active surveillance [7]. RDTs, introduced since the early 1990s for the detection of malaria parasites, exhibit high sensitivity and specificity compared with microscopy and Polymerase Chain Reaction [8, 9].

The diagnosis of malaria parasite *P. falciparum* infection using *P. falciparum* antigen has been widely accepted as a rapid antigen test for *P. falciparum*[10]. Its accuracy has also been put at 86% - 99% compared with microscopic detection of malaria parasites in blood smears [11]. Multiple studies are been advocated to test the performance of RDTs in diverse clinical settings in both malaria endemic and non-endemic countries. In Nigeria and Ogun State, a number of studies on malaria [12, 13, 14, 15 and 16] had given insight into the transmission patterns in any given area which are useful tools for control purposes.

Few studies had investigated the use of HRP-2 antigen based RDTs, with reported sensitivity compared to gold standard thick blood film (TBF) microscopy of > 80% and specificity of >90% for parasitaemia of > 100 parasites/μL of blood [17]. Odimayo *et al.*[18] had reported lower sensitivity of 70%. All these techniques were based on detecting the circulating antigens in individuals living in malaria endemic areas. Therefore, the study was conducted to evaluate the performances of Microscopy and Rapid Methods as diagnostic tools in assessing malaria parasites in persons attending primary health facilities, Ogun State, Nigeria.

**Materials and Methods**

**Study setting**

The study was conducted in seven primary health care (PHC) facilities in four local government areas (LGA) as presented in Figure 1 namely; PHC Enugada (N7.15542Ў, E3.32981Ў), PHC Iberekodo (N7.18172Ў, E3.34046Ў) and PHC Sabo (N7.16843Ў, E3.32015Ў) in Abeokuta North LGA, PHC Oke-ilewo (N7.13512Ў, E3.33939Ў) in Abeokuta South LGA, PHC Owode-egba (N6.94391Ў, E3.50269Ў) and PHC Ofada (N6.86379Ў, E3.42645Ў) in Obafemi-owo LGA and PHC Obantoko (N7.17890Ў, E3.39567Ў) in Odeda LGA. Total population of the four LGAs was estimated to be 947,695 (2006 Census figures).

**Figure 1:** The selected Primary Health Centres (PHCs) in Ogun State.

**Study design, approval and laboratory techniques**

This study was undertaken between October 2012 and January 2013. Consent was obtained from the Chairmen and Medical Health Officers of the selected Local Government Areas. Three hundred and eighty-four (384) persons were selected using the stratified sampling method. Participants were clinically evaluated and referred to the laboratory for malaria diagnosis by microscopy and RDT analysis after consent by the participants and care givers in the study. The method of [18] was employed in blood collection by venipuncture technique. Thick and thin blood films were stained approximately using Giemsa’s staining method and analysed microscopically at 100× objective and the features in the thin film seen were used to identify *Plasmodium* species. The degree of parasitaemia was determined by counting and calculating the percentage of infected Red Blood Cells (RBC). Parasitized red cells in thick films was determined by counting a total of eight hundred (800) red cells taking note of the number that contained parasites
(excluding gametocytes) in at least five (5) fields on the thin blood film. A blood smear was considered negative if no parasite was seen after 10 minutes of search or examination under 100 high power fields of microscope. The study was blinded, since results of microscopy were not shared with the SD Bioline test until all samples were processed.

**Malaria diagnosis with SD Bioline**

All the samples were tested with the SD Bioline assay. The SD Bioline used was based on lateral flow immunochromatographic RDT in a cassette format. The cassette had three lines; a control line which indicated the validity of the test, a HRP-2 line that showed an infection with *P. falciparum* and a third that showed a Pan-line (pLDH) which indicates mixed infection of any of the *Plasmodium* species. A single indication revealed no infection with neither of the parasites. The test kits lot number was 090147 with an expiration date of 15 November, 2013. The readings were carried out at daylight within 15 to 30 minutes according to the manufacturer’s direction. One positive test line was recorded as positive for *P. falciparum*, two positive lines was captured as *P. falciparum* and second Plasmodium specie (*P. malariae*, *P. ovale* or *P. vivax*) and the non-detection of both test lines with control line only was recorded as negative.

**Data analysis**

Data from the microscopy and RDT methods were analysed using Statistical Package for Social Sciences (SPSS) version 16 for significant relationship at 95% confidence level. Sensitivity, specificity, positive and negative predictive value were calculated for *P. falciparum* and Pan.

**Results**

From the 384 persons examined for malaria parasite (Table 1), 273 were positive by microscopy, thus giving a prevalence of 71.1%. PHC Enugada had the highest prevalence of 84.4%, while PHC Obantoko had the least prevalence of 66%. The prevalence of malaria parasite was not significant among the different primary health centres studied (*p*=0.635). Abeokuta-North LGA had the highest mean intensity of 45.7%; Abeokuta-South LGA - 44.7%, Odeda LGA - 41.5% and Obafemi/Owode LGA - 39.5% (Table 2).

Males 110(73.8%) were more infected than females 163(69.4%) as presented in Table 3. Malaria prevalence according to sexes was significant (*p*=0.0347). The different species observed are presented in Figure 2. *P.falciparum* with 95.6% observation was the predominant parasite followed by *P. malariae* (3.3%), *P. ovale* (0.7%) and *P. vivax* (0.4%). The age-group 0-10 years had the highest malaria prevalence 94(81%) while the age group 51-60 years had the least 6(54.5%) as shown in table 3. The results were significant (*p*=0.011).

**Table 1: Malaria parasite prevalence by microscopy and RDT method in the different primary health centres**
**Figure 2:** Frequency of the different Plasmodia as observed from the study

A total of 273 (71%) was found to be positive by microscopy and 92(24%) by RDT. The comparative performance of the rapid test and standard microscopy showed that 64 RDT cases corresponded with microscopy and these are known as Number of True Positive (NTP) cases, the Number of True Negative (NTN) cases were 83. The Number of False Positive (NFP) cases where the RDT detected malaria parasite and the standard microscopy did not detect malaria parasites were 28 and the Number of False Negative (NFN) where the RDT detected negative and the standard microscopy detected malaria parasites were 209, (Table 4). The sensitivity and specificity of *P. falciparum* for RDT analysis was 29.9% and 79.8% respectively. The positive predictive value was 69.1% and the negative predictive value was 43%, (Table 4). The overall accuracy for the detection of *P. falciparum* by RDT was 38.3%. The sensitivity and specificity of *Pan* was 0% and 91.2%. Positive Predictive Value (PPV) and Negative Predictive Value (NPV) was 0% and 100%, (Table 5).

### Table 4: Comparative performance characteristics of rapid test and standard microscopy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Rapid Diagnostic Test</th>
<th>Microscopy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
</tr>
<tr>
<td>Microscopy</td>
<td>Positive</td>
<td>64 (NTP)</td>
<td>209 (NFN)</td>
</tr>
<tr>
<td>Total</td>
<td>Negative</td>
<td>28 (NFP)</td>
<td>83 (NTN)</td>
</tr>
<tr>
<td></td>
<td>92</td>
<td>292</td>
<td>384</td>
</tr>
</tbody>
</table>

### Table 5: Evaluation of SD Bioline using microscopy as the gold standard

<table>
<thead>
<tr>
<th>Parameters</th>
<th><em>P. falciparum</em></th>
<th>Pan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>29.9%</td>
<td>0%</td>
</tr>
<tr>
<td>Specificity</td>
<td>79.8%</td>
<td>91.2%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>69.1%</td>
<td>0%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>43%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Discussion**

The high rate of malaria prevalence in the blood samples examined was quite worrisome. This is a reflection of the high rate of malaria parasitaemia in endemic malaria regions. The overall malaria prevalence of 71.1 % found in the study area was higher than the 53.5% obtained in Ogun State by [15], and in other parts of Nigeria [20, 21, and 22]. However, the prevalence was lower than report by [14] which revealed an overall prevalence of 85.1% in Abeokuta; and [23] who in 2012 reported 80.4% in some parts of Abia State. Nigeria. This wide range of difference may be attributed to difference in climatic factors and behavioural patterns of people in the area which promote mosquito breeding and susceptibility of the people to vector bites.

The frequency of observations of *P. falciparum*, *P. malariae* and *P. ovale* recorded in the study are in agreements with previous studies [24], and confirms that *falciparum* malaria is almost entirely confined to the tropics and subtropics and is therefore not surprising that *P. falciparum* recorded the highest prevalence rate. In Tanzania, malaria prevalence studies conducted in urban areas of Dodoma and Iringa by [25] indicated that *P. falciparum* was the predominant malaria parasite (Iringa = 100%, Dodoma=97.8%). Also [26] recorded predominance of *P. falciparum* in the prevalence and intensity of malaria in blood donors in Nnewi, Anambra State, Nigeria. *Plasmodium vivax* is rare because this population lacks the Duffy blood group antigens on their red blood cells. The presence of *Plasmodium vivax* in the study area could have been as a result of migration by travellers.

Malaria prevalence among the sexes was statistically significant (*p*<0.05), as males were also more infected than the females. Studies had shown that females have better immunity to malaria and varieties of other parasitic diseases and this was attributed to hormonal and genetic factors ([27]). This may equally be attributed to the fact that males
expose themselves to the bites of mosquitoes and other vectors more than females, especially when the weather is hot and during farm work. Exception is found during pregnancy and reproductive ages, when females are more vulnerable to malaria attacks due to immune suppression [28]. Malaria prevalence was statistically significant in the various age groups. Children were more affected. This may be attributed to low-transferred maternal immunity or infection acquired through the mother.

It is expected that appropriate malaria RDT should have a high sensitivity (95%) and specificity (97%), and ability to detect low parasite density infections. This is contrary since this study observed a sensitivity of 29.9% and specificity of 79.8% of the product used. Mason et al., [29] and Gillet et al., [30] had observed in separate studies that the performance of SD Bioline can best be compared with other malaria RDTs by considering the antigens, HRP-2 and pLDH separately. The specificity of HRP-2 as reported in this study is in agreement with other studies except for the WHO recommendation. The loss in specificity as per WHO recommendation can however be as a result of self-treatment, because in our setting most participants may have treated themselves initially for malaria before coming for medicare to the primary health care facilities. This self-treatment might have cleared their parasitaemia, but have residual circulating HRP-2 antigens [31].

The low sensitivity recorded indicated a high rate of false negative results which was not expected. The high false negative results recorded in this study should not be glossed over since it has the potential for damaging the credibility of malaria control programmes. Also, failure to detect malaria parasitaemia could prevent a clinician from prescribing anti-malarial therapy. It is therefore important for the laboratories to understand the likely causes of false negative results in malaria testing using malaria RDT, especially in an endemic setting like ours.

The positive predictive value of 69.1% recorded is in the range of previous studies of 64 to 71% [32 and 33] and this outcome provides an argument to use the RDT as initial screening as the platform consider patients with a positive result as truly malaria infected patients and the clinicians can therefore proceed with the treatment. It can also be argued that in a highly endemic setting, the first treatment to be considered for most cases presented at the out-patient department is malaria treatment.

Although this RDT has been designed to detect both P. falciparum and other species infections and is being marketed as a species-specific combination test, the results obtained when compared with malaria microscopy, showed that RDT failed to pass this test due to false-positive and false-negative test results and inability to distinguish P. falciparum from other species. The product insert of the RDT used here claims to have 95.5% sensitivity and 99.5% specificity for the detection both of P. falciparum as well as P.f/ Pan Infection. However, our study conducted under actual field conditions gives a much lower sensitivity estimate for detection of P.f/ Pan infection at 0%, and a sensitivity estimate of 29.9% for detection of P. falciparum. Although all kits were stored at 4°C, it was not possible to avoid their exposure to temperatures >30°C consistently during the point-of-care testing in laboratory, a fact that could have affected the performance of RDT-based tests.

RDTs for malaria were developed to overcome the limitations of microscopy and to reduce irrational treatment of acute febrile illnesses. These had revolutionized malaria diagnostics, and made it possible for healthcare workers as well as patients to actually see the test results before treatment but the low sensitivity of the RDT in this study had renewed the call that gold standard still remain the best diagnostic method in determining malaria parasites in human population.

Acknowledgement

All Chairmen and Staff of the Local Governments, Medical Personnel of the primary health facilities
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