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EVALUATION OF COMMERCIALLY AVAILABLE RAPID DIAGNOSTIC TEST KITS FOR THE DIAGNOSIS OF PLASMODIUM FALCIPARUM INFECTION IN NIGERIAN CHILDREN

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ABSTRACT:
The use of rapid diagnostic tests (RDT's) as an alternative to microscopy for the diagnosis of malaria has been adopted as an effective diagnostic tool in many endemic countries. However, the differential sensitivity and specificity of these commercially available RDTs is a major challenge that affects their diagnostic accuracy. This study was conducted to evaluate the performance of three commercially available RDTs for their abilities to detect *Plasmodium falciparum* in Nigeria children. A total of 184 (71 males, and 113 females) children presenting with fever together with signs and symptoms of malaria at the outpatient unit of Ekiti State Teaching Hospital, Ado Ekiti in Nigeria, were recruited into the study. Blood was aseptically collected by venipuncture into EDTA bottle and tested for malaria infection by three RDT, microscopic examination and nested Polymerase Chain Reaction (PCR). The sensitivity, specificity and predictive values of the methods were calculated using PCR as the gold standard. The prevalence of malaria by nested PCR and microscopy was 79.9% and 77.7% respectively, while for the RDTs it was 66.8%, 73.4% and 69.0% for SD Bioline, Carestart, and Micropoint respectively. The sensitivity and specificity of microscopy was 95.8% and 75.0% respectively. The corresponding values for the RDTs were: SD bioline 84.4% and 29.5%; Carestat 83.7% and 30.6%; and for Micropoint 85.8%. Using Kappa coefficient as a measure of agreement, microscopy showed a high measure of agreement (k = 0.73) while each of the RDTs showed poor measures of agreement. The study concluded that diagnosis of malaria cannot completely rely on RDTs in our study area.

Keywords: Malaria, Microscopy, RDT, PCR, Children, sensitivity, specificity.

INTRODUCTION:

Malaria still remain a major cause of morbidity and mortality globally, inflicting significant and increasing burden on the global economy, with sub-Saharan Africa accounting for about 90% of all cases reported [1,2]. It is caused by one or more infections with *Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* with infection of *P. falciparum* resulting in most severe form of malaria. Currently the control of malaria primarily relies on early and reliable parasite - based diagnosis and reliable treatment [3, 4]. In most endemic countries including Nigeria, laboratory diagnosis is based on inexpensive and sensitive examination of stained blood smears under light microscope [5]. However, there are a number of shortcomings of microscopy; it is time consuming, labor intensive, the need for good expertise and highly experienced microscopist among others [6].

Many other methods including Polymerase Chain Reaction (PCR), immunochromatographic assay, mass spectrometry, and flow cytometry have all being described for the diagnosis of malaria [6,7]. They all have the disadvantages which include the requirement of expensive and sophisticated equipment which is not affordable for use in the endemic malaria communities in resource limited countries. To address the limitations of microscopy and PCR-based techniques, other methods are being explored. Fast diagnostic methods based on the detection of the parasite antigen using monoclonal antibodies incorporated into test strips called rapid detection tests (RDTs) have been introduced. Two major parasites antigens, histidine-rich protein 2 (HRP2) and the parasite lactate dehydrogenase (pLDH) are the target proteins for the numerous numbers of RDTs that are currently available commercially [8]. While quality RDTs are currently recommended as the standard diagnostic tools for routine malaria diagnosis, the sensitivity and specificity of these RDTs under different conditions and in different locations are still unclear [9]. RDTs have been shown to be useful in rapid diagnosis of malaria parasite in human blood when high quality microscopy is not readily available [10]. The world Health Organization (WHO) recommended that either RDT or microscopy should be used in diagnosing all suspected cases of malaria infection before treatment [11].

Generally, the HRP2 based RDTs have higher sensitivity for *P. falciparum* detection and they are also less expensive than the *Plasmodium* lactate dehydrogenase (pLDH) or Aldolase based RDTs which detects also the non-*falciparum* [12]. Since more than 80% of malaria in Nigeria is due to *P. falciparum*, it makes the HRP2 based RDT a more preferred test. One of the disadvantages

of the HRP2 based RDT is the persistence of the HRP2 protein in the blood for extended period after successful elimination of the parasite with an effective antimalarial treatment leading to false positive results and limited specificity [13,14]. In addition, the spread of HRP2 deleted parasites that produces false negative results may have a major negative impact on the sensitivity of PfHRP2-based RDTs in sub-Saharan Africa [15,16].

Many WHO recommended HRP2 RDT based kits are currently available and these kits are being used in hospitals and health centers where neither microscopic nor PCR test methods are available. In addition, home management of malaria (HMM) with treatment based on RDT results, is recommended to reduced unnecessary use of antimalarial as one of the strategies for improving access to prompt and effective malaria case management [17,18].

In this study, three HRP2-based commercially available RDTs; SD BIOLINE Malaria Ag Pf, (Standard Diagnostics, Seoul, Korea); CareStart™ Malaria HRP2 (*Pf*) (Access Bio, Inc, USA) and Micropoint *Pf*HRP-2 were assessed for their *P. falciparum* diagnostic performance. The RDTs were assessed in comparison with microscopy and PCR assay in order to provide information on the diagnostic performance of these commercially available RDTs in Nigeria.

METHODOLOGY:

This study was carried out at the Ekiti State Teaching Hospital, Ado-Ekiti situated in the south-Western geopolitical zone of Nigeria, with the city representing a typical urban setting in Nigeria. The study design was a double-blind clinical diagnostic assay of malaria using microscopy, three RDT kits which were purchased from a pharmaceutical shop at a city in Nigeria and nested PCR as the gold standard. The study was approved by the ethical committee of Ekiti State Teaching Hospital, Ado – Ekiti, and conducted in accordance with the Declaration of Helsinki of 1975.

Simple random sampling technique was used to recruited participants for the study. The study population comprised of children with clinical signs of malaria and for whom test for malaria parasite have been requested for by clinicians. Clinical diagnosis was based on fever (temperature = 37.5°C and above) or history of fever, alongside typical symptoms associated with acute malaria infection. Children whose parents/guardians willingly gave informed consent were recruited into the study.

Blood was aseptically collected into EDTA bottle from which RDT was performed with the three (SD- Bioline, CareStat, Micropoint) HRP2-based RDTs following the manufacturer's protocol [19].Thick blood smears were prepared and

stained with 10% Giemsa solution (Sigma-Aldrich, USA) for 30 minutes, allowed to air dry and subsequently examined using oil immersion objective lens. All the fields were examined and parasites counted against 200 White Blood Cells (WBCs) [10, 20]. Genomic DNA was extracted from whole blood using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) and nested PCR method was used for the amplification of the *P. falciparum* 18sRNA gene using protocol previously described [21]. The PCR products were visualized under UV light on 2% agarose gel after electrophoresis in 0.5X Tris borate EDTA buffer and ethidium bromide staining.

All data were analyzed using descriptive statistics. Statistical group analysis was performed with SPSS, version 16.0 for windows.

The sensitivity, specificity and predictive values of each of the methods (RDTs, microscopy) were calculated using PCR as the standard. Sensitivity was defined as the probability that a truly infected individual will test positive and specificity as the probability that a truly uninfected individual will test negative. Cohen's kappa coefficient was used to compare the measure of agreements between microscopy and the RDTs versus nested PCR results as the reference standard. All statistical analysis was calculated at 95% level of significance.

RESULTS:

A total of 184 children comprising of 113 (61.4%) females and 71 (38.6%) males were recruited into the study. The age range of the children was 0.5 -12years with a median age of ±6years and an interquartile range (IQR) of 6.7±0.3 years. The median weight and IQR was 21±1.5kg and 23.0kg respectfully. The mean body temperature was 38.3±0.5oC while the mean PCV of the participants was 25±1.0 % respectively.

The number of *P. falciparum* positive cases detected by microscopy was 77.7 % (143/184), while nested PCR detected 79.9 % (147/184). The three RDTs detected 66.8 % (SD-Bioline, 73.4% (Carestat) and 69.0 % (Micropoint) malaria positive cases (Table 1).

Table 2 shows the varied performance of each diagnostic test across the different age groups. The detection rate of all the diagnostic test was higher in younger children aged 0-5 years (Microscopy 78.4%; PCR 81.9%; SD Bioline 68.1%; Carestart 73.3%; Micropoint 70.7%) compared to older children aged 6-12 years (Microscopy 77.6%; PCR 76.5%; SD Bioline 64.7%; Carestart 75.3%; Micropoint 66.2%) but the differences were not statistically significant. Similarly, the prevalence based on gender detected by all the methods was not statistically significant though prevalence was higher among the females (Table 3).

Comparison of RDTs diagnostic methods with PCR and Microscopy

The comparison of the three commercially available RDTs selected for this study revealed a higher carestat result of 113 out of 147 detected by PCR when compared to the others. Both SD Bioline and Micropoint showed a higher negativity (17/37) rate agreement with PCR compared to Carestat (14/37). Table 4 shows the performance of the Three RDTs with respect to their positive and negative rates in comparison to PCR.

Sensitivity, specificity and the predictive values of each diagnostic method:

The sensitivity and specificity of RDTs and microscopy using PCR as the detection standard is shown in table 5. Microscopy had a higher sensitivity (95.8%), specificity (75.0%) Positive Predictive value (PPV) (93.2%) and Negative Predictive Value (NPV) (83.3%) compared to the three tested RDTs.

Among the RDTs, Micropoint had the highest sensitivity of 85.8% followed by SD Bioline and Carestart with 84.4% and 83.7% compared to nPCR. All the three RDTs had very low specificity compared to Microscopy. The RDTs specificity in descending order was 33.3%, 30.6% and 29.5% for Micropoint, Carestart and SD Biolone respectively (Table 5). Carestart had the highest PPV of 76.9% while Micropoint had the highest NPV of 51.4%.

Kappa coefficient was used to compare the agreement between microscopy and RDTs using PCR results as the gold standard. This study found a good agreement between microscopy and PCR (k=0.73) while among the RDTs the agreement with PCR was poor (SD Bioline k=0.16; Carestat k=0.16; Micropoint k=0.21).

Table 1: Results for Giemsa microscopy, PCR and malaria RDTs for the detection of *Plasmodium falciparum* (N = 184)

Methods	Positive, (%)	Negative, (%)
Giemsa microscopy	143 (77.7)	41 (22.3)
Nested PCR	147 (79.9)	37 (20.1)
SD Bioline	123 (66.8)	61 (33.2)
Carestat	135 (73.4)	49 (26.6)
Micropoint	127 (69.0)	57 (31.0)

Table 2: Prevalence of *P. falciparum* by age group based on different diagnosis methods

Methods	0-5-year (%) N = 116	6-10 years (%) N = 68	p-value
Microscopy	91 (78.4)	52 (77.6)	0.52
PCR	95 (81.9)	52 (76.5)	0.24
SD Bioline	79 (68.1)	44 (64.7)	0.38
Carestart	85 (73.3)	50 (75.3)	0.55
Micropoint	82 (70.7)	45 (66.2)	0.32

Table 3: Prevalence of *P. falciparum* by gender group based on different diagnosis methods

Methods	Male (%) N = 113	Female (%) N = 71	p-value
Microscopy	85 (75.9)	58 (81.7)	0.23
PCR	87 (77.0)	60 (84.5)	0.15
SD Bioline	74 (65.5)	49 (69.0)	0.37
Carestart	80 (70.8)	55 (77.5)	0.21
Micropoint	78 (69.0)	49 (69.0)	0.56

Table 4: Performance of the different RDTs in comparison to PCR and Microscopy

	RDT	PCR Positive (N = 147)			PCR Negative (N = 37)		
Microscopy		**Positive**	**Negative**	**Total**	**Positive**	**Negative**	**Total**
SD-Bioline	Positive	99	5	104	5	14	19
	Negative	38	5	43	1	17	18
Carestat	Positive	108	5	113	5	17	22
	Negative	29	5	34	1	14	15
Micropoint	Positive	103	6	109	4	14	18
	Negative	34	4	38	2	17	19

Table 5: Comparison of the sensitivity, specificity, positive predictive value, negative predictive value of microscopy and RDTs versus PCR

Method	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95 % CI)	Kappa
Microscopy	95.8 (92.5-99.1)	75.0 (61.6-88.4)	93.2 (89.1-97.3)	83.3 (71.2-95.5)	0.73
SD Bioline	84.4 (78.0-90.9)	29.5 (18.1-41.0)	70.6 (63.2-77.9)	48.6 (32.5-64.8)	0.16
Carestart	83.7 (77.5-89.9)	30.6 (17.7-43.5)	76.9 (70.0-83.7)	40.5 (24.7-56.4)	0.16
Micropoint	85.8 (79.8-91.9)	33.3 (21.1-45.6)	74.2 (67.1-81.2)	51.4 (35.3-67.5)	0.21

DISCUSSION:

This study evaluated the diagnostic performance of three commercially available RDTs and microscopy using nested PCR as the gold standard method for the diagnosis of *P. falciparum* among children. The sensitivities obtained for microscopy, SD Bioline, Carestart and Micropoint were 95.8%, 84.4%, 83.7% and 85.8% respectively. This confirms previous report that RDTs can be very useful and reliable in the management of patients with suspected malaria, especially in rural health centers where microscopic diagnosis cannot be readily performed and in community case management of malaria where treatment is provided by trained community health workers [22,23,24]. Although the sensitivity of these diagnostic methods is good and comparable the same cannot be said of their specificity. The specificity obtained for microscopy was relatively high (75.0%) but those of the RDTs were very low (SD Biolone 29.5%, Carestart 30.6% and Micropoint 33.3%). The implication of these results is that the true positive rate (sensitivity) of all the methods are good, the true negative rate (specificity) of the RDTs are poor. Approximately half of the samples that were negative were actually taken as positive by the RDTs. Studies have shown that HRP-2- based RDTs can be influenced by several factors, including antigenic variability of the target protein, antigen persistence in the bloodstream following elimination of parasites, parasite density below the RDT threshold of detection and parasites lacking hrp2 genes [16,25]. In malaria endemic areas where transmission is perennial, studies have shown that HRP2 antigen could persist in the bloodstream for more than 5 weeks after successful treatment [26,27].

Another possibility that cannot be excluded is that many individuals carried low parasite density that may not be detected by microscopy despite

the quality control like using double reading of two experience microscopist. These findings point towards the possibility of over diagnosis when using HRP-2 tests for the management of malaria in children living in area of intense and seasonal transmission. Besides all the aforementioned factors, RDTs performance could be affected by storage, handling and weather [28]. Generally, in Nigeria, RDTs are sold in many places with little or no proper good storage facility. Many sellers do not strictly follow the storage and handling conditions stipulated by the manufacturers. The low specificity observed in this study may therefore not be the true performance of these RDTs as the RDTs used for the study were purposely bought from pharmaceutical stores in the city.

Another important finding from this study is the high rate of malaria infection observed in the study population. Although the study was conducted during high malaria transmission period, it is still an indication that there is still high transmission of malaria in this area despite different malaria intervention that has been introduced [10]. The malaria infection rate was higher among younger children (0-5 years old), compared to older ones (6-10-years-old) although the difference in the prevalence was not statistically significant. This observation is consistent with the finding of some studies in endemic areas where it is well known that immunity to malaria increases with age [29,30]. In addition, male children were found to have a higher infection rate than their female counterparts. Several studies have reported similar findings, which may be due to female children being less biologically vulnerable to infectious diseases than male children [31,32].

In conclusion, our data showed high sensitivity of microscopy and commercially available RDTs but low specificity of the RDTs in the diagnosis of malaria in our study population. The implication of this is that overreliance of malaria diagnosis on RDTs may lead to misdiagnosis in the study area. There is need for a more coordinated malaria control approach by all stakeholders that will include making sure that available RDTs in the market are stored according to manufacturer's instructions. There must be a renewed interest from stakeholders to scale up the control effort in this area.

Conflict of interest: The authors declare that there is no conflict of interest.

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