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COMPARISON OF PERFORMANCE CHARACTERISTICS OF TWO MALARIA RAPID DIAGNOSTIC TESTS IN DETECTING MALARIA INFECTION AMONG FEBRILE PATIENTS IN URBAN AREA OF LAGOS, NIGERIA

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ABSTRACT

Antigen-based malaria rapid diagnostic tests (mRDT) play an important role in confirmation of malaria cases in all levels of healthcare in Nigeria because they are fast, easy to perform, and do not require special equipment or electricity. This study aimed at assessing the performance of two mRDTs in detecting histidine-rich protein 2 antigen in blood and urine of persons with uncomplicated falciparum malaria. Malaria parasite microscopy was the malaria diagnosis gold standard in this study. Matched blood and urine samples from 1,026 febrile persons were tested with two commercially available malaria antigen-detecting kits (SD Bioline® and First Response®). Based on blood samples, similar malaria positivity rates were obtained by microscopy 181 (17.6%), SD Bioline 186 (18.1%) and First Response 183 (17.8%). Detection of HRP2 antigens in urine was significantly lower than HRP2 detection in blood ($P<0.001$) regardless of brand of mRDT used [SD Bioline® (5.2% vs 18.1%) and First Response® (4.6% vs 17.8%)]. The performance characteristics of SD Bioline® and First Response® using blood samples were similar ($P>0.05$): sensitivity 96.7% vs 95.0%; specificity 98.7% vs 98.7%, Positive Predictive Value (PPV) 92.5% vs 94% and Negative Predictive Value (NPV) 99.3% vs 86.4%. SD Bioline ® and First Response® had low sensitivities (27.1% and 24.3% respectively) in detecting HRP2 in urine. Detection of HRP2 in urine and blood by the two kits was not dependent on the level of parasitaemia. The performance of the two brands of mRDTs in detecting HRP2 in whole blood and urine were similar ($P>0.05$). The two kits serve as good alternatives to malaria microscopy. The high NPV of both kits with urine specimen indicates that urine has the potential to be used for malaria but is not currently recommended to be used on blood-based malaria test kits.

Keywords: Histidine Rich Protein 2, Malaria Rapid Diagnostic Test, Performance characteristics, Urine.

INTRODUCTION

Malaria is caused by infection of red blood cells with protozoan parasites belonging to the genus *Plasmodium* transmitted by female Anopheles' mosquito (Regev-Rudzki *et al.,* 2013). Of the five species of *Plasmodium* that infect man (*P. falciparum, P. vivax, P. ovale*, *P. malariae* and *P. knowlesi), P. falciparum* is the most prevalent in Nigeria, accounting for over 90% of malaria infections (Federal Ministry of Health, 2015; Bichara *et al.*, 2017). In Nigeria, the burden of malaria is a major contributor to the economic burden of disease in communities where it is endemic and it is responsible for annual economic loss of 132 billion naira (Jimoh *et al.*, 2007; Rumun and Terungwa, 2015). In 2018, Nigeria accounted for 25% of global malaria cases, with over 56 million cases and 95,000 deaths (WHO, 2019a).

The common symptoms associated with malaria include fever, headache, chills, anorexia, vomiting, malaise and joint pains but these are non-specific as they are also observed in other infectious diseases caused by bacteria and viruses (Rasheed *et al.,* 2009; Tahita, *et al.,* 2013). Thus, clinical diagnosis of malaria leads to overdiagnosis of malaria in health facilities (Uzochukwu *et al.,* 2009; Oladosu and Oyibo, 2013). The World Health Organization has therefore recommended routine parasite-based confirmation of suspected cases of malaria by either microscopy or mRDT (WHO, 2015). In malaria endemic countries including Nigeria, mRDTs have been deployed by malaria control programmes as a tool for parasite-based diagnosis because they are fast, easy to perform, does not require special equipment or electricity, and can also be carried out by non-specialized

health care workers.

In Nigeria, it is estimated that 60% of outpatient visits and 30% hospitalizations are attributable to malaria (Federal Ministry of Health, 2015). In Nigeria, mRDTs have the capacity to improve the quality of malaria diagnosis in health facilities considering the high number of patients visiting the hospitals for care. High workload negatively affects the quality of malaria diagnosis by microscopy because it is subject to human error due to fatigue compared with mRDTs (Burchett *et al.,* 2017). The mRDTs bridge diagnosis gap in areas where malaria microscopy in not feasible, thereby checkmating the irrational use of antimalarial medicines. It is therefore important that mRDTs perform optimally, in terms of their sensitivity and specificity, to be relied on in health facilities for the rapid diagnosis of malaria.

Malaria antigen-detecting rapid diagnostic tests are based on the principle of immunochromatographic detection of malaria parasite antigen such as histidine–rich protein II (HRP-2) or specific Plasmodium lactate dehydrogenase (pLDH). The HRP-2 antigen is produced only by *P. falciparum* and is produced by all the stages of this malaria parasite species (Hayward *et al.,* 2000; Desakorn, 2005). Histidine-rich protein 2 is a soluble protein secreted into the blood stream, and is therefore detectable in an infected person even when parasites are sequestered in capillaries of deep tissues (Rubach *et al.,* 2012). In clinical malaria, patients shed elevated levels of proteins, including *Plasmodium falciparum* proteins such as HRP-2, in urine in detectable quantities (Genrich**,** *et al.,*2007; Ho *et al.,*2014). This study is aimed at evaluating two mRDTs recommended for use in Nigeria in detecting the presence of malaria antigens in blood and urine samples.

MATERIALS AND METHODS

Study Site

Matched blood and urine samples were collected from two study sites in Lagos: a missionary hospital in Amukoko, Ajeromi Ifelodun Local Government Area (LGA) and a secondary public health facility in Surulere LGA. Amukoko is a densely populated urban area of Lagos with population density of 687,316 and human population in Lagos as at 2019 population was estimated to be 13,903,620 (Twumasi *et al.,* 2020). Amukoko is located on the Longitude ($6^0$27' 52"N) and Latitude (3^0 20' 44" E). It is about 14 km from Lagos center, it is bounded on the North by Apapa LGA and on the East by Amuwo–Odofin LGA. Amukoko is the 50^{th} community instituted in Ajeromi–Ifelodun LGA of Lagos state. Surulere General Hospital, Randle is located at the Surulere LGA, Lagos on the Longitude (6.5082^0 N) and Latitude (3.3574^0 E) with an Area of 23-kilometer squares and 503,975 inhabitants with a population density of 21,864 inhabitants per square kilometer, (Twumasi *et al.,* 2020).

Study Population

Febrile patients with axillary temperatures ≥37.5°C or history of fever in the last 48 hours attending the out-patient clinics of the two clinics and who consented to the study were recruited into the study. Patients who did not meet the inclusion criteria and who had signs suggestive of severe malaria or other severe illness were excluded from the study.

Sample collection

Matched blood and urine samples were collected from suspected malaria cases. Prior to sampling, the axillary temperature of each patient was measured with the aid of a mercury-type clinical thermometer. The age, sex and history of fever in last 48 hours of study participants were recorded. Two milliliters of venous blood from each patient was collected in EDTA vacutainer for malaria diagnosis and determination of total white blood cell (WBC) count. Urine was collected into a sterile universal container from each of the study participants. Thick and thin blood films made on same slide were stained with 3% Giemsa stain and examined for the presence of malaria parasites as described by WHO (2010). At the start of each test procedure, individual patient blood samples were thoroughly mixed and tested for malaria antigen (HRP2) reactivity separately with SD-Bioline and First Response mRDT kits. Positive (200 parasites/μl of blood) and negative malaria parasite control panels were run along with the samples. The laboratory workup was carried out at the International Centre for Malaria Microscopy, College of Medicine, University of Lagos, Idi – Araba, a WHO Quality Assurance Centre for

mRDTs and Malaria Microscopy.

Ethical consideration

The study was approved by the Health Research Ethics Committee of the College of Medicine, University of Lagos, Lagos, Nigeria. All the study participants consented to the study before they were recruited into the study.

Data analysis

The performance characteristics of the mRDTs were determined by calculating sensitivity, specificity, positive and negative predictive values (Kyabayinze *et al.,* 2008).

$$\text{Sensitivity} = \frac{True\ positive}{True\ positive + False\ Negative}\ x\ 100$$

$$\text{Specificity} = \frac{True\ Negative}{True\ Negative + False\ Positive}\ x\ 100$$

Positive Predictive Value (PPV) =

$$\frac{True\ positive}{True\ positive + False\ Negative}\ x\ 100$$

Negative Predictive Value (NPV) =

$$\frac{True\ Negative}{True\ Negative + False\ Positive}\ x\ 100$$

Personal identifiers were removed from the data before entry into a Microsoft Excel and replaced with codes. Access to study data was limited to the investigators. Data were analyzed using EPI INFO version 3.5.1. Proportions were compared using chi square. P-values less than 0.05 were considered significant at 95% confidence interval.

RESULTS

Socio-demographic characteristics of study participants

A total of 1,026 participants were enrolled into this study. There were more females 624 (60.8%) than males 402 (39.2%). The mean age of the study participants was 25.5±16.7 years. Most of the participants were aged 20-29 years 271(26.4%), followed by those aged 30-39 years, 164 (16.0%). The least age group was children aged 5-9 years, 81(7.9%).

Table 1: Baseline Demographic Characteristics of Study Participants

Character	N (%)
Number of participants	1026
Age (years)	
Mean ± SD	25.5 ± 16.7
<5	144 (14.0)
5-9	81 (7.9)
10 -19	138 (13.5)
20 – 29	271 (26.4)
30 – 39	164 (16.0)
40 – 49	119 (11.6)
≥ 50	109 (10.6)
Gender	
Male	402 (39.2)
Female	624 (60.8)

Malaria prevalence in the study population

Malaria prevalence varied widely based on type of sample analyzed regardless of the type of diagnostic method. Malaria prevalence based on microscopy, the gold standard, was 17.6%. The prevalence based on SD-Bioline and First Response malaria antigen test kits using blood specimen were 18.1% and 17.8% respectively. Lower prevalence rates were obtained using urine as specimen for malaria diagnosis on blood-based mRDT [SD Bioline 5.2%; First Response 4.6%] compared to using blood samples.

Table 2: Malaria Prevalence by Microscopy and the two mRDTs

Malaria test	Specimen Used	Malaria Prevalence (%)
Microscopy	Blood	181 (17.6)
SD-Bioline mRDT	Blood	186 (18.1)
First Response mRDT	Blood	183 (17.8)
SD-Bioline mRDT	Urine	53 (5.2)
First Response mRDT	Urine	47 (4.6)

Kit Sensitivity and specificity

The sensitivity of SD Bioline and First Response malaria test kits using whole blood as diagnostic specimen were 96.7% and 95.0% respectively while their specificity was the same, 98.7%.

Table 3: Performance of SD Bioline and First Response using Blood Sample

		Microscopy			Performance characteristics			
Kit type		Neg	Pos	Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
SD Bioline	**Neg**	834	6	840	96.7	98.7	94.1	99.3
	Pos	11	175	186				
	Total	845	181	1026				
First Response	**Neg**	834	9	843	95	98.7	94	98.9
	Pos	11	172	183				
	Total	845	181	1026				

The sensitivity for SD- Bioline and First Response using urine as diagnostic specimen were 27.1% and 24.3% respectively while their specificity was 99.5% and 99.6% respectively.

Table 4: Performance of SD Bioline and First response Using Urine Sample

		Microscopy			Performance characteristics			
Kit type		**Neg**	**Pos**	**Total**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
SD Bioline	**Neg**	841	132	973	27.1	99.5	92.5	86.4
	Pos	4	49	53				
	Total	845	181	1026				
First Response	**Neg**	842	137	979	24.3	99.6	93.6	86
	Pos	3	44	47				
	Total	845	181	1026				

Comparison of kits

The comparison of the performance characteristics of the two brands of mRDT showed that the two mRDT brands were similar (P = 0.05) in terms of sensitivity, specificity, PPV and NPV.

Table 5: Comparison of the performance of SD Bioline and First Response in detecting HRP2 antigen from Blood and Urine

Performance	Blood			Urine		
	SD Bioline	First Response	P	SD Bioline	First Response	P
Sensitivity (%)	96.7	95	0.429	27.1	24.3	0.548
Specificity (%)	98.7	98.7	-	99.5	99.6	1.00
PPV (%)	94.1	94	0.967	92.5	93.6	0.869
NPV (%)	99.3	98.9	0.441	86.4	86	0.784

However, there were significant differences (P<0.001) when the sensitivity and NPV values of the two mRDT brands obtained from analyzing blood and urine specimen were compared. The values of specificity of SD Bioline obtained from analyzing blood and urine were similar (p = 0.082) but this was not the case with First Response (p = 0.044).

Table 6: Comparison of HRP-2 Detection in Blood and Urine using SD Bioline and First Response

Performance	SD Bioline			First Response		
	Blood	Urine	P	Blood	Urine	P
Sensitivity (%)	96.7	27.1	<0.001	95	24.3	<0.001
Specificity (%)	98.7	99.5	0.082	98.7	99.6	0.044
PPV (%)	94.1	92.5	0.911	94	93.6	0.805
NPV (%)	99.3	86.4	<0.001	98.9	86	<0.001

Rapid kits comparison with Gold standard

The detection of HRP2 in parasitized blood or urine specimen by the two brands of mRDT was not dependent on the parasite density. The detection of HRP2 in blood was >90% at 1-200 parasites/µl of blood and equally have highest detection positivity of malaria antigen at parasitemia range ≥500 parasites/µl of blood. However, the detection of HRP2 in urine were low irrespective of the type of kit used as there are irregularities in the detection positivity of malaria antigen at different ranges of parasite densities

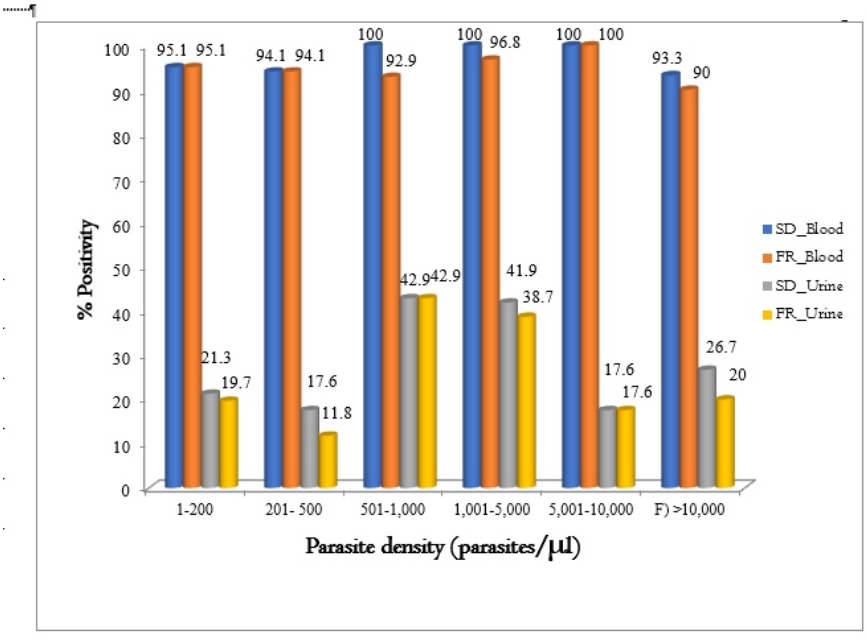

Figure 1: Detection of Plasmodium falciparum HRP2 antigen from blood and urine using mRDT kits based on parasite density

DISCUSSION

The prevalence of malaria among febrile patients in this study based on microscopy (parasite density) was 17.6%, however, the prevalence based on the RDTs were similar to the rate determined by microscopy. The prevalence reported in this study was lower than the reports of Oladosu and Oyibo (2013) and Orish *et al.* (2016) who reported 20.7% and 21.3% prevalence respectively, among febrile children aged 0-12 years. The low prevalence might be as a result of scale up of malaria intervention programmes in Lagos state in the last 5 years. Another possible reason could be the difference in the study populations. In our study, study participants included children and adults while Oladosu and Oyibo (2013) and Orish *et al.* (2016) worked with children ≤12 years. The inclusion of adults who have been noted to have considerably lower malaria prevalence than children could have brought down the prevalence reported in this study (Aina *et al.,* 2013; Olukosi *et al.,* 2018).

The use of other body fluids other than blood for the detection of malaria parasites would bring a great relief to communities with blood taboos (Mharakurwa *et al.,* 2006). Other workers have experimented with detection of malaria infection with other body fluids. Anchinmane and Shedge (2016) detected malaria antigens in urine using malaria RDTs. Mharakurwa *et al.* (2006) reported that *P. falciparum* infection can be detected in urine and saliva using polymerase chain reaction method. Katzin *et al.* (1991) detected malarial antigens in urine by Western blot method. In this study, HRP2 antigens were detected in urine of persons infected with falciparum malaria, although the positivity rate was much lower than HRP2 detection in blood, hence, using urine for malaria parasite detection in persons infected with falciparum malaria in place of blood is not recommended.

In this study, the performance characteristics of SD-Bioline and First Response in malaria diagnosis were similar regardless of the specimen used. This finding is consistent with the prequalification assessment of malaria test kits conducted by the Federal Ministry of Health, which led to their recommendation for use in routine diagnosis of malaria in Nigeria (Federal Ministry of Health, 2015). The significantly lower sensitivity of blood-based malaria test kits when urine is used has also been reported by other workers. Genton *et al.* (1998) reported 84% sensitivity for a blood based mRDT (ParaSight®-F test) with blood and 26% with urine as specimen for malaria testing. Similar findings were also reported by Buppan *et al.* (2010), Anchinmane and Shedge (2016) and Samal *et al.* (2017). The lower sensitivity might be due to the degradation or proteolytic cleavage of HRP2 excreted in urine (Ehrich *et al.,* 1985; Mharakurwa *et al.,* 2006). The positive predictive value of the two kits were similar regardless of the specimen used. A high PPV of >90% is an indication that urine has good potentials of being a specimen for malaria diagnosis. Similar findings were also reported by Buppan *et al.,* (2010) and Samal *et al.,* (2017). An improvement in the sensitivity of the kits may enable low levels of malaria antigen found in urine to be detected (Genton *et al.,* 1998).

The high specificity of the kits using blood specimen observed in this study was similar to the reports of other workers who reported high specificity of over 97% for SD Bioline (Tadese et al., 2016; Azazy et al., 2018) and over for 93% for First Response (Ghouth et al., 2012; Ayogu et al., 2016). This is one of the strong points of mRDTs that led to recommendation of the use of mRDT in malaria endemic areas for routine malaria diagnosis. As malaria prevalence drops locally and globally (WHO, 2019a), the contribution of malaria to number of febrile cases also drops. It is therefore important to exclude malaria from other febrile illnesses in order to improve the rational use of antimalarial medicines.

In this study, a linear relationship between malaria positivity and level of parasitaemia was not observed in blood and urine specimen of parasitized individuals. The secretion of HRP2 antigen is not uniform across the different strains of *P. falciparum*. Some strains of *P. falciparum* do not express HRP2 protein and may cause false-negative results with HRP2-based mRDTs (WHO, 2019b). Occasionally, high levels of HRP2 are expressed during early stage of asexual cycle alongside a fast rise in parasite concentration at the ring stage, then a slower buildup at both trophozoites and schizonts stages (Howard *et al.,*

1986 and Desakorn, 2005). Immature gametocytes express HRP2 antigens much more than mature gametocytes (Hayward *et al.,* 2000; Zanghi *et al.,* 2018). The sensitivity of HRP2-based kits is also improved by cross-reaction with HRP3 antigen, an antigen homologous to HRP2 (Beshir *et al*., 2017). This property may be responsible for detection of malaria at low parasitaemia.

CONCLUSION

SD-Bioline and First Response malaria antigen test kits can serve as alternatives to malaria microscopy. The performance characteristics of the two mRDTs in the detection of HRP2 antigens in blood and urine were similar. The high negative predictive value of both kits when urine specimen was used indicates that urine has the potential to be a diagnostic specimen for malaria if there is the possibility of increasing the sensitivity of these tools to detect low levels of HRP2 in urine. The detection of HRP2 antigen was not dependent on the level of parasitaemia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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