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Performance of hrp-2 Based Rapid Test and Microscopy in the Diagnosis of *Plasmodium falciparum* in Benue State, Nigeria

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ABSTRACT

Microscopy and RDTs are the most frequently used diagnostic tools for malaria case detection and management in every malaria endemic community. Prompt and accurate diagnosis of plasmodium infections is important for malarial case containment. The requirement of expertise and difficulties in the identification of malarial parasites using microscopy has made RDTs popular as they are rapid and easy to perform with minimum expertise. Most RDTs for malaria case management in Nigeria target the histidine rich protein-2 antigen (PfHRP2) to detect Plasmodium falciparum. This study was therefore conducted between April and August, 2018 to evaluate the performance of HRP-2 based RDTs and microscopy in Benue State, Nigeria. Finger prick blood samples were collected from 510 volunteered participants. Giemsa stain thick films and pfhrp2 based RDTs kits were used to determine malaria parasites and plasmodium antigens in blood samples respectively. The occurrence of malaria by microscopy was higher (89%) compare to RDT (21%) (P>0.05). Female had the highest malaria infection (89.9%) by microscopy while male had the highest (45.8%) malaria infection by RDT, in all there was no significant difference in the infection rates (P>0.0%).There was no significant difference in the distribution of malaria across the different age groups for both microscopy and RDT (P>0.05). HRP2 based RDT failed to identify 78.6% cases of malaria identified by microscopy resulting to false negatives. Compared to microscopy, Ag Care Start ™ PfHRP2 based RDT had a poor sensitivity (21.4%) and specificity (82.1%) which does not meet the WHO recommendation of the minimum of 95% sensitivity and 97% specificity for RDTs. HRP2 based RDT missed 78.6% of malaria cases detected by microscopy. The poor performance of HRP-2 based RDT (Care Start ™ malaria PfHRP-2 Ag RDT) in this study area could be as a result of exposure of the kits to high temperatures (>30°) or deletion of hrp2 genes. There is need to investigate the role of malaria RDT storage conditions and HRP2 gene deletion with respect to malaria RDT performance in the study area.

KEYWORDS: *Performance, Diagnosis, Plasmodium falciparum, Microscopy, Rapid diagnosis test*

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I. INTRODUCTION

Malaria remains one of the most deadliest infectious diseases of humanity, which causes significant mortality and morbidity than any other parasitic infection in the tropics, particularly in Africa (Skeet, 2005). WHO recommended that all

suspected malaria cases should be confirmed with a parasite base diagnostic assay before treatment(WHO, 2010). Accurate and early diagnoses are very crucial and have become increasingly important both for individual case management, disease surveillance and effective treatment of malaria (McMorrow *et al.*, 2011; Landier *et al.*, 2016). Accurate diagnosis is also vital to enable identification of malaria negative patients in order to avoid wrong use of anti-malarial drugs which could encourage drug resistance (WHO, 2013; Mfuh *et al.*, 2019). Before the advent of malaria rapid diagnostic tests (RDTs), diagnosis of malaria was based on microscopy of thick blood smears with the quantification and differentiation of *plasmodium species* at affordable cost (Souse-Figueiredo *et al.*, 2012). However, the major drawback of routine microscopy in malaria studies is the requirement for expertise in the parasite identification, lack of equipment and electricity (Haditsch, 2004; Bell *et al.*, 2006). The challenges of microscopic diagnosis has necessitated the use of rapid diagnostic test (RDT) to ensure prompt and early diagnosis, processing with minimal expertise, very high specificity and sensitivity (McMorrow *et al.*, 2011; Wongsrichanalai, 2001).

A number of most widely used RDTs are based on detection of the histidine rich protein 2 (HRP2) product of the pfhrp-2 gene (Baker *et al.*, 2005). The diagnosis of *P. falciparum* malaria using PfHRP2 as *P. falciparum* antigen has been widely accepted as a rapid antigen test (Onyenekwe *et al.*, 2007). Hrp2 based malaria RDTs have the abilities to improve the quality of malaria case management, surveillance and reduce morbidity and mortality, especially in remote areas (McMorrow *et al.*, 2011; Berzosa *et al.*, 2018). The test performance of these kits is largely dependent on the design and quality characteristics, such as detection sensitivity, specificity, insufficient quantity of antibodies used in the RDT and thermal stability (Cheng *et al.*, 2014). The performance of HRP2 based malaria RDTs in Nigeria for malaria diagnosis has not been consistent and a few studies have investigated the use of HRP2 antigen based RDTs with reported sensitivity of 23.4% to 97.7%, among which were false negative samples (Oyeyemi *et al.*, 2015; Otuu *et al.*, 2015; Ukpai and Odukaesieme 2014; Adekunle *et al.*, 2014; Adefolalu *et al.*, 2013). False negative RDTs are dangerous to malaria case management since it will delay antimalarial treatment, potentially endangering life and the patient will be a source for on-going malaria transmission (Cheng *et al.*, 2014).

RDTs can only be a useful diagnostic tool if it achieves greater than 95% sensitivity and specificity of 97% (WHO, 2000). Most health facilities in this part of the country use HRP2 based RDT kits for malaria case detection. This study therefore evaluated the performance of HRP-2 based rapid diagnostic tests using microscopy as a gold standard among participants attending selected health care facilities in Benue State, Nigeria.

II. METHODOLOGY

Study Area and Study Design

Benue State is located in the North Central Zone of Nigeria. The second largest river in Nigeria (River Benue) is found within the Benue valley. Rain fall is between 900- 1000 MM annually. Rainy season starts from April to October while dry season starts from October to March. There are intensive agricultural activities during the rainy seasons (Amuta *et al.*, 2015). A cross sectional study was carried out in Makurdi, Katsinal- Ala, and Otukpo Local Governments. This study was conducted between April and August, 2018. One general hospital was chosen in each of the selected Local Government Areas of Benue State, Nigeria.

Sample Size

A total of 510 volunteered participants were included for the study. One hundred and fivety three (153) from Makurdi, one hundred and seventy (170) from Katsina Ala and one hundred and eighty seven (187) from Otukpo.

Microscopic Examination of Thick Blood Films

Both sexes and all ages attending the selected health care facilities in Benue State, Nigeria were included in the study. Thumb prick blood samples were obtained from the volunteered participants for the study. Thick blood films were prepared as described in Ojurongbe *et al.* (2013). The slides were stained with 10% Giemsa for 10 minutes; air dried and examined using oil immersion with a high power objective lens (X100) for characteristic features of malaria parasites.

Rapid Diagnostic Test (RDT)

The HRP-2 based RDT (Care Start ™ malaria PfHRP-2 Ag RDT) was performed according to the manufacturer's instructions. A test strip coated with the central histidine-rich repeat region of HRP2 was used. A drop or 50 µl of blood samples from the site of the finger prick were obtained with the capillary tube and applied to the test strip and

developed using the buffer provided by the manufacturer. Test strips were examined and interpreted after 20 minutes.

Informed Consent and Ethical Approval

Informed consent was obtained from the volunteered participants and ethical approval with reference number MOH/STA/204/VOL.1/79 was obtained from the Benue State Ministry of Health and Human Services.

Data Analysis

Chi-square analysis was used to determine the significant difference in the malaria infection rate between the two diagnostic tools (RDT and microscopy). The infection rate between male and female and between the age groups was also determined using the same statistical tool. In all, P values less than 0.05 were considered statistically significant. Diagnostic performance was also determined by calculating sensitivity, specificity, negative and positive predictive values.

III. RESULTS

Table 1 shows malaria distribution base on sex and age groups. Females were slightly more infected (89.9%, 214/238) compared to the males (88.2%, 240/272) using microscopy while males were more infected (54.2%) than female (45.8%) using RDT. The differences in the infection rate among gender were not statistically significant (P> 0.05). For age distribution, the teenagers (11- 20 years) were more infected (89.6%, 412/460), followed by adults (>21 years) (85.4%, 35/41) and school age children (1- 10 years) with 77.8% (7/9) prevalence. There was no significant difference (x^2=2.33, df= 4, P>0.05) in the malaria infection rate among the different age groups by microscopy.

Table 1: Distribution of *P. falciparum* by Microscopy among Subjects in Relation to Sex and Age

	No. Examined	No. Positive by Microscopy (%)	No. Positive by RDT (%)
SEX			
Female	272	240 (88.2)	58 (54.2)
Male	238	214 (89.9)	49 (45.8)
Total	**510**	**454 (89)**	**107 (21.0)**
AGE			
1-10	9	7 (77.8)	2 (1.9)
11-20	460	412 (89.6)	91 (85.0)
>21	41	35 (85.4)	14 (13.1)
Total	**510**	**454 (89.0)**	**107 (21.0)**

Sex: x^2= 1.95, df= 2, P> 0.05 (Microscopy) and **Sex:** x^2= 2.39, df= 2, P> 0.05 (RDT)

Age: x^2=2.33, df= 4, P>0.05 (Microscopy) and **Age:** x^2=19.06, df= 4, P>0.05 (RDT)

The overall prevalence of malaria infection by microscopy was 89% (454/510) and 21% (107/510) by HRP-2 based RDT. Though, there was a high variation in the prevalence rate among the two diagnostic tools, however, there was no significant difference (x^2=0.98, df=2, P> 0.05). Based on microscopy, General Hospital Otukpo had the highest prevalence (90.9%, 170/187), followed by General Hospital Makurdi (88.2%, 135/153) and General Hospital Kastina- Ala (87.6%, 149/170) as shown in **Table 2**.

Table 2: Prevalence of *Plasmodium falciparum* by Microscopy and RDT in the Study Area.

General Hospital	Number Examined	No. Positive By Microscopy (%)	No. Positive By RDT (%)
Makurdi	153	135 (88.2)	37 (24.2)
Katsina Ala	170	149 (87.6)	32 (18.8)
Otukpo	187	170 (90.9)	38 (20.3)
Total	510	454 (89.0)	107(21.0)

A number of subjects with true positive, true negative, false positive and false negative results are presented in **Table 3**. The percentage of false negative 70% (357/510) subjects was high while false positive was low 1.9% (10/510). The performances of the Care Start ™ malaria *P.f.* (HRP-2) Ag RDT kit was expressed by calculating the sensitivity, specificity, positive and negative predictive values for *P. falciparum,* taking microscopy result as a gold standard as shown in **Fig 1.**

Table 3: Comparative Performance Characteristics of Rapid Test and Standard Microscopy

Parameters	Microscopy		
	Positive	Negative	Total
RDT Positive	97 (TP)	10 (FP)	107
Negative	**357 (FN)**	46 (TN)	403
Total	454	56	510

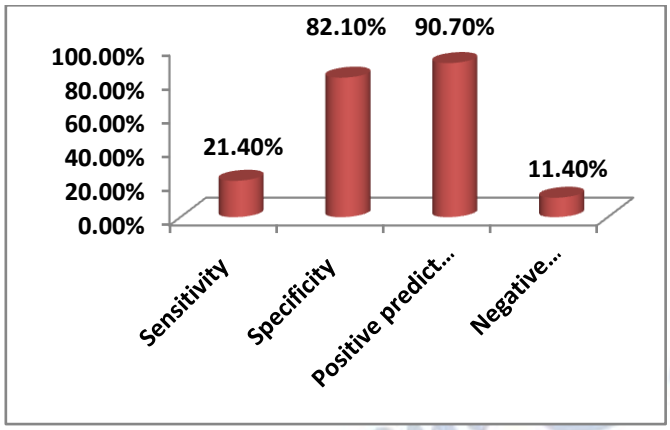

Fig 1: Evaluation of Care Start ™ malaria *P.f.* (HRP-2) Ag RDT using Microscopy as the Gold Standard

IV. DISCUSSION

Accurate and prompt diagnosis of malaria is the only way to effectively treat, manage and eventually eliminate malaria in our communities. RDT and microscopy are the most frequently used malarial diagnostic tools; hence the need to evaluate the performance of HRP2 based RDT and microscopy. Microscopy was able to identify 89% cases of malaria while HRP2 based RDT identified only 21%.HRP2 based RDT missed 78.6% (357/454) of malaria cases detected by microscopy. The poor performance of HRP2 malaria RDT compared to microscopy could be due to storage conditions that were not suitable for the RDT kits. The monoclonal antibodies that are used to bind antigen and produce test result are reported to be sensitive to a combination of heat and humidity, when not protected by appropriate packing, transportation and storage (McMorrow *et al.*, 2011). The parasite factor, environmental factors, operational factor, design and quality characteristics of the RDT kits used in the study area could also explain the high variation in the frequency of infection between the two diagnostic tools (Cheng *et al.*, 2014). The high rate of positive smear in this study was consistent with previous studies in Abuja (Otuu *et al.*, 2015) Ogun (Adekunle *et al.*, 2014) and Osun (Oyeyemi *et al.*, 2015). In contrast, the prevalence in this study is higher than the 14.6%, 31% and 39.6% reported in Lagos (Olaniyan *et al.*, 2013) Cameroon (Mfuh *et al.*, 2019) and Sokoto (Bala *et al.*, 2014). The 21% prevalence of malaria by HRP-2 base RDT correlated with other studies in Ogun (Adekunle *et al.*, 2014), Osun (Oyeyemi *et al.*, 2015); Lagos (Olaniyan *et al.*, 2013) but lower than others in Abuja (Otuu *et al.*, 2015) and Niger (Adefolalu *et al.*, 2013).

The slightly higher infection rate among female compare to males by microscopy suggest that both sexes had equal chances of infection except during pregnancy for the females during which they have suppress immunity (Predhan and Ghosh, 2013; Mclean *et al.*, 2015). There were high variations in infection rates across gender and age groups by microscopy and RDT. Teenagers between the ages of 11- 20 years had the highest infection (89.6%), while the ages between I – 10 years had the least malaria infection rate of 77.8% by microscopy.

The false positive cases detected in this study could be due to the presence of pfhrp2 antigen that persists in peripheral blood stream for weeks after parasites clearance (WHO, 2013). High numbers of false positives had also been identified in Nigeria (Adekunle *et al.*, 2014) and Ghana (Amoah *et al.*, 2016). High percentage 70% (357/510) of participants were diagnose negative by RDT but confirmed positive by microscopy. The 70% RDT false negative results in this study could be essentially linked to isolates with low parasite density (Trouvay *et al.*, 2013), excess of antigen as well as antibodies that block the detection antibodies target sites (Gillet *et al.*, 2009), the prozone effect (Maltha *et al.*, 2012), and possible deletion of HRP2 gene in the sample (Gamboa *et al.*, 2010; Koita *et al.*, 2012).

WHO (2000), recommended the minimum of 95% sensitivity and 97% specificity for malaria RDTs to be considered an effective diagnostic tool. Contrary to World Health Organisation recommendation, this study recorded a low sensitivity of 21.4% and specificity of 82.1% for Care Start ™PfHRP-2 RDT kit. The poor sensitivity of the malaria RDT in this study portends a great danger for malaria case management. This poor RDT sensitivity will cause continuous malaria transmission, since much malaria infected cases in the population will be left untreated. Most health facilities usually rule out malaria in patients that test negative with RDT kit without further confirmation. However, the present experience suggests that such a practice at this point of care will dimishe treatment efforts and endangers malaria control. Low sensitivity and high false negative cases were recorded in Osun (Oyetunde *et al.*, 2015) and Ogun (Adekunle *et al.*, 2014).

V. CONCLUSION

The performance of malaria HRP2 based RDT indicated low sensitivity with high rates of false negative results. It is possible that other factors like

storage conditions, high temperatures, prozone effect and deletion of hrp2 gene may have contributed to the low sensitivity obtained in this study. Further studies that aim to elucidate the role of storage conditions, high temperatures, prozone effect and deletion of hrp2 gene are needed to explain the low performance of HRP2 malaria RDT observed in this present study.

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