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INVESTIGATION OF THE DIAGNOSTIC ACCURACY OF MALARIA RAPID DIAGNOSTIC TEST IN PATIENTS WITH RECURRING MALARIA SYMPTOMS

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ABSTRACT

Accurate diagnosis and proper treatment are central to breaking malaria transmission cycle and eradication of malaria especially in an endemic country as Nigeria. Malaria rapid diagnostic test (RDT) is a simple and effective diagnostic tool. There is rise in the use of RDT in malaria diagnosis in Nigeria, however, it's accuracy in patients with recurring malaria symptoms is unclear. This study evaluated the efficacy of *Plasmodium falciparum* histidine-rich protein 2 based rapid diagnostic test (RDT) in the diagnosis of malaria in patients with recurring malaria symptoms. Microscopy was used as the reference method for diagnosis. Giemsa and Field stained thick and thin smear were used to count and detect malaria parasite. The results showed that out of 100 participants examined, a total prevalence of 85 percent (Giemsa stain)/ 83 percent (Field's stain) was recorded for microscopy while and 36 percent was recorded for RDT. Additionally, we observed that there was no significant difference ($p>0.05$) in the parasite density observed in both Giemsa and Field's staining technique. Conclusively, our study revealed that RDT is not effective in diagnostic tool in patients with recurring malaria symptoms, therefore, microscopy is still the gold- standard for malaria diagnosis.

Keywords: Malaria, *Plasmodium falciparum*, Rapid diagnostic test, Microscopy

INTRODUCTION

Malaria remains a global public health concern especially in sub-Saharan Africa. An estimated 249 million new cases and 608,000 deaths was reported in 2022 (WHO, 2023). Despite interventions like vector control, treatment, and vaccines, malaria continues to pose a considerable risk with heightened morbidity and mortality (Okagu *et al*., 2021; Omar *et al*., 2021). Four of the African countries account for nearly half of all malaria cases worldwide - Nigeria (26.6%), the Democratic Republic of Congo (12.3%), Uganda (5.1%), and Mozambique (4.1%) (WHO, 2023). Nigeria and partners have implemented mass long-lasting insecticidal nets (LLINs) campaigns, replacement campaigns, intermittent preventive treatment, and expanded malaria case management, using rapid diagnostic test (RDT) for diagnosis. (Fagbamigbe *et al*., 2019) yet 97% of Nigeria population is at risk of Malaria.

Accurate malaria diagnosis is essential for malaria eradication in Africa. World Health Organisation (WHO) recommended basing malaria treatment on parasite diagnosis (Bharti *et al*., 2016; WHO, 2018). However, in malaria control and elimination, accurately diagnosing the disease remains a significant hurdle that varies in effectiveness depending on the specific epidemiological setting (Mahende *et al*., 2016). Malaria diagnosis is primarily accomplished through microscopy due to its ability to detect active malaria cases, determine parasite density, and evaluate treatment effectiveness (Oboh *et al*., 2021; WHO, 2018). Malaria parasites in research settings are detected, identified, and quantified using stained thick and thin blood smear protocol as described by WHO (WHO, 2015). However, the use of microscopy has some limitations. These limitations include: operator dependence, the ongoing need for thorough training, the use of substandard reagents and poor power supply as experienced in Nigeria. Therefore, in resource-poor countries, implementing quality assurance practices can be challenging (Anchinmane *et al*., 2011; WHO, 2018).

Current guidelines for malaria treatment by the WHO require parasitological confirmation by malaria RDT (RDT) and/or microscopy (WHO, 2015). RDT uses immune chromatographic materials impregnated with monoclonal antibodies against *Plasmodium* species to detect malaria parasite antigen in the blood of infected individuals. The most commonly used RDT kits target histidine-rich protein 2 (HRP-2) antigen (Gillet *et al*., 2011). In endemic countries, the use of mRDT kits is widespread, due to quick results, affordability, and minimal training needed for outcome interpretation (Mayxay *et al*., 2004). Interestingly, reliability on RDT, have increased in settings with power outages and a scarcity of skilled malaria microscopists and quality reagents including Nigeria (Ita *et al*., 2018; Azikiwe *et al*., 2012).

Malaria diagnosis can be optimally achieved using RDT, but the rising number of false-negative results creates a challenge for malaria control efforts. (Mouatcho *et al*., 2013). PfHRP2/3 gene deletions and asymptomatic infections with low parasite densities below the detection limit can cause mRDT to yield false negatives (Motshoge *et al*., 2016). False-negative RDT in untreated patients harbouring malaria parasites can affect the transmission of malaria (Feleke *et al*., 2022). In patients with recurrent malaria, false negatives can occur due to drug resistance or inadequate treatment. Thus, the present study sought to compare the diagnostic accuracy of RDT and microscopy in patients with recurring malaria symptoms attending Ahmadu Bello University Medical Centre, Zaria, Nigeria.

MATERIALS AND METHODS

Ethical issues and selection standards

The Medical Director of Ahmadu Bello University Medical Centre, Zaria provided approval before the commencement of this study. Participants or caregivers was given a thorough explanation of the study protocol and procedures before their consent was obtained. Notwithstanding, critically ill patients who may require hospital admission and pregnant women were not included in the study.

Study design and population

The study was conducted at Ahmadu Bello University Medical Centre, Zaria, Nigeria. A total of 100 consenting

infected patients were recruited in the study. The sociodemographic data of each study participant was gathered using a pre-tested semi-structured questionnaire.

Collection of Blood sample

Venous blood sample (3 mL) was collected by venipuncture procedures from 100 patients with recurring *P. falciparum* malaria symptoms into EDTA tube for testing the diagnostic accuracy between RDT and microscopy.

Malaria diagnostic accuracy test

The blood sample from each study participant was subjected to RDT and microscopy. For RDT, the *P. falciparum*-specific HRP-2 test kit (Care Start1, Access Bio Inc) was used following the instructions of the manufacturer. For microscopy, both Field and Giemsa-stained thick blood smear microscopic slides were used to determine the blood parasite level. Blood samples were collected in EDTA vacutainers and two thick films were made for each sample. 5 drops of blood was spread on a slide and air dried. After 1 hour of air drying, one of the films was stained with Field stain while the other was stained with 10% Giemsa (pH = 7.2) for 30 min. The stain was then gently rinsed using distilled water and the slide was air dried at room temperature. The film was examined using oil immersion objective lens at ×100 magnification. The parasite density of each sample was estimated using the formula:

$$Parasite\ density\ per\ \mu L\ of\ blood = \frac{Number\ of\ parasites\ counted\ x\ Patient's\ actual\ WBC\ count}{Number\ of\ WBC\ counted\ on\ the\ film}$$

RESULTS AND DISCUSSION

A total of 100 individuals were examined; the baseline data showed that 63 were male while 37 were females. The age range of participants were between 9months to 67 years with an average age of 26 years. Participants < 20 years were 22 while > 20 were 78 as shown in Table 1.

Table 1: Baseline characteristics of participants in the study (*n*=100)

Parameters	Number(N)	Percentage (%)
Age group (years)		
<20	22	22
>20	78	78
Sex		
Female	63	63
Male	37	37
Total	**100**	**100**

Table 2 showed the history of fever and medication of patients. 53 participants had fever less than 2 weeks prior to this study, 24 participants had fever more than 2 weeks while 23 participants had fever months before this study. 60 participants treated malaria less than 2 weeks prior, 22 participants more than 2 weeks while 18 participants treated fever months prior to this study.

Table 2: History of fever and medication (*n*=100)

Parameters	Number(N)	Percentage (%)
History of fever		
<2 weeks	53	53
>2 weeks	24	24
Months ago	23	23
Last Medication		
<2 weeks	60	60
>2 weeks	22	22
Months ago	18	18
Total	**100**	**100**

Figure 1 displayed a bar chart comparing the prevalence of malaria by Rapid diagnostic Test and microscopy. The result showed that 36 participants tested positive using RDT while 85 and 83 tested positive using microscopy (Giemsa and Field's stain respectively).

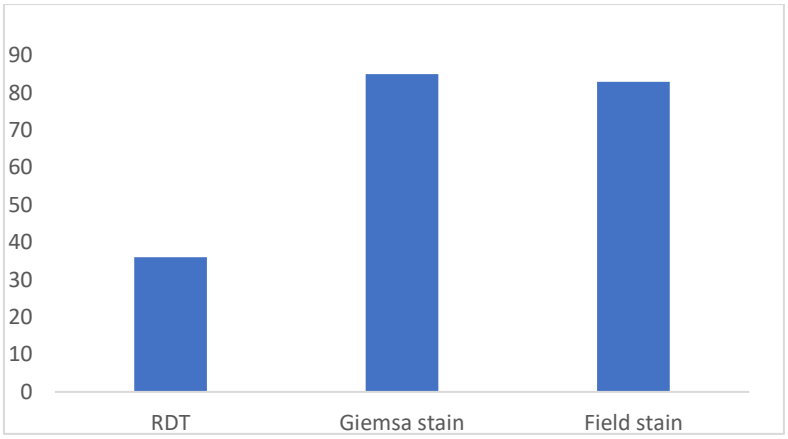

Figure 1: Malaria prevalence with RDT and Microscopy

Figure 2 detailed the parasite density as observed in Giemsa and Field's staining microscopic technique. The difference between the parasite density in each staining technique was not statistically significant ($p>0.05$).

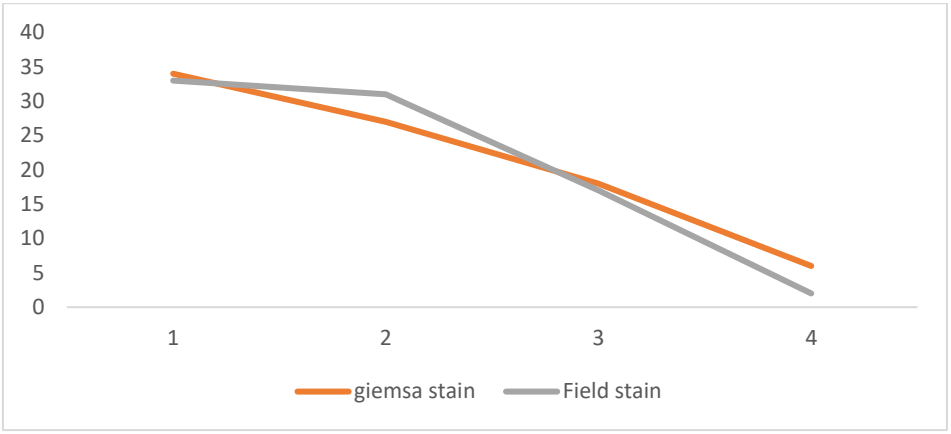

Figure 2: Comparison of parasite density between Field stain and Giemsa stain

Discussion

Our finding showed that the malaria prevalence by microscopy was 85% using Giemsa stain and 83% using Field's staining technique. This corroborated the report that malaria is still a public health burden in Nigeria (WHO, 2023) and consistent with previous report on malaria prevalence in ABUMC, Zaria (Shaibu *et al*.,2019). Malaria prevalence by microscopy reported in this study further buttress that microscopy is still the gold standard for malaria diagnosis.

Malaria prevalence determined using RDT was 36%. Previous reports showed that in patients with a recent history of malaria infection, residual circulating antigen can remain in the blood for up to 28 days after treatment and the parasites have been completely cleared, leading to false positive results from RDT (Iqbal *et al*., 2004; Maltha *et al*., 2013, Girma *et al*., 2019). However, our findings showed otherwise. Malaria parasites were detected using microscopy despite most participants treating malaria in recent time while detection using RDT was low.

This observation may be attributed the increased report of antimalarial drug resistance (Adamu *et al*., 2020) which could lead to treatment failure and recurring malaria as observed in patients in this study. Therefore, RDT should be used along with microscopy in patients with recurring malaria symptoms.

CONCLUSION

In conclusion, while malaria rapid diagnostic test (RDT) is efficacious for malaria diagnosis in high endemic region including Nigeria, it should be complemented with microscopy especially in patients with recurring malaria symptoms. This will prevent false negatives and help reduce the transmission cycle in patients carrying the parasite.

LIMITATION OF STUDY

This study did not put into consideration possible genetic polymorphism of *Plasmodium falciparum* histidine-rich protein 2/3 genes.

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