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Comparative Assessment of Diagnostic Performance of Urine Malaria Test Strip Against Rapid Diagnostic Tests Kit in Febrile Children Attending General Hospital Mahuta, Kebbi State, Nigeria

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Abstract

Malaria caused by species of plasmodium, is transmitted to humans by infected anopheles mosquitoes. There have been around 627,000 deaths of malaria worldwide, 78% of deaths in children under the age of 5, which has led to a large number of death (> 90%) in sub -Saharan Africa. Precise and rapid diagnosis is one of the important steps to reduce the incidence and mortality of malaria. Rapid diagnostic test (RDT) of malaria in blood is currently used, but other specific diagnostic test kits using body fluid have been developed. This study was carried out to assess the performance of one-step Urine Malaria Test™ (UMT) against RDT in the detection of a poly-histidine antigen (HRP2) in the urine samples of thermal and febrile patients for the diagnosis of malaria. Blood and urine samples were collected from 100 suspected malaria patients of which 85 were malaria blood smear positive cases (test samples) and 15 malaria negative cases (controls). The urine and blood samples of each patient were evaluated for rapid UMT and RDT. Using microscopy as gold standard, the sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of the UMT rapid malaria test for urine and RDT for blood were calculated. The accuracy of RDT rapid malaria test for malarial parasite detection was 95% for blood and 84 % for UMT. Rapid malaria test processed with urine may be a useful non-invasive and cost effective malaria diagnostic technique in the nearest future.

Keywords: Diagnostic, Evaluation, Urine, malaria test, febrile children.

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INTRODUCTION

Malaria is caused by the *Plasmodium* parasite, which is transmitted to humans through the bite of an infected female Anopheles mosquito. These different *Plasmodium* species have different clinical significance and infect humans in different combinations around the world (Nevill, 2010). Despite WHO's interventions to combat malaria, it remains a public health priority, especially in sub-Saharan Africa, where Nigeria is located (WHO, 2014). There are around 627,000 deaths due to malaria annually and 78% of these deaths occurred in children under the age of 5 and took place in the south of the Sahara (WHO, 2021). Reliable diagnosis of malaria requires laboratory confirmation of the presence of *Plasmodium* species in the blood of febrile patients. In east Africa, 90% of the malaria occurred as a result of *Plasmodium falciparum*, Accuracy of outpatient malaria diagnosis has become increasingly important due to emerging drug resistance and the use of alternative and expensive antimalarial drugs (Nevill, 2010; Sowunmi *et al.,* 2011).

It is estimated that accurate diagnostic tests for malaria have the potential to prevent 400 million cases of unnecessary treatment, save 100,000 lives each year, avoid waste already scarce resources, and positively impact the timely treatment of malaria (Reyburn *et al.,* 2010). Current malaria diagnostic methods require the use of blood for diagnosis using RDT and microscopy. Thick smear microscopy based on demonstrating the presence of *Plasmodium* in blood remains the gold standard for diagnosing malaria (Moody, 2002). However, this method depends on the operator and requires early and continuous education to maintain high quality tests; this quality assurance practice is often difficult to implement in resource-poor and malaria-endemic countries. In addition, the use of microscopy in health facilities has been reported in diagnosing malaria (Kahama *et al*., 2011; Harchut *et al.,* 2013). In other to reduce these deficiencies, many countries have developed and deployed rapid malaria diagnostic test kits for the rapid diagnosis of clinical malaria. Also, these malaria diagnostics are reported to be more sensitive, accurate, relatively inexpensive and rapid. However it's biggest disadvantage is complete dependency on blood samples for diagnosis. As such, these techniques introduce the risk of accidental infection of diseases such as hepatitis B and C, HIV and other blood-related diseases common in malaria-endemic areas (Rafael *et al*., 2011). These techniques also require rigorous training and biosafety precautions to ensure proper containerization and disposal of used needles. The use of needles also reportedly limits malaria diagnosis in some African communities where blood drawing is still considered taboo (Achary *et al.,* 2015).

Urine Malaria Test (UMT) is a recombinant monoclonal antibody and immunochromatographic lateral flow assay that detects *Plasmodium falciparum* specific histidine-rich protein 2, a polyhistidine protein or febrile patients Fragments shredded in urine. HRP2 is produced by the merozoite and gametophyte forms of *Plasmodium*. The blood then carries HRP2 to the kidneys, where it is passed to the bladder as part of the urine. Urine collection is non-invasive, simple, safe, stress-free, painless, and can be done by individuals with limited training, including patients (Togbo *et al*., 2014). Collection does not require special equipment, it allows multiple or consecutive collections outside the hospital (Wellington *et al.,* 2017).

Therefore, there is a need to develop a non-invasive, simple, rapid, easy-to-operate, and reliable diagnostic method to achieve rapid and accurate diagnosis of malaria. Therefore, the aim of this study was to compare the diagnostic characteristics of the novel UMT with

currently used blood RDTs to validate the utility of the UMT as a malaria diagnostic tool in Nigeria.

MATERIALS AND METHODS

Study area

General Hospital Mahuta is a state-owned hospital, located in Mahuta town, the headquarters of Fakai Local Government Area in Kebbi State, Nigeria, which is located between the Latitude 11.55250 N and Longitude 4.98436 E.

According to the 2006 national census, the population was 121,212. Majority of the inhabitants are civil servants, businessmen, farmers, teachers and students (Mohammed *et al.*, 2021).

This study area has two seasons; the dry season (October to March), and the rainy season (April to September). Human malaria can be described as moderately endemic in the dry season and high in the rainy season, peaking at the beginning and near the end of the rainy season. Mahuta General Hospital provides care for over 1,500 patients annually (Mohammed *et al.*, 2021). Participants in this study were patients who visited this hospital for consultation during the study period.

Study design

This is a cross-sectional comparative study conducted at Mahuta General Hospital from June 2022 to August 2022. A structured questionnaire was administered to each participant, and interviews were conducted with those unable to read or write. Samples (urine and blood) were collected once from patients who agreed to participate in the study.

Sample size

The study's sample size was determined using standard formula developed by (Leslie, 1965) to calculate the minimum sample size using the p-value from previous studies.

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where n is number of sample (sample size), Z the Standard normal deviate at 95% confidence interval of 1.96, P is the prevalence from previous study carried out in Fako Division, Cameroon (7.0%) (Nforbugwe *et al.,* 2020) and d is the allowable margin of error (0.05).

Therefore, the minimum sample size will be

$$n = \frac{(1.96)^2\, 7.0(1-7.0)}{(0.05)^2}$$

$$= 100$$

Inclusion criteria for selecting participants

All patients ≤14years having axillary temperatures of >37.5^{0}C or with a history of fever in the previous 48 hours whose relatives gave consent to the study were included.

Exclusion criteria for selecting participants

All patients over 14 years of age were excluded. Patients without consent were also excluded. Participants with hematuria, leukocytes, urobilinogen were excluded after initial urine screening using urinalysis dipsticks, as these parameters are characteristic of many kidney diseases and may skew study results. In addition, those presenting with signs and symptoms of rheumatoid arthritis (joint pain, joint inflammation, limited movement, discomfort, and

joint tenderness) were also not considered, as rheumatoid arthritis has been shown to produce false effects on RDT Positive result. Finally, patients already taking antimalarial drugs were also excluded.

Ethical approval

Ethical approval for this study was obtained from the Kebbi State Department of Health and Mahuta General Hospital Research Ethics Review Board with numbers; MOH/KSREC/VOL.1/57 and GHM/SUB/011/VOL, respectively.

Specimen collection

The main samples collected are blood and urine. Capillary blood was collected by finger puncture. Following standard procedure, approximately 50 µL of blood are used to make a thin, thick blood film. Blood was screened by malaria RDT and microscopy. Urine samples was collected in a leak-proofed container for UMT analysis.

Microscopic examination and quantification of parasites

A capillary blood sample drawn from the patient's finger prick was used for thin and thick smear were prepared for microscopy in accordance with WHO standard microscopy techniques and read at 100 objective lense magnification with oil immersion lens (Moll *et al.,* 2008). The slides were independently read by two trained and experienced laboratory technologists. Microscopy was considered positive only when asexual parasite forms - trophozoites and schizonts (not individual gametophytes) were detected, as asexual forms indicate active infection. Parasitaemia was categorized as low (<1000parasites per µL of blood), moderate (1000-4999 parasites per µL of blood) and high (>5000parasites per µL of blood). Determine the parasite density by counting the number of parasites seen per 200 leukocytes and use the formula to calculate the parasite density per microliter based on the putative average count of 8,000 leukocytes per microliter using the formula reported by Moody, 2002; Moll *et al.,* (2008). Parasitemia per microliter = number of parasites × 8,000 = 200 leucocytes

Rapid Diagnostic Test

A commercially available RDT kit (CareStart™ Malaria HRP2) Combo, ACCESSBIO, INC., New Jersey, USA) was used to detec*t Plasmodium*. For testing, remove the RDT test device from the foil bag and place it on a flat, dry surface and marked with a patient identifier. Using 5 µl capillary pipette, the blood sample were transferred into RDT specimen well. Two drops of buffer were then dispensed theirin. After 20 minutes,the RDT kit was read and interpret them as positive, negative or indeterminate (Maltha *et al*., 2010).

Urine Malaria Test

A commercially available urine diagnostic test UMT strip (Fyodor Biotechnologies, Inc. Baltimore MD USA, catalog number UMT-5, Urine Malaria TestTM Kit) was also used to diagnose the presence of malaria. The UMT strip was dipped in 200 µL of urine for two minutes to allow the sample to wick and saturate the strip. The strip was then removed, placed on its foil pouch packaging and incubated for 20 minutes. The results were reported as negative, positive, or invalid: if two visible lines appeared on the strip (even if very faint) the test was positive; if only the control line appeared, the test was negative. Test results reported as invalid, i.e., failure to observe a control line or the presence of a darkly stained background that obscured the test lines, were repeated to resolve the discrepant event (Moll *et al*., 2008).

RESULTS

Demographics and clinical characteristics of the study population.

Out of a total of 100 individuals who were screened in this study, 49 (49.0%) of the participants were females, while 51 (51.0 %) were males with the Age range of 1 to 14 years. The highest malaria prevalence (36.0%) was seen in the 6-11 age group while the least was found in the age group of 1-5 and 12-14 with the prevalence of 30.0% and 34.0% respectively (Table 1).

Table 1:

Age range	Male	Female	Total
1 - 5	13 (25.4)	17 (34.6)	30 (30.00)
6 - 11	16 (31.3)	20 (40.8)	36 (36.00)
12 - 14	21 (41.1)	13 (26.5)	34 (34.00)
Total	51 (51.00)	49 (49.00)	100 (100.00)

Demographic and clinical characteristics of the study population

Comparison of the performance of RDT and UMT with microscopy

Out of 100 blood samples collected, 85 test samples were positive for malaria by microscopic examination while 15 were negative. With microscopy as a gold standard, the RDT processed by blood, giving a sensitivity of 94.0%, specificity of 100%, efficiency/accuracy of 95% PPV of 100% and NPV of 75%.

When microscopy was compared with UMT processed by urine, the sensitivity, specificity, efficiency/accuracy, PPV and NPV was 81%, 100% ,84%, 100% and 48% respectively (Table 2).

The two malaria diagnostic test methods that were evaluated against GM, displayed close similarities in their diagnostic performance taking their specific efficiency/accuracy and sensitivity into consideration.

Table 2: Comparison of performance of RDT and UMT with microscopy

	Microscopy							
	Pos	Neg	Total					
RDT Blood Result.				Sen	Speci	Accu	PPV	NPV
Positive	80	0.00.	80					
Negative	05	15	20	94%	100%	95%	100%	75%
Total	85	15	100					
UMT test result Malaria Test Result								
Positive	96	0.00	69					
Negative	16	15	31	81%	100%	84%	100%	48%
Total	85	15	100					

(RDT= Rapid Diagnostic Test, UMT = Urine Malaria Test, POS = positive, Neg = Negative, Sen = Sensitivity, Speci = Specificity, Acc= Accuracy, PPV= Positive Predictive Value, NPV= Negative Predictive Value)

DISCUSSION

Determination of the presence of parasites may be valuable in communities where blood is contraindicated and reducing compliance issues associated with blood collection. For these reasons, many researchers have tried using body fluids such as urine and saliva to detect malaria (Mharakurwa *et al*., 2006). Western blotting could be use detect malaria antigens in urine (Katzin *et al*., 1991). Similarly, a study by Mharakurwa *et al*. (2003) showed that *P. falciparum* infection could be detected in urine and saliva by PCR techniques.

Diagnosis is currently performed by microscopy, which requires good training and simple laboratory facilities. In contrast, rapid immunochromatographic tests do not require laboratory, electricity, or any special equipment. They used monoclonal or polyclonal antibodies to target the parasite antigen histidine-rich protein 2 (HRP2) of *P. falcifarum*. Histidine-rich protein 2 (PfHRP2) is a water-soluble protein produced by the asexual stage and gametophyte of *Plasmodium falciparum* and expressed on the surface of the erythrocyte membrane. Parasite lactate dehydrogenase (pLDH), a soluble glycolytic enzyme produced by the asexual and sexual stages of all four live human malaria parasites, is present in and released from parasite-infected red blood cells (Kakkilaya, 2003).

The RDT and UMT rapid malaria test uses the principle of immunochromatography help in detecting the malaria antigens pLDH and Pf HRP2 in the blood of malaria patients, especially febrile children, as these malaria antigens are also released in the urine (Genton *et al*., 1998). Therefore, in this study, we processed blood and urine samples from malaria patients using the RDT and UMT with a view to detecting malaria parasite.

In this study, RDT and UMT were very similar in sensitivity (94% and 81%) and specificity (100% and 100%) respectively. There are also similarities in the different diagnostic features of the two techniques. These findings are similar to report of Tagbo *et al.* (2014). This suggests that UMT can aid in the clinical management of suspected malaria cases in our setting.
Our study shows a higher prevalence of malaria (85.00 %) than 20% reported by Tagbo *et al.* (2014). This difference may be attributable to differences in study sites, and our study only involved patients with febrile symptoms, whereas asymptomatic participants were considered in the study conducted by Tagbo *et al*. (2014).

The recent study showed that, RDT malaria test processed by blood gives a sensitivity of 94%, specificity 100%, efficiency/accuracy 95%. These findings are similar to the percentages; sensitivity (96 %), specificity (100%) and accuracy (97%) reported by Anchinmane *et al.* (2016) in their study area. When compared with microscopy as the gold standard, 69 out of the 85 microscopic malaria positive cases were positive through the usage of UMT wherein urine samples were employed. In this current study, UMT recorded an appreciable sensitivity (81%), specificity (100%), efficiency/accuracy (84%). These results are higher than sensitivity (17.3 %), specificity (100%) and accuracy (38 %) reported by Anchinmane *et al.*, (2016) in their study.

CONCLUSION

The UMT kits studied showed modest agreement with blood smear microscopy and a relatively low level of sensitivity compared to blood-based RDT test kits for malaria diagnosis. Detection of parasite antigens in urine would be an affordable, rapid, non-invasive method that is safe for patients and technicians in under-resourced settings. More research is needed to determine the cost-effectiveness and outcome differences between the different brands of rapid malaria immunochromatographic tests devices available. Time is now needed to study an updated rapid malaria test that can more accurately detect malaria antigens in urine.

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