ISSN: 2689-8616

Research Article

Advancements in Journal of Urology and Nephrology

Comparative Evaluation of the Diagnostic Performance Characteristics of a One-Step Urine Malaria Test (UMT) against Rapid Diagnostic Tests (RDT) in Febrile Patients from Fako Division, Cameroon

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Submitted: 11 March 2022; **Accepted:** 17 March 2022; **Published:** 23 March 2022

Citation: *Nforbugwe Achu Che Awah, Bihnwi Rengerline Nchotu, Agnes Djema Bongah, Nguedia Jules Clement Assob. (2022). Comparative Evaluation of the Diagnostic Performance Characteristics of a One-Step Urine Malaria Test (UMT) against Rapid Diagnostic Tests (RDT) in Febrile Patients from Fako Division, Cameroon. Adv J Uro Nephro, 4(1), 17-22.*

Abstract

Background: *Presently, all malaria diagnostic methods like: microscopy and Rapid Diagnostic Tests are invasive as they depend on blood samples for malaria diagnosis. Hence this study was aimed at comparing the diagnostic performance characteristics of the novel UMT to the currently used Blood RDT, and to find out the efficacy of this UMT in detecting low parasitemia in the study population.*

Methodology: *A cross sectional study involving 200 febrile participants, with no signs and symptoms of rheumatoid arthritis, kidney diseases, no history of hematuria, >15/µl leucocytes and urobilinogens of >1 mg/dl in their urine, were recruited from the month of April to August 2017 in the Limbe and Buea Regional Hospitals. The main samples requested for analyses were urine and blood.*

Results: *Using the blood smear microscopy as standard, out of the 200 participants, 93 (46.5%) were positive for P. malaria. UMT had a sensitivity and specificity of 82.41% and 83.48 while that of RDT was 84.09% and 83.03 respectively falciparum (CI: 72.80 to 92.05%, Kappa 0.665, p =0.001). The UMT had a lowest limit of detection of 140 parasites/µl which was similar to RDT. The PPV and NPV of UMT and RDT were (81.74% and 85.98%) and (80.04% and 87.28%), respectively. There was a close agreement between the RDT and UMT when compared to microscopy (83.5% and 83.0% respectively).*

Conclusion: *The UMT kit that was evaluated in comparison to the blood based RDT, showed a lot of similarities using the blood smear microscopy as gold standard. Hence, it can be used in our setting for the prompt and accurate diagnosis of malaria in febrile patients.*

Keywords: Fyodor Urine Malaria Test, Malaria, Febrile, Sensitivity, Specificity

Introduction

The disease, Malaria is caused by the *Plasmodium species*, which is transmitted to humans by the bite of an infected female *Anopheles* mosquito. These different *Plasmodium* species have different clinical implications and infect humans in different combinations around the world. Despite the interventions put in place by the World Health Organization to fight Malaria, it still remains a public health priority especially in sub-Saharan Africa [1]. The 2014 World Health Organization report stated that there were about 584,000 malaria deaths annually worldwide, with 78 % of these deaths occurring in children under 5 years old, this largely (>90 %) occurred in Sub-Saharan Africa [2].

Reliable diagnosis of malaria requires laboratory confirmation of the presence of malaria parasites in the blood of a febrile patient. In eastern Africa, where 90% of the malaria is due to *Plasmodium falciparum*, accuracy of malaria diagnosis at the outpatient level is becoming increasingly important due to emerging drug resistance and the use of alternative, costly antimalarial drugs [3,4]. It is estimated that accurate diagnostic tests for malaria have the potential

to prevent 400 million unnecessary treatment cases, save 100, 000 lives per year, waste of already scarce resources and impacts positively on the prompt treatment of malaria [5].

Current malaria diagnostic methods necessitate the use of blood for diagnosis, using either RDT and/or microscopy. Although these methods for malaria diagnosis have been reported to be more sensitive, accurate, relatively cheaper and rapid, their greatest disadvantage is their dependency on blood samples for diagnosis. These techniques therefore, introduce the risk of accidental infections from diseases such as Hepatitis B, Malaria, HIV and other blood related diseases which are common in malaria-endemic areas [6,7]. These techniques also necessitate rigorous training and biological safety precautions, so as to ensure proper containerization and disposal of used needles. The use of needles has also been reported to also serve as a limitation for malaria diagnosis in certain African communities which still regard blood withdrawal as a taboo [7].

The UMT, is a recombinant monoclonal antibody and an immunochromatographic lateral flow assay, that detects *P. falciparum* specific Histidine-Rich Protein 2, a poly-histidine protein or fragments shedded in the urine of febrile patients. HRP2 is produced by merozoite and gametocyte forms of the malaria parasite. The blood then transports HRP2 to the kidneys, where it is passed on to the bladder as part of the urine. The collection of urine is non-invasive, simple, safe, stress free, painless, and can be done by individuals with limited training, including patients. No special equipment is needed for collection and it allows for multiple or serial collections outside of the hospital [8].

There is therefore a need for the development of a non-invasive, simple, rapid, easy to perform, and reliable diagnostic methods, for the prompt and accurate diagnosis of malaria. Hence, the objectives of this study were to compare the diagnostic characteristics of the novel UMT to the currently used Blood RDT in an attempt to validate the use of UMT as a diagnostic tool for malaria in the South West Region of Cameroon. The study was also aimed at finding out the efficacy of UMT in detecting low parasitemia in the study population.

Methodology

Buea is the capital of the Southwest Region of Cameroon and is located in the eastern slopes of Mount Cameroon. Limbe is the divisional capital of Fako, which host a number of touristic sites and is located at the foot of Mount Cameroon which is about 870km above sea level. This study area has two seasons —the dry season (between October and March), and the rainy season (between April and September). Human malaria can be described as mesoendemic in the dry season and hyperendemic in the rainy season, with peaks at the beginning and towards the end of the rainy season [9]. The population in this study area experiences an estimated 3.93 infective bites person/night and it has been reported that *P. falciparum* accounts for up to 96% of malaria infections in this area [10,11]. The Buea and Limbe Regional Hospitals provide care to over 10,000 patients annually. Participants were patients who were visiting these hospitals for consultation during this study period.

Study Design

It was a cross sectional study that was conducted in the Limbe and Buea Regional Hospitals from April to August 2017. Structured questionnaires were given to each participant and interviews were done for those who could not read or write. Samples (urine and blood) were collected once from patients who accepted to take part in the study after giving their consents.

Sample Population

A sample size of 200 was used. Those who participated in this study were: febrile patients of 0-70years and above, with axillary temperatures>37.50C or with a history of fever in the previous 48 hours. Pregnant women and most importantly those whose consent were given also participated. Those who were excluded from this study were: patients with a history of hematuria, >15/µl leucocytes and urobilinogens of ≥1 mg/dl in their urine since these parameters are features of many kidney diseases with probable high levels of antibody that may cause false positive results. These parameters were excluded using the CYBOWTM Urinalysis test strips. More to this, those who were presenting with signs and symptoms of Rheumatoid Arthritis (painful joints, inflamed joints, limitation in motion, malaise, and tenderness of the joints) since Rheumatoid Arthritis has been proven to give False Positive results for RDT and patients who were already on antimalarial drug.

Sampling Technique

The systematic random sampling technique was used, with daily attendance as sampling frame. Recruitment of participants was done daily from Mondays to Fridays in the Limbe and Buea Regional Hospitals. Data was collected from the administration of structured questionnaires and interviews. Furthermore, each participant was given an identification number so as to avoid confusion in the course of the study.

Laboratory Analysis

Specimen Collection

The main specimens were blood and urine. Capillary blood was collected by finger pricking. 50ul of this blood was used to make a thin and thick blood film. Microscopic analysis was prioritized over other methods of malaria parasite determination. Urine was collected in a leak-proofed container for UMT analysis.

Microscopic Examination and Quantification of Parasites

The prepared blood films were air-dried and stained with 10% Giemsa (1 in 20 dilutions) for 25 – 30 min [12]. Two trained and experienced microscopists who did not have prior knowledge of the patients' clinical history, read the slides independently and an average parasitaemia density was obtained. Slides were considered positive only when asexual parasite forms – trophozoites and schizonts (not gametocytes alone) – were detected, since asexual forms are indicative of active infection. Parasite densities were determined by using the formula [13]. A blood film was assumed negative when the examination of 100 thick film fields did not show

the presence of asexual forms of *P. falciparum.* Parasitaemia was categorized as low (<1000parasites/ul), moderate (1000-4999parasites/ul blood) and high (>5000parasites/ul blood).

Parasitaemia per microlitre = number of parasites × 8, 000
200 leucocytes.

Rapid Diagnostic Test (RDT)

A commercially available RDT kit (*CareStart*™ Malaria HRP2) Combo, ACCESSBIO, INC., New Jersey, USA) was used to detect malaria parasites, according to the manufacturer's instructions, using 5µl of capillary blood. The membrane strips were read and interpreted after 20 min as either positive, negative or indeterminate [14].

Urine Malaria Test

A commercially available urine diagnostic test, UMT strip (Fyodor Biotechnologies, Inc. Baltimore MD USA, catalog number UMT-5, Urine Malaria Test™ Kit),) was also used to diagnose the malaria infection following the manufacturer's instructions. The results were then carefully interpreted as positive, negative or indeterminate [15].

Ethical Consideration

The research protocol was read and approved by the Faculty of Health Sciences. Ethical clearance for this study was obtained from the University of Buea, FHS-Institutional Review Board. Administrative clearance was obtained from the, Regional Delegation of public health for the South West Region. Administrative authorizations were obtained from the Limbe and Buea Regional Hospitals. An informed consent form was made stating the special measures involved to ensure no harm was caused to the participants. The risks and benefits were well stated in this form. The consents of these participants were obtained by signing the consent and accent forms.

Data Management and Statistical Analysis

Data was entered into Microsoft excel 2010 software program and double checked for errors before being exported to SPSS version 22 (IBM Inc). Frequency tables were used to present demographic characteristics. Measures of descriptive statistics were used to compute means, median and SD of Age. For determination of sensitivity, specificity, PPV, NPV, PLR, NLR, a 2x2 cross tabulation of each UMT and RDT against microscopy was done. ROC curves were plotted to evaluate the accuracy of UMT and RDT against microscopy as the gold standard. Linear regression analysis was done to determine the relationship between age and parasite load. The detection limit was calculated from the sample with the lowest parasitaemia with the true positive result. Statistical significance was considered at $P<0.05$.

Results

Demographics and Clinical Characteristics of Patients

From a total of 786 individuals who were screened, 200 who met the inclusion criteria were enrolled between April and August 2017, which are spanning periods of high malaria transmission in the study area. In this study, 54.5% (109) of the participants were females and 45.5% (91) were males. The mean age was 27 years with the range of Nine months to 86 years. The highest malaria prevalence (23.5%) was seen in the 21-30 age group while the least (6.5%) was found in the 41-50 age group and the 61-70 age group. All the participants presented with fever (≥37.5°C) on enrollment, with a mean body temperature of 38.2°C. Headaches, body pains, nausea and chills were the most commonly reported symptoms. The participants presented with other diseases like: diabetes, hypertension, typhoid, HIV/AIDS, TB, gastritis and asthma. In this study, 77% of the population used the Insecticide Treated Mosquito bed-nets while 23% did not.

Table 1: Distribution of Malaria Prevalence in the Study Population according to Age and Gender

Parameters		N	Prevalence of Malaria n (%)	Chi-Square	p. value
Gender	Male	109	54(49.5)	0.891	0.345
	Female	91	39(42.9)		
Age	<20	62	34(54.8)	5.782	0.216
	20-29	49	19(38.8)		
	30-39	27	14(51.9)		
	40-49	18	5(27.8)		
	>50	44	21(47.7)		
Total		200	93(46.5)		

The participants were screened for malaria parasites using Giemsa Microscopy. Among the participants, 93 were positive for *P. falciparum* malaria, giving an overall prevalence of 46.5%. No significant association was observed between the prevalence of malaria and sex ($P=0.345$). Likewise, no significant association was observed between the prevalence of malaria and age ($P=0.216$) (see Table 1).

Out of the 93 samples that were positive, as determined by GM, 75 were also found positive by the UMT while 74 were found to be positive for RDT. Out of the 107 samples that were negative, as determined by Giemsa Microscopy, 91 were found negative by the UMT while 93 were found Negative for RDT. (see Table 2).

Table 2: Summary of the Results Obtained using the UMT and RDT against Microscopy

Parameters	Microscopy			Total n (%)
		Positive n (%)	Negative n (%)	
UMT	Positive	75(80.6)	16(15.0)	91(45.5)
	Negative	18(19.4)	91(85.0)	109(54.5)
	Total	93(46.5)	107(53.5)	200(100.0)
RDT	Positive	74(79.6)	14(13.1)	88(44.0)
	Negative	19(20.4)	93(86.9)	112(56.0)
	Total	93(46.5)	107(53.5)	200(100.0)

Among the two diagnostic test methods that were evaluated against GM, there were close similarities in their diagnostic performance characteristics, taking into considerations their specificity, sensitivity, PPV, NPV, PLR and NPV See in Table 3. From this study, there was a close agreement between the RDT and UMT when compared to microscopy (83.5% and 83.0% respectively See Table 3.

Table 3: Comparing the Performance Characteristics of UMT and RDT against Microscopy

Parameters	UMT % (CI)	RDT % (CI)
Sensitivity	82.41 (77.4 – 92.8)	84.41 (78.6-93.0)
Specificity	83.48 (78.6 – 88.5)	83.03 (78.5 – 88.3)
PPV	81.46 (71.4 – 89.5)	80.05 (72.2 – 89.2)
NPV	85.06 (80.2 – 93.7)	87.00 (80.03 – 92.9)
PLR	4.99 (3.8 – 8.1)	4.98 (3.8 – 8.1)
NLR	0.21 (0.19 – 0.25)	0.19 (0.17 – 0.23)
Parameters	UMT % (CI)	RDT % (CI)
Agreement between tests	83.0 (79.3 – 93.7)	83.5 (77.5 – 93.8)

Sensitivity = [true positive /(true positive + false negative) × 100]; specificity = [true negative/(true negative + false positive) × 100; PPV = [true positive/(true positive + false positive) × 100]; NPV = [true negative/(true negative + false negative) × 100]; Agreement = [true positive + true negative/N × 100].

The lowest parasite density detected was 140parasites/µl. The proportion of the participants who did not have detectable parasites in their blood was 53.5% (107) out of the 200 participants who were sampled. Most of the participants who were positive for malaria had parasite densities >200parasite/ul. While the least proportion of the sampled population had a parasite density of <100. The highest parasite density (57,560trop/mm3) was seen in the 21-30 age group.

Table 4: Sensitivity of UMT with Microscopy at Different Parasite Densities

Parameters	Parasite density (parasites/ul			Overall
	<100	<200	>200	
Sensitivity	0%	40%	95%	81%
95% CI	0%-0%	5%-50%	89%-99.7%	73%-89%
Specificity	85%	85%	85%	85%
95%CI	78%-92%	78%-92%	78%-92%	78%-92%
PPV	0%	16%	100%	82%
95%CI	0%-0%	0.6%-32%	78%-100%	75%-90%
NPV	97%	87%	0%	83%
95%CI	93%-100%	80%-93%	0%-0%	77%-90%

Discussion

Analytical sensitivity (AS) which is the least detectable number of parasites of UMT, from the study was 140 parasites/µL. This AS was also similar to that of RDT. This detection limit was high compared to other methods such as Microscopy (50 parasites/µL) and PCR (10 – 50 parasites/µL) [16]. The UMT had the lowest limit of detection of 140 parasites/µl, and a 40% sensitivity at ≤200 parasites/µl . This was similar to a work done by Tagbo Oguonu et al, in 2014 who had as lowest limit of detection of 120 parasites/µl, and a 50% sensitivity at ≤200 parasites/µl [17]. Many reasons can be suggested for the relatively poor sensitivity at lower parasitaemia levels, which may be related to parasite antigen production, antigen content in urine, cross reactivity with other antibodies in patients and time of urine void. Nwakanma et al. noted that the amount of malaria antigen was low in urine and dependent probably on the time of collection of the samples. They suggested that first void morning urine might probably give better sensitivity than later timed samples [18]. This may not be practicable in clinical practice where the results are required for immediate treatment. With the probable variability in malaria antigen quantity, it is likely that the expected amount of antibody impregnated in the urine-specific test kits as well as the quantity of body fluid required may be higher than those of blood-specific test kits thus necessitating a probable further optimization of the Fyodor UMT to enhance test sensitivity in low parasitaemia. It is known that the property of the antibody impregnated in the nitrocellulose pad of the immunochromatographic test kits also determine the sensitivity. Immunochromatographic tests in which IgG antibody is used as the coating antibody to capture HRP-2 antigen are likely to give higher rates of false positivity than a test system in which IgM antibody is coated onto the strips [8].

From this study, there was a close similarity in the sensitivity (84.09% and 82.41%) and specificity (83.03% and 83.48%) of the RDT and UMT respectively. There was also a similarity in the different diagnostic characteristics of both techniques. These findings were also similar to the work that was done by Tagbo Oguonu et al, in 2014 [17]. This indicates that the UMT could aid in the clinical management of suspected malaria cases in our setting.

Our study demonstrated a high Pre-Test Probability (Prevalence) of Malaria, as 46.50 %, with results similar to studies by Tagbo et al., with a prevalence 20%. Explanation to the slight discrepancies in values is attributed to differences in study site and our study involving febrile symptomatic compared to asymptomatic case involved in studies that study. The pre-test probability of disease in a patient who tests positive by the UMT is 45.5% while that for RDT was 44%. For example, upon receiving a negative UMT result for a suspected malaria case, the clinician now knows that this patient's probability of having malaria parasites detectable by microscopy is unlikely, only 4%. In contrast, a positive UMT result would indicate that the probability of detecting malaria parasites in this patient by microscopy is 46%.

Hence, the UMT could potentially expand malaria testing in the health care settings, particularly in hard-to-reach locations or health care facilities where blood draw is difficult or impractical for microscopy, and advance the current global effort toward universal diagnosis in cases of fever suspected of being malaria.

The high degree of sensitivity from the population from 0-20 years of age, may suggest that the UMT is able to detect acceptable level of antigens especially in areas of high malaria transmission. Also the false positivity related to the presence of the gametocyte is indicative of the ability to detect sexual form of *P. falciparum* a factor which is useful in absolute sensitivity tests against the clinical episodes that was used in this study. However, in areas of low malaria endemicity, this level of false positives may create drug wastage, which the current malaria control efforts seek to reduce. False positive results may be attributed to the ability of all histidine-rich protein 2 (HRP2) antigen malaria test kits to detect the parasite antigen even after the malaria illness. The presence of rheumatoid factor and schistosomiasis in a patient may also lead to false positivity, and will need to be further evaluated [19]. These factors are known to affect the blood type malaria RDTs, but little is known about such influence on the urine malaria test kits. It may be assumed that since both (blood and urine-based) test kits are specific for HRP2 such effect may also occur with the UMT.

The false negative results that were gotten from UMT are comparable to those of blood-specific malaria RDTs. Many factors have been described to contribute to the false negative results with HRP2-based rapid test kits. These include parasite and host factors such as deletion or mutation of HPR2 gene and an illustration of the prozone effect observed with immunochromatographic tests such as malaria RDT [19, 20].

Some of the limitations observed with the use of the UMT was the delay in provision of urine by some subjects, particularly among children. This may be a delay factor in the promptness of testing and treatment. Again, the prevalence of malaria for our study was limited to a single plasmodia species; *P. falciparum*, hence not revealing the true prevalence of malaria in the community as there could be infections with other plasmodium species.

Abbreviations

WHO: World Health Organization.
PCR: Polymerase Chain Reaction
PfHRP2: *P. falciparum* Histidine-Rich Protein 2
RDTs: Rapid Diagnostic Tests
U.T.M: Urine Based Malaria Test Kit
P. f: *Plasmodium falciparum*
PLR: Positive Likelihood Ratios.
PPV: Positive Predictive Value
NPV: Negative Predictive Value
NLR: Negative Likelihood Ratios

Conclusion

The Urine Malaria Test kit that was evaluated in comparison to the blood based RDT, showed a lot of similarities with blood smear microscopy as gold standard. Hence, it can be used in our setting for the prompt and accurate diagnosis of malaria in febrile patients.

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