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RESEARCH ARTICLE

Detection of Malaria Parasite Protein in Urine of Patients with Acute Uncomplicated Malaria Using Rapid Diagnostic Test Kits

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ABSTRACT

Objectives: The invasive nature of the current malaria diagnostic techniques impairs compliance to diagnosis, especially for on-field detection. Adapting non-invasive methods of biological sample collection for rapid diagnosis of malaria infections may provide a more efficient approach to case management and epidemiological studies of malaria. This study was designed to evaluate the detection of *Plasmodium falciparum* Histidine-rich Protein II (PfHRP-2) in urine samples and optimization as diagnostic markers for *P. falciparum* infection.

Methods: One hundred (100) microscopically confirmed patients with *Plasmodium falciparum* infection and 25 *P. falciparum* negative controls were recruited for the study. Blood samples of all participants were tested for the presence of PfHRP-2 using Rapid Diagnostic Test (RDT) kits. In addition, urine samples of the confirmed malaria-infected patients were analyzed for PfHRP-2 using the CareStart™ and Global Devices (USA) Malaria kits. The diagnostic performances of the RDT kits were evaluated.

Results: Overall, the two brands of malaria rapid diagnostics demonstrated 71% sensitivity (95%CI=62.1-79.9%) and 96% specificity (95%CI=88.3-103.7%) for PfHRP-2 detection in urine.

The sensitivities of the tests in urine at asexual parasitemia ≤ 2000 μL^{-1} and asexual parasitemia > 2000 μL^{-1} were 69.6% (95%CI=56.3-82.9%) and 72.2% (95%CI=60.3-84.2%) respectively. Global Devices and CareStart™ kits had individual sensitivities of 80% (95%CI= 65.7-94.3%) and 67.1% (95%CI= 56.1-78.1%) respectively for PfHRP-2 detection in urine (P= 0.072).

Conclusion: Findings revealed that urine-based RDTs have limited capacities for malaria diagnosis due to their low sensitivity and require more optimizations to meet required diagnostic standards. *J Microbiol Infect Dis 2022; 12(3):97-107.*

Keywords: *Rapid Diagnostic Test (RDT), non-invasive technique, Plasmodium falciparum Histidine-rich protein II (PfHRP-2), urine, sensitivity*

INTRODUCTION

Malaria remains a significant cause of morbidity and mortality in regions where the disease is endemic [1]. The most recent estimate of malaria cases in 2020 is 241 million, with global mortality of 627000 deaths [2]. Therefore, all efforts towards malaria control in high-burden countries are geared towards quality-assured vector control,

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chemoprevention, prompt and sensitive diagnosis, and effective treatment of confirmed malaria cases [3]. The purpose of these elimination and control strategies is to achieve a drastic reduction in malaria-related morbidity and mortality [3].

Adoption of diagnosis of all suspected malaria cases by parasitological confirmation as recommended by WHO [4] has dramatically improved case management in malaria-endemic regions [5]. The importance of an accurate diagnosis of malaria is evident. Giving patients antimalarial drugs based only on a possible clinical diagnosis leads to misdiagnosis and the unnecessary use of antimalarials while compromising the quality of care. [6]. The development, deployment, and use of rapid diagnostic tests (RDTs) for malaria has aided in more accurate, timely diagnosis and early treatment of malaria and, in turn, contributed to a reduction in morbidity and mortality of malaria [7]. The availability of rapid diagnostic tests also provides a valuable diagnostic helpful option for epidemiological surveillance, detection of parasites in asymptomatic subjects who are potential reservoirs for transmission, and as a guide for the rational use of antimalarial therapies for effective chemotherapy [8].

Although microscopy remains the gold standard for the diagnosis of malaria to date, parasite protein-based rapid diagnostic tests have a comparative advantage over other techniques due to the ease of application, rapid turnover of results, cost-effectiveness, portability, and availability without requiring a source of electricity [9,10]. Despite these advantages, the common feature of all malaria diagnostic techniques is the invasive nature of specimen (blood) collection. Blood collection imposes several challenges to healthcare workers, researchers, and patients, including increased risk of needle injuries, pain, and accidental infection from diseases such as HIV/AIDs and hepatitis [9,11]. In addition, the cultural objection to considering blood withdrawal as taboo, the fear of small children and some adults for blood collection, and the requirement of repeated sampling during post-treatment follow-up studies are indices that constrict proper epidemiological data gathering of the disease [12]. More worrying is the current situation of the Corona Virus Disease (COVID-19) pandemic, a disease with similar pathologic manifestation as malaria [13]. Due to the similarities in their symptoms, differentials in diagnostics between the two diseases become almost impossible when confounded by the limitation of invasive sample collection, especially in rural areas within the malaria-endemic regions. In order to circumvent these challenges and meet national and global targets for the control and elimination of malaria, it is crucial to improve current diagnostic techniques by developing and deploying novel, rapid, sensitive, non-invasive, and user-friendly field diagnostic tests. This will improve compliance to routine malaria diagnosis and, more importantly, may enhance home management of the disease, especially in the COVID-19 era, when a provision of standardized healthcare is overstretched and not readily available. It will also improve field diagnosis for epidemiological surveys.

The potential of other biological fluids such as saliva, urine, or sweat to be used as non-invasive samples for malaria diagnosis using the conventional parasite antigen-based rapid diagnostic test kits designed for blood samples remains under-explored. Malaria rapid diagnostic test kits detect Plasmodium antigens such as *Plasmodium falciparum* histidine-rich protein II [10,14], Plasmodium Lactate dehydrogenase [7,14], or *Plasmodium falciparum* aldolase [7] in the patient's blood samples. Previous studies have described the detection of parasite biomarkers, *Plasmodium falciparum* histidine-rich protein II, and Plasmodium Lactate dehydrogenase in patients' saliva with varying degrees of sensitivity using enzyme immunoassays, rapid diagnostic test kits, or molecular tools [12,15,16]. In addition, the possibility of excretion of malarial antigens and antibodies into the urine during malaria infection has been described [9], and *Plasmodium falciparum* histidine-rich protein II has been confirmed in urine samples of patients with acute uncomplicated malaria using Urine Malaria Test™ [17,18] and QDx™ rapid malaria test [19]. Unfortunately, the sensitivity of detection of *P. falciparum* histidine-rich protein II in urine with different optimization strategies was low in the reported studies [17-19]. More studies are still required to provide high-quality surveillance data for decision-making on the eligibility of urine as a non-invasive malaria diagnostic technique. In this study, urine was assessed for its suitability as a non-invasive

medium for malaria diagnosis using the conventional rapid diagnostic test kits designed for blood samples to identify variance in detection levels in different products. Two rapid diagnostic test kits, CareStart™ Malaria PfHRP-2, USA (WHO prequalified) and Global Devices Malaria *P. falciparum/P. vivax* (USA), were comparatively evaluated for *P. falciparum* histidine-rich protein II in the urine of patients with uncomplicated *P. falciparum* malaria. Both kits were originally designed to detect malaria parasite protein in the blood of malaria-infected patients. The diagnostic performances of the two rapid diagnostic test kits were also analyzed at various levels of parasitemia as determined by microscopy.

METHODS

Study site

The study was conducted from September to December 2016 at the Malaria Clinic of the Malaria Research Laboratories, Institute of Advanced Medical Research and Training, College of Medicine, University of Ibadan, Nigeria. It was part of a Drug Therapeutic Efficacy Study. Ethical approval was obtained from the Oyo State Ministry of Health, and written informed consent was obtained from the participants, parents, or guardians before enrolment into the study.

Patients. Patients aged four months and above with microscopically confirmed pure *P. falciparum* infection were eligible to participate in this study. Other criteria for inclusion were temperature ≥37.5 ^{0}C or recent history of fever in the 24-48 hours preceding presentation, absence of concomitant illnesses, and no history of antimalarial drug use two weeks prior to presentation. Written informed consent was obtained from the patients or the parent/guardian of each child prior to enrolment in the study. Demographic information, such as the age and gender of each participant, was recorded. The disease history was taken, and complete physical examinations were performed by the attending physician. Participants' enrollment, collection, and analysis of samples by microscopy and rapid diagnostic test kits are illustrated in Figure 1. Matched blood and urine samples were obtained from each participant (n=100) at presentation before treatment. Twenty-five participants with thin and thick-film negative slides were enrolled to serve as a control, and matched blood and urine samples were obtained from these participants.

Microscopy

Thick and thin blood films were prepared from blood samples of participants collected by finger pricking at enrolment and allowed to air-dry. The dried blood films were stained with 10% Giemsa stain (Thin films were fixed with methanol prior to staining) and examined under a microscope with an oil immersion objective at 1000× magnification for the presence of malaria parasites. Parasite densities were determined with results from the thick films. Asexual parasites were counted against 500 white blood cells, and parasite densities, expressed as asexual forms of parasites per microliter of blood, were estimated from these counts assuming 6,000 white blood cells/μL. Slides were declared falciparum malaria negative after screening at least 200 consecutive fields.

Blood and urine samples for rapid diagnosis of malaria

Five microliters of a blood sample from each participant were obtained by finger prick bloodletting into capillary tubes for rapid malaria diagnosis and analyzed immediately with CareStart™ Malaria PfHRP-2 (Accessbio, USA) RDT kits. Urine samples were collected from participants at the same hour of the enrollment day. Each participant voided urine into 5mL universal bottles, which were placed immediately on ice after collection. The urine samples were analyzed with two brands of rapid diagnostic test kits (CareStart™ Malaria {Accessbio, USA} and Global Devices Malaria {USA}) for PfHRP-2 detection.

Rapid diagnostic tests with blood

The participants' blood samples were tested with CareStart™ Malaria PfHRP-2 (Accessbio, USA) RDT kits according to the manufacturer's instructions. Briefly, five μL of blood sample were dispensed into the sample well of the RDT cassette. Two drops (60 μL) of the buffer were dispensed into the buffer well, and the results were read in 20 minutes. The results were reported as positive for *P. falciparum* infection if two visible bands appeared on the cassette (bands along the control and the *P. falciparum* region) and negative if only a visible band along the control region appeared. Non-appearance of any visible band on the control

region of the cassette indicated invalid results, which were repeated on new cassettes.

Rapid diagnostic test with urine

Five microliters of urine samples were analyzed with two brands of rapid diagnostic test kits (CareStart™ Malaria {Accessbio, USA} and Global Devices Malaria {USA}) for PfHRP-2 detection. CareStart™ Malaria PfHRP-2 kits detect only PfHRP-2, while Global Devices malaria kits detect both PfHRP-2 and P. vivax Lactate Dehydrogenase. Participants were assigned by random purposive allotment into one of the two diagnostic test groups in a ratio of 2.7:1 (CareStart™: Global Devices). Analysis of urine samples with CareStart™ Malaria PfHRP-2 kits followed a similar protocol as stated for blood samples above.

Analysis of urine with the Global Devices Malaria kit also followed a similar protocol to CareStart™ kits, except that the results were read after 10 minutes of adding the buffer, and the result interpretation was slightly different. Two bands appeared on the cassettes for *P. falciparum-positive* patients (on the control and *P. falciparum* regions). Three bands would appear for mixed infection: in the control region and on both the P. vivax and *P. falciparum* regions, while malaria-negative results had only one band along the control region. Results were regarded as invalid if no band appeared on the control region.

Band intensity rating of the rapid diagnostic tests

The color intensities of the bands on the rapid diagnostic test cassettes for *P. falciparum-positive* participants were rated. Each cassette was assigned any of the numbers 1 to 5 in increasing order of band intensity while *P. falciparum* negative detection was assigned a 0 (zero) score. The assignments were as follows: Very Faint (1), Faint (2), Moderately thick (3), Thick (4), Very thick (5).

Data analysis

Data were analyzed using the statistical package for social sciences (SPSS) version 20. Diagnostic performance was assessed using standard measures of diagnostic efficiency, including sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) of the rapid malaria test for urine and blood. Kappa (κ) coefficient was used to calculate the agreement between diagnostic tests. In addition, an association between parasite densities and rapid diagnostic test kits band intensities was tested using Spearman correlation analysis.

RESULTS

Demographic Features

A total of 125 participants were recruited into the study: One hundred (100) patients had microscopically confirmed *P. falciparum* mono-infection, while 25 healthy volunteers with microscopically negative blood films were recruited as negative controls. The baseline characteristics of the recruited participants are summarized in Table 1. Overall, the mean age of all participants was 9.9 ± 4.9 years (range 0.3 – 32). The number of participants aged <5 years, 5-15 years, and >15 years were 18 (14.4%), 95(76%), and 12 (9.6%), respectively. The mean axillary body temperature of *P. falciparum*-infected patients was 37.5 ± 1.2oC (range 35.3 – 41oC). The geometric mean asexual parasitemia in the infected patients was 3575 μL^{-1} (range: 24 – 471556). These parameters were similar in the groups whose urine was tested with CareStart™ malaria and Global Devices malaria kits ($P > 0.05$; Table 1). In addition, there was no significant difference between the geometric mean of parasite density of the participants whose blood or urine samples were tested with either CareStart™ Malaria PfHRP-2 kit or the Global Devices Malaria *P. falciparum/P. vivax* ($P = 0.22$).

Diagnostic performances of RDT with blood samples.

Of the 100 participants with microscopically confirmed falciparum malaria, 98 (98%) blood samples were positive for the presence of *P. falciparum* Histidine-rich protein II using CareStart™ malaria rapid diagnostic test kits. Asexual parasitemia of the two patients with negative RDT results was 389 and 407 μL^{-1}. The sensitivity of CareStart™ rapid diagnostic test kit, when compared with microscopy, was 98% (95% CI=95.3%-100%), while its specificity was 100%, with a kappa agreement of 0.95. The accuracy of the diagnostic test was 98.4%, while its positive and negative predictive values were 100% and 92.6%, respectively.

Diagnostic performances of RDT with urine samples

Of the 100 participants who had confirmed *P. falciparum* infection by microscopy, 71% (71/100) of the urine samples were positive for *P. falciparum* when tested with the rapid diagnostic test kits. The overall sensitivity of the rapid diagnostic test kits for detecting *P. falciparum* HRP-2 in urine compared with microscopy evaluation was 71% (62.1-79.9%). The sensitivity of the RDT in whole blood was significantly higher than in urine (98% versus 71%, P = <0.001), while its specificity when compared with microscopy was 96% (95% CI=88.3%-103.7%) with a kappa agreement of 0.47. The accuracy of the tests was 76%, while the positive and negative predictive values were 98.6% and 45.3%, respectively. However, at asexual parasitemia of ≤ 2000 μL^{-1}, the sensitivity of the rapid diagnostic test kits was 69.6% (95% CI=56.3-82.9), while its sensitivity at asexual parasitemia of > 2000 μL^{-1} was 72.2% (95%CI=60.3-84.2%). Asexual parasitemia in the 29 patients with urine-negative RDT ranged from 118 to 138106 μL^{-1}. All blood samples except one of these 29 patients were positive for RDT.

Comparative analysis of efficiency of CareStart™ test kits and Global Devices Malaria test kits in urine samples

The sensitivity of the CareStart™ test kits for detection of *P. falciparum* HRP-2 in urine was 67.1% (95% CI=56.1%-78.1%) and the specificity was 95.2% (95% CI=86.1%-104.3%) with an agreement of 0.46. The test accuracy was 73.6%, while the positive and negative predictive values were 97.9% and 46.5%, respectively. The Global Devices Malaria kit demonstrated a detection sensitivity of 80% (95% CI=65.7%-94.3%), and the specificity was 100% in urine. The accuracy was 82.4%, while the positive and negative predictive values were 100% and 60%, respectively. There was no significant difference between the sensitivities obtained from the two kits tested with urine (P=0.072). In addition, there was no significant difference between the geometric means of the parasite densities of those whose urine was positive for PfHRP-2 using the rapid diagnostic test kits and those that were negative (P=0.55).

Influence of Packed Cell Volume, Temperature, and parasitemia on RDT positivity

Logistic regression analysis revealed that chances of malaria RDT positivity with blood samples increased with decreasing packed cell volume (OR= 0.98, CI 0.72-1.35, P = 0.92) and increased with increasing parasitemia (OR= 1.001, CI 0.99 - 1.01, P = 0.6). Similarly, the odds of urine RDT positivity increased with decreasing packed cell volume (OR = 0.95, CI= 0.87 - 1.06, P = 0.43) and increased with increasing temperature (OR: 1.158, CI= 0.80 - 1.68, P = 0.4) and parasitemia (OR= 1.01, P = 0.16).

Frequency distribution of urine RDT kits band intensities and parasite density.

Among the 100 RDT kits used for testing the urine of the participants who were *P. falciparum* positive by microscopy, 29 cassettes showed no band (false negative). Eleven cassettes developed very faint bands; 13 cassettes showed faint bands, 15 cassettes showed moderately thick bands, 14 cassettes showed thick bands, and 18 very thick bands. The mean distribution of parasite densities across the various categories of the RDT band intensities is shown in Figure 2, while the association between the parasite densities and the RDT band intensities is shown in Table 3.

DISCUSSION

The desirable protocol for sample collection for malaria diagnosis should be simple, non-invasive, without pain, and practicable for all health workers [15]. Now more crucial than ever, it should also be self-administered with ease. The present study exploited the possibility of using urine as a sample for field malaria diagnosis. The diagnostic performances of two malaria rapid diagnostic test devices, originally designed to detect PfHRP-2 protein in malaria parasite-infected blood, were evaluated for the same antigen in urine samples.

In the present study, the high sensitivity and specificity obtained with the CareStart™ Malaria RDT kit using blood correspond with the WHO's sensitivity and specificity of greater than 95% and greater than 90%, respectively, for any rapid diagnostic test [8]. Furthermore, the specificity of the CareStart™ Malaria RDT kit using blood samples was comparable to that of a previous study conducted in the same endemic area (97.6%).

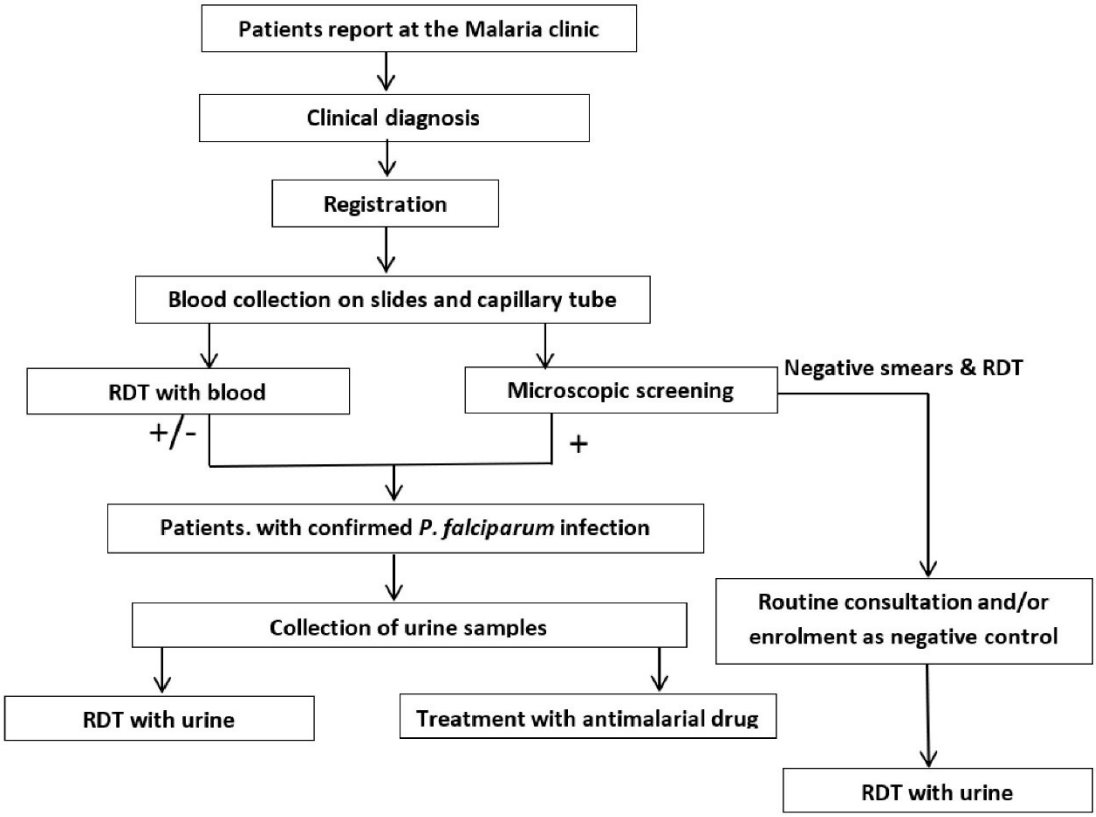

Figure 1. Flow chart showing the patient enrollment process, sample collection, and test performed on samples.

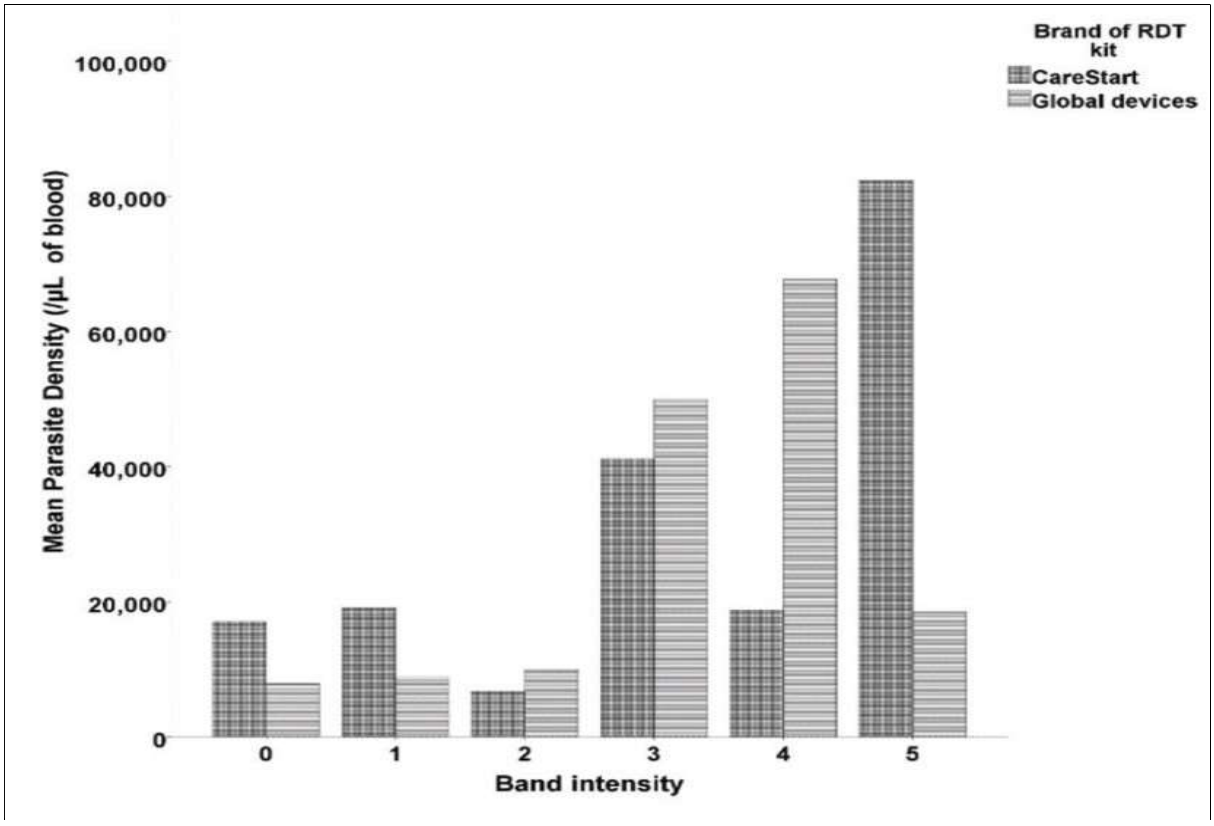

Figure 2. Mean parasitemia in the participants with acute uncomplicated malaria versus the band intensity on the rapid diagnostic test kits.

Table 1. Demographic profile of enrolled participants with acute uncomplicated *falciparum* `malaria and negative control volunteers.

Parameters	Malaria RDT kit types			P value
	Global devices malaria	CareStart™ malaria	All	
***P. falciparum*-positive individuals (by microscopy)**				
Number	30	70	100	
Gender (Male/Female)	19/11	41/29	60/40	0.82
Age (year)				
Mean ± S.D	9.1 ± 4.1	10 ± 4	9.7 ± 4	0.53
Range	3 – 17	2.9 – 20	3 – 20	
No. < 5 years	4	7	11	0.95
5-15 years	23	58	81	0.69
> 15 years	5	3	8	0.73
Weight (Kg)				
Mean ± S.D	25 ± 11.5	25.8 ± 10	25.5 ± 10.5	0.73
Range	5 – 54	10 – 63	5 – 63	
Temperature (°C)				
Mean ± S.D	37.6 ± 1.2	37.5 ± 1.2	37.5 ± 1.2	0.49
Range	35.7 – 39.6	35.3 – 41	35.3 – 41	
No. > 37.4°C	16	34	50	0.83
Duration of illness (day)				
Mean ± S.D	3.8± 2.7	3.4± 2.1	3.5± 2.3	0.36
Range	1– 14	1– 10	1 - 14	
Parasite positivity by microscopy (%)	30	70	100	
Microscopically-negative volunteers (Negative Control)				
Number	4	21	25	
Gender (Male/Female)	3/1	8/13	11/14	1.0
Age (year)				
Mean ± S.D	11 ± 9	10.4 ± 7.7	10.5 ± 7.7	0.35
Range	0.3 – 22	3 – 32	0.3 – 32	
No. < 5 years	1	6	7	0.28
5-15 years	2	12	14	0.24
> 15 years	1	3	4	0.85
Weight (Kg)				
Mean ± S.D	14.0 ± 8.2	26.0 ± 15.5	24.3 ± 15.1	0.21
Range	5 – 54	10 – 63	5 – 63	
Temperature (°C)				
Mean ± S.D	36.8 ± 1.1	36.8 ± 1.1	36.8 ± 1.1	0.98
Range	35.7 – 39.6	35.3 – 41	35.8 – 39.9	
No. > 37.4°C	1	4	5	0.79

ALL- all participants. SD- Standard Deviation. RDT- Rapid Diagnostic Test

Table 2. Comparison of diagnostic performances of the rapid diagnostic tests in blood and urine samples of participants with acute uncomplicated malaria and negative control volunteers.

RDT Kits	Microscopy			Sensitivity (95% CI)	Specificity (95% CI)	Agreement (κ)	Accuracy (%)	PPV (%)	NPV (%)
	Positive	Negative controls	Total						
CareStart™ blood									
Positive	98	0	98						
Negative	2	25	27	98	100	0.95	98.4	100	92.6
Total	100	25	125						
CareStart™ &Global Devices (urine)									
Positive	71	1	72						
Negative	29	24	53	71	96	0.47	76	98.6	45.3
Total	100	25	125						
CareStart™ urine									
Positive	47	1	48						
Negative	23	20	43	67.1	95.2	0.46	73.6	97.9	46.5
Total	70	21	91						
Global Devices									
Positive urine	24	0	24						
Negative	6	4	10	80	100	0.49	82.4	100	60
Total	30	4	34						

The gold standard is microscopy.
PPV - Positive predictive value. NPV - Negative predictive value

However, the sensitivity of the test kit in that study was lower (78.4%) [20]. It thus suggests that there are inherent differences in detection thresholds despite using a test device from the same manufacturer. This may be a result of possible exposure to improper storage conditions, which may reduce the performance quality of products [10].

Overall, the two RDT kits used in this study to diagnose malaria in urine had a high specificity of 96%, conforming to WHO specifications, although with a sensitivity of 71%. Nevertheless, these results are comparable with a similar study that tested for PfHRP-2 in urine using RDT apparatus [17,19,21]. The Global Devices Malaria kits had a higher specificity and equal sensitivity to the Urine Malaria Test™ (UMT) dipstick, which detected PfHRP-2 in a study in the same endemic area [17] with sensitivity and specificity of 83.75% and 83.48%, respectively. A similar study that detected PfHRP-2 in urine using CareStart™ Malaria PfHRP-2 reported a lower specificity (64.6%) but higher sensitivity of 96.6% [21] compared to the results obtained in the present study. Furthermore, Anchinmane and Shedge reported a sensitivity and specificity of 17.3% and 100%, respectively, for detecting PfHRP-2 in urine using the PfHRP-2 and Lactate dehydrogenase-based QDx® Rapid Malaria Test kit [19]. This current finding suggests that the use of urine-based RDTs should be limited to screening out true malaria-negative individuals, whether symptomatic or asymptomatic, especially at points of entry such as airports (for medical check-ups of foreign workers/ tourists from malaria-endemic countries) and not a replacement for current routine malaria diagnostics due to its low sensitivity. The combination of the CareStart™ Malaria kit with Global Devices Malaria kits had higher sensitivities at asexual parasitemia above 2000 μL^{-1} of blood.

The reasons for the reduced sensitivity of the CareStart™ RDT in urine compared to its antigen detection sensitivity in the blood may not be farfetched. This may be partly due to the kits' design, as both kits used were commercially designed to detect PfHRP-2 in blood samples. The apparent accuracy of RDT

in detecting malaria parasites is a function of several factors, including target antigen concentration in the host biological fluid [22]. Other factors may include the dynamics of the antigen-antibody flow along the nitrocellulose strip and the presence of a target epitope to bind with the antibodies in the test sample [22]. Interestingly, appreciably high parasite densities (microscopy) were observed in some participants whose urine samples tested negative for PfHRP-2 using the rapid diagnostic test kits. These false negative outcomes may be ascribed to the proteolytic cleavage of the proteins that are excreted in the urine [16,17], which may affect the performance of rapid diagnostic test kits. Another possible reason for the false negativity may be due to the occurrence of *P. falciparum* with deleted or mutated HRP-2 antigen-coding genes [23].

Table 3. Associations between patients' demographic parameters, urine RDT performance and parasite densities.

Variables		R	P value
1	2		
Age	RDT Band intensity	-0.76	0.45
Age	Parasite Density	-0.17	0.09
Sex	RDT Band intensity		0.78
Sex	Parasite Density		0.12
Parasite density	RDT Band intensity	0.16	0.11
Parasite density	Urine RDT kits Positivity		0.55
Illness duration	Parasite Density	-0.07	0.47
Illness duration	RDT Band intensity	0.148	0.14

RDT kit band intensities correlate positively with parasitemia quantified by microscopy. This would suggest that the secretion of the protein, PfHRP-2 into the urine is directly proportional to the parasitemia. A previous study also indicated that a positive correlation exists between the concentration of the proteins in the blood and parasite biomass [24]. Although, in the present study, few RDT cassettes that were tested with the urine of patients with low parasitemia had high band intensities and vice versa. These outliers suggest that the interpretation of the band intensity on the RDT cassettes could extend beyond the circulating parasitemia to include sequestered parasite biomass [25]: It could be that the periods of blood sampling coincided with the time course of asexual parasites sequestration in deep tissues resulting in a reduction in their peripheral blood levels [25]. Studies from the same study region [26] and other geographical locations [27] have demonstrated early mobilization of asexual parasitemia from deep tissues to peripheral circulation following artemisinin-like treatment. Other reasons may be the immune responses of the host and competition between clones in multiclonal infections [28].

The inverse relationship between duration of illness and parasite density further explains the greater tendency for the parasites to sequester into deep tissues as the days of untreated illness increase, thus reducing the parasites in peripheral circulation during the sampling period. It is noteworthy that the duration of illness, however, correlates positively but not significantly with the band intensity. This is supported by reports of the persistence of PfHRP-2 in the blood for as long as two weeks after complete parasite clearance [9,14]. The parasite densities were similar across the age group distribution and the intensities of the bands on the rapid diagnostic test kits. Age and sex showed no influence on the parasitemia and the intensities of the bands on the RDT kits.

Although the assessment of the rapid diagnostic test performances in urine samples revealed that Global Devices malaria RDT kits demonstrated higher sensitivity and specificity than the CareStart™ RDT kit, the sensitivities of the malaria RDTs in the urine reported in this study are still below optimum. This presents a challenge to using urine as a diagnostic fluid for diagnosing *P. falciparum* malaria. The timing of urine collection has also been previously implicated as a determinant of test sensitivity [29]. Though first void morning urine was suggested to give better sensitivity than those collected at later times, this method may not be realistic in clinical practice where the results of diagnosis are required immediately to effect prompt treatment.

Malaria rapid diagnostic tests with urine samples are associated with some biases which may affect test performances: (i) low level of parasitemia may present a false negative RDT result (ii) persistence of histidine-rich protein II in urine long after parasite clearance may yield a false *P. falciparum* positive result [9] (iii) the concentration of parasite protein in urine is lower than that in the blood which results in lower sensitivity of the RDT with urine[22], hence the design of urine-based RDTs should be optimized to detect lower thresholds of antigens due to potential for dilution of parasite antigens in urine (iv) conditions such as anemia, hematuria, and pregnancy could influence the performance of test [30]

Finally, future studies should incorporate a proteomic analysis of the parasite protein to determine the amount of PfHRP-2 in the urine. With the quantification of parasite proteins, a better comparison of the parasite density, RDT kit positivity, and band intensity can be made. In designing kits specific for urine samples and other biological fluids for malaria diagnosis, efforts should be made to circumvent the possible biases and limitations that may reduce their performance.

CONCLUSION

The study's findings revealed that although *P. falciparum* infections can be detected in the urine of malaria-infected patients, urine-based RDTs are not sensitive enough to replace the current routine blood-based malaria Rapid Diagnostic Tests. Blood-based malaria diagnostics are still encouraged for routine malaria diagnosis and epidemiological surveillance studies as the associated pains are mild, tolerable, and temporary, with broad applications to adults and children. In addition, urine-based RDTs may help screen healthy people in cases/places where blood-based malaria tests are not readily available or impracticable. However, this and other non-invasive diagnostic methods require more optimizations to meet required diagnostic standards.

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