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Research Article

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A comparison of microscopic and rapid diagnostic test methods for diagnosis of malaria parasite

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Keywords

Malaria, Rapid diagnostic tests, Microscopy, Sensitivity, Specificity.

Abstract

Early and accurate diagnosis of febrile patients is essential for the proper treatment of uncomplicated malaria and prevention of severe malaria. This necessitated the introduction of rapid diagnostic tests (RDTs) for malaria for patients in endemic regions with poor power supply and shortage of qualified manpower required for microscopy. However, there is very little evidence to guide decision-makers on the sensitivity and specificity of these RDTs. Therefore, the aim of this study was to compare the use of microscopy and RDTs in the diagnosis of malaria parasite. The hospital-based retrospective study was conducted by reviewing laboratory registers and records of 387 patients with febrile illness lasting for 1-3 days who were clinically diagnosed of malaria fever and sent to the laboratory for confirmation. The overall prevalence of malaria parasite with smear microscopy of 39.3% was higher than in RDT test 37.0% but there was no significant difference between smear microscopy and RDT test results (P=0.685). This study found a low proportion of false positive and negative test results with the RDT, which is a characteristic of a test method that can give a quality result. Using smear microscopy as the gold standard, RDT was found to have a sensitivity of 94.0% and specificity of 99.6% with positive predictive and negative predictive values of 99.3% and 96.3% respectively. The sensitivity of RDT test was higher in males (93.8%) than in females (93.2%) but there was no significant difference between the two genders (P=0.819). The mean age of participants with negative RDT results (32.68) was significantly higher than those with RDT positive result (20.50) (P<0.0001). The highest sensitivity of the RDT (97.4%) was in those under 18 years. However, there were no significant differences among the different age groups (p<0.05). Similarly, the mean PCV of participants with negative RDT results (32.07) was significantly higher than those with RDT positive result (28.15) (P<0.0001). Antigen-based malaria RDT is as reliable as smear microscopy for the diagnosis of malaria parasite in malaria endemic areas.

Introduction

Over one million children die annually from malaria, especially in sub-Sahara Africa with over 500 million people affected by malaria worldwide (Amazu et al., 2009). Improving access to effective treatment for malaria is one of the initiatives to reduce malaria associated morbidity and mortality in malaria endemic countries (Alba et al., 2010). Cases of suspected malaria requires parasite-based diagnosis, except for children in high-prevalence areas and certain other situations, to achieve the improved access to effective treatment for malaria (Bell et al., 2001; WHO, 2006). However, this laudable initiative was impeded by lack of rapid and accurate laboratory detection of malaria parasites in those seeking the test in primary health facilities where there is lack of power supply for the conventional microscopic identification of parasites and lack of expertise to carry out the test, which remains the gold standard in laboratory diagnosis of malaria (Nandwani et al, 2005). This led to the introduction of rapid diagnostic tests (RDTs) for malaria for patients in endemic regions, especially in settings with poor power supply and shortage of qualified manpower in Africa. RDTs come as ready to use kits with all necessary reagents and accessories. They are easy to perform without the requirements of extensive training or sophisticated equipment for performing the test or to interpret the results. Results are ready in about 15-20 minutes (Moody, 2002). The kits detect malaria parasites' protein *i.e.* histidine on the red blood cells whole blood for antigen method or the presence of antibodies against histidine in the human serum for antibody method (Moody, 2002).

However, there is very little evidence, especially from malaria endemic areas to guide decision-makers on the sensitivity and specificity of these RDTs. Therefore, the aim of this study was to compare the use of microscopy and RDTs in the diagnosis of malaria.

Objectives

1. To compare the use of microscopy and RDTs in the prompt and accurate diagnosis of malaria
2. To determine the performance of rapid diagnostic test among different age groups
3. To determine the performance of rapid diagnostic test based on gender
4. To determine the performance of rapid diagnostic test based on parked cell volume (PCV) level

Methods

A retrospective study was conducted to compare the use of microscopy and RDT in the diagnosis of malaria by reviewing laboratory registers and records of 387 patients, who visited Cottage Hospital Otuasega, Ogbia local government area, Bayelsa state, South-South Nigeria with febrile illness lasting for 1-3 days and were clinically diagnosed of malaria fever and sent to the laboratory for malaria diagnosis between March and October 2016.

Venous blood samples were collected from the 387 patients into BD EDTA vacutainers by trained laboratory staff. Thick and thin films were made from the EDTA samples within 10 minutes of sample collection. The films were air-dried and thereafter stained with 10% Giemsa solution, washed after 10 minutes of staining using clean water. The stained slide was air-dried; a drop of immersion oil was applied and examined microscopically for malaria parasites using 100X objective lens.

This study used CareStart™ Malaria RDT, which is an immunochromatographic test coated with monoclonal antibody specific for Plasmodium falciparum's Histidine Rich Protein-2 (HRP-2). A drop (5µL) of the EDTA whole blood samples was added to the RDT cards and two drops of buffer (60µL) was added to form a blood-buffer mixture. The mixtures go through the card across the lines of bound antibody and gives a result within 20 minutes. A positive result indicates the presence of two bands (test line and control line). For a negative result, only the control line appeared.

Results

Characteristics of the Study participants

The participants were 387 made up of 247 females (63.8%) and 140 males (36.2%). The mean age (± standard deviation) of all participants was 28.2 ± 21.3 years with ages ranging from 2 month to 100 years and with 133 of them of ages ≤18years, 126 between 19 to 35 years old and 128 above 35 years of age (Figure 1 & 2).

Figure 1: Gender of general population

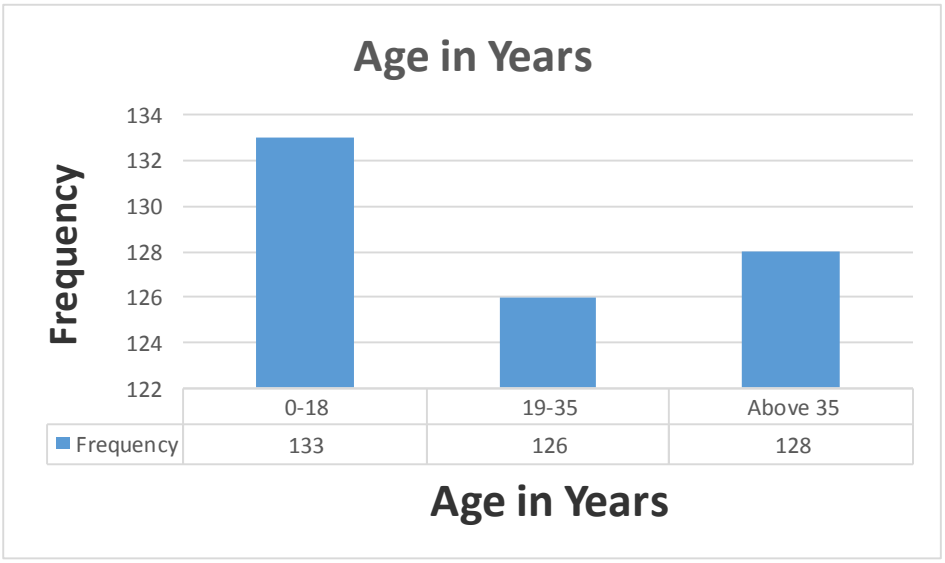

Figure 2: Age Distribution and frequency of Participants.

Performance of the RDT among different age groups

The mean age of participants with negative smear microscopy results (32.6) was significantly higher than those with smear microscopy positive result (21.3) (P<0.0001). Similarly, the mean age of participants with negative RDT results (32.7) was significantly higher than those with RDT positive result (20.5) (P<0.0001) (Table 1).

Table 1: Mean age of participants tested with smear Microscopy and RDT

Test Method	Results	N	Mean	Std. Deviation	P value
Malaria Parasite Examination by Microscopy	Positive	152	21.3	19.7	P<0.0001
	Negative	235	32.6	21.0	
Malaria Parasite Examination by RDT	Positive	143	20.5	19.2	P<0.0001
	Negative	244	32.7	21.1	

The sensitivity of the RDT appeared to decrease with the ages of the participants of this study with those under 18 years, 19 - 35 years and above 35 years recording sensitivities of 97.4%, 90.9% and 90.3% respectively. However, there were no significant differences among the different age groups ($p<0.05$) (Table3). The Positive Likelihood Ratio of the RDT was zero among all the age groups 19 -35 and above 35 years old since there was no case of false positive result. The lowest negative likelihood ratio of 0.03 and highest disease prevalence of 57.1 were in those under 18 years of age. The lowest disease prevalence among the participants (24.2) was recorded among those above 35 years of age (Table 2 & 3).

Performance of the RDT in relation to smear microscopy

A total of 152 participants tested positive for malaria by blood smear microscopy while 142 tested positive for malaria parasite by RDT. These give prevalence of 39% and 37% of malaria parasite among the participants using microscopy and RDT respectively. The overall prevalence of malaria parasite with smear microscopy was higher than in RDT test but there was no significant difference between smear microscopy and RDT test results (P=0.685). Taking smear microscopy as the gold standard, the performance of the RDT was computed to determine its sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV). The sensitivity of the RDT test was found to be 94.0% and specificity of 99.6% while the PPV and NPV were 99.3 and 96.3 respectively (Table 2 & 3).

Table 2: No. of subjects with true/false negative and true/false positive RDT's test results by age

Categories	True positive	False positive	True negative	False negative	Total
General Population	142 (36.7)	1 (0.3)	235 (60.5)	9 (2.6)	387
under 18 years	74 (56.4)	1 (0.3)	56 (42.1)	2 (1.5)	133
19 - 35 years	40 (31.7)	0 (0.0)	82 (65.1)	4 (3.2)	126
above 35 years	28 (21.9)	0 (0.0)	97 (75.8)	3 (2.3)	128

Table 3: Diagnostic performance of rapid diagnostic test (RDT) among different age groups

Diagnostic parameters	General population	Under 18 years	19 - 35 years	above 35 years
Sensitivity	94.0	97.4	90.9	90.3
Specificity	99.6	98.3	100	100
Positive predictive value	99.3	98.7	100	100
Negative predictive value	96.3	96.6	95.4	97.0
Positive likelihood ratio	221.9	55.5	NC	NC
Negative likelihood ratio	0.1	0.03	0.1	0.1
Disease prevalence	39.0	57.1	34.2	24.2
Diagnostic odd ratio	2219	1850	NC	NC

NC: Not computed since no value was recorded for false positive

Performance of the RDT based on gender

Fifty-eight percent (58%) of clients diagnosed of malaria using smear microscopy were female and 42% were male. Similarly, 57% of clients diagnosed of malaria using malaria RDT test were female and 43% were male. This shows a sexual distribution in the malaria positive results given by both smear microscopy and RDT with females appearing to be more infested by malaria parasite than males in the study area.

The sensitivity of RDT test was higher in males (93.8%) than in females (93.2%) but there was no significant difference between the two genders (P=0.819). The specificity of RDT test was higher in females (100%) than in males (98.7%) but there was no significant difference between the two genders (P = 0.173 (Table 4 & 5).

Table 4: No. of subjects with true/false negative and true/false positive RDT's test results by Sex

Categories Total	True positive	False positive	True negative	False negative
General Population 387	142 (36.7)	1 (0.3)	235 (60.5)	9 (2.6)
Male 140	60 (56.4)	1 (0.3)	75 (42.1)	4 (1.5)
Female 247	82 (31.7)	0 (0.0)	159 (65.1)	6 (3.2)

Table 5: Diagnostic performance of rapid diagnostic test between gender

Diagnostic parameters	General population	Male (139)	Female (248)	p-values
Sensitivity	94.0	93.8	93.2	p=0.850
Specificity	99.6	98.7	100	
Positive predictive value	99.3	98.4	100	
Negative predictive value	95.9	94.9	96.4	
Positive likelihood ratio	219.5	71.3	NC	
Negative likelihood ratio	0.1	0.1	0.1	
Disease prevalence	39.3	45.7	35.6	
Diagnostic odd ratio	2195	713	NC	

NC: Not computed since no value was recorded for false positive

Performance of the RDT based on PCV level

The mean PCV ± standard deviation of all participants was 30.3 ± 7.5%. The mean PCV of participants with negative smear microscopy results (32.3 ± 7.1%) was significantly higher than those with smear microscopy positive result (28.1 ± 7.4%) (P<0.0001). Similarly, the mean PCV of participants with negative RDT results (32.1 ± 7.1%) was significantly higher than those with RDT positive result (28.2 ± 7.5) (P<0.0001).

Table 6: Diagnostic performance of smear microscopy and RDT by PCV level

Test Method	Test Result	Mean PCV	Standard deviation	p-values
Microscopy	Negative	32.33	7.09	P<0.0001
Microscopy	Positive	28.07	7.41	
RDT	Negative	32.07	7.14	P<0.0001
RDT	Negative	28.15	7.48	

Discussion

This study found a low proportion of false positive and negative test with the RDT, which is a characteristic of a test method that can give a quality and reliable result.

The sensitivity of the RDT of 94.0% reported in this study is lower than 96%, 97%, 97.6% and 100% reported by Hopkins et al., (2008); Msellem et al (2009); Nicastri et al (2009) and Ajumobi et al (2015) but higher than the 62.5% reported by Osei-Yeboah et al (2016) in studies within and outside Nigeria. This means that the probability of the RDT to detect

positive cases among those that are positive with smear microscopy is as high as 94.0%. However, the sensitivity from this study is not up to the 95% sensitivity recommendation by World Health Organization (WHO, 2000).

The specificity of 99.6% found in this study is higher than 93%, 88%, 98.5% and 92.7%% reported by Buchachart et al (2004); Msellem et al (2009); Ajumobi et al (2015) and Osei-Yeboah et al (2016) but less than the 100% reported by Dougnon et al (2015). This means that the probability of the RDT to detect negative cases among those that are negative with smear microscopy is as high as 99.6% and therefore prevent unnecessary treatment of those who are not having the parasite but only those with the parasite. Also, the high specificity of the RDT will improve the cost effectiveness of malaria diagnosis since it is unlikely to miss out those not having malaria parasite.

This study found the proportion of individuals with positive RDT results among the general population who had positive malaria smear microscopy (positive predictive value - PPV) was 99.3% while the proportion of individuals with negative RDT results among those who had negative malaria result with smear microscopy (negative predictive value - NPV) was 96.3%. The high PPV means that any individual who has a positive RDT test result would have high probability of having a positive malaria test result with smear microscopy or being malaria positive. Also, the very high positive diagnostic likelihood ratio is because of low false positive test indicating that the RDT has a very good likelihood of presenting a positive test in infected clients compared to their uninfected counterparts and therefore can serve as useful tool in early diagnosis of malaria in clients to avert the burdens of late diagnosis.

The highest sensitivity of the RDT among those under 18 years old found in this study agrees with that reported by Nkrumah et al (2011); Osei-Yeboah et al (2016) where high sensitivities of RDT was found in children compared to adults. The high sensitivity among this age group could be due to lower immunity and therefore less interference by antibodies among them.

The findings of this study of PCV of participants with negative smear microscopy and RDT results being higher than those with positive results agreed with the report by Ayodele (2014). This could be so because malaria parasites need to infect the red blood cells of their human host for their continued survival and reproduction. This causes destruction of both red blood cells parasitized and non-parasitized and their sequestration. Also, high circulating tissue necrotic factor leads to ineffective erythropoiesis (Abdalla et al, 2004; Akhtar et al, 2012). These bring about a reduction in PCV level below the normal range, hence severe anaemia is common in malaria endemic areas (Abdalla et al, 2004).

Conclusions

We conclude that malaria RDTs are as reliable as smear microscopy in the diagnosis of malaria, especially the detection of *P. falciparum.* RDTs are helpful in the diagnosis of malaria parasite in low resource settings where major laboratory equipment for the diagnosis of malaria are unavailable.

We recommend that only antigen based kits be used for malaria parasite test in the tropics where malaria is endemic.

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