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Original Article

Evaluation of two new rapid diagnostic tests (RDTs) for the diagnosis of malaria

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Abstract

Rapid diagnostic tests (RDTs) address the need for accurate diagnosis of malaria, particularly in resource limited settings. In this study, two malaria RDTs were compared with gold standard microscopy: OnSite Pf/Pv test detecting Plasmodium falciparum-specific histidine rich protein-2 (Pf HRP2) and P. vivax-specific parasitic lactate dehydrogenase (pLDH) antigens; and SD Bioline anti-Pf/Pv test detecting anti-HRP2 and anti-pLDH antibodies for the diagnosis of P. falciparum and P. vivax infections, respectively. For OnSite test, the overall sensitivity was found 96.2%, specificity 98.2%, positive predictive value (PPV) 98.2%, negative predictive value (NPV) 96.4% and agreement with microscopy was found to be 0.94. On the other hand SD Bioline test, the overall sensitivity was 75.4%, specificity 83.7%, PPV 84.3%, NPV 74.5% and agreement with microscopy was 0.59. These data revealed that the RDT based on antigen detection (Onsite test) was more reliable than that based on the antibody detection (SD Bioline test).

Key words: Malaria, Rapid Diagnosis, Plasmodium falciparum, Plasmodium vivax, RDT.

Introduction

Malaria is still considered a major public-health problem in the thirteen eastern districts of Bangladesh, bordering India and Myanmar with 98% malaria cases. According to a nationwide survey in 2007, Plasmodium falciparum caused 90.18% infections, P. vivax caused 5.29% and the remaining (4.53%) was due to the mixed infections by the two species[1].

Prompt and accurate diagnosis is critical for the effective management of malaria. The global impact of malaria has spurred interest in developing effective diagnostic strategies for resource-limited areas where malaria is a substantial burden. Malaria is diagnosed predominantly by assessing the clinical signs and symptoms, along with compound microscopy as the current gold standard for detecting parasitemia[2]. However, well-trained, competent microscopists along with careful maintenance of functional infrastructures and effective quality control (QC) systems are the prerequisites for microscopy[3].

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Lack of resources acts as a major barrier to reliable and scheduled diagnosis in many malaria-endemic countries such as Bangladesh. In the last 10 years, the quest for newer and easier diagnostic methods for malaria has picked up momentum and the development of lateral flow immunochromatographic-based rapid diagnostic tests (RDTs) has opened a new avenue[4]. RDTs offer a simple and rapid complement to malaria diagnosis by microscopy. Thus, it can be useful for the diagnosis of malaria in circumstances where microscopy-based diagnosis may be limiting or unreliable.[2] Based on this fact, the National Malaria Control Programme (NMCP) of Bangladesh has been using RDTs to improve the management of malaria in endemic areas where facilities for microscopy is often lacking[5].

In this study, two malarial RDTs: OnSite Pf/Pv test (CTK Biotech, USA) and SD Bioline anti-Pf/Pv test (SD Bioline, Korea) have been evaluated in comparison to microscopy to observe whether they can be used as alternatives to microscopy, especially in resource limited settings. The Onsite test detects Pf HRP-2 and pLDH antigens; and SD Bioline test detects anti-Pf HRP-2 and anti-pLDH antibodies for the diagnosis of P. falciparum and P. vivax infections, respectively. In 2011, a study has been conducted in Bangladesh where four RDTs (Paracheck, Falcivex Pf, Onsite

Pf and Onsite Pf/Pv) based on antigen detection were evaluated against microscopy and real-time PCR[5]. However, our study evaluated two RDTs based on two different modes of action. Of the two RDTs, Onsite Pf/Pv test was based on antigen detection whereas SD Bioline anti-Pf/Pv test was based on antibody detection.

Methods

Study area and sample size

A total of 106 blood samples were used in this evaluation which were collected in between May 2009 to June 2010. Samples were taken from febrile patients referred for malaria diagnosis at Matiranga Upazila Health Complex (UHC), Khagrachari district; and at Ramu UHC and Ukhia UHC of Cox's Bazar district. Subjects within the 3-60 years of age were considered eligible for the study. Subjects those participated willingly in this study were enrolled and no sex or ethnic barrier was considered. The OnSite Pf/Pv test (device) and SD Bioline anti-Pf/Pv test (device) were evaluated at the Parasitology laboratory, ICDDR,B using stored blood samples. Results were compared with those obtained from the compound microscopy.

Sample collection and processing

Five ml of blood was collected from each adult patient through venepuncture maintaining asepsis. In case of children, three ml of blood was taken. For microscopy, 2 drops of samples were used to prepare thick and thin blood smears. The remaining samples were preserved in sterile EDTA (Ethylene-diaminetetraacitic acid) tubes and were placed in an ice bag for transportation to the Parasitology Laboratory, ICDDR,B. The samples were then stored at-20^{o}C.

Microscopy

For microscopy, both thick and thin blood film with whole blood sample of each patient were prepared by standard procedure as described by Kilian et al[6]. The smear was stained with 10% Giemsa dye in phosphate buffer saline and was examined using a compound microscope under X1000 magnification. This was done at the field site by an experienced microscopist. Blood films were defined as negative upon the absence of parasite. Declaring a smear positive or negative was routinely based on the examination of at least 200 fields in the Giemsa-stained thick film and species identification was done by examining the thin film under X1000 magnification. When at least one parasite was found, a smear was recorded as positive. Slides were cross-checked by another experienced microscopist posted at the corresponding Upazila Health Complex.

Rapid diagnostic tests

The OnSite Pf/Pv test (device) and SD Bioline anti-Pf/Pv test (device) were evaluated at the Parasitology laboratory, ICDDR,B. Tests were done and results were considered positive according to the respective manufacturer's instruction leaflet. Briefly, 5 µl and 20 µl of whole blood were added on the test device for OnSite and SD Bioline, respectively. Then 3-4 drops of lysis buffer were added. After 20-30 minutes the result was read for OnSite test, while the result was read within 10 minutes for SD Bioline test.

Statistical analysis

All quantitative data were recorded in a data sheet and were processed using SPSS software version 17.0 (SPSS Inc. USA). Sensitivity and specificity as well as the positive predictive value (PPV), negative predictive value (NPV) and Kappa (k) value were calculated considering the results of microscopy as gold standard.

Results

According to microscopy, out of 106 suspected patients, 27 (25.5%) and 22 (20.8%) were positive for P. falciparum and P. vivax, respectively. Two patients had mixed infection (1.9%) of both these parasites. Thus, a total of 51 (48.1%) patients were positive for malaria parasites by microscopy. However, according to OnSite RDT, 27 (25.5%) and 22 (20.8%) were positive for P. falciparum and P. vivax, respectively, with only one (0.9%) case of mixed infection. According to SD Bioline RDT, 35 (33.0%) were P. falciparum infected and 16 (15.1%) were P. vivax infected while 6 (5.7%) were infected with both (Table I).

Table I: Comparison of OnSite and SD Bioline diagnosis results with results of Microscopy for malaria

Microscopy		OnSite			SD Bioline			
	N	Pf	Pv	MI	N	Pf	Pv	MI
N	55 (51.9%)	54	1	0	0	41	13	0	1
Pf	27 (25.5%)	2	24	0	1	5	19	0	3
Pv	22 (20.8%)	0	0	22	0	3	1	16	2
MI	2 (1.9%)	0	2	0	0	0	2	0	0
Total	106	56	27	22	1	49	35	16	6
		(52.8%)	(25.5%)	(20.8%)	(0.9%)	(46.2%)	(33.0%)	(15.1%)	(5.7%)

N= Negative, Pf= Plasmodium falciparum positive, Pv= Plasmodium vivax positive, MI= Mixed Infection (both P. falciparum and P. vivax positive)

OnSite RDT showed 96.2% sensitivity and 98.2% specificity (PPV 98.2% and NPV 96.4%) for detection of any Plasmodium infection when compared with microscopy. However, it showed sensitivity and specificity for the detection of P. falciparum 93% and 98.7%, respectively (with 96.4% PPV and 97.4% NPV). For P. vivax, 91.7% sensitivity and 98.8% specificity (95.7% PPV and 97.6% NPV) were reported. On the other hand, SD Bioline test showed 75.4% sensitivity and 83.7% specificity (PPV 84.3% and NPV

74.5%) for the detection of any Plasmodium infection, with 82.8% sensitivity and 77.9% specificity (58.5% PPV and 92.3% NPV) for the detection of Plasmodium falciparum; and 75% sensitivity and 95.1% specificity (81.9% PPV and 92.9% NPV) for the detection of P. vivax. Overall agreement with microscopy (k value: determined by using SPSS 17.0) was found 0.94 and 0.59 for OnSite test and SD Bioline test, respectively (Table II).

Table II: Comparative indicators of OnSite and SD Bioline tests for malaria

Indicators	OnSite	SD Bioline				
	Overall	Pf	Pv	Overall	Pf	Pv
Sensitivity	96.2	93	91.7	75.4	82.8	75
(95% CI)	(85.7-99.3)	(75.8-98.8)	(71.5-98.5)	(61.9-85.5)	(63.5-93.5)	(52.9-89.4)
Specificity	98.2	98.7	98.8	83.7	77.9	95.1
(95% CI)	(89.0-99.9)	(91.9-99.9)	(92.5-99.9)	(69.8-92.2)	(66.8-88.3)	(87.3-98.4)
PPV	98.2	96.4	95.7	84.3	58.5	81.9
(95% CI)	(88.2-99.9)	(79.8-99.8)	(76.0-99.8)	(70.9-92.5)	(42.2-73.3)	(58.9-94.0)
NPV	96.4	97.4	97.6	74.5	92.3	92.9
(95% CI)	(86.2-99.4)	(90.2-99.6)	(90.8-99.6)	(60.7-84.9)	(82.2-97.1)	(84.5-97.1)
Kappa	0.94	0.93	0.92	0.59	0.54	0.72

CI= Confidence Interval, PPV= Positive Predictive Value, NPV= Negative Predictive Value, Pf= Plasmodium falciparum, Pv= Plasmodium vivax

Microscopic images for Plasmodium falciparum and P. vivax:

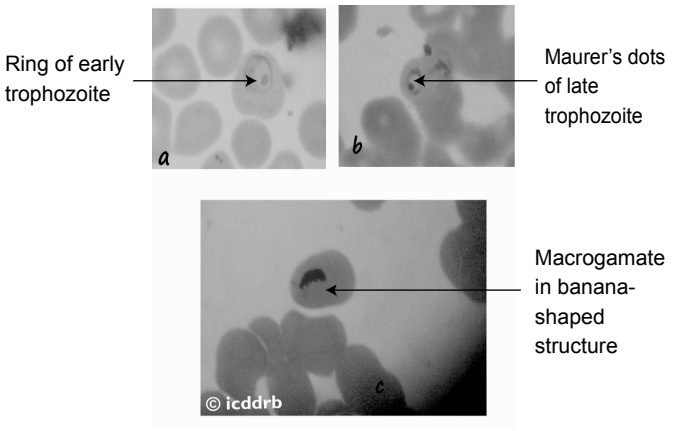

Figure 1: Different stages of Plasmodium falciparum. a. Early trophozoite, b. Late trophozoite, c. Macrogamete (X 1000 magnification under compound light microscope).

a. Early trophozoite stage was designated by a ring form structure inside RBC (Red Blood Cell). For Plasmodium falciparum, the size of early trophozoite ranged from 1/5 to 1/3 of RBC diameter. Appearance of the infected red cells remained unchanged.

b. Late trophozoite stage was designated by the onset of big and few blue-mauve Maurer's dots in RBC cytoplasm.

c. Macrogamate stage of P. falciparum was designated by a banana shaped structure in RBC cytoplasm.

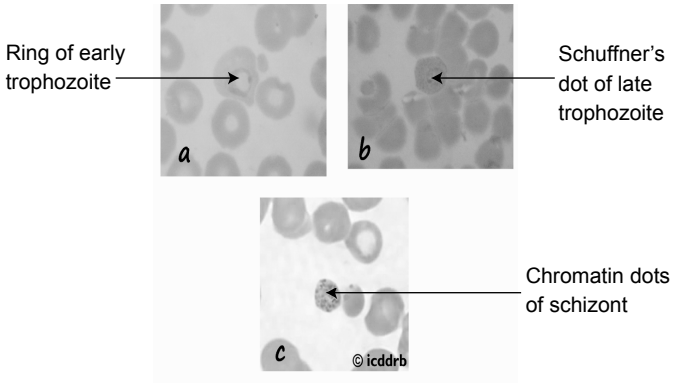

Figure 2: Different stages of Plasmodium vivax. a. Early trophozoite, b. Late trophozoite, c. Schizont (X 1000 magnification under compound light microscope).

a. Early trophozoite stage was designated by a ring form structure inside RBC. For Plasmodium vivax, the size of early trophozoite ranged from 1/4 to 2/3 of RBC diameter. Appearance of the infected red cells became enlarged and pale.

b. Late trophozoite stage was designated by the onset of small and numerous pink Schuffner's dots in RBC cytoplasm.

c. Schizont stage was designated by chromatin dots in RBC cytoplasm. At this stage the parasite reproduces itself so its chromatin could be detected by microscopy.

Discussion

Our study results revealed that the Onsite test was more efficient than the SD Bioline test as an alternative to microscopy. Overall sensitivity of SD Bioline test was markedly lower (75.4%, CI: Confidence Interval, 95%) than OnSite test (96.2%, CI, 95%) for the diagnosis of malaria. The sensitivity of SD Bioline test was also reported lower in other similar studies in Nigeria[7,8]. The underlying reason is that the OnSite test is based on a direct method which detects antigens and SD Bioline test is an indirect method which detects the presence of antibody in sample. High sensitivity of OnSite test indicated that there had been significant correlation between the results of OnSite test and microscopy. For OnSite test, there was little difference between the sensitivity for P. falciparum

(93%) and P. vivax (91.7%). In case of SD Bioline test, sensitivity for P. vivax (75%) was much lower than that for P. falciparum (82.8%). It is known that the anti-pLDH antibodies are likely to be less temperature stable compared to Pf HRP2 specific antibodies and looses sensitivity more rapidly in uncontrolled storages[9]. The sensitivity should be >95% to prove the usefulness for RDT[10].

Moreover, overall specificity of OnSite test (98.2%, CI, 95%) was higher than that of the SD Bioline test (83.7%, 95% CI). The specificity of SD Bioline test was reported low in a study conducted in rural Nigeria[7] but in another study in asymptomatic malaria in children of Nigeria it was reported even up to 100%[7]. In case of OnSite test, specificity for both P. falciparum and P. vivax were 98.7% and 98.8%, respectively. In case of SD Bioline test, specificity for P. falciparum (77.9%) was lower than that for P. vivax (95.1%). The reason behind the low specificity may be due to the detection of false positive P. falciparum infection in the suspected cases. This might be due to any previous history of infection with P. falciparum.

NPV was also higher for OnSite test than SD Bioline test. Similar to the study, high NPV for OnSite test has been reported in a recent study in Bangladesh[5]. The NPV was also reported low (68.0%) for SD Bioline test in another study conducted in 2003[8] but in another study conducted in 2007, it was significantly higher (83.2%)[7]. In case of Onsite test, the high NPV thus permitted us to assertively diagnose negative test patients as non-malarial patients[6]. Hence, the possibility of missing a positive case is less by OnSite test than SD Bioline test. As the sensitivity, specificity, PPV and NPV was found to be higher for Onsite test, the agreement with microscopy was also higher for OnSite test than SD Bioline test. This again indicated the superiority of OnSite test over SD Bioline test.

In this study, recently introduced OnSite Pf/Pv test and SD Bioline anti-Pf/Pv test were evaluated for their diagnostic capacity compared to microscopy. National Malaria Control Programme (NMCP) of Bangladesh is now using Pf HRP-2 based Paracheck RDT which detects only P. falciparum infections[5]. Although P. falciparum causes the majority of malaria cases in Bangladesh, malaria prevalence survey in 2007 showed that 10% cases are due to pure P. vivax infection or mixed infections of these two species. So, now NMCP is aiming at RDTs that can detect multiple infections[5]. As Onsite Pf/Pv and SD Bioline anti-Pf/Pv test can detect multiple infections, this study could provide additional support for NMCP. Moreover, this study evaluated two RDTs that were based on different modes of action. Thus, the findings of this study can provide an effective guideline to NMCP for monitoring their ongoing programme.

In conclusion, the OnSite Pf/Pv test was found to be more reliable than the SD Bioline anti-Pf/Pv test as a diagnostic tool for malaria revealing that, RDTs based on antigen detection are more precise than the RDTs based on antibody detection. When the facilities for microscopy are found to be poor, OnSite test can be a potential alternative to microscopy. As now, National Malaria Control Programme (NMCP) of Bangladesh is aiming at RDTs that can detect multiple infections[5]. Onsite Pf/Pv could be a method of choice for application in resource limited settings. Thus, the findings of this study can provide an effective guideline to NMCP for monitoring their ongoing programme.

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