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COMPARISON OF RAPID IMMUNOCHROMATOGRAPHIC ASSAYS (ICT MALARIA P.F. /P.V. TEST AND OPTIMAL TEST) WITH MICROSCOPY FOR DETECTION OF MALARIA PARASITES

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ABSTRACT

This study was conducted to compare the ability of developed immunochromatographic assays, ICT malaria P.f./P.v. test and OptiMAL test with standard microscopy for the diagnosis of malaria parasites. Blood samples were obtained from 150 patients clinically suspected of having malaria. A total of 59 samples (39.3%) were positive by blood films while 64 (42.7%) were positive by ICT p.f./p.v. and 52 (34.7%) by OptiMAL tests. The blood film indicated that 32.2% (19 of 59) of patients were positive for *P. vivax* and 67.8% (40 of 59) were infected with *P. falciparum*. ICT P.f./P.v. test showed 23.4% (15 of 64) were positive for *P. vivax* and 76.6% (49 of 64) were infected with *P. falciparum*. Similarly, OptiMAL test detected 30.8% (16 of 52) were positive for *P. vivax* and 69.2% (36 of 52) were infected with *P. falciparum*. ICT P.f./P.v. test had sensitivities 78.9%, 87.5% and specificities 100%, 87.3% for *P. vivax* and *P. falciparum* respectively. OptiMAL test showed sensitivities 84.2%, 80% and specificities 100%, 96.4% for *P. vivax* and *P. falciparum* respectively. Thus these rapid immunoassays (ICT P.f./P.v. and OptiMAL) tests can be used as supplementary test to traditional light microscopy for the diagnosis of malarial parasites.

KEYWORDS: ***Plasmodium*, Microscopy, ICT malaria P.f./P.v., OptiMAL**

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INTRODUCTION

Currently, the vast majority of malaria cases in the world are detected by light microscopy of stained blood smears which remains the gold standard for malaria diagnosis[1]. Routine microscopic examination is laborious, time taking and requires a well maintained microscope along with an experienced microscopist. New techniques, such as hybridization with DNA probes are too sophisticated for routine use. Recently, efforts have been made to develop malaria rapid diagnostic devices (MRDDs) to facilitate malarial diagnosis[2]. Keeping these facts in mind a study was done to compare microscopic examination of blood smears with newly developed rapid immunochromatographic assays. ICT malaria P.f./P.v. test is a rapid immunochromatographic assay for the detection of *P. falciparum* Pf HRP 2 antigen and a pan-malarial antigen, manufactured in a test card form[3,4]. The OptiMAL dipstick test is also a rapid immunochromatographic assay which detects P. falciparum-specific parasite Lactate Dehydragenase enzyme (pLDH) and a parasite Lactate Dehydrogenase (pLDH) common to the four *Plasmodium species*[5].

MATERIALS AND METHODS

Study setting and population

The study was conducted in the month of September 2012 at a tertiary care hospital South Odisha, India. The malaria transmission in Odisha is perennial but peaks from August to October after seasonal rains. Present study included first 150 self-presenting patients more than 14 years of age and either sex who qualified as clinically presumptive malaria (i.e. an axillary temperature greater than or equal to 37.5°C or history of fever in the previous 48 hours) were recruited to the study. The exclusion criteria included individuals with other known causes of non-malarial febrile illnesses and pediatric age group. After obtaining informed consent demographic data were recorded and venous blood sample was collected in the Microbiology Department venous blood was collected from all 150 patients in a sterile vacutainer containing anticoagulant potassium EDTA.

Malaria parasites detections

Blood slide preparation and microscopy

Thick and thin smear blood films were prepared within 30 minutes and stained with standard Giemsa stain. All the slides were examined for malaria parasites by light microscopy independently by two microscopists. When there is a difference of opinion, a third microscopist's opinion was taken into account. Microscopists in the laboratory were blinded to the rapid immunochromatographic test results. A thin blood smear was examined for 15 minutes and for a thick blood smear, 500 fields were visualized[6]. Malaria parasites detected in either by thick or thin blood films were considered as positive.

Rapid immunochromatographic diagnostic tests

All rapid test kits were stored according to manufacturer's recommendations i.e. at 4 to 30°C. All 150 patients were tested simultaneously with both rapid immunochromatographic kits according to manufacturer's instructions. ICT Malaria P.f/P.v. test card (AMRAD-ICT Sydney, Australia) was considered *P. falciparum* positive if control line along with Pf HRP 2 specific and/or pan malarial antigen lines were visible and if only control and pan malarial antigen lines were observed, the sample was counted as positive for *P. vivax*. OptiMAL malaria test (TCS Biosciences Ltd., Buckingham, UK) was considered positive for *P. falciparum* when one control band and two test bands appeared and was positive for *P. vivax* when one control band and one test band appeared in the test kit. Tests with no band as control line were considered invalid.

RESULTS

A total of 150 blood samples were tested for malaria parasites by the ICT malaria P.f./P.v. test and OptiMAL test methods and the results

were compared to those obtained from examination of thin and thick smear blood film. The blood film results indicated that 59 (39.3%) patients were infected with malaria and the rest 91 (60.7%) were malaria negative. Among the positive patients *P. vivax* was detected in 19 cases (32.2%) and *P. falciparum* in 40 cases (67.8%). Correspondingly, the ICT malaria P.f./P.v. test results showed that 64 (42.7%) of the patient samples were positive for malaria parasites and 86 (57.3%) were malaria negative. Infection with *P. vivax* accounted for 23.4% (15 of 64), while infection with *P. falciparum* accounted for 76.6% (49 of 64). Similarly, the OptiMAL test results indicated that 52 (34.7%) were malaria positive and 98 (65.3%) were malaria negative. Among the positive patients *P. vivax* accounted for 16 (30.8%) of cases and *P. falciparum* in 36 cases (69.2%) as shown in Table-1.

Table-1
A summary of findings in 150 patients for Giemsa microscopy, ICT P.f./P.v. and optiMAL test.

Number of Cases	Giemsa stained Blood film	ICT malaria P.f/P.v.	optiMAL
Positive	59	64	52
P. vivax	19	15	16
P. falciparum	40	49	36
Negative	91	86	98
Total	**150**	**150**	**150**

The blood film identified four *P. vivax* positive samples that were not detected by ICT P.f./P.v. test, however there was 100% agreement between blood film results and ICT results for other 15 samples containing *P. vivax.* Fourteen cases of *P. falciparum* detected by ICT P.f./P.v. were not detected by blood film and five cases of *P. falciparum* detected by blood film were not detected by ICT P.f./P.v. test method (Table-2).

Table-2
Comparison of ICT malaria P.f./P.v. test with peripheral blood smear examination for malaria parasite detection.

Name of the Species	ICT malaria P.f./P.v. results	Blood film results		
		Positive	**Negative**	**Total**
P. vivax	Positive	15 (100%)	0	15 (100%)
	Negative	4 (3%)	131 (97%)	135 (100%)
	Total	**19**	**131**	**150**
P. falciparum	Positive	35 (71.4%)	14 (28.6%)	49 (100%)
	Negative	5 (4.9%)	96 (95.1%)	101 (100%)
	Total	**40**	**110**	**150**

The sensitivity and specificity for *P. vivax* with ICT malaria P.f./P.v. test was 78.9% and 100% respectively, like wise for *P. falciparum* 87.5% and 87.3% respectively. Microscopy revealed three *P. vivax* positive cases that were not detected by OptiMAL test. Similarly eight *P. falciparum* blood film positive cases were not detected by OptiMAL test. Four cases of *P. falciparum* detected by OptiMAL test were not detected by blood film, as shown in Table-3. The sensitivity and specificity for *P. vivax* with OptiMAL test was 84.2% and 100% respectively, similarly for *P. falciparum* 80% and 96.4% respectively.

Table-3
Comparison of OptiMAL test with peripheral blood smear examination for malaria parasite detection.

Name of the Species	OptiMAL test results	Blood film results		
		Positive	**Negative**	**Total**
P. vivax	Positive	16 (100%)	0	16 (100%)
	Negative	3 (2.3%)	131 (97.7%)	134 (100%)
	Total	**19**	**131**	**150**
P. falciparum	Positive	32 (88.9%)	4 (11.1%)	36 (100%)
	Negative	8 (7%)	106 (93%)	114 (100%)
	Total	**40**	**110**	**150**

DISCUSSION

Because drug resistant *P. falciparum* infections are now common and potentially lethal, presumptive antimalarial treatment is no longer feasible and early diagnosis is essential. Recent advances, have supplemented microscopy with a standardized antigen detection test using high technology production method and low technology applications. Both ICT malaria P.f./P.v. test and OptiMAL test belong to the rapid immunochromatographic assays. We employed these tests and compared it with conventional blood film examination for diagnosis of *P. falciparum* and *P. vivax* infection. In our study the sensitivity for *P. vivax* by using ICT P.f./P.v. and OptiMAL were 78.9% and 84.2% respectively with 100% specificity in both cases. This confirms the observation of Huong *et al*. in 2002[7]. They had found the sensitivity and specificity to be 73.7% and 100% respectively for *P. vivax* with OptiMAL test. Manson et al. obtained sensitivity for *P. vivax* with ICT P.f./P.v. and OptiMAL to be 2.9% and 47.1% respectively and specificity 100% and 96.9% respectively[8]. The lower percentage of sensitivity might have occurred due to low specificity of the IgM monoclonal antibody used in the ICT P.f./P.v. for the pan malarial antigen or could be due to regional variations in the genetic determinants of pLDH and ICT pan malarial antigen, leading to a failure to be recognized by the test kit monoclonal antibodies [8,9]. The sensitivity for P. falciparum by testing with ICT P.f./P.v. and optiMAL were 87.5% and 80% respectively. These value are similar to the study by Gatti *et al*. in 2002 and Chayani *et al.* in 2004[9,10]. The lower sensitivity of OptiMAL explained by the fact that, it detects pLDH which is produced only by living parasites, where as the ICT P.f./P.v. detects both living as well as dead parasites and the parasites not yet cleared from the host[11]. The specificity for *P. falciparum* with ICT P.f./P.v. and OptiMAL were 87.3% and 96.4% respectively. This observations was more or less similar with Tjitra *et al.* in 2001 and Cooke *et al.* in 1999[1,12]. They found specificity for ICT P.f./P.v. and OptiMAL were 89.8% and 92%

respectively. The lower percentage of specificity with ICT P.f./P.v. may be explained by the fact that in patients had previous recent infection with malaria for which the HRP 2 antigen level in the blood had not been decreased or on medication[13]. Other possibilities are, there may be circulating rheumatoid factor in serum of patients[14]. This evaluation has shown that, the immunochromatographic assays are rapid, simple, stable, reproducible, sensitive and effective for the diagnosis of malaria. The sensitivity and specificity of these tests are close to microscopic examination of blood smears but does not require highly skilled personnel to perform or interpret results. The OptiMAL has the added advantage that it can detect all four *Plasmodium species* and can be used to follow the efficacy of drug therapy since it detects an enzyme produced only by living parasites[10]. This present study was limited by the small sample size. The limitation of immunochromatographic assay was that they could not be used to determine the level of parasitaemia and showed low sensitivity for the detection of *P. vivax*. There were also batch quality variations in the form of visibility of antigen lines in malaria rapid diagnostic immunoassays. There was also difficult to interpret a positive test result in our holo-endemic setting with many asymptomatic carriers.

CONCLUSION

Overall performance of rapid immunochromatographic assays showed slightly low sensitivity and specificity compared to 'gold standard microscopy' for the diagnosis of malaria. Both the rapid diagnostic tests revealed low sensitivity against detection of *P. vivax*. Since pLDH antigen based tests detect the presence of live parasites, efficacy of drug therapy can be monitored. Thus rapid immunoassays can be used as supplementary to light microscopy, in areas where the existing laboratory service is inadequate or of an unacceptable standard, in areas where there are treatment based on clinical diagnosis, rapid immunoassays can reduce unnecessary use of antimalarial drugs which are more expensive and toxic and in complicated malaria cases where peripheral parasitaemia may be negative, but the rapid immunoassays might be expected to provide evidence of antigenaemia. However, the selection of rapid immunoassays should be regularly reevaluated, to ensure that the appropriate test kits can be used.

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