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Short Communication

Comparision of microscopy and RDTs techniques for laboratory detection of malaria

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The development of rapid and specific diagnostic test to identify individuals infected with malaria is important to control severe public health impact of this disease.This study evaluated the ability of rapid malaria diagnostic test (BIOTEC Malaria P.v/P.f rapid device) to detect *Plasmodium falciparum* and *Plasmodium vivax* malaria during out break in Chabahar province of Sistan and Balouchestan in Iran. Whole blood samples were obtained from 250 suspected malaria patients. Results from these kit were compared to the reading obtained from 100 fields of Giemsa-stained thick smear blood film.This test is rapid (15 min) for malaria detection. The device is coated with monoclonal antibodies against *P. falciparum* histidine rich protein-2 (PfHRP2) and enzyme parasite lactate dehydrogenase isomer of *P. vivax* (PLDH) . A total of 55% samples (138 of 250) were positive by blood films, while 54% samples (136 of 250) were positive by malaria P.v/P.f rapid device. The blood film indicated that 81% (112 of 138) of patients were positive for *P.vivax* and 19% (26 of 138) of patients were positive for *P. falciparum*. The malaria P.v/P.f rapid device showed that 79.6% (110 of 138) were positive for *P.vivax* and 1.4% (2 of 138) were negative. 19% (26 of 138) were positive for *P. falciparum*. These results demonstrated that the malaria P.v/P.f rapid device had sensitivity of 98.5% and specificity of 100% when compared to traditional blood films. The malaria P.v/P.f rapid device showed an excellent correlation with traditional blood films in identification of both *P. vivax* and *P. falciparum* malaria. At present for diagnosis of malaria in endemic area, this kit is rather expensive when compared to microscopy. Neverthless, it is very useful for remote areas where the necessary facilities for microscopic examination are not accessible.

Key words: RDTs, PLDH, PfHRP2, malaria.

INTRODUCTION

In order to control the severe public health impact of malaria, the development of rapid and specific diagnostic tests to identify individuals infected with this disease is important. Early diagnosis and treatment of parasitemia is vital for the control of malaria, one of the most prevalent parasitic diseases world wide. Microscopic examination of thick blood film is currently the standard method for malaria diagnosis. Microscopic examination howerver, requires well trained and experienced malaria microscopists and it is also rather time consuming (Iqbal et. al., 2001, Edrissian et. al., 2001). The World Health Organization (WHO) has repeatedly emphasized the urgent need for simple and cost effective diagnostic tests for malaria to over come the deficiencies of both light microscopy and clinical diagnosis (Russell et. al., 2002). Using rapid diagnostic tests (RDTs) to distinguish fevers caused by other illnesses is important (WHO, 2006). The

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availability of a simple and accurate test could greatly aid in the diagnosis of malaria in travelers returning to their home countries (Jelinek et. al., 1999). RDTs can detect *Plasmodium falciparum* infection even when the parasite is sequestered in deep vascular compartment. In case of mixed infection, one specie often suppresses the other, thereby making detection of the suppressed one difficult (Ritabrata et. al., 2005). As health programs in malaria endemic countries implement artmisinin combination therapy (ACT). ACT is currently much more expensive than older anti malarials such as chloroquin (CQ). RDTs can help target ACTs to patients who really have malaria. Finally, avoiding unnecessary use of ACTs on patients who do not have malaria may help prevent or delay drug resistance, making ACTs effective for a longer period (WHO, 2006).

MATERIALS AND METHODS

The study was performed from June to November 2008 in Chabahar of south province Sistan and Blouchestan of Iran. The blood samples were collected during a malaria out break from individuals living in urban areas and villages in active and passive malaria surveillance program.

Patient and sample collection:

From 250 suspected malaria patients with symptoms including fever and/or chills of several days, samples were collected. At the start of the study, informed consent and demographic information were obtained from all individuals who participated in the study. Sample collection was performed in active and passive surveillance program. In passive sample collection, finger prick blood samples were collected from suspected malaria patients who were referred to the health centers of Chabahar while active collection sample, was done house to house in the villages and urban centres of Chabahar. In addition, each individual was asked about signs and symptoms of malaria as well as any drug used within the last 2 weeks. Three drops of finger prick blood samples were used for the thick smear, 1 drop was used for thin blood smear and 5 µl was used for rapid diagnostic test (RDT).

Microscopy of Geimsa –stained blood films

Thick and thin blood smears were stained with Geimsa stain (pH 7.2) for 20 -30 min and examined by two experienced microscopists who had no knowledge of patient disease to avoid any bias. The smears were analyzed by x1000 magnification for the presence of malaria parasites. The research was blinded as results from the microscopic examination were not allowed to interfered with the RDTs results until all samples were examined.

The BIOTEC malaria *P. vivax*/*P. falciparum* (Pv/Pf) rapid device

All whole blood samples were tested with this kit. The BIOTEC malaria Pv/Pf rapid device was coated with monoclonal antibidies against the interacellular metabolic enzyme, plasmodium lactate dehydrogenase (PLDH), specific *Plasmodium vivax* and *P. falciparum* Histidine Rich Protein-2 (P.f HRP2) antigens. Detection system for *P. falciparum* was based on the detection of *P. falciparum* specific histidine rich protein-2 (P.f HRP2) which is a water soluble protein that is released from infected red blood cells (RBCs). The detection system of *P. vivax* was based on the presence of *P. vivax* specific PLDH. The test used clearing buffer in a dropper bottle and desiccant pounch and 5 µl sample loop. After retrieving 5 µl sample with calibrated loop, this sample was delivered to the sample pad in the sample port A. Immediately, four drops of clearing buffer was added into port B. Finally, the results was read at the end of 15 min (Table 2)

Principles of the method

As the test sample flows through the membrane assembly, after addition of the clearing buffer, the coloured colloidal gold conjugates of anti Pf HRP2 antibody and anti *P. vivax* specific PLDH antibody complexes the HRP2/PLDH antigen in the lysed sample. This complex moves further on the membrane to the test region where it was immobilised by the monoclonal anti Pf HRP2 antibody and/or monoclonal anti *P. vivax* specific PLDH antibody coated on the membrane. This led to the formation of a pink-purple coloured band in the respective regions which confirmed a positive test results. Absence of a coloured band in the test region indicated a negative test result for the corresponding antigen.

RESULTS

A total of 250 blood samples were analyzed for malaria parasites by the RDT method and the results were compared to results obtained from reading thin and thick blood smear. The blood smear results indicated that 55% (138 of 250) of patients were infected with malaria. Among the positive patients samples, *P. vivax* was present in 81% (112 of 138) and *P. falciparum* was present in 19% (26 of 138). The RDTs results indicated that 54% (136 of 250) of the patients were infected with malaria. Among the positive patients samples, *P. vivax* was present in 79.6% (110 of 136) and *P. falciparum* was present in 19% (26 of 136). However, 2 cases detected by the blood film *P. vivax* were not detected with RDT. The BIOTEC malaria Pv/Pf rapid device had sensitivity of 98.5% (95%[CI], 96.5 to 100%) and specificity of 100% (95%[CI]) when compared to traditional blood films for detection of malaria. The BIOTEC malaria Pv/Pf rapid device had sensitivity of 98.2% (95%[CI], 96.2 to 100%), specificity of 100% (95%[CI]), positive predictive value of 100% (95%[CI]) and negative predictive value of 98.6% (95%[CI], 96.6 to 100%) when compared to traditional blood films for detection *P. vivax*. The BIOTEC Malaria Pv/Pf Rapid Device had sensitivity, specificity, positive predictive value and negative predictive value of 100% (95%[CI]) when compared to traditional blood films for detection *P. falciparum* (Table 1).

DISCUSSION

This study compared the microscopy method and BIOTEC rapid device for detection of malaria. The important finding of this study was that BIOTEC rapid device, had sensitivity of 98.5% and specificity of 100% compared to microscopy method for detection of malaria. Out of a total

Table 1. Comparison of microscopy and RDT methods for the detection of malaria.

Microscopy	No of samples	RDT		Sensitivity	Specificity	Positive predictive value	Negative predictive value
		Positive	Negative				
Positive	138	136	2	98.5%		100%	
Negative	0	0	138		100%		98.6%
Total	138	136	140				

Table 2. Comparison of microscopy and RDT methods for the detection of *Plasmodium falciparum* and *Plasmodium vivax*.

Microscopy		Microscopy	RDTs	sensitivity CI 95%	Specificiy CI95%	Positve predictive value CI95%	Negative predictive value CI95%
		Frequency					
P.V	Positive	112	110	98.2% (96.2 -100)%	100%	100%	98.6% (96.6 - 100)%
	Negative	0	2				
P.F	Positive	26	26	100%	100%	100%	100%
	Negative	0	0				
Control	Positive	0	0				
	Negative	138	138				
Total		276	276				

of 250 suspected malaria patients, 55% were positive by microscopy method and 54% of them were positive by BIOTEC rapid device. Although in this study, two samples were positive by microscopy method as trophozoit of *P. vivax* was seen in their blood films, these two samples were negative by BIOTEC rapid device. It is possible that the two patients infected with malaria started treatment with anti malaria drugs when symptoms of malaria appeared. As PLDH are produced only from living parasities and medication could have led to the death of parasities, PLDH test was negative (Carol et. al., 1998). Alternatively, the results showed that the BIOTEC rapid device had high sensitivity and specificity compared to microscopy method for detection of *P. falciparum* and *P. vivax*. Therfore, this test can be useful for remote areas of Iran which have no facilities for microscopy method and prevent presumptive treatment using anti-malaria drugs such as chloroquin and artmisinine. This could delay drug resistance and make the drugs effective for a longer period.

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