Int J Infect. September 2014;1(3): e21441. DOI: 10.17795/iji-21441

Published online 2014 September 20. **Research Article**

The Comparison of Microscopic Method and Rapid Diagnostic Test in Detecting *Plasmodium* species

Alireza Salimi Khorashad [1,*]; Masoud Salehi [1]; Bita Roshanravan [2]

[1]Infectious Diseases and Tropical Medicine Research Center, Boo-Ali Hospital, Zahedan University of Medical Sciences, Zahedan, IR Iran
[2]Department of Chemical and Environmental Engineering, Faculty of Engineering, University Putra Malaysia, 43400 Serdang, Selangor D.E., Malaysia

[*]*Corresponding author*: Alireza Salimi Khorashad, Infectious Diseases and Tropical Medicine Research Center, Boo-Ali Hospital, Zahedan University of Medical Sciences, Zahedan, IR Iran. Tel: +98-5413424393, Fax: +98-5413424393, E-mail: salimi35ali@gmail.com

Received: June 23, 2014; **Revised:** July 23, 2014; **Accepted:** July 23, 2014

Background: Since Iran is one of the malaria endemic areas, diagnosis of this disease is important. Although the microscopic study of stained peripheral blood smears is as a gold standard of malaria diagnosis, this method requires a microscope, the equipment, and trained people. Because of some problems associated with microscopic method in some situations, rapid diagnostic test (RDTs) can be a suitable alternative. Nevertheless, then diagnosis of malaria should be approved by microscopic

Objectives: The rapid diagnosis of disease and treatment of patients is necessary to identify the contributing factors and break the cycle of transmission. In order to achieve this goal, we need a method that requires no special equipment such as microscopes, paint trays, and specially trained technicians for microscopic detection method

Patients and Methods: Blood sample were drawn from the finger of 178 patients with suspected malaria by a technician. Two methods of microscopic detection and RDT kits with immune chromatographic procedures were employed to diagnose malaria. Finally, all slides and cassettes of RDT kits were transferred to Zahedan for reviewing and control.

Results: After data collection, the results of 178 samples were reported according to the *Plasmodium* species. RDT detected 71.4% of *Plasmodium vivax* while microscopic method had detected all the cases (100%). Although there was a significant difference between two methods of diagnosis in detecting *P. vivax* ($P < 0.05$), the results were the same in diagnosing *Plasmodium falciparum* (100%).

Conclusions: Our study showed that only 71.4% of all positive samples for *P. vivax* were detected by RDT. The results were the same for both methods in diagnosis of *P. falciparum*.

Keywords: Malaria; Iran; Diagnostic

1. Background

Malaria is one of the most dangerous diseases. Approximately 300 million to 500 million people are facing this disease. About one million deaths are happening each year among young children in Africa because of *Plasmodium falciparum*. Iran is a malaria endemic area and malaria is considered as a major health-medical problem in this area (1, 2). Incidence of malaria in recent years has been decreased due to the efforts of health authorities in Sistan and Baluchistan Province. Although microscopic examination of a stained peripheral blood smear (PBS) is considered as the gold standard for malaria diagnosis, this method requires time, equipment, and trained personnel. Due to the increasing mortality from malaria and the problems of microscopic method, rapid diagnostic tests (RDTs) can be an alternative way for malaria diagnosis in critical situations; however, the result should be approved by microscopic method (3). The RDTs are based on immune chromatography, *P. falciparum*-specific histidine rich protein 2 (pfHRP-2), and solubility in water and can identify the type of *P. falciparum*. In addition, Plasmodium lactate dehydrogenase (PLDH) is produced in sexual and nonsexual stages in all *Plasmodium* species (4-9). RDTs have some limitations such as low sensitivity in people who have no clinical symptoms, positive tests in a large number of people due to incomplete treatment, no distinction between non-falciparum species in mixed infections, and absence of various stages of parasite identification in blood, which limit the use of RDTs in research work.

2. Objectives

The rapid diagnosis of disease and treatment of patients is necessary to identify the contributing species to break the cycle of transmission and to help solving the major issues in disease controlling in the region. In order to achieve this goal, we need a method that requires no special equipment such as microscopes, paint trays, and specially, trained technicians for microscopic detection method, which would be more efficient in areas where access to electricity is not possible. Therefore, this proposed method can seriously contribute to the malaria-controlling program in the southern areas of Sistan and Baluchistan Province, Iran, where the population is scattered and is constantly facing the natural disasters such as earthquake and floods.

3. Patients and Methods

This study was conducted from October 2009 to March 2010. First, the blood samples were taken from the fingers of 178 patients with suspected malaria fever who were referred to rural and urban health centers affiliated with Zahedan University of Medical Sciences. Both diagnostic method, namely, microscopic detection methods and RDT with immune chromatography technique, were performed by a technician under the supervision of a physician. We used malaria 102 (p.f/p.v) poct kits (InTec Products Inc., Xiamen, China). Finally, all the lamellas and RDT kit cassettes were transferred to Zahedan for review and controlling purposes. In microscopic method, first two thick and thin PBS were prepared and the results were recorded after staining with Giemsa and microscopic diagnosis. The emphasis was on the principle of quality controlling of the field by seeing the slides and microscopic observation of at least 200 subjects in order to ensure the result of microscopic slides. In RDT, 2 μL to 50 μL of fingertip blood sample was drawn from a patient who had been tested with microscopic method and was placed at a recommended site on tape (especially the well on the cassette, built for putting the blood and buffer) by using a RDT kit by a dispenser. Then a buffer containing hemolysis compounds and proprietary antibody was added whereas three band kits were used: the first band for controlling, the second one for pfHRP-2 antigen, and the third one as an indicator of non-falciparum antigens. Based on the instructions provided in the kit, control band discoloring was necessary and showed that the test was valid. The lack of control band discoloring illustrated the invalidity of the test. Control band discoloring and lack of discoloring of the wristbands was the sign of negative tests. Finally, by submitting and comparing the experimental results of microscopic method and RDT, the results were analyzed by statistical software.

4. Results

After data collection, the results of 178 samples were reported by following positive and negative results separations and the type of malaria (Table 1).

Table 1. Comparison of Microscopy and Rapid Diagnostic[a,b]

Test Method	*Plasmodium vivax*, positive results	*Plasmodium falciparum*, positive results	Mix, positive results	Negative
Microscopic Method	42 (23.6)	5 (2.8)	5 (2.8)	126
RDT Method	30 (16.8)	5 (2.8)	5 (2.8)	138
Percentage Difference Between Two Methods	12 (71.4)	0	0	12 ()

[a] Abbreviation: RDT, rapid diagnostic test.
[b] Data are presented as No. (%).

5. Discussion

Due to the high mortality rate of malaria, limitation of the microscopic method in the malaria control program and the need for special equipment, the use of a rapid diagnostic method with microscopic methods seems necessary. Therefore, many efforts have been made to detect malaria outside the range of microscopic techniques. These methods are nucleic acid probes and immunofluorescence, diffusion gel, counterimmunoelectrophoresis, radioimmunoassay, enzyme immunoassay, immunochromatography test (ICT), hemagglutination test, indirect immunofluorescence, and western blot (10-12). Polymerase chain reduction (PCR) is used to identify the four *Plasmodium* species in the cases where the parasite level is low; moreover, it can be used in mixed infection (13-15). The aforementioned diagnostic methods need equipment and facilities. ICT is considered for qualitative detection of malaria antigen. For each slide, about 20 to 60 minutes in microscopic method and five to 30 minutes with RDT method is needed (1, 4). Sensitivity of both microscopic and RDT studies shows that although this method is simple and accessible, has no significant sensitivity. It also has major differences with other studies in this field; in 2003, World health organization showed that if the number of parasites was about 100/μL of blood, the sensitivity of RDT could be similar to that of microscopic method. Regarding *P. falciparum*, if the number of parasites was more than 100/μL of blood, the sensitivity of PLDH and PfHRP-2 would be over 95% (16, 17). Specificity investigation of these two methods by WHO in 2000 revealed that RDT has a specificity of 100% in diagnosing *P. falciparum* (6). In the study by Bell et al. on RDT sensitivity in detecting *P. falciparum*, the sensitivity of PfHRP-2 was more than PLDH and aldolase (7). For sensitivity and specificity investigation of PfHRP-2 and PLDH tests in detecting *P. falciparum*, Iqbal J et al. illustrated that the sensitivity of PfHRP-2 was more than PLHD; however, specificity of PLDH was more than PfHRP-2 (1, 4, 16, 17). In addition, the Edrisian et al.

indicated that the specificity and sensitivity of ICT were around 100% and 93%, respectively (5). Evaluation of the stability level of RDTs by Bell and Peeling demonstrated that PLDH and aldolase had lower stability in comparison to PfHRP-2 Whereas the temperature also increased and immediately lowered their sensitivity in uncontrollable conditions (7). False-negative cases were reported in 2003 (WHO), for instance, in very low level of parasite ($< 100/\mu L$), the kit is corrupted or damaged and the sensitivity is lost. Reported false-positive cases are due the presence of rheumatoid factor, incomplete treatment, and delay in clearance of blood circulating antigens that are either free or complex. Singh et al. by studying 344 patients with symptomatic *P. falciparum* and *P. vivax* revealed that sensitivity and specificity were 97.5% and 88% for *P. falciparum* and 72% and 99% for *P. vivax,* respectively (3). In the current study, we used the gold standard of malaria diagnosis (microscopic method), which revealed a sensitivity of 100% for *P. falciparum* and 71.4% for *P. vivax*. It illustrates a significant difference in the sensitivity level of attempted test regarding diagnosing *P. vivax*. Since the detection mechanism for *P. falciparum* is pfHRP-2, enzyme reaction and PLDH enzyme reactions are related to *P. vivax*, our results are in agreement with that of Bell et al. They believed that the sensitivity of the Pf HRP-2 was more than PLDH and aldolase (7). Due to the quality of the kit manufacturers, the difference could be seen because the used kit in this study was made in china; therefore, more attention should be paid to the selection of high quality kit. Bell et al. study on the stability level of RDTs revealed that PLDH and aldolase were less stable in comparison to PfHRP-2 in terms of temperature rise and they lost their sensitivity rapidly in the uncontrollable conditions (7-9). Since the present study was performed in tropical areas, the results might be different in sensitivity of considered tests with similar studies conducted on the mentioned subject. Our study showed that only 71.4% of all positive samples for *P. vivax* in microscopic method were detected via RDT. The results were the same for both methods in diagnosis of *P. falciparum*.

Acknowledgements

We are grateful to Infectious Disease and Tropical Medicine Research Center in Zahedan for their cooperation in the implementation of this project with 2002 code and 3188 numbers. In addition, we would like to thank all laboratory staff in section of Malaria Diagnosis in Zahedan University of Medical Sciences.

Funding/Support

This study was financially supported by Zahedan University of Medical Sciences.

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