Research Journal of Recent Sciences ISSN 2277-2502
Vol. **1 (ISC-2011),** 361-364 **(2012)** *Res.J.Recent Sci.*

Short communication

Evaluation of one Rapid Method for Diagnosis of Malaria The Optional and Better Replacement of Microscopy

Panchal Hetal K.[1] and Desai Pratibha B.[2]
[1]Dolat Usha Institute of Applied Sciences, Valsad; Veer Narmad South Gujarat University, Surat, Gujarat, INDIA
[2]Shree Ram Krishna Institute of Comp. Edu. and Applied Sciences; Veer Narmad South Gujarat University, Surat, Gujarat, INDIA

Available online at, www.isca.in
(Received 27th September 2011, revised 16th January 2012, accepted 28th January 2012)

Abstract

Microscopy has been the most trustable technique for the diagnosis of malaria in India. Reduction of morbidity and mortality rate of malaria highly influenced by earlier and proper diagnosis. This study was carried out at Valsad, Gujarat. It involved use of microscopy i.e. field's stain and detection of Plasmodium falciparum - HRP II antigen, Plasmodium vivax - pLDH antigen detection by one rapid diagnostic Test (RDT) SD Bioline. Present study was carried out from 966 EDTA anticoagulated samples collected from clinical laboratories and hospitals of Valsad. Microscopic examinations of stained thick and thin films, shows 8.39%, 13.97%, 0.21% were detected as Plasmodium falciparum, P. vivax and mix respectively. Whereas with Rapid Diagnostic test using HRP II, p-LDH antigens 9.05% and 13.87% were detected as P. falciparum, P. vivax respectively. The study shows reasonable harmony between microscopy and RDT. Among two methods RDT was found to have high sensitivity (97.70%) and specificity (98.93%) compared to microscopy. Though the microscopic method is cost effective but laborious and needs an expertise. The RDT results were highly accurate and can be used where microscopy is inaccurate or in case of unavailability of expert.

Keywords: Malaria, malaria diagnosis, rdt, microscopy.

Introduction

Malaria is most important parasitic disease in tropical areas. Around 300 million malaria cases reported each year in the world, causes 1 to 3 million deaths. Nearly 3 billion people lives with the risk of malaria. In India during running year 2011 total malaria cases reported were 336,545. Among them 53.75% were due to Plasmodium falciparum and rest of the cases were due to P. vivax[1]. The disease is caused by Plasmodium species namely P. falciparum, P. vivax, P. ovale and P. malariae transmitted through biting of female Anopheles mosquito[2,3]. Valsad district of Gujarat state, India is considered as one of the malaria endemic area[4].

Microscopic examination of thick and thin blood smears stained with Romanosky's stain is the most common technique to diagnose malaria since last hundred years[5,6]. Microscopy continues to be the gold standard for identification of Plasmodium species in the laboratories[7]. The method is easy to apply and cost effective in the laboratories where skilled professionals are available who can even detect very low level of parasite like 10 to 50 parasites/µl. So the sensitivity of microscopy may fluctuate depending upon the skill of technician. In these consequences WHO has recognized the need for simple and cost effective diagnostic test for malaria to overcome the deficiencies of microscopy and clinical diagnosis[3]. To overcome this problem one most easy, cheaper, faster and reliable method available is Rapid Diagnostic Test (RDT). RDT detects P. falciparum Histidine-Rich-Protein II (HRP II) antigen and parasite Lactate Dehydrogenase (pLDH) antigen present in all four Plasmodium species[8,9]. It does not require any special equipment and give results within 15 to 30 minutes[10,11]. To estimate the impact of RDT SD Bioline on malaria diagnosis, we analyzed all samples to see the difference between conventional diagnostic microscopy and RDT SD Bioline.

Material and Methods

Total 966 samples were collected from various clinical laboratories of Valsad, Gujarat during December 2010 and July to September 2011. Approximately 1 ml blood sample was collected from each patient in a vaccutainer containing an anticoagulant EDTA. All samples were tested by both diagnostic methods microscopy and RDT. Thick and thin smears were prepared on slide, stained with Field's stain B and A for 5 and 12 seconds respectively[12]. Thick smears were used to confirm malaria and to count parasites/µl. Smears were considered negative if no parasite was observed in 200 consecutive fields of thick smear in oil immersion objective. Parasites were counted against 200 to 500 leucocytes. For the parasite estimation it was assumed that 8000 leucocytes present in 1 µl of blood [13,14]. Thin smears were used to identify and differentiate parasites. RDT SD Bioline malaria antigen detection test was purchased from SD Bio Standard Diagnostic Pvt. Ltd. The test cassette

contains a membrane strip, precoated with one monoclonal antibody against Plasmodium falciparum HRP II antigen and other polyclonal antibodies specific to pLDH of all 4 human malaria Plasmodium species as two separate lines. The test is one step, rapid, qualitative and differential for Plasmodium falciparum and Plasmodium vivax, the two prominent parasites found in India. For all samples, the cassettes were removed from pouch, approximately 5µl blood samples were placed in small, circular wells with loop, and 4 drops of assay diluent were placed in square assay diluent wells. Results were recorded at the end of 15 minutes and maximum within 30 minutes.

Results and Discussions

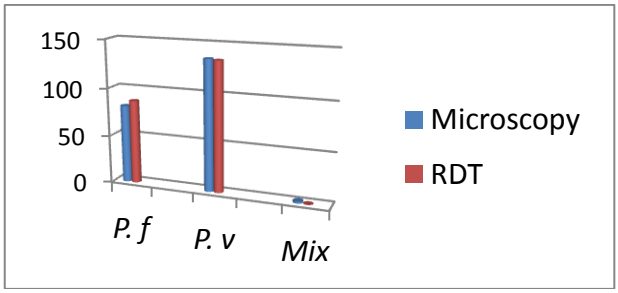
Out of 966 blood samples 213 were recognized positive by both tests. Total 218 and 221 cases were found positive by Microscopy and RDT respectively. Detail results are shown in Table 1 and 2. Figures showing observation of ICT and microscopy are shown as figure 1 and 2 respectively. The sensitivity and specificity of the test found was also high about 97.70% and 98.93% respectively. 08 samples were detected as false positive and 05 samples were detected as false negative. Comparison of microscopy and RDT results are also shown in graph 1.

Table - 1
Results of Microscopy and RDT analysis

Technique	Positive for *P. f*	Positive for *P. v*	Positive for mix	Negative	Total
Microscopy	81 (8.39%)	135 (13.97%)	02 (0.21%)	748 (77.43%)	966
RDT	87 (9.05%)	134 (13.87%)	00 (0.00%)	745 (77.12%)	966

Table -2
Results of Microscopy and RDT analysis

Microscopy/RDT	Positive	Negative	Total
Positive	213	05	218
Negative	08	740	748
Total	221	745	966



Graph-1
Comparative Results of Microscopy and RDT

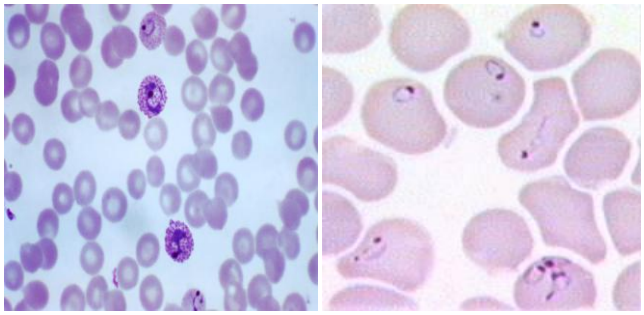


Figure- 1
Microscopic Observation of Malarial Parasites

Figure-2
Rapid Diagnostic Test Results

In this study RDT has shown high level of agreement with microscopy. The sensitivity and specificity of the test was also very high. Out of 08 false negative patients 05 were previously treated with chemoprophylaxis against malaria. Even in microscopic analysis of these samples the parasites/µl counts were as low as like 60, 311, 170, 296, 318 etc. Compared with microscopic diagnosis, the SD Bioline RDT was found false positive in 05 patients. This may be due to persistent antigenemia following treatment of malaria in 03 patients with recent history of malaria. Because in some cases antigenemia may remain positive 3-28 days after disappearance of circulating parasites[15]. Other 02 false positive results may be due to rheumatoid factor, hepatitis etc [16,17,18,19]. This may be due to a non specific reaction of rheumatoid factor, hepatitis antigens with coated antibodies.

Our study was valuable because the sample size was quite large about to access the acceptability of RDT. Microscopy involves good time and tough microscopic observation. Skilled professional is required to observe the same. Sometime when parasitemia is very low even a keen observation may lead to false negative diagnosis. It is also not easy to differentiate different Plasmodium species without ample experience. In rural areas where skilled malaria detecting experts are not available diagnosis may be delayed and lead to improper diagnosis of malaria and there

by treatment which some time even lead to death. In this situation alternatively we suggest RDT as optional method to diagnose malaria. RDT can be performed within couple of minutes. It is easy to perform that even a new lab technician or a layman can also perform it and interpret the results. Even the RDT was able to differentiate between P. falciparum and P. vivax. It is unable to differentiate P. vivax, P. ovale and P. malariae but in India malaria is mainly caused due to P. vivax and P. falciparum. The sensitivity and specificity of the test found was also high. It suggests RDT as a better option of microscopy in diagnosis of malaria.

Conclusion

In India malaria is mainly found due to P. falciparum and p. vivax. Both can be differentiated well by RDT. Results obtained by RDT are suggesting that it can be used for malaria diagnosis. It makes diagnosis faster, better and reliable. Even can be used at areas where experts are not available or results are needed in emergency.

Acknowledgment

The authors wish to thank to staff of all clinical laboratories of Valsad district, who gave their valuable support. The authors also wish to thank management and staff of Shree RamKrishna Institute of Computer Education and Applied Sciences, Surat; Dolat Usha Institute of Applied Sciences, Valsad.

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