Volume : 2 | Issue : 11 | Nov 2013 • ISSN No 2277 - 8160

Research Paper | Medical Science

A Comparison of Microscopic Examination and Rapid Diagnostic Tests Used to Diagnose Malaria

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ABSTRACT

Malaria presents a diagnostic challenge. Microscopy remains the gold standard. However, microscopy is labour intensive and requires expertise and time, which causes therapeutic delay. Rapid malaria diagnostic test detects plasmodium parasite and is fast, reliable and simple to use. Objective: To compare the results of peripheral smear microscopy and pLDH/HRP2 based immunochromatographic RDT for diagnosis of malaria. Material & Methods: Study was conducted on clinically suspected cases of malaria. Microscopic examination was done on thin and thick smear, stained by field's stain and pLDH/HRP2 based immunochromatographic RDT were performed and the results were compared. Results: Out of 4664 specimens 569(12.2%) were positive by RDT and 511(10.956%) were positive by microscopy. Out of 569 positive cases, 495 (87%) were P. vivax and only 74 (13%) were P. falciparum infection. Among 59 microscopy negative & RDT positive cases 41(69.5) % patients were positive for P. vivax & 18(30.5%) for P. falciparum. The sensitivity of RDT was 99.8% and its specificity was 98.57%. P value of 0.0605 indicates that there is no significant difference between diagnostic capacity of RDT test & microscopy. Conclusion: The results indicate that pLDH/HRP2 based immunochromatographic RDT demonstrated acceptable sensitivity and specificity. So it should be used as screening tool.

KEYWORDS : Malaria, plasmodium, immunochromatographic RDT

Introduction:

Malaria is an extremely complex disease that has been responsible for deaths and social disruption since the beginning of recorded human history. In such areas malaria diagnosis is often made only on the basis of clinical symptoms although this is alarmingly inaccurate [1]. In such areas malaria diagnosis is often made only on the basis of clinical symptoms. The current malaria control strategies are mainly based on early diagnosis and a correct treatment of the cases. These are essential to reduce the fatal outcome of the disease.

Microscopy remains the gold standard for diagnosis of malaria in clinical practice. Microscopy is regarded as the most suitable diagnostic instrument for malaria control because it is inexpensive to perform, and it is able to differentiate plasmodium species and quantify parasites. Microscopy is considered the most accurate method for the diagnosis of malaria, but almost exclusively when performed by experienced persons. [2-4] However, microscopy is labour intensive, requires expertise and time, which causes therapeutic delays.

Blood film microscopy the current standard test for diagnosis of malaria has several limitations that necessitate field evaluation of alternative diagnostic methods. The traditional method for the detection of the malaria parasite is the examination of thick and thin blood smears by microscopy. The shortcomings of microscopy for malaria diagnosis are well known [5, 6].

The need for a simple, sensitive diagnostic test has led to the development of rapid diagnostic tests (RDTs) among other alternative techniques. RDTs may have their place because expert microscopy in malaria-endemic countries is hard to establish and cost of RDTs has been greatly reduced. [7]

Aims and Objective:

To compare the results of peripheral smear microscopy and immunochromatographic RDT for diagnosis of malaria.

Materials and Methods:

The study was cross-sectional and was conducted from June 2012 to August 2012 at V. S. General Hospital.

The suspected cases of malaria (fever with chills and rigor); both male and females of all age group were included in the study. Cases with fever with chills, cough, and breathlessness or symptoms of Urinary tract infection were excluded.

For RDT: 5 μl blood was collected by finger prick and tested by pLDH/HRP2 based immunochromatographic RDT by SD Bioline Malaria Ag P. vivax/P. falciparum. It is a rapid immunochromatographic test for qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) specific to P. falciparum and P. vivax simultaneously in human whole blood. [8, 9]

For Microscopy: Blood sample were collected by venepuncture in EDTA tube to make thick and thin smears. The smears were stained with field's stain and examined under 100x magnifications for plasmodium species and quantization. A malaria blood film was considered negative after 100 high power (40x) fields had been examined and no parasite observed.

Observation & interpretation :

Out of total 4664 specimens 569(12.2%) were positive by RDT and 511(10.956%) were positive by microscopy.

Table 1. Type of Plasmodium infection, n= 569

Types of plasmodium sp.	n	%
P. falciparum	74	13
P. Vivax	495	87

Among all positive cases 310(54.48%) were male & 259(35.32%) were female. Male: Female ratio was 1.5: 1. Mean age group was 21 to 30 years. Total 151 patients were in age group 21 to 30 years.

Table 1 shows Out of 569 positive cases, 495 (87%) were P. vivax and only 74 (13%) were P. falciparum infection.

Table 2. Comparison of slide microscopy and rapid diagnostic tests by species detected.

		RDT positive		Microscopy positive	
		P. vivax	P. falciparum	P. vivax	P. falciparum
June	OPD	70	2	68	1
	Ward	27	0	23	0
July	OPD	79	1	70	1
	Ward	42	9	36	8
Aug	OPD	186	29	174	23
	Ward	91	33	84	23
		495	74	455	56
Total		569		511	

Table 2 shows that maximum cases (339) were noted in month of August. Among them 277 were positive for P. vivax and 62 were positive for P. falciparum.

Among 58 microscopy negative & RDT positive cases 41(69.5) % patients were positive for P. vivax & 18(30.5%) for P. falciparum.

The performances of RDTs were evaluated based on the following criteria: sensitivity, specificity, positive prediction value (PPV), negative prediction value (NPV), and diagnostic performance of the RDT in comparison with the microscopic method (gold standard).

The study shows sensitivity & specificity of RDT was 99.80% and 98.58% respectively. P value of 0.0605 indicates that there is no significant difference between diagnostic capacity of RDT test & microscopy.

Discussion

Malaria can be a life-threatening disease in a vulnerable population if not treated. Therefore, a quick and accurate diagnosis is very important. To prevent unnecessary antimalarial treatments, it is important to confirm clinical suspicions with a good laboratory test.

In our study sensitivity & specificity of RDT was 99.80% and 98.58% respectively. Reports from Rajini Kurup and Rena Marks indicate that RDTs have showed 100% sensitivity & 71% specificity by same kit.[10] but other study by Jos´e-Luis Portero and Maria Rubio-Yuste showed the sensitivity was 69.7% and the specificity 73.7%.[11]

The HRP2 (histidiine rich protein 2) is a water-soluble antigen produced by both asexual stages and young gametocytes of P. falciparum. Because malaria treatment often targets asexual stages only, HRP2-based RDTs may yield false-positive results for active infection up to a couple of weeks after adequate malaria treatment as a result of the persistence of antigens produced by residual circulating gametocytes. [9] Persistent HRP-2, on the other hand, could be an advantage in detecting low-level, fluctuating parasitaemia in chronic malaria.

Plasmodial gametocytes also produce pLDH (lactate dehydrogenase) and so a pLDH test may remain positive despite clearance of the asexual parasite forms in circulation.

Most RDTs only record the presence or absence of antigens but cannot measure the parasite density. These should, therefore, only be considered as an extended means of parasite based diagnosis where microscopy is absent.

The possible explanations for 58 discrepancies of RDT positive and microscopy negative cases may include:

Sequestration of parasites: erythrocytes containing mature parasites clump together in the microvasculature and are, therefore, not seen in the peripheral circulation and blood films, while antigen continues to be released.

Poor blood film preparation generates artifacts commonly mistaken for malaria parasites, including bacteria, fungi, stain precipitation, and dirt and cell debris

Persistence of antigens produced by residual circulating gametocytes after adequate malaria treatment . Because malaria treatment often targets asexual stages only [12, 13] and

In case of chronic malaria, where antigen is still circulating due to presence of hepatic schizogony. But parasite can't be seen in peripheral blood due to absence of erythrocytic schizogony.[14]

Low parasitaemia [15]

Conclusion:

As compared with microscopy, HRP2 and pLDH based RDTs demonstrated acceptable sensitivity and specificity.

RDTs are sensitive, simple to use and provide quick results without the need for good microscopic equipment and electricity, making them a good alternative to microscopy in endemic areas. In areas where there is a high incidence of malaria, the lack of facilities undermines the benefits of RDTs.[16]

A combination of RDTs and microscopy for malaria diagnosis should be considered, prioritizing RDT as a screening tool.

REFERENCES

1. WHO: New perspectives: Malaria diagnosis[http://www.who.int/tdrold/publications/publications/pdf/malaria_diagnosis.pdf] webcite 2000. | 2. World Health Organization (WHO), 2006a. The Role of Laboratory Diagnosis to Support Malaria Disease Management.Focus on the Use of Rapid Diagnostic Tests in Areas of High Transmission: Report of a WHO Technical Consultation, October 25–26, 2004. | 3. Proux S, Hkirijareon L, Ngamngonkiri C, McConnell S, Nosten F,2001. Paracheck-Pf: a new, inexpensive and reliable rapid test for P. falciparum malaria. Trop Med Int Health 6: 99–101. | 4. Zurovac D, Midia B, Ochola SA, English M, Snow RW, 2006b. Microscopy and outpatient malaria case management among older children and adults in Kenya. Trop Med Int Health 11: 432–440. | 5. Makler MT, Palmer CJ, Ager AL: A review of practical techniques for the diagnosis of malaria.Ann Trop Med Parasitol 1998, 92:419-433. | 6. Moody A: Rapid diagnostic tests for malaria parasites.Clin Microbiol Rev 2002, 15:66-78. | 7. Perkins MD, Bell DR: Working without a blindfold: the critical role of diagnostics in malaria control.Malar J 2008, 7(Suppl 1):S5. | 8. Kakkilaya BS. Rapid diagnosis of malaria. Lab Medicine. 2003 Aug;8(34):602-8. | 9. Hopkins H, Kambale W, Kamya MR, Staedke SG, Dorsey G, Rosenthal PJ, 2007. Comparison of HRP2- and pLDH-based rapid diagnostic tests for malaria with longitudinal follow-up in Kampala, Uganda. Am J Trop Med Hyg 76: 1092–1097. | 10. Rajini Kurup, Rena Marks A comparison of microscopic examination and rapid diagnostic tests used in Guyana to diagnose malaria Malaria Reports 2012; volume 2:e2 | | 11. Jos´e-Luis Portero, Maria Rubio-Yuste, Accuracy of an immunochromatographic Diagnostic Test (ICTMalaria Combo assette test) Compared toMicroscopy among under Five-Year-Old Children when diagnosing Malaria in Equatorial Guinea Malaria Research and Treatment Volume 2010, Article ID 858427 | 12. Moody A. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev 2002;15: 66-78. | 13. World Health Organization. The role of laboratory diagnosis to support malaria disease management: Focus on the use of rapid diagnostic tests in areas of high transmission, report of a WHO technical consultation, 25-26 October 2004. Geneva: world Health Organization; 2008. pp 4-48. | 14. Dondorp AM, Desakorn V, Pongtavor -npinyo W, et al. Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. PLoS Med 2005; 2:e204. | 15. Bell DR, Wilson DW, Martin LB. False-positive results of a Plasmodium falciparum histidine-rich protein 2-detecting malaria rapid diagnostic test due to high sensitivity in a community with fluctuating low parasite density. Am J Trop Med Hyg 2005;73:199-203. | 16. Mboera L, Fanello C, Malima R, et al. Comparison of the Paracheck-Pf rest with microscopy, for the confirmation of Plasmodium falciparum malaria in Tanzania. Ann Trop Med Parasitol 2006; 100:115-22. Dondorp AM, Desakorn V, Pongtavor - npinyo W, et al. Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. PLoS Med 2005; 2:e204. |