International Journal of Contemporary Pediatrics
Gandhi V et al. Int J Contemp Pediatr. 2018 Jan;5(1):92-96
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20175565

Original Research Article

Is rapid diagnostic test (malaria Pv/Pf Ag card test) reliable in diagnosing malaria

Vishruti Gandhi, Prasad Muley*, Niyati Parikh, Hardik Gandhi, Akash Mehta

Department of Pediatrics, SBKS Medical Institute and Research Center, Sumandeep Vidyapeeth, Piparia, Vadodara, Gujarat, India

Received: 29 November 2017
Accepted: 18 December 2017

***Correspondence:**
Dr. Prasad Muley,
E-mail: muleyprasad123@gmail.com

ABSTRACT

Background: Malaria is a protozoan disease transmitted by the bite of infected female anopheles mosquitoes is one of the most important parasitic diseases of human with transmission in 109 countries, affecting more than one billion people worldwide. This study was planned to compare the gold standard i.e. peripheral blood smear examination and the newer rapid diagnostic test (malaria plasmodium falciparum/ plasmodium vivax antigen card) to know the diagnostic accuracy of Rapid Diagnostic Test (RDT) kits.
Methods: All the suspected cases of WHO defined malaria between 1month to 18 years of age were enrolled in the study.
Results: Out of 96 clinically suspected cases of malaria 63 were confirmed by peripheral smear. The age range of participants ranged from 4 months to 17 years. On peripheral smear examination, out of 96 clinically suspected cases, 37 (38.5%) cases were positive for *P. vivax*, 23 (23.9%) were positive for *P. falciparum* and 3 (3.1%) were positive for both parasites by microscopy. Sensitivity and specificity of RDT for *Plasmodium Vivax* is 92.5% and 96.4% respectively. Sensitivity and specificity of RDT for *Plasmodium Falciparum* is 96.2% and 90%.
Conclusions: The rational use of RDTs as a complement to microscopy might give substantial health benefits through earlier treatment, reduction in morbidity and mortality and more rationalized approach for choosing anti-malarial drugs, which in terms may prevent drug resistance.

Keywords: Malaria, *P. falciparum*, *P. vivax*, Peripheral smear, RDT

INTRODUCTION

Malaria is a protozoan disease transmitted by the bite of infected female anopheles mosquitoes is one of the most important parasitic diseases of human with transmission in 109 countries, affecting more than one billion people worldwide and causing 1 to 3 million deaths each year.[1] Approximately, 2.48 million malaria cases are reported annually from south Asia of which 75%cases are contributed by India alone.

It is also very important to differentiate between types of malarial parasite as presumptive treatment of malaria encourages the development and spread of drug resistant *P. falciparum* parasites.[2] Early diagnosis and prompt treatment of malaria with efficient drugs is required for effective malaria control. Haematological changes are invariably seen in malaria of which anemia and thrombocytopenia are the most important features. However, a definitive diagnosis can be established only on demonstrating malarial parasite or its products in blood. Over the years many new diagnostic tests as quantitative buffy coat, rapid diagnostic test, genetic probes and PCR have been developed in an attempt to improve the diagnosis of malaria, however microscopy has remained the gold standard against all other tests

have been evaluated.[3] The detection of malarial antigens (HRP-2, LDH) by variety of Rapid diagnostic test available is of great value especially in severe and complicated malaria wherein blood smears may be negative. Hence, this study was planned to compare the gold standard i.e. peripheral blood smear examination and the newer rapid diagnostic test (malaria plasmodium falciparum/ plasmodium vivax antigen card) to know the diagnostic accuracy of rapid diagnostic kits.

METHODS

For all the clinically suspected cases of WHO defined malaria fulfilling the eligibility criteria on basis of predefined inclusion and exclusion criteria (given vide infra) were subjected to rapid diagnostic test (RDT) and peripheral smear examination.[4]

Inclusion criteria

- Between age of 1 month to 18 years of age were enrolled in the study.
- Patients whose parents or guardians were willing to give consent were included in the study.

Exclusion criteria

- Patients presenting with signs and symptoms suggestive of diseases which could give false positive card test results eg: rheumatoid arthritis, immunocompromised status were excluded from the study.

All the enrolled subjects were subjected for routine investigation. Peripheral thin and thick blood smear examined for identification of malaria parasite.

Preparation of blood smear was done as per standard routine protocol.[5]

- A drop of blood not larger than a pin head taken on a grease-free clean slide at a distance of about half an inch from the right end.
- A spreader was held at an angle of 45 degrees in contact with the drop of blood and was lowered to an angle of 30 degrees and pushed gently to the left, till the blood is exhausted. As the blood exhausted, the film began to form “tails” which ended near the center of the slide.
- The film was allowed to dry and labelled for identification.

Slide was subjected to dehaemoglobinization before staining with giemsa stain.

Rapid diagnostic test was carried out throughout the study by Malaria p.f/p.v Ag card of Transasia Bio-Medical Ltd. was used. The test kit contains the following components to perform the assay:

- Malaria card made of nitrocellulose
- Micro pippet, with mark on 5 micro litres.
- Assay buffer
- Sample loop
- Instruction manual

RDT is a visual, rapid immunoassay for the qualitative differential detection of Pf and Pv malarial antigens of human blood based on sandwich immunoassay principle. The first step of the test procedure involves mixing the patient’s blood with a lysing agent in a strip or well. This ruptures the red blood cells, releasing more parasite protein. Labelled antibody, either in the well or on the strip, may then bind with the target antigen. The resulting mixture of blood products and antigen labelled antibody complex then passes along the nitrocellulose strip. The free labelled antibody captures the parasite antigen if present, which will in turn, be captured by the test band antibody.

The accumulation of microscopic dye particles on the thin band produces a visible line if sufficient antigen-labelled antibody complex is present. The control band will become visible as sufficient labelled antibody accumulates on the line. A visible control line indicates that labelled antibody has traversed the full length of the strip, pass the test line. The intensity of the test band varies depending on the amount of antigen present.[5]

For the rapid diagnostic test, a positive test result will be evident when a thin, solid red band appeared on the dip stick along with the control band. Positive results will be recorded with appearance of bands in the respective areas of Pf and Pv along with the control band.

RESULTS

Out of total 96 clinically suspected malaria cases 63 were confirmed by peripheral smear. The age of the participant involved in the study ranged from 4 months to 17 years.

Out of 63 peripheral smear positive malaria cases, 42 (66.7%) were >5 years of age, 20 (32.7 %) were 1-5 years of age and 1 (1.6%) were < 1 year of age, of which 42 (66.7%) were male and 21 (33.3%) were females. Amongst 63 smear positive cases common symptoms were abdominal pain (34.9%), vomiting (33.3%), cough and cold (19%), convulsion (12.7%), constitutional symptoms (11.1%), loose stools (7.9%), headache (6.3%), altered sensorium (6.3%) respectively (Table 1). Commonest sign was anemia seen in 54 cases (85.7%) which was followed by splenomegaly (74.6%), hepatomegaly (34.9%), icterus (12.7%), koilonychias (4.8%), oedema (4.8%) respectively (Table 1).

Out of 54 cases with anemia most of Pv positive cases (50%) had mild anemia while Pf positive cases 57.1% had moderate anemia.

Table 1: Co-relation of symptoms and signs in children with peripheral smear positive malaria (n=63).

Symptoms/signs	No. of cases	% of cases
Abdominal pain	22	34.9
Vomiting	21	33.3
Cough and cold	12	19.0
Constitutional symptoms	7	11.1
Loose stool	5	7.9
Headache	4	6.3
Altered sensorium	4	6.3
Anemia	54	85.7
Splenomegaly	47	74.6
Hepatomegaly	22	34.9
Icterus	8	12.7
Convulsion	8	12.7
Oedema	3	4.8
Koilonychia	3	4.8

On peripheral smear examination, out of 96 clinically suspected cases, 37 (38.5%) cases were positive for *P. vivax*, 23 (23.9%) were positive for *P. falciparum* and 3 (3.1%) were positive for both parasites by microscopy.

Table 2: Diagnostic confirmation of clinically suspected cases of malaria (n=96).

	RDT	Peripheral smear
	No (n=96)	No (n=96)
P. Falciparum	27 (28.13%)	23 (23.96%)
P. vivax	34(35.41%)	37 (38.54%)
Both	5 (5.20%)	3 (3.12%)
Negative for malaria	30 (31.25%)	33 (34.38%)

In present study peripheral smear positive cases of *P. vivax* and *P. falciparum* were 37 (38.54%) and 23 (23.96%) respectively. Malaria cases diagnosed by rapid diagnostic test (RDT) were *P. vivax* 34 (35.41%) and *P. falciparum* 27 (28.13%).

Table 3: Comparison between RDT and peripheral smear positive plasmodium vivax (n=96).

		Peripheral smear		Total
		Positive Pv + mixed (n=40)	Negative (n=56)	
RDT_PV	Positive count	37(38.5%)	2 (2.1%)	39 (40.6%)
	Negative count	3 (3.1%)	54 (56.3%)	57 (59.4%)
Total	Count	40 (41.6%)	56 (58.4%)	96 (100.0%)

Table 4: Comparison between RDT and peripheral smear positive plasmodium falciparum (n = 96).

		Peripheral smear		Total
		Positive Pf + mixed (n=26)	Negative (n=70)	
RDT_PF	Positive count	25 (26.0%)	7 (7.3%)	32 (33.3%)
	Negative count	1 (1.1%)	63 (65.6%)	64 (66.6%)
Total	Count	26 (27.1%)	70 (72.9%)	96 (100.0%)

Table 5: Comparison of RDT with peripheral smear in diagnosis of malaria.

RDT versus microscopy	Malaria species	
	P. vivax	***P. falciparum***
True positive (n)	37	25
False positive (n)	2	7
False negative (n)	3	1
True Negative (n)	54	63
Sensitivity (%)	92.5%	96.2%
Specificity	96.4%	90%
PPV (%)	94.8%	78.1%
NPV (%)	94.7%	98.4%

Table 3 shows that out of 40 peripheral positive *P. vivax* cases 37 (38.5%) were positive by RDT and 3 cases were negative by RDT which were positive for peripheral smear.

Table 4 depicts that out of 26 peripheral positive P. falciparum cases 25 (26.0%) were positive by RDT and 1 case was negative for RDT and positive for perioheral smear.

Sensitivity and specificity of RDT for P. vivax is 92.5% and 96.4%, similarly sensitivity and specificity for P. falciparum is 96.2% and 90% respectively.

Positive predictive value and negative predictive value for *P. vivax* is 94.8% and 94.7%, and for *P. falciparum* positive predictive value is 78.1% and negative predictive value is 98.4%

DISCUSSION

Malaria is a parasitic infection of global importance and is a major public health problem in India. The surveillance activities against malaria are aimed at early diagnosis and prompt treatment of cases to reduce attributable morbidity and mortality.

There are four principal methods for diagnosing malaria. These are symptomatic, microscopy, antigen test and molecular methods. In the present study 96 cases were enrolled.

In the present study out of 96 clinically suspected cases of malaria, microscopy showed 63 positive cases for malaria (23 Pf, 37 Pv and 3 mixed infection), whereas RDT was positive for 66 cases (27 Pf, 34 Pv, and 5 mixed infection. The use of malaria RDTs is recommended by WHO when reliable microscopy is not available.

In the present study majority of patient belonged to *P. vivax* category (58.7%) followed by *P. falciparum* (36.5%) and mixed infections in 4.8% cases. Finding was similar to that of the other studies.[5-7] All the confirmed smear positive patients presented with fever, and had variety of signs and symptoms such as anemia (85.7%), abdominal pain (34.90%), cough and cold (19%), splenomegaly (34.9%) was similar to other various studies.[7,8]

Anemia was found in 85.7% (n=54) of cases. Out of which *P. vivax* had 47.6%, and *P. falciparum* had 33.3%, finding was similar to Jain et al.[5] Overall sensitivity for RDT in the present study was 96.7% and specificity is 85.7% is comparable to other various studies.[9-11]

Present study showed that sensitivity and specificity for *P. vivax* is 92.5% and 96.4% respectively is similar to studies conducted by Palmer CJ et al, Pande PR et al and S A Khan et al.[12-14] Sensitivity and specificity for *P. falciparum* is 96.2% and 90% respectively is similar to other studies conducted in various other setups.[9,10,14,15]

RDT missed one case of *P. falciparum* and three cases of *P. vivax* which were positive on microscopy. The probable reasons for these false negative results are following, that

- RDT is not sensitive below a parasitic index of 100 parasite/ul
- RDT detects pLDH which is produced by living parasites
- the blood samples judged positive by pathologist may have been dead parasites and not yet cleared from the host.

CONCLUSION

Although microscopy is a gold standard for diagnosing malaria, it is time consuming as one test requires 60 minutes, in comparison to that RDT is simple more objective, requires no equipment, but only drawback is it is quite expensive. RDT does not have any subject bias whereas in microscopy the results are affected by the skill and workload of the microscopists. The rational use of RDTs as a complement to microscopy might give substantial health benefits through earlier treatment, reduction in morbidity and mortality and more rationalised approach for choosing anti-malarial drugs, which in terms may prevent drug resistance. It was observed that RDTs had sensitivity of 96.2% for Pf, 92.5% for Pv and specificity of 90% Pf and 96.4% Pv. RDTs can be considered for a wider role in management and control of malaria as an accurate diagnostic tool after refinement.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. White NJ, Breman JG. Malaria and Babesiosis-Diseases caused by red blood cell parasite. In: Harrisons's principles of internal medicine. Eds. Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL. 16th edn. New York: Mc Graw- Hill; 2005;1:1218-32.
2. Rane SR, Bapat VM, Holla VV. Malaria, still a threat to life: a post mortem study. Indian J Pathol Microbiol. 2003;46(1):17-9.
3. World Health Organization. Global health observatory data repository. 2013. Available at who.int/gho/data/node.main.A364
4. WHO. World Health Organization global malaria programme. Available at who.int/iris/bitstream/10665/208815/1/WHO_HTM_GMP_2016.6_eng.pdf
5. Adewoyin AS, Nwogoh B. Peripheral blood film: a review. Ann Ib Postgrad Med. 2014;12(2): 71-9.
6. Jain M, Kaur M. Comparative study of microscopic detection methods and hematological changes in malaria. Indian J Pathol Microbiol. 2005;48(4):464-7.
7. Sitalakshmi S, Srikrishna A, Devi S, Damodar P, Mathew T, Varghese J. Changing trends in malaria-a decade's experience at a referral hospital. Indian J Pathol Microbiol. 2003;46(3):399-401.
8. Muddaiah M, Prakash PS. A study of clinical profile of malaria in a tertiatry referral centre in South Canara. J Vector Borne Dis. 2006;43(1);29-33.
9. Patil BS, Swamy HM, Shankarappa RS. Clinical study of falciparum malaria. JAPI. 2000;48(1):109.
10. Malik S, Khan S, Das A, Samantaray JC. Plasmodium lactate dehydrogenase assay to detect malarial parasites. NAtl Med J India. 2004;17(5):237-9.
11. Bharti PK, Silawat N, Singh PP, Singh MP, Shukla M, Chand G, et al. The usefulness of a new rapid

diagnostic test, the first response malaria combo (pLDH/HRP2) card test, for malaria diagnosis in the forested belt of central India. Malaria J. 2008;7(126):1-6.
12. Kumar A, Sharma VP, Thavaselvam D, Sumodan PK. Clinical trials of a new immunochromatographic test for diagnosis of *Plasmodium falciparum* malaria in Goa. Indian J Malariol. 2000;33(44):166-72.
13. Carol JP, John FL, Winslow. Evaluation of optimal test for rapid diagnosis Pv and Pf malaria. J clin Microbial. 1998;36(1):203-6.
14. Pande P. Evaluation of immunochromatographic rapid diagnostic test versus peripheral blood smear for diagnosis of malaria in Nepal. Med J Shree Birendra Hospital. 2010;9(1):9-13.
15. Khan SA, Anwar M, Hussain S. Comparison of optimal malaria test with microscopy for the diagnosis of Malaria. J Pathol. 2004;54:404-8.
16. Mishra MN, Misra RN. Immunochromatographic methods in malaria diagnosis. MJAFI. 2007;63:127-9.

Cite this article as: Gandhi V, Muley P, Parikh N, Gandhi H, Mehta A. Is rapid diagnostic test (malaria Pv/Pf Ag card test) reliable in diagnosing malaria. Int J Contemp Pediatr 2018;5:92-6.