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Original Research Article

Comparison of diagnostic methods of malaria by peripheral smear, centrifuged buffy coat smear and rapid antigen detection test

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ABSTRACT

Background: Malaria is common, life threatening infection in endemic area and presents diagnostic challenge to laboratories in most endemic areas. A rapid and accurate diagnosis is a pre requisite for effective treatment, especially for potentially fatal cases of falciparum infection.
Methods: Total 200 patients presented with fever and chills, were taken for study and performances of peripheral blood and centrifuged buffy coat smear were compared against the result of rapid antigen detection test (standard method).
Results: out of 200 cases, 55 were positive by rapid detection test.30 of P. vivax, 24 of P. falci and 1 was mixed infection. Peripheral smear had 85.5% sensitive and 100% specific compared to RDT which was 100 % sensitive and specific whereas centrifuged buffy coat was 92.7% sensitive and 99.3% specific.
Conclusions: Easy, rapid, most sensitive and specific diagnostic method will help in early diagnosis and lead to decrease in morbidity and mortality.

Keywords: Centrifuged buffy coat smear, Malaria, Peripheral smear, Rapid detection kit

INTRODUCTION

Malaria is endemic throughout most of the tropics. Of the approximately 3.4 billion people worldwide who are exposed annually, 1.2 billion are at high risk; the World Health Organization (WHO) states that more than 207 million developed symptomatic malaria in 2012.[1] Malaria is a major health problem in India, being one of the biggest burdens in terms of morbidity and mortality among all infectious diseases.[2]One of the most important problems in controlling malaria is limited access to effective diagnosis and treatment. The earliest symptoms of malaria are very nonspecific and variable such as fever with chills, rigor, nausea and vomiting, headache, body ache, fatigue and abdominal discomfort. Hence, there is difficulty to clinically diagnose malaria but treatment has to be started immediately in order to avoid complications.[3] Thus, the non-specific nature of clinical presentation of malaria may lead to overtreatment of malaria and missing diagnosis of malaria in low transmission areas. Therefore, precise diagnosis and species identification is very essential.[4]

Diagnostic modalities which are available for malaria are conventional peripheral blood smear examination, concentration techniques such as buffy coat smears and fluorescent (QBC) technique, rapid antigen detection test e.g. Optimal, SD Bioline, Para HIT-f, Para check, ICT, Para screen and Molecular diagnostic methods such as Polymerase chain reaction (PCR). These techniques vary in their sensitivity, specificity,

positive and negative predictive values, time consumption, cost effectiveness and ease of procedure etc.[5] Keeping in mind the seriousness of the condition and the current availability of diagnostic facilities across India, the present study is undertaken to compare between the peripheral blood smear examination, centrifuged buffy coat smear examination and rapid antigen detection test using histidine rich protein-2 antigen and plasmodium lactate dehydrogenase enzyme in patients with symptoms of fever with chills.

METHODS

A total number of 200 patients presented with fever with chills were taken for the study. Leishman stained peripheral blood smear examination, centrifuged buffy coat smear examination and rapid antigen detection test using histidine rich protein-2 antigen and plasmodium lactate dehydrogenase using SD BIOLINE malaria antigen Pf/Pan were performed on all the 200 patients. Patients already received recent antimalarial therapy or who had been treated with antimalarial drugs were excluded from the study. With aseptic precautions, from each patient-approximately 2 mL of venous blood sample was collected in EDTA and subjected to various techniques for the diagnosis of malaria like-peripheral smear examination, centrifuged buffy coat smear and antigen detection test (SD Bio Line Malaria Antigen Pf/Pan kit).

Peripheral blood smear

Smears were prepared and stained with Leishman stain. After staining, smears were examined at 100 x. 200 oil immersion fields were examined before smear was reported as negative for malaria.[6]

Centrifuged buffy coat smear

Smears were prepared by taking blood in Wintrobe's tube, which was centrifuged at 3000 rpm for 12-15 min. Then supernatant plasma was separated and discarded from the Wintrobe's tube, a buffy coat and an equal thickness of RBCs layer just below was aspirated and used to make smears which were stained by Leishman stain.

After staining, the smears were examined at 100X magnification. 100 oil immersion fields were examined before smear was reported as negative.

SD bio line malaria antigen

Pf/Pan rapid test which is one step, rapid and differential test for the detection of HRP-2 specific to P. falciparum and pLDH pan specific to plasmodium species. Rapid antigen detection test was taken as the standard reference in the present study.[7-9] Sensitivity, specificity, PPV and NPV of peripheral smear and centrifuged buffy coat smear were compared with rapid antigen detection test results.

RESULTS

Out of 200 cases, 55 cases were malaria positive by rapid antigen detection test, which was considered as the standard reference in the present study.

Of the 200 cases tested, 55 cases were positive by SD Bio Line malaria antigen P.f./Pan rapid test, 47 cases were positive by peripheral smear examination and 52 cases were positive for malaria by centrifuged buffy coat smear examination (Table 1).

Table 1: Results of all three diagnostic methods.

	PS	CBCS	Antigen detection test (AG)	Cases	Interpretation
	Negative	Negative	Negative	144	PS+CBCS+AG negative
	Negative	Positive	Positive	4	CBC+AG positive
	Negative	Negative	Positive	4	Only AG positive
	Negative	Positive	Negative	1	Only CBCS positive
	Positive	Positive	Positive	47	PS+CBCS+AG positive
Total	47	52	55	200	55 cases positive

Table 2: Comparison of peripheral smear examination with rapid antigen detection test.

PS	Rapid Antigen detection test		Total
	Positive	Negative	
Positive	47	0	47
Negative	8	145	153
Total	55	145	200

Peripheral blood smear examination showed sensitivity, specificity, positive and negative predictive values of 85.5%, 100%, 100% and 94.7% respectively in comparison with rapid antigen detection test (Table 2).

Centrifuged buffy coat smear examination showed sensitivity, specificity, positive and negative predictive

values of 92.7%, 99.3%, 98.1% and 97.3% respectively in comparison with rapid antigen detection test (Table 3).

Table 3: Comparison of centrifuged buffy coat smear examination with rapid antigen detection test.

CBCS	Rapid antigen detection test		Total
	Positive	Negative	
Positive	51	1	52
Negative	4	144	148
Total	55	145	200

DISCUSSION

Malaria is a parasitic infection of global importance and is a major public health problem in India accounting for sizeable morbidity, mortality and economic loss.[10] The surveillance activities against malaria are aimed at early diagnosis and prompt treatment of cases to reduce attributable morbidity and mortality.[10]

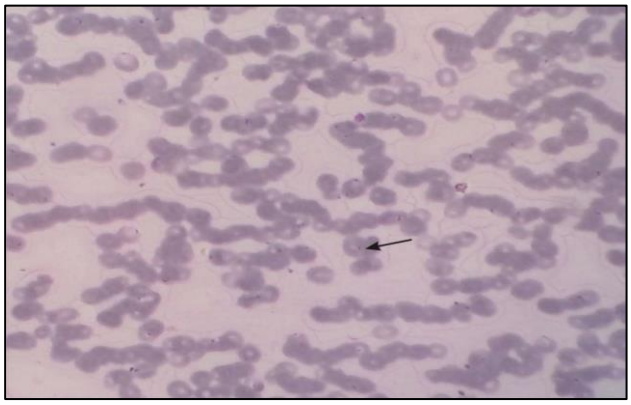

Figure 1: Ring form of P. falciparum on peripheral smear.

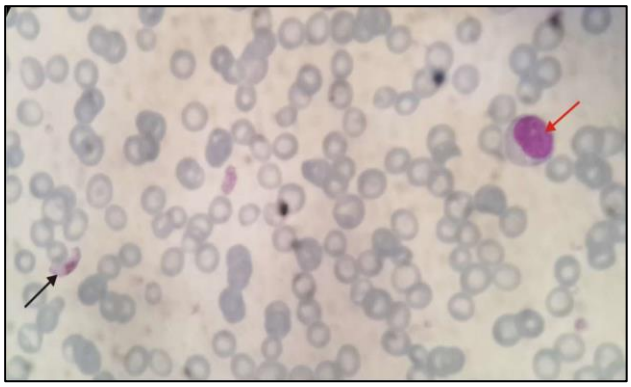

Figure 2: Gametocyte of P. falciparum (black arrow) with monocyte (red arrow) on peripheral smear.

The absolute necessity for rational therapy in the face of rampant drug resistance places importance on the accuracy of malaria diagnosis.[11] The traditional diagnostic method used is the blood smear examination of fever cases. Though it is the time tested and optimum method for diagnosis of malaria, known as gold standard, there are some operational difficulties, which results in delay of radical treatment in many cases. This delay can be dangerous in cases of Plasmodium falciparum.[10] Rapid detection and effective treatment is a pre-requisite for reducing the morbidity and mortality due to malaria. Newer techniques like Antigen detection assays are rapid, simple and easy to interpret.[12] Previous studies showed that RDT based on malaria antigen method is as specific as the traditional microscopy and even appears more sensitive than microscopy.[7,8,13]

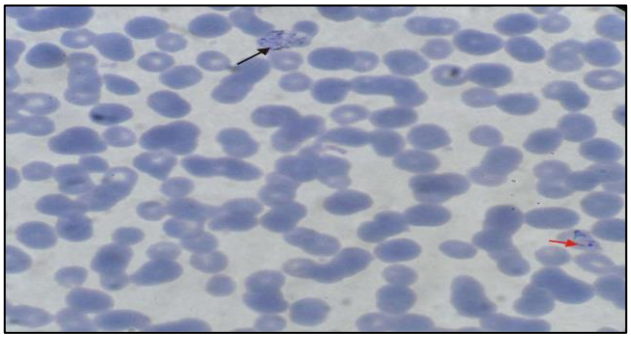

Figure 3: Schizont (black arrow) and ring form (red arrow) of P. Vivax on peripheral smear.

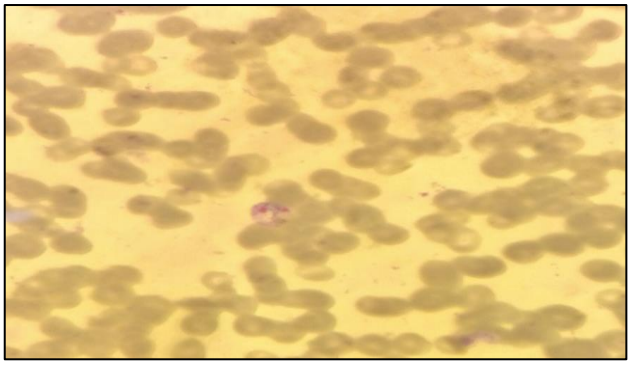

Figure 4: Schizont of P. Vivax on peripheral smear.

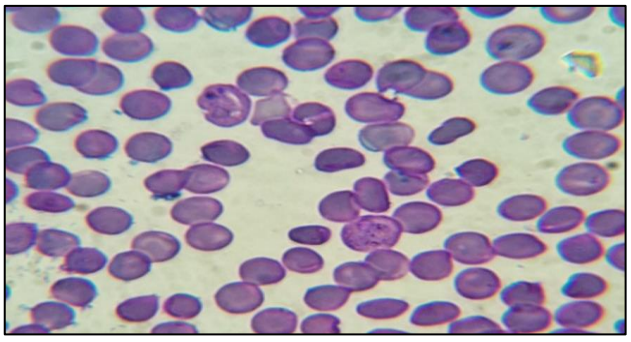

Figure 5: Schizont of P. Vivax on centrifuged buffy coat smear.

The present study was designed to compare the sensitivity, specificity, positive predictive value and negative predictive values of Leishman stained PS and

CBCS in clinically suspected cases of malaria by using rapid antigen detection test as the standard reference.[7,8]

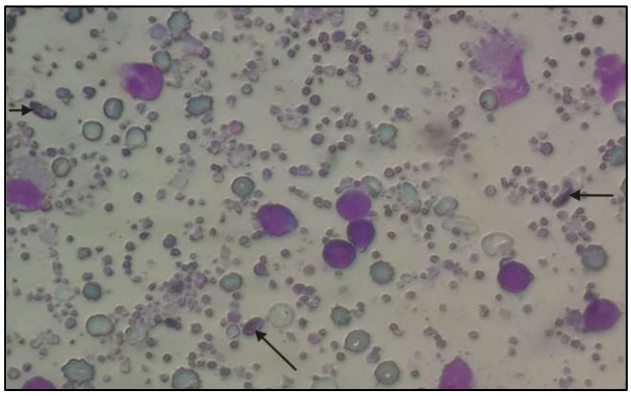

Figure 6: Gametocyte of P. falciparum on centrifuged buffy coat smear.

The present study was conducted in which 200 cases presented with fever with chills were taken for the study. All the 200 cases were tested for malaria by using following diagnostic methods-

- Leishman stained peripheral smear examination
- Centrifuged buffy coat smear examination
- Rapid antigen detection test (using SD BIOLINE Pf/Pan malaria antigen Kit).

In the present study, we failed to detect 4 cases of P. vivax and 4 cases of P. falciparum by peripheral smear examination, may be due to sequestration of parasites coupled with low parasitemia. The sensitivity, specificity, PPV and NPV values of Peripheral smear in present study were 85.5%, 100%, 100% and 94.7% which are consistent with Bhandari PL et al (86.79%, 100%, 100%, 87.03%) and Akhtar S et al (85%, 96%, 96.2%, 86.6%).[4,7]

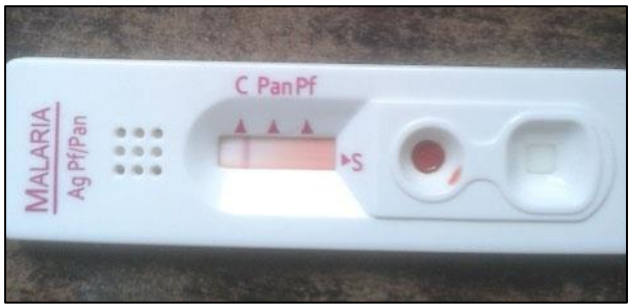

Figure 7: SD bio line malaria antigen Pf/Pan rapid test- negative.

In the present study, we failed to detect 2 cases of P. vivax and 2 cases of P. falciparum by centrifuged buffy coat smear examination may be due to sequestration or due to low parasitemia. One case was diagnosed positive for P. vivax by centrifuged buffy coat smear due to misinterpretation which was diagnosed as negative by peripheral smear examination and rapid antigen detection test.[14-16] The sensitivity, specificity, PPV and NPV values of centrifuged buffy coat smear in present study were 92.7%, 99.3%, 98.1% and 97.3% which are consistent with Akhtar S et al.[7]

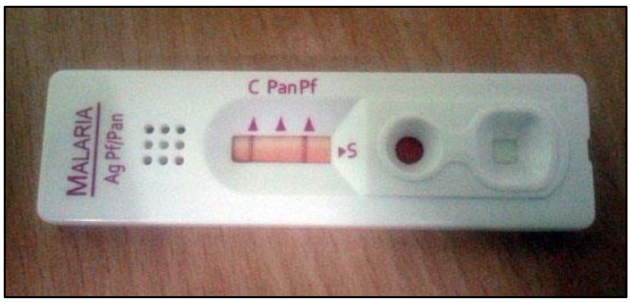

Figure 8: SD bio line malaria antigen Pf/Pan rapid test- P. falciparum +ve.

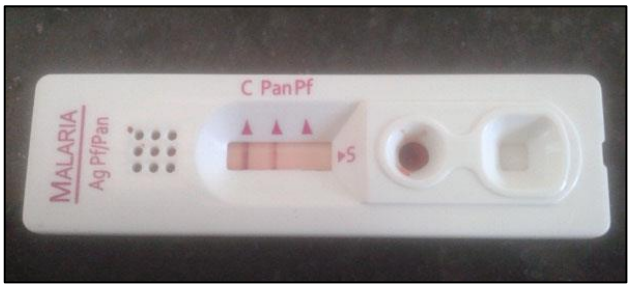

Figure 9: SD bio line malaria antigen Pf/Pan rapid test- P. vivax +ve.

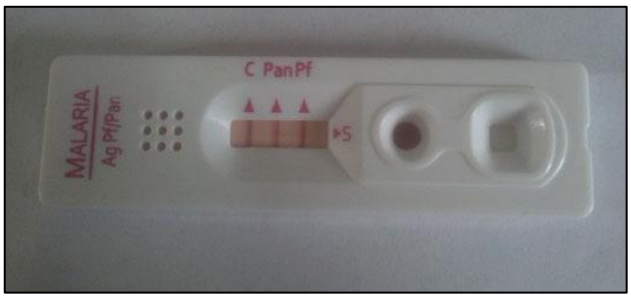

Figure 10: SD bio line malaria antigen Pf/Pan rapid test- mixed +ve.

Importance of centrifuged buffy coat smear over peripheral smear

- By adding the process of centrifugation to the peripheral smear technique, 4 more cases were detected, improving its sensitivity to a great extent from 85.5% to 92.7% -a total of 7.2% extra cases were detected. Earlier studies of Duangdee C et al and Davis R et al showed that there was a significant increase in the sensitivity of malaria detection in blood samples where parasitized red cells have been enriched through centrifugation and it could detect malarial parasites in patients whose conventional smear shows negative parasitemia[17,18]

- Among PS positive cases, CBCS had an exceptional sensitivity of 100%
- Among PS negative cases, however CBCS could detect 4 out of 8 cases, but it over detected one case. This false positivity may still be accepted due to its ability to detect 50% more of cases which were not detected by peripheral smear. This underlies the importance of CBCS test in suspected malaria cases who are negative by peripheral smear examination
- It is cheap, easy to perform and cost effective
- Equipment required for this technique is available in peripheral laboratories. Hence, it can be used in rural area
- The sensitivity, specificity, PPV and NPV values of centrifuged buffy coat smear in present study were 92.7%, 99.3%, 98.1% and 97.3% compared to peripheral smear whose sensitivity, specificity, PPV and NPV values were 85.5%, 100%, 100% and 94.7%.

Importance of rapid antigen detection test over peripheral smear

- By undergoing rapid antigen detection test, negative cases by peripheral smear were greatly benefited because 8 cases which were not detected by peripheral smear, they were detected by rapid antigen detection test either due to low parasitemia or due to sequestration of parasite. Thus, improving the sensitivity from 85.5% to 100%. Earlier studies of Azikiwe CCA et al and Aubouy A et al showed that rapid antigen detection test is as specific as PS and appears even more sensitive than PS[8,19]
- Among PS positive cases, rapid antigen detection test had an exceptional sensitivity of 100% and as specific as PS
- Though PS is cost effective, but it is time consuming (30-40minutes), require electricity and microscope, require trained and skilled microscopist. Whereas, rapid antigen detection test is simple, single step rapid (3-5minutes), does not require expertise.

Importance of rapid antigen detection test over centrifuged buffy coat smear examination

- By undergoing antigen detection test, negative patients of centrifuged buffy coat smear would be greatly benefitted because 4 cases which were not detected by CBCS, they were detected by antigen detection test. Thus, improving its sensitivity from 92.7% to 100%
- It is simple, rapid (3-5 minutes), easy to perform, sensitive and specific
- Since the test requires no laboratory or technical equipment, a diagnostic facility can be set up in rural areas and require little training to interpret the results
- Though the cost of the test may prevent its routine use but if complications associated with malaria considered, it may be a better option in emergency situations, area where workload is high which delays results and in places where experienced microscopist is not available. It can be performed at bedside in 3-5 minutes, therefore delay in treatment is usually avoided.

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REFERENCES

1. WHO. World Malaria Report, 2013. Geneva, Switzerland. Available at http://www.who.int/malaria/world_malaria_report_2013/en/.
2. Park K. Malaria. In: Park's textbook of social and preventive medicine.18th edn; Banarsidas Bhanot: Jabalpur; 2004:201-203.
3. White NJ, Breman JG. Malaria. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, ed. Harrison's principles of internal medicine. 18th ed. McGraw Hill, New York; 2012:1688-1705.
4. Bhandari PL, Raghuveer CV, Rajeev A, Bhandari PD. Comparative study of peripheral blood smear, quantitative buffy coat and modified centrifuged blood smear in malaria diagnosis. Indian J Pathol Microbiol. 2008;51:108-12.
5. Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria diagnosis: a brief review. Korean J Parasitol. 2009;47(2):93-102.
6. Paniker CKJ. Malaria and Babesia. In: Ghosh S, ed. Textbook of medical parasitology. 7th edn. New Delhi: Jayapee Brothers Medical Publishers; 2013:63-83.
7. Akhtar S, Maimoon S, Wilkinson A, Gowardhan V, Mahore S. Feasible choices in diagnostic methods of malaria. J Vector Borne Dis. 2010;47:151-4.
8. Azikiwe CCA, Ifezulike CC, Siminialayi IM, Amazu LU, Enye JC, Nwakwunite OE. A comparative laboratory diagnosis of malaria: microscopy versus rapid diagnostic test kits. Asian Pacific Journal Tropical Biomedicine. 2012:307-10.
9. Vyas S, Puwar B, Patel V, Bhatt G, Kulkarni S, Fancy M. Study on validity of a rapid diagnostic test kit versus light microscopy for malaria diagnosis in Ahmedabad city, India. East Mediterr Health J. 2014;20(4):236-41.

10. Kumar KR, Sudarshan KS. Clinical evaluation of a rapid diagnostic kit (Para check Pf) for diagnosis of Plasmodium falciparum. Indian J Prev Soc Med. 2004;35(1):10-4.
11. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test. Am J Trop Med Hyg. 2007;77(6):119-27.
12. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test. Am J Trop Med Hyg. 2007;77(6):119-27.
13. Ujah IOA, Ikeh EI, Glew RH. Evaluation of the Optimal test for rapid diagnosis of malaria. African Journal Clinical Experimental Microbiol. 2003:4(1):14-7.
14. Kuladeepa VA, Sukesh. Quantitative buffy coat (QBC) test and other diagnostic techniques for diagnosing malaria: review of literature. National J Med Res. 2012;2(3):386-8.
15. Kakkilaya BS. Malaria site- all about malaria. (Monograph on internet) India; 2009. Available at URL:http:/www.malariasite.com.
16. Mckenzie FE, Sirichaisinthop J, Miller RS.Gasser RA, Wongsrichanalai C. Dependence of malaria detection and species diagnosis by microscopy on parasite density. Am J Trop Med Hyg. 2003;69(4):372-6.
17. Duangdee C, Tangpukdee N, Krudsood S, Wilairatana P. Use of buffy coat thick films in detecting malaria parasites in patients with negative conventional thick films. Asian Pac J Trop Biomed. 2012;2:301-3.
18. Davis R, Flanigan T, Wilson E. Passive gravitational sedimentation of peripheral blood increases the sensitivity of microscopic detection of malaria. Asian Pacific J Tropical Med. 2013:552-5.
19. Aubouy A, Dechavanne S, Dasseya R, Massougbodji A. Value of rapid tests for diagnosis of malaria in Benin. Med Trop (Mars). 2010;70(5-6):485-9.

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