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A Comparative Study of Standard Peripheral Blood Smear and Antigen Detection Test in Diagnosis of Malaria in a Tertiary Care Hospital in Jalandhar

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ABSTRACT

Background: Malaria can cause fatal complications if the diagnosis and treatment are delayed. So, quick detection and early treatment of malaria are the best policies for the management of disease. Microscopy has been the gold standard for malaria diagnosis for decades. Recently, many new rapid diagnostic tests (RDTs) are being widely used. We have made an attempt to compare peripheral smear and rapid antigen detection methods for the diagnosis of malaria. **Material and methods:** A total of 500 blood samples were collected from patients presenting with symptoms of malaria. Thin and thick blood smears were prepared and stained with Leishman's stain. *Plasmodium falciparum* (Pf) HRP-2 antigen and *Plasmodium vivax* (Pv) specific pLDH detection was done using rapid test device for malaria diagnosis. **Results:** Out of the 500 blood samples tested, only 3.4% were positive for malaria. Difference between the positivity for Plasmodium species by both methods was 0.6%. Sensitivity, specificity, positive predictive value and negative predictive values were 85%, 99.6%, 89.5% and 99.4%, respectively with peripheral blood film (PBF) method. **Conclusion:** The study highlights that the RDT, for diagnosis of malaria, is as reliable as microscopy. Microscopy is simple, economical, sensitive and specific but it is time consuming and requires a microscope. In places where such facilities are not available, rapid, simple and easy to interpret antigen detection test can be done.

Keywords: *Plasmodium falciparum*, *Plasmodium vivax*, malaria diagnosis, rapid diagnostic test

Malaria is one of the important vector-borne disease in India and can be fatal if not treated promptly. The early diagnosis and treatment of malaria is essential to prevent complications, especially in cerebral malaria. The disease occurs in over 90 countries worldwide, and over 600 million clinical cases and 3.1 million malaria-related deaths are estimated to occur per year. Malaria is caused by five Plasmodium species with different geographic distribution; *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) are more common in India. It is important to differentiate between types of malarial parasite causing the illness as treatment of malaria based on assumption would encourage the development and spread of drug-resistant Pf parasites. Conventional peripheral smears, quantitative buffy coat (QBC) and rapid diagnostic tests (RDTs) are commonly available diagnostic tests for malaria.

Changing patterns of accepted morphological appearances of Plasmodium species, possibly due to drug pressure, strain variation or approaches to blood collection, have created diagnostic problems that cannot easily be resolved merely by reference to an atlas of parasitology. Fortunately, new technology provides additional diagnostic options, which can be reviewed and compared to more traditional methods. The various techniques to diagnose malaria are conventional peripheral smear, QBC, antigen-based rapid diagnostic kits and molecular studies (polymerase chain reaction).

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Conventional peripheral blood smear examination for demonstration of malaria parasites is the gold standard method for diagnosing malaria. However, this technique is time consuming and requires skilled personnel. QBC is sensitive but has drawbacks such as high cost, imperfect speciation and is associated with false-positive results due to artifacts, such as cell debris. RDTs detect parasitic antigens like histidine-rich protein-2 (HRP-2), Plasmodium lactate dehydrogenase (pLDH) and pan-specific aldolase. RDTs are easy to perform and yield quick results but they are expensive, associated with false-positive results due to persistent antigenemia, and cross-reactions with autoantibodies such as rheumatoid factor and false-negative result in severe malaria, which could be attributed to immune-complex formation, prozone phenomenon and other causes. All these techniques vary in there sensitivity, specificity, positive and negative predictive values. For this reason, the present study was done to compare the peripheral blood smear test with malaria antigen card test.

MATERIAL AND METHODS

The prospective study was conducted in the Dept. of Pathology in a tertiary care hospital from April 2018 to March 2019. Only 500 blood samples from patients presenting with symptoms of malaria were included in the study. Prior to study, permission from ethical committee was taken.

Thick and thin smears were prepared from finger prick and approximately 5 mL of venous blood was collected in an anticoagulant tube containing ethylenediaminetetraacetic acid (EDTA) from each patient during the peak of fever and transported to the laboratory.

Peripheral Blood Smear

Thick and thin blood smears were prepared and stained with Leishman's stain as per standard method. The slides were examined using light microscope and the average time spent on each slide depended on parasite density. Thick smears were reported negative when no parasite was observed on examination of 200-300 oil immersion fields (OIF) while a thin smear was reported negative when no parasites were observed in 100 OIF. The red blood cells in the tail end of the thin smear were examined for the species identification and stages of the parasites.

Rapid Diagnostic Tests

Pf HRP-2 antigen and Pv specific pLDH detection was done using rapid test device for malaria (Pv/Pf). The kits were all from the same batch and were used before the expiry date and performed according to the instruction manual by the manufacturer.

The test goes by the principle of agglutination of antibodies/antisera with respective antigen in immune-chromatography format using nano gold particles as an agglutination revealing agent.

As the test sample moves through the membrane assembly of the device following the addition of the clearing buffer, the colored colloidal gold conjugates of the agglutinating sera for HRP-2 and the agglutinating sera for Pv specific pLDH complexes the HRP-2/pLDH in the sample. This complex moves ahead on the membrane and reaches the test region and undergoes immobilization by the agglutinating sera for Pan Malaria specific pLDH and/or agglutinating sera for HRP-2 coated on the membrane. This gives rise to the formation of pink-purple colored bands. This validates a positive test result. A band appears under Pf at the test region in falciparum positive samples while a band appears under Pv in vivax malaria positive samples. If a band appears under Pf as well as Pv in the test region, it suggests mixed infection.

If colored band is absent in the test region, it points to a negative test result. The unreacted conjugate and unbound complex move further on the membrane and undergo immobilization by agglutinating sera for rabbit globulin coated on the membrane at the control region, giving rise to a pink-purple band. The control band formation is based on the "Rabbit globulin/agglutinating sera for rabbit globulin" system. This is independent of the analyte detection system.

Therefore, it promotes the formation of a consistent control band signal that does not depend on the analyte concentration. The control band helps validate the test performance.

The procedure and interpretation of test results were conducted according to the manufacturer's literature guideline.

STATISTICAL ANALYSIS

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each method using standard formulae.

Sensitivity = TP/TP+FN, Specificity = TN/TN+FP, PPV = TP/TP+FP, NPV = TN/TN+FN

(TP – True Positive, TN – True Negative, FP – False Positive, FN – False Negative)

RESULTS

Out of the 500 blood samples tested, only 17 (3.4%) were positive for malaria. Out of 17 positive cases, Pv was diagnosed in 15 cases (88.23%), Pf was identified in 1 case (5.88%) and 1 smear (5.88%) showed mixed infection with both Pv and Pf. RDT showed 14 positive cases (2.8%) of malaria, of which 12 (85.71%) cases were of Pv, and 1 case each (7.14%) was of Pf and mixed infection with falciparum and vivax. Difference between the positivity for Plasmodium species by both methods was 0.6% (Table 1).

Sensitivity, specificity, PPV and NPV were 85%, 99.6%, 89.5% and 99.4%, respectively with peripheral blood film (PBF) method (Table 2).

DISCUSSION

Malaria is today one of the major causes of human suffering, both in terms of increasing morbidity/mortality and stunting intellectual/economic growth. Accurate diagnosis and early treatment of malaria is essential to reduce mortality and morbidity. During the last decade, several new rapid diagnostic techniques have been developed and evaluated widely. They aim at prompt and accurate diagnosis of malaria parasite that helps in early initiation of appropriate antimalarial drug to prevent the complications. Leishman's or Giemsa-stained thick smears are considered to be the 'Gold standard' in diagnosis of malaria. In the present study, the PBF positivity for malaria was 3.4% whereas other workers reported it to be 16.84% and 18.28%, respectively. As per Punjab Malaria Elimination Campaign (2017-2021) in Punjab, Annual Parasite Incidence (API) is less than 1 case per 1,000 population at risk and in 2016, API in Jalandhar was reported between 0 to 0.1. In India, 40% of cases are as result of Pv malarial infection, 44.3% are due to Pf malarial infection and 10-15% are due to mixed malarial infection.

In our study, among malaria positive cases by PBF method, 88.23% were due to Pv infection and 5.8% positive cases each were of Pf and mixed infection. Almost similar results were reported by other workers as 98.85% for Pv, 0.57% each for Pf and mixed infection by PBF method. RDT showed 14 positive cases (2.8%) of malaria, of which 12 (85.71%) cases were of Pv and one case each (7.14%) was of Pf and mixed infection with falciparum and vivax. However, other workers with RDT method reported it to be 94.17% for Pv, 2.11% for Pf and 3.7% for mixed infection.

In the present study, we observed 87% sensitivity, 99.6% specificity, 82.35% PPV and 99.58% NPV in antigen card test comparing with the peripheral blood smear (Table 2), whereas other workers have observed 100% sensitivity, 99.5% specificity, 98.59% PPV and 100% NPV in antigen card test comparing with the peripheral blood smear. In the present study, we reported 85% sensitivity for Pf and Pv. It was found that at lower parasitemia, the sensitivity dropped considerably. Sensitivity was 100% at parasitemia level 240 parasites/μL or more. Thick smear provides enhanced sensitivity of blood film technique and seems to be better than thin film to detect low levels of parasitemia and relapse or recrudescence. RDT missed 3 cases of Pv which were positive on microscopy. These false-negative results may be due to the fact that RDT is not sensitive below a parasitic index of 100 parasite/μL and it detects pLDH produced by living parasites. The blood samples judged positive

Table 1. Comparison of Microscopy and Rapid Diagnostic Test Method

	P. vivax (Positive)	*P. falciparum* (Positive)	Mixed infection (Positive)	Negative
PBF (n = 500)	15 (3%)	01	01	483
RDT (n = 500)	12 (2.4%)	01	01	486
Difference between two methods	3 (0.6%)	0	0	

Table 2. Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of PBF and RDT Methods for Diagnosis of Malaria

Method of test	Sensitivity	Specificity	PPV	NPV
PBF	85%	99.6%	89.5%	99.4%
RDT	87.5%	99.6%	82.35%	99.58%

by pathologist may have been dead parasites not yet cleared from the host.

CONCLUSION

The study highlights that RDT for diagnosis of malaria is as reliable as microscopy. Microscopy is simple, economical, sensitive and specific but it is time-consuming as one test requires 30-40 minutes and it is subject to bias; results are affected by the skill and workload of the microscopists. RDT is simple, and requires no equipment; however, its drawback is that it is quite expensive and its sensitivity and specificity are debatable at low parasitemia. 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 antimalarial drugs, which may prevent drug resistance.

SUGGESTED READING

1. Paul SN, Murmu S, Singh US, Kumar S. A comparative study of peripheral blood smear and rapid diagnostic test for diagnosis of malaria in Jharkhand, India. IJPMR. 2016;4(4):1-2.
2. Yan J, Li N, Wei X, Li P, Zhao Z, Wang L, et al. Performance of two rapid diagnostic tests for malaria diagnosis at the China-Myanmar border area. Malar J. 2013;12:73.
3. 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(1):92-6.
4. Bhat SK, Sastry AS, Nagaraj ER, Mannur S, Sastry AS. Laboratory diagnosis of malaria by conventional peripheral blood smear examination with Quantitative Buffy Coat (QBC) and Rapid Diagnostic Tests (RDT) - A comparative study. International Journal of Collaborative Research on Internal Medicine & Public Health. 2012;4(10):1746-55.
5. Mendiratta DK, Bhutada K, Narang R, Narang P. Evaluation of different methods for diagnosis of *P. falciparum* malaria. Indian J Med Microbiol. 2006;24(1):49-51.
6. Parija SC, Dhodapkar R, Elangovan S, Chaya DR. A comparative study of blood smear, QBC and antigen detection for diagnosis of malaria. Indian J Pathol Microbiol. 2009;52(2):200-2.
7. Tyagi BK. Vector-borne diseases: Epidemiology and Control. Preface. Jodhpur: Scientific Publishers; 2008.
8. Kumar KR, Sudarshan KS. Clinical evaluation of a rapid diagnostic kit (Paracheck-Pf) for diagnosis of *Plasmodium falciparum* in Karnataka state of India. Indian J Prev Soc Med. 2004;35(1):10-4.
9. Elimination Campaign. 2017-2021. Action Plan & Road Map for Malaria Elimination in Punjab, India. Department of Health and Family Welfare, Punjab. (online) (Cited 2019 July11). Available at: https://www.punjabnvbdcp.in/Download/Punjab%20Malaria%20Elimination%20Campaign%202017-2021.pdf
10. World Health Organization, Regional Office for South-East Asia. (2000). Implementation of collaborative actiities on roll back malaria in the South-East Asia Region: report of an intercountry meeting, New Delhi, 4-6 May 1999. WHO Regional Office for South-East Asia. Available at: https://apps.who.int/iris/handle/10665/205327
11. Kulkarni P, Varna I. Rapid diagnostic tests versus peripheral smear in malaria: a comparative study. Trop J Pathol Microbiol. 2016;2(3):179-82.
12. Cooke AH, Chiodini PL, Doherty T, Moody AH, Ries J, Pinder M. Comparison of a parasite lactate dehydrogenase-based immunochromatographic antigen detection assay (OptiMAL) with microscopy for the detection of malaria parasites in human blood samples. Am J Trop Med Hyg. 1999;60(2):173-6.

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COVID-19: Presence of Viral RNA in Specimens Always Correlates with Viral Transmissibility

No, in a ferret model of H1N1 infection, the loss of viral culture positivity but not the absence of viral RNA corresponded to the end of the infectious period. Real-time reverse transcriptase PCR results continued to be positive 6-8 days after the loss of transmissibility. (*Lancet Infectious Diseases*)

In SARS, Live Virus was Detected for 4 Weeks

No, for SARS coronavirus, viral RNA can be detected in the respiratory secretions and stool of some patients after onset of illness for more than 1 month, but live virus could not be detected by culture after Week 3. (*Lancet Infectious Diseases*)

It's Easy to Differentiate Between Infective and Non-infective Virus

The inability to differentiate between infective and non-infective (dead or antibody-neutralized) viruses is a huge limitation of nucleic acid detection. However, given the difficulties in culturing live virus from clinical specimens during a pandemic, using viral RNA load as a surrogate is reasonable for generating clinical hypotheses. (*Lancet Infectious Diseases*)