

Comparative Study of Peripheral Blood Smear and Rapid Diagnostic Kits for Antigen Detection for Diagnosis of Malaria

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Rapid diagnosis of malaria is an important tool for effective treatment & to prevent complication of malaria & also to reduce mortality and morbidity caused by malaria. This study was taken to compare the efficacy of two methods available in our institutional set up i.e. thick & thin smear & Rapid diagnostic test kit to detect pLDH & HRP in blood of patient. A total of 235 samples were collected from patients of pyrexia suspected to be malaria by sign/symptoms for traditional microscopy: Thick & thin smears were prepared & stained with Leishman's stain, taking thick smears as gold standard. Antigen detection was done using commercially available kits, out of 235 samples thick smear were positive in 46 cases & thin smear in 28 cases & antigen (Malariagen) were positive in 34 cases. Thin smears had a sensitivity & specificity of 60.86 % and 100% respectively. Antigen detection had a sensitivity & specificity of 73.91 % and 100% respectively.

Leishman's stained thick smear although cost effective, is difficult to interpret unless seen by experienced microscopists & its time consuming & labour some technique were as antigen detection is rapid, simple, nonmicroscopic method, easy to interpret and can be done bedside of patients or during night calls or emergency.

Key words: Malaria, diagnosis, Antigen detection, sensitivity, specificity, efficiency.

Malaria is a protozoal disease caused by infection with parasites of the genus *Plasmodium* and transmitted to man by certain species of infected female anopheles Mosquito. It is a serious endemic disease in many parts of the world. Humans are infected by at least four species of *Plasmodium*: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. *P. falciparum* accounts for more than 60% of worldwide malarial infections.

Malarial attack comprises of three distinct stages viz. cold stage, hot stage and sweating stage. Onset is with lassitude, headache, nausea and chill and rigors and Temp 39-41°C. Headache is severe and vomiting is present. Hot stage patient feels burning hot. Sweating stage fever comes down with profuse sweating¹.

Malaria presents a diagnostic challenge to the medical community worldwide. Malaria has occurred in more than 109 countries¹. It is estimated that 247 million episodes of malaria have occurred in 2006 worldwide and 1.1 to 2.2 million deaths were due to malaria every year¹. In India, in the year 2006 there were approximately 1.67 million cases of malaria

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reported of which 75 million were caused due to *P. falciparum* and deaths were 1487¹⁻³. In India about 27% population lives in malaria high transmission areas (> 1 case/1000 population) and about 58% in low transmission (0 to 1 case/1000 population) areas¹. Due to serious complication of *P. falciparum* it is necessary for a prompt and accurate diagnosis and treatment of malaria. The diagnostic technique available in our institutions were conventional thick and thin smear and antigen detection tests detecting parasitic antigens like histidine-rich protein 2 (HRP-2), *Plasmodium* lactate dehydrogenase (pLDH), both techniques vary in their sensitivity and specificity, positive and negative predictive values and efficiency. So keeping in mind the seriousness of condition and current availability of diagnostic facilities in our hospital, we decided to conduct the comparative study of the commonly employed diagnostic technique in diagnosis of malaria i.e. thick and thin smear and antigen detection using pLDH and HRP.

Comparison of Leishman's stained thin (PBS) and Rapid antigen detection for diagnosis of malaria

MATERIAL AND METHODS

The present study was conducted in department of microbiology SSIMS and RC at Davangere. A total of 235 cases were studied during 1 year duration from January 01 to December 09. These cases were majority from Department of Medicine, Pediatric, OBG and few from Casualty also. Most of the cases were pyrexia suspected to be malaria.

Sample collection

The specimen collected was 2 ml of EDTA blood. Blood should be collected before

starting anti malarial treatment. Blood is collected from earlobe or finger in older children and adults; and from great toe in infants. It can be collected anytime during fever because high density of malaria parasites appear in circulation during paroxysm of fever.

Thick and thin smears

Blood samples were subjected to two types of blood Smears which are useful in searching for and identification of malarial parasite. It is recommended that both types of films be prepared in single slide. Thick films are more reliable in searching for parasite, as large volume of blood is examined under each microscopic field. Thick blood film is much better to detect low levels of parasitemia and reappearance of circulating parasites during infection recrudescence or relapse. Therefore scanty parasites are found about 20 times more rapidly in thick films than in thin films. The thin smear is more valuable for identifying the species of parasite present. They are seen more clearly in thin smear. Thin smears are often preferred for routine estimation of the parasitemia because the organisms are easier to see and count. The ability to count parasites in sequential blood films enables the response to therapy to be monitored, particularly for *P. falciparum* infections. Thick smears were reported negative after examination of 200 to 300 (OIF) oil immersion field and thin smears was given negative when no parasites were observed in 200 (OIF)⁴.

Antigen detection using pLDH and HRP

There are many rapid diagnostic tests for malarial parasites as explained by other investigators⁵⁻⁶ and kits are commercially available in the market. Antigen detection Kits used were NANO malaria *P. vivax* and *P. falciparum* (P.V/P.F) antigen detection test is a single use, unitized immuno chromatographic test

Table 1. Sensitivity, specificity, positive predictive value, negative predictive value and efficiency of thin smear and antigen detection methods for diagnosis of malaria

Diagnostic Test	Sensitivity (%)	Specificity (%)	Positive Predictive Value (PPV) (%)	Negative Predictive Value (NPV) (%)	Efficiency of the test (%)
Thin Smear	60.86	100	100	91.3	92.34
Antigen Detection	73.91	100	100	94.02	94.89

that uses antibodies to *P. vivax* and *P. falciparum*, in whole blood.

Principle of the test the malaria; the malaria *P. vivax* and *P. falciparum* is a rapid immuno chromatographic screening test for detection of *P. vivax* and *P. falciparum* in whole blood. The method employs antibodies to Histidine Rich Protein of *P. falciparum* and pLDH antibody of *P. vivax*. One set of these antibodies is conjugated to colloidal gold and other set is immobilized on the nitro cellulose membrane. The whole blood sample is applied to the sample well followed by addition of running buffer. Running buffer lyses red blood cells and release *P. vivax* and *P. falciparum* antigen. The released antigen binds to the gold conjugated anti HRP antibody and anti pLDH antibody. In a *P. vivax* and *P. falciparum* positive patient, the dye conjugated – immune complex migrates on the nitrocellulose membrane and is captured by antibody immobilized in the test zone and produces pink/ purple band. The result was read after 15 min as per the manufacturer's instructions. It was interpreted as positive for *P. falciparum* if only T1 band was seen along with control C band. If only T2 and C bands were seen, it was interpreted as positive for Plasmodium vivax. If T1, T2 and C bands appeared it indicated sample was positive or both *P. vivax* and *P. falciparum*.

RESULTS

The entire 235 sample were evaluated by Leishman's stained thin peripheral blood smear and rapid diagnostic test kits for antigen detection. Malarial parasite was detected 34 cases by rapid diagnostic kit were as peripheral smear detected 46 cases, out of 46 case thick smear detected in all 46 were as thin smear detected in only 28 cases. The total incidence of malaria was found to be 19.57 % (46\235). Most of the cases were either due to *P. vivax* or *P. falciparum*.

DISCUSSION

Plasmodium falciparum infection causes high morbidity and Mortality. so to prevent life threatening complications of *P. falciparum*⁷. Rapid detection and effective treatment is most important.

The recommended method and current gold standard used for routine laboratory diagnosis of malaria is the microscopic examination of stained thick and thin smears. In the most capable hands, this method can be expected to detect 50 parasites\ μl and to identify at species level 98% of all parasites seen. Hanscheid⁵ discussed the problems that can arise when different methods for diagnosis of malaria are compared.

Leishman's stained thick smears are considered to be the "gold standard" in diagnosis. However interpretation of thick smears is laborious and time consuming and also depends on preparation of smear, staining technique, and finally on the microscopists^{8,9} who interpret the result. blood film method remains standard method for diagnosing malaria since it detects all plasmodium species and allows visualization of parasite growth stages, which is essential for making therapeutic decisions.

In our study we compared thick and thin blood smear with rapid diagnostic kit. The sensitivity of Leishman's stained thin smear was found to be lowest (60.86 %) however; this method had a high specificity and positive predictive value (100% and 100 %) Efficiency of thin smear is 92.34%. Microscopy is a sensitive method. Skilled microscopists can detect 5 to 10 parasites / μl of blood. Warhurst and Williams¹⁰ reported that examination of thin blood films is only 1\10 as sensitive as examination of thick blood films for the quantification of malarial parasites.

Malaria antigen detection had sensitivity, specificity and PPV of 73.91%, 100%and 100 % respectively. Efficiency of antigen detection test is 94.89% this test was based on detection of pLDH and HRP. The values obtained for this kit-based procedure were much lower than those observed for other test based on similar principle¹¹⁻¹². This low sensitivity could be attributed to low parasitemia levels as observed by Iqbal *et al.*¹³ who observed 75% sensitivity at parasitemia < 100/ μl . The specificity was comparable to other observers using the tests based on similar principle¹¹⁻¹². However, the test was found to be user friendly, more rapid, non microscopic method and interpretation was much easier compared to smear. There by saving training and time.

A negative rapid diagnostic test cannot

at present be accepted at face value and will need to be confirmed by microscopic examination .the possibility of gene deletion isolates that do not express HRP-2 has been postulated.

Several workers report usefulness of rapid diagnostic tests as a means of monitoring the course of a parasitemia during therapy^{14,15}. Srinivasan *et al.*¹⁶ investigated the significance of parasites that remain in the peripheral blood after several days of therapy, as detected by microscopy.

The new generation of rapid diagnostic test offers a realistic practical chance to move the diagnosis of malaria away from the laboratory and nearer to the patient .A sensitivity of >100parasites/µl obtainable for *P. falciparum* diagnosis for both HRP-2 and pLDH is as good as most clinical laboratory staff in nonspecialised laboratories could expect to attain microscopically with limited exposure to malaria cases¹⁷. The added assurance that life threatening parasitemia with *P. falciparum* will not be missed, particularly for inexperienced laboratory staffs during night calls. The ability to detect the majority of the non-falciparum malaria cases also makes these tests ideally suited as major backup procedures for malaria diagnosis.

CONCLUSION

Malaria presents a diagnostic challenge to the medical community worldwide. And it is a serious endemic disease in many parts of the world. We need to employ more sensitive tests, which are rapid to detect low levels of parasitemia in population. Therefore we recommend QBC were facilities are available. If adequate laboratory facilities are not available than Rapid antigen detection can be done, which are rapid, simple, nonmicroscopic and easy to perform.

REFERENCES

1. PARK'S Text book of Preventive and Social Medicine 20TH ed. 2009; 222.
2. WHO (2008), World Malaria Report 2008.
3. Govt. of India, Annual Report 2007-08, Ministry of Health and Family Welfare, New Delhi 2008
4. Parija SC. Textbook of medical parasitology. 3rd ed. Chennai: AIPHD; 2006.
5. Hanscheid T. Diagnosis of malaria: a review of alternatives to conventional microscopy. *Clin Lab Haemataol.* 1999; **21**: 235-245.
6. Moody A. Rapid diagnostic tests for malarial parasites. *Clin Microbiolo Rev.* 2002; **15**: 66-78
7. Singh N, Chand G, Nagpal AC. Risk of malaria among professionals working in forest of central India. JAPI 2005(In press)
8. Dowling MA, Shute GT. A comparative study of thick and thin blood films in diagnosis of scanty malaria parasitemia. *Bull world Health organ* 1966; **34**: 249-67.
9. Payne D. Use and limitations of light microscopy for diagnosing malaria at primary health care level. *Bull World heath Organ.* 1988; **66**: 621-6.
10. Warhurst, D. C., and J. E. Williams., Laboratory diagnosis of malaria. *J. Clin. Pathol.* 1996; **49**: 533-538.
11. Iqbal J, Hira PR, Sher A, Al-Enezi AA. Diagnosis of imported malaria by plasmodium lactate dehydrogenase (PLDH) and histidine-rich protein 2 (PfHRP-2) based immunocapture assays. *Am J Trop Med Hyg.* 2001; **64**: 20-3
12. Palmer CJ, Lindo JF, Klaskala WI, Quesada JA, Kaminsky R, Baum MK et al. Evaluation of the OptiMAL test for rapid diagnosis plasmodium vivax and plasmodium falciparum malaria *J Clin Microbiol* 1998; **36**: 203-6.
13. Iqbal J, Sher A, Hira PR, Al-Owaish R. Comparison of the OptiMAL test with PCR for diagnosis of malaria in immirgrants. *J Clin Microbiol* 1999; **39**: 3644-6.
14. Oduola, A. M., G. O. Omitowoju, A. Sowunmi, M. T. Makler, C. O. Falade, D. E. Kyle, F. A. Fehintola, O. A. Ogundahunsi, R. C. Piper, B. G. Schuster, and W. K. Milhous., *Plasmodium falciparum*: evaluation of lactate dehydrogenase in monitoring therapeutic response to standard anti-malarial drugs in Nigeria. *Exp. Parasitol.* 1997; **87**: 283-289.
15. Moody, A., A. Hunt-Cooke, E. Gabbett, and P. Chiodini., Performance of the OptiMAL® malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the Hospital for Tropical Diseases, London. *Br. J. Haematol.* 2000; **109**: 891-894.
16. Srinivasan, S., A. H. Moody, and P. L. Chiodini., Comparison of blood-film microscopy, the OptiMAL® dipstick, Rhodamine 123 and PCR for monitoring anti-malarial treatment. *Ann. Trop. Med. Parasitol.* 2000; **94**: 227-232. 66
17. laboratory Milne, L. M., P. L. Chiodini, and D. C. Warhurst., Accuracy of routine diagnosis of malaria in the United Kingdom. *J. Clin. Pathol.* 1994; **47**: 740-742.