ORIGINAL RESEARCH PAPER
Volume - 15 | Issue - 08 | August - 2026 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARATIVE STUDY BETWEEN MALARIAL RAPID ANTIGEN TEST & PERIPHERAL BLOOD SMEAR MICROSCOPY AMONG PATIENTS ATTENDING TERTIARY CARE CENTRE

Pathology

Dr. Divya Singh Resident

Dr. Sumit Prakash Rathore* Senior Professor Department Of Pathology, Jhalawar Medical College, Jhalawar, Rajasthan*Corresponding Author

ABSTRACT

INTRODUCTION- In highly endemic areas, rapid and efficient diagnostic methods are needed for early diagnosis and management of malaria. Non-testing before treatment often results in extensive overuse of anti-malarial drugs, which has economic implications, drug resistance and may even border on safety as far as the individual is concerned. The present study was therefore aimed at assessing the validity of antigen detecting MRDTs by comparing the test results with that of conventional Giemsa stained thick and thin blood film microscopy. **MATERIAL AND METHODS –** This cross-sectional study was conducted on 150 febrile patients who were directed to the laboratory of Malaria Control Cell of Government Hospital Jhalawar, Rajasthan. **RESULTS** - Maximum patients examined were in between the age groups of 11-40 yrs. though malaria was present in 16(32.65% cases of 11-20 yrs age. The sensitivity of RDT as 89.80%, specificity is 100%, positive predictive value was 100%, negative predictive value 95.28% and diagnosed accuracy was 96.67%. The sensitivity of Peripheral Smear Test as 95.45%, specificity is 100%, positive predictive value was 100%, negative predictive value 98.15% and diagnosed accuracy was 98.67%. **CONCLUSION -** The study concludes that both peripheral smears and MRDTs are effective for malaria diagnosis, with MRDTs having slightly higher sensitivity in detecting positive cases, including a higher incidence of mixed infections and Plasmodium falciparum cases.

KEYWORDS

INTRODUCTION

In highly endemic areas, rapid and efficient diagnostic methods are needed for early diagnosis and management of malaria. Non-testing before treatment often results in extensive overuse of anti-malarial drugs[1], which has economic implications, drug resistance and may even border on safety as far as the individual is concerned.[2] Therefore since 2010, the World Health Organisation (WHO) recommended that anti-malarial treatment can be initiated only after parasitological confirmation of suspected cases.[3]

Malaria Rapid Diagnostic Test (MRDT) has several advantages for screening activities. It is easily accessible, cost-effective, easy to perform and interpret, easy to transport and provides results immediately.[4] It is thus suitable for usage in malaria-endemic areas, settings with limited health personnel and facilities, and during outbreaks.[3] Its usage has been reported to significantly reduce referrals and inpatient's length of hospital stay.[5]

On the other hand microscopy requires electricity, well-trained technicians and rigorous maintenance of functional infrastructures.[6]

However, concern about the accuracy of MRDTs has made its wide-scale usage a debatable issue.[7] Discrepancies have been observed in MRDT sensitivities in several observational studies.[8] The validity of RDT kits is commonly measured by its sensitivity and specificity. It is a very critical situation wherein anti-malarial drugs are denied to patients with malaria due to False Negative (FN) test results. On the other hand, drugs are dispensed unnecessarily for treating patients due to False Positive (FP) test results.[9]

The present study was therefore aimed at assessing the validity of antigen detecting MRDTs by comparing the test results with that of conventional Giemsa stained thick and thin blood film microscopy.

MATERIAL AND METHODS

The present cross-sectional study was conducted from April 2024 to October 2025 among 150 febrile patients who were directed to the laboratory of Malaria Control Cell of Government Hospital Jhalawar, Rajasthan. The study period corresponds to the peak season of the outbreak of malaria in Jhalawar. Institutional Ethics Committee Approval was taken for the conduct of the study.

Clinically suspected cases of malaria which had both peripheral smear and Rapid diagnostic tests performed on the same blood sample and also those who were willing to give consent for participation was included in this study. Seriously ill patients and those refusing or unable to provide informed consent were excluded.

Sample Collection: During this period, 300 blood samples was received for malaria diagnosis from clinically suspected cases. Blood samples was collected in EDTA vacutainer tube. Peripheral smears was made on a clean glass slides with a drop of blood, air dried and stained with Leishman stain. Smears was thoroughly examined under oil immersion for the presence of malaria parasite.

MICROSCOPIC EXAMINATION OF THICK AND THIN SMEARS: The most commonly used, fairly rapid and accepted method for diagnosis of malaria is microscopic examination of stained blood smears. It remains the gold standard for diagnosis of malaria.

The peripheral blood smear provides comprehensive information on the species, the stages and the density of parasitemia. The efficiency of the test depends on the quality of the equipment and reagents, and type of quality of the smear, skill of technician, the parasite density, and the time spent on reading the smear. The test take about 20 to 60 minutes depending on the proximity of the laboratory and other factors mentions above. Before reporting a negative result, atleast 200 oil immersion visual fields at a magnification of 100x should be examined on both thick and thin smears, which has a sensitivity of 90%. The smear can be prepared from blood collected by venipuncture, finger prick and ear lobe stab. Blood sample was taken before antimalarial drugs were started.

The thick smear of correct thickness is the one through which newsprint is barely visible. It is dried for 30 minutes and not fixed with methanol. This allow the red blood cells to be hemolyzed and leucocytes and any malaria parasites present was the only detectable elements. A number of Romanowsky stains like Field's, Giemsa's, Wright's and Leishman's are suitable for staining the smears. Thick films are ideally stained by the rapid Field's technique or Giemsa's stain for screening of parasites. The thick film is first de-hemoglobinised in water and then stained with Giemsa.4% Giemsa in buffered solution at pH7.1. Immerse the slide (at least 12 hour old) in stain for 30 minutes. Rinse with fresh water, drain dry and examine.

Field's stain method for thick blood films: This is made from 2 components. Field's A is a buffered solution of azure dye and Field's B is a buffered solution of eosin. Upto the preparing smear is similar to above. Then slide is dipped into Field's stain A for 3 seconds then dipped into tap water for 3 seconds and gently agitated. The slide is dipped into Field's B for 3 seconds and washed gently in tap water for a few seconds until the excess stain is removed. The slide is drained vertically and left to dry.

Thin film examination is the gold standard in diagnosis of malarial infection. Air dry the thin smear for 10 minutes. After drying, the thin smear should be fixed in methanol. This can be done by either dipping the thin smear into methanol for 5 seconds or by dabbing the thin smear

with a methanol-soaked cotton ball. While fixing the thin smear, all are should be taken to avoid exposure of thick smear to methanol. Thin blood films stained by Giemsa's or Leishman's stain are useful for specification of parasites and for the stippling of infected red cells and have a sensitivity of 200 parasites/µl.

In p. vivax infection, almost all RBC stages of parasite were represented in the blood smear. In p. falciparum infection, usually only ring forms or gametocytes were seen. presence of mature trophozoites and schizonts of p. falciparum in blood smear were indicative of a serious infection.

If *P. falciparum* is identified, it is necessary to estimate parasite density. Since gametocytes are non-pathogenic, they should be excluded while counting parasites. Two methods for estimation of parasite density:

1. In a thick smear, number of parasites was counted till 200 white blood cells are observed. Number of parasites present per µl of blood will calculated from below formula:

Total leukocyte count/µl x Number of parasites / 200

2. In a thin smear, number of parasites amongst 1000 red cells is counted and reported as a percentage. Number of parasites in µl of blood can be calculated if red cell count in millions/µl is known; if it is not known, then it can be arbitrarily taken as 5 million/µl.

Number of parasites in 1 µl of blood = Red cell count in million/cmm x parasite percentage

Each case of fever underwent both MRDT and blood smear examination. Microscopic examination of stained blood smears was taken as "gold standard" for detection of malaria parasitaemia. This method has been reported to detect a parasitaemia as low as 0.0001%. All these samples underwent Rapid Diagnostic test using Antigen based Pf (HRP-II) and PV (pLDH) specific kit. Procedure was performed as per manufacturer's instructions. About 5 µl of blood was put in sample well with the help of disposable loop provided with the kit. 4 drops of assay diluent provided with the kit was added to second well.

Results were interpreted after 15 -20 minutes. Results were interpreted as negative when only control band appeared with two negative test bands and as mixed infection when control band and two test bands appeared. It was interpreted as Plasmodium Vivax infection when PV band appeared along with control band. Plasmodium Falciparum was diagnosed when Pf band and control band appeared.

All the collected data was managed and analyzed with standard software of Biostatics (SPSS Version 20). Statistical analysis of data was done with Chi-square[10] analysis (for quantitative analysis), Student t-test with assistance of qualified statistician. All values was compared at 5% or 0.05 and 1% or 0.01 levels of significance. Sensitivity, specificity, Positive Predictive value (PPV) and Negative predictive value (NPV) was calculated using standard formulae considering Peripheral smear diagnosis as gold standard.

RESULTS

Maximum patients examined were in between the age groups of 11-40 yrs. though malaria was present in 16(32.65% cases of 11-20 yrs age.

Table -1: Validity of rapid diagnostic test for detection of all types of malaria.

Test results	PS* positive	PS negative	Total
RDT** positive	44	0	44
RDT negative	5	101	106
Total	49	101	150

*Peripheral smear, **Rapid diagnostic test

The sensitivity of RDT as 89.80%, specificity is 100%, positive predictive value was 100%, negative predictive value 95.28% and diagnosed accuracy was 96.67%.

Table 2: Validity of Peripheral Smear Test for detection of all types of malaria.

Test results	PS positive	PS negative	Total
RDT positive	42	0	42
RDT negative	2	106	108
Total	44	106	150

The sensitivity of Peripheral Smear Test as 95.45%, specificity is 100%, positive predictive value was 100%, negative predictive value 98.15% and diagnosed accuracy was 98.67%.

Table 3: Comparison of Rapid Diagnostic Test & Peripheral Smear Test for detection of all types of malaria.

Test results	PS* positive	PS negative	Total
RDT** positive	44 (TP)	5 (FP)	49
RDT negative	0 (FN)	101 (TN)	101
Total	44	106	150

DISCUSSION

Malaria is a major public health problem worldwide and can cause death if not diagnosed and treated early. In order to avoid complications due to malaria and for the effective management of malaria, prompt and accurate diagnosis is essential.[11] Newer techniques like QBC and antigen detection tests are rapid, simple and easy to interpret.

In the present study RDT with Pf and PV specificity were used. Past studies have also proven that the cost of malaria treatment can be reduced by 24% by using RDT and 46% by microscopy against presumptive treatment.[12] In the present study out of 150 patients 49 (32.67%) were positive and 101 (67.33%) were negative to RDT whereas 44 (29.33%) were positive and 106 (70.67%) were negative on microscopic examination. Similar findings were also reported in a study conducted by Kulkarni et al.[13]

Previous studies have shown sensitivity and specificity ranging from 84 to 100% for RDT.[14-15] In the present study we found 89.80% and 100% respectively. In our study peripheral smear were negative in 14 cases that showed positivity with RDT.

These peripheral smears were retrieved and studied again. In few cases parasite density was very low and occasional parasite was noted after careful screening of the smears and few cases were partially treated cases before visiting this hospital. Compared to Peripheral smear RDTs are more sensitive and specific for diagnosis of P Falciparum and mixed infections.

This is important because Falciparum causes severe disease and has high mortality requiring urgent intervention, whereas P. Vivax needs to be treated with primaquine to prevent relapses of malaria.

The advantages of RDTs are that it is simple, easy to perform, no instruments or electricity required and interpretation is also easy. But the disadvantage is parasite density cannot be assessed and cannot be used to assess response to treatment as it can be positive for 7-14 days after treatment.[16]

The advantages of peripheral smears are it is cheaper than RDT, parasite density can be assessed and it can also be used as quality control measure to check efficiency of RDTs.

CONCLUSION

The study concludes that both peripheral smears and MRDTs are effective for malaria diagnosis, with MRDTs having slightly higher sensitivity in detecting positive cases, including a higher incidence of mixed infections and Plasmodium falciparum cases. Nonetheless, one must carefully evaluate their accuracy, particularly in cases of negative outcomes. A hybrid approach that uses both modalities may result in the most accurate diagnostic.

REFERENCES

1. Ali H, Ahsan T, Mahmood T, Bakht SF, Farooq MU, Ahmed N. Parasite density and the spectrum of clinical illness in falciparum malaria. J Coll Physicians Surg Pak. 2008;18:362–8.
2. Bastiaens GJ, Schaftenaar E, Ndaro A, Keuter M, Bousema T, Shekalaghe SA, Malaria diagnostic testing and treatment practices in three different Plasmodium falciparum

transmission settings in Tanzania: before and after a government policy changeMalar J 2011 10:76.
3. Wiseman V, Kim M, Mutabingwa TK, Whitty CJ, Cost-effectiveness study of three antimalarial drug combinations in TanzaniaPLoS Med 2006 3:e373.
4. Batwala V, Magnussen P, Hansen KS, Nuwaha F, Cost-effectiveness of malaria microscopy and rapid diagnostic tests versus presumptive diagnosis: implications for malaria control in Uganda Malar J 2011 10:372.
5. Harvey SA, Jennings L, Chinyama M, Masaninga F, Mulholland K, Bell DR, Improving community health worker use of malaria rapid diagnostic tests in Zambia: package instructions, job aid and job aid-plus-training Malar J 2008 7:160.
6. Boyce RM, Muiru A, Reyes R, Ntaro M, Mulogo E, Matte M, Impact of rapid diagnostic tests for the diagnosis and treatment of malaria at a peripheral health facility in Western Uganda: an interrupted time series analysis Malar J 2015 14:203.
7. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH, A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT)Am J Trop Med Hyg 2007 77:119-27.
8. English M, Reyburn H, Goodman C, Snow RW, Abandoning presumptive antimalarial treatment for febrile children aged less than five years--a case of running before we can walk? PLoS Med 2009 6:e1000015.
9. Ishengoma DS, Francis F, Mmbando BP, Lusingu JP, Magistrado P, Alifrangis M, Accuracy of malaria rapid diagnostic tests in community studies and their impact on treatment of malaria in an area with declining malaria burden in north-eastern Tanzania Malar J 2011 10:176.
10. Chandy C.J: Malaria. Nelson Book Of Pediatrics, 20th edition, 2016, eds Kliegman RS, Stanton BF, St Geme JW, Schor NF:1709-1721.
11. World Health Organization. WHO Guidelines for malaria 2022. Available at: https://www.who.int/ publications/i/item/guidelines-for-malaria. Accessed on 12 December 2021.
12. Osei-Kwakye K, Asante KP, Mahama E, Apanga S, Owusu R, et al. (2013) The Benefits or Otherwise of Managing Malaria Cases with or without Laboratory Diagnosis: The Experience in a District Hospital in Ghana. PLoS ONE 8(3): e58107.
13. Kulkarni P, Varna I. Rapid diagnostic tests versus peripheral smear in malaria: a comparative study. Trop J Pathol Microbiol. 2016;2(3): 179-82.
14. Hada S, Das ML, Singh YL. Diagnostic methods of malaria in Eastern Nepal: a comparative study of traditional and two rapid diagnostic tests. Nepal Med Coll J 2011; 13(4): 261-266.
15. Ouattara A, Doumbo S, Saye R, Beavogui AH, Traoré B, Djimdé A, Niangaly A, Kayentao K, Diallo M, Doumbo OK, Thera MA. Use of a pLDH-based dipstick in the diagnostic and therapeutic follow-up of malaria patients in Mali. Malar J. 2011 Nov 24;10:345.
16. Mendiratta DK, Bhutada K, Narang R, Narang P. Evaluation of Different Methods for Diagnosis of P. Falciparum Malaria. Indian J Med Microbiol. 2006 Jan;24(1):49-51.