ORIGINAL RESEARCH PAPER
Volume-9 | Issue-3 | March-2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A COMPARATIVE LABORATORY DIAGNOSIS OF MALARIA: MICROSCOPY VERSUS RAPID DIAGNOSTIC TEST KITS

Microbiology

Dr Sangeeta Bishnoi Research Scientist, Department of Microbiology, RNT Medical College, Udaipur, Rajasthan

Dr Anshu Sharma Senior Professor & Head of Department, Department of Microbiology, RNT Medical College, Udaipur, Rajasthan

Dr Seema Sonkariya* Senior Resident, Department of Microbiology RNT Medical College, Udaipur, Rajasthan
*Corresponding Author

ABSTRACT

Introduction: Malaria is one of the highest killer diseases affecting most tropical countries. It affects over 500 million people worldwide and over one million children die annually from malaria. Over the years many new tests have been developed in an attempt to improve the diagnosis of malaria, but conventional method by smear microscopy remains the gold standard against which all other tests have been evaluated. Rapid diagnostic tests (RDTs) for malaria could be considered for most patients in endemic regions.
Objective: To compare microscopy and rapid diagnostic tests (RDTs) in the diagnosis of malaria.
Material & Methods: Blood samples of 7434 clinically suspected cases of malaria of all ages and both sexes were collected between June 2018 and November 2018 at a tertiary care teaching hospital in Southern Rajasthan. One step malaria antigen rapid test was done in all the patients. Patients who were positive on rapid diagnostic kit, thick and thin smear was prepared among those. Results were simply presented as percentage positive of the total number of patients under study.
Results: Out of 7434 samples, 400 samples were positive in rapid card test (antigen detection) and out of 400 samples, 240 samples are positive for malaria in microscopy examination. Out of total 400 rapid card test positive samples 208 (52%) patients were male and 192 (48%) were female patients while out of total 240 microscopy positive samples 129 (53.75%) patients were male and 111 (46.25%) were female patients. In present study 51% were P. falciparum, 37.5% P. vivax and 11.5% mixed infection by rapid card test (n=400) while in the microscopy out of 240, 48.75% were P. falciparum, 39.58% were P. vivax and 11.66% were mixed infection.

KEYWORDS

Rapid diagnostic tests, Microscopy, Malaria

INTRODUCTION

Malaria is one of the highest killer diseases affecting most tropical countries. It affects over 500 million people worldwide and over one million children die annually from malaria.[1]

In India about 92% of malaria cases and 97% of malaria deaths reported from north-eastern states, Chattishgarh, Jharkand, Madhya Pradesh, Orissa, Andhra Pradesh, Maharastra, Gujarat, Rajasthan, west Bengal and Karnataka.[2] In 2017 in India there were 0.67 million malaria cases, among which 0.44 million cases were due to P. falciparum and annual parasite index was 0.53.[3]

Malaria is caused by parasites P. Vivax, P. Falciparun, P.malariae and P.ovale. Man develops disease after 10 to 14 days of being bitten by an infective mosquito. There are two types of parasites of human malaria, plasmodium vivax, plasmodium falciparum, which are commonly reported from India. Inside the human host, the parasite undergoes a series of changes as a part of complex life cycle. The parasite completes life cycle in liver (pre erythrocytic schizogony) and red blood cells (erythrocytic schizogony). Infection with P. falciparum is the most deadly form of malaria.[4,5] Malaria affects mainly poor, underserved and marginalized populations in remote rural areas which are characterised by inadequate control measures and limited access to health care.[5]

The most important problems in controlling malaria in India are its diverse clinical presentations and limited access to effective diagnosis and treatment.[6] Laboratory confirmation of malaria infection requires the availability of a rapid, sensitive, and specific test at an affordable cost. Over the years many new tests have been developed in an attempt to improve the diagnosis of malaria, but conventional method by smear microscopy remains the gold standard against which all other tests have been evaluated. However, the microscopy requires technical expertise and availability of a good quality microscope.[7] Rapid diagnostic tests (RDTs) for malaria could be considered for most patients in endemic regions, especially in poor power settings where there is shortage of qualified manpower in Africa. However, there is very little evidence, especially from malaria endemic areas to guide decision-makers on the sensitivity and specificity of these RDTs.[8] RDTs are commercially available in kit forms with all necessary reagents and the ease of performance of the procedures, does not require extensive training or equipments to perform or to interpret the results. Results are read in 12–15 min.[9] Therefore, present study was aimed to compare the two methods of microscopy and RDTs in the diagnosis of malaria.

Subjects and Methods

Study design, settings and participants:

It was a hospital based prospective study conducted over a period of six months from June 2018 to November 2018, in the Clinical Microbiology Laboratory, Department of Microbiology of a tertiary care hospital in Southern Rajasthan. Patients with clinical suspicion of Malaria who presented in the Outpatient Department constituted the study population. During the study period total 7434 blood samples were processed for the malaria testing from patients who presented in outpatient department. Patients having fever and diagnosed as leptospirosis, dengue fever, enteric fever, pneumonia, urinary tract infection and sepsis and Patients who were currently taking antimalarial therapy or who had been treated with antimalarial drugs within the past 2 weeks were excluded from the study

Data collection

All the study subjects were informed about study by patient information sheet. Informed written consent was obtained from all patients, who were taken for the study. Under aseptic conditions 3 ml of venous blood was collected in ethylene diamine tetra acetic acid (EDTA) tube.1.2mg of EDTA anhydrous salt was added per ml of blood.[10]

Peripheral smear preparation

Thick and thin blood smears were prepared and stained with Geimsa stain according to the standard guidelines described elsewhere.[11,12]

After staining, the smears were examined at x1000 magnification. Atleast100-200 fields, each containing 20 WBCs were examined before thick smear was reported as negative for malaria. The red blood cells in the tail end of the thin smear were examined for the species identification and stages of the parasites.[11,12]

Malaria rapid diagnostic test:

One step malaria Pf/Pv antigen test (Whole blood) by Recombigen

Laboratories Pvt. Ltd. was used in the study.

Principle:
The malaria antigen test contains a membrane strip, which is pre-coated with one monoclonal antibodies as one line across a test strip. One monoclonal antibody (test line 1) is specific to the *P. falciparum* histidine rich protein-2 (Pf HRP-2) and another monoclonal antibody (test line 2) is *P. vivax* specific to *P. falciparum* histidine rich protein 2(Pf HRP-2) and *P. vivax* specific to the lactate dehydrogenase of *Plasmodium falciparum* and *Plasmodium vivax*.

5µL of whole blood will be added into the sample well ("S" small well) with the help of sample loop provided with kit. Then 4 drops or 140-170 µL of assay buffer added into developer well ("A"). The test result will be read at 20 minutes. Interpretation of test results of both the rapid tests were carried out according to the manufacture's literature guideline.

Statistical analysis
Data were analyzed and statistically evaluated using SPSS software, version 17 (Chicago II, USA). Quantitative data was expressed in mean, standard deviation while qualitative data were expressed in percentage. 'p' value less than 0.05 was considered statistically significant.

Ethical issues
All participants were explained about the purpose of the study. Confidentiality was assured to them along with informed written consent. The study was approved by the Institutional Ethical Committee.

Observations & Results
During the study period total 7434 samples were processed. Out of which 400 samples were positive in rapid card test (antigen detection) and out of 400 samples, 240 samples are positive for malaria in microscopy examination.

Out of total 400 rapid card test positive samples 208 (52%) patients were male and 192 (48%) were female patients. Therefore male : female ratio was 1.1 : 1 Males were affected more than female while out of total 240 microscopy positive samples 129 (53.75%) patients were male and 111(46.25%) were female patients. Therefore, male: female ratio was 1.16 :1. (Table 1). In our study, according to data 41-60 years of age group was affected more than the others i.e. 35% followed by age group of 21-40 years (30%) while in the microscopy Maximum cases were observe in 41-60 years (33%) and minimum cases were in 1-20 years (18.75) of age group. (Table 1).

In present study 51% were P. falciparum, 37.5% P. vivax and 11.5% mixed infection by rapid card test (n=400) while in the microscopy out of 240, 48.75% were P. falciparum, 39.58% were P. vivax and 11.66% were mixed infection. (Table 2)

DISCUSSION
Malaria is a major vector-borne disease in India. Extensive geographical areas, climatic diversity and variable malaria epidemiology in India is associated with high parasite genetic diversity and rapidly evolving drug resistance.[13] There are four principal methods for diagnosing malaria. These are symptomatic, microscopic, antigen test and molecular methods. Symptomatic diagnosis is the most common, and people in poorer countries often use symptoms alone to diagnose malaria. In other areas, too, symptomatic diagnosis is often the initial one, followed by one of the other methods. However, it should be noted that many other disease present symptoms very similar to malaria, and diagnosis by symptoms alone can be misleading and even harmful, treating for malaria where other treatment is called leaves the actual disease uncured and the patient very critical condition. It is there for imperative to follow up symptomatic diagnosis with one of the other more accurate methods.[8]

WHO currently makes the tentative recommendation that parasite based diagnosis should be used in all cases of suspected malaria with possible exception of children in high prevalence area and certain other situations.[14,15]

Immunochromatographic method to detect the presence of malaria parasite appears to be the most rapid and requires minimum or no training at all. Immunochromatographic method relies on the migration of liquid across the surface of a nitrocellulose membrane.[22] The test is based on the capture of parasite antigen from the peripheral blood using monoclonal antibodies prepared against malaria antigen target and conjugated to either a liposome containing selenium dye or gold particles in a mobile phase[8]. Microscopy is the most widely tool used to diagnose malaria at peripheral levels. In capable hands it is very sensitive for parasitaemia <50L (0.001%)[16] and it can give important information to the clinician like species, parasites stages and parasite density. However, good quality of microscopy is difficult to implement and maintain. It is labor intensive and requires highly skilled personnel and regular quality control. The use of malaria RDTs is recommended by WHO when reliable microscopy is not available. In non-endemic settings, where microscopic expertise is lacking due to low incidence, malaria RDTs are of value for the diagnosis of malaria and they provide information about the involvement of P. falciparum. In a recent external quality control session, 72.7% of 183 Belgian laboratories offering malaria diagnosis declared to use RDTs as a tool for diagnosis, and their use was recommended if performed in conjunction with microscopy[17]. Maltha J et al[17] also showed that P. falciparum, and Plasmodium vivax showed 94.6%, and 92.9% degree of sensitivity using RDTs in malaria parasites concentration of >1000/L, respectively, but, they showed percentages lower than an average of 58% sensitivity in malaria concentration of <100/L. It should naturally be expected that their sensitivity will drop to almost zero at concentration of 0.001% (<50/L) at which microscopy would also be negative. Our work also shows clearly that the antigen based method has a better correlation with both the gold standard i.e. microscopy and the clinical settings.

During the study period total 7434 blood samples were processed for the malaria testing out of which 400 samples was positive in rapid card test and out of 400, 240 (60%) samples was positive in microscopic examination. Vyas S. et al[18] reported malaria positivity in rapid card test 20% and in microscopy 12.7%, Azikiwe CCA et al[8] reported 64% positivity in rapid card test and 59% in microscopy. In our study the higher positivity in rapid card test than the microscopy may be due to the persistently circulating antigen and prior use of antimalarial drugs. False positive results in RDT can be due to various factors such as persistence of HRP2 antigen in patient blood weeks after a successful treatment. Plasmodia gametocyte also produces pLDH thus test could be positive despite clearance of asexual forms of parasite; It could also be due to interaction with rheumatoid factor found in patients with rheumatoid arthritis.[19]

In our study, 400 samples were tested positive in RDT out of which 208 (52%) were male and 192 (48%) were females. In microscopy out of 240 positive samples, 129 (53.75%) were males and 111(46.25%) were females. Gupta P et al[13] reported 2:1 male:female ratio while Jelia S et al[20] reported 78% males and 22% females. Rajeshwar K et al[21] reported 66.15% were males and 33.84% females in Karanatka, India. This indicates that malaria is more common in males because males are more frequently exposed to the risk of acquiring malaria than females because of their outdoor life which they lead[20]. In addition to leisure activity sleeping arrangement may also affect malaria transmission.

In present study, out of 400 cases maximum were in the age group of 41-60 years Followed by age group of 21-40 years (30%). Jelia S et al[20] reported 38% were between the age group of 21-30 years. Similar study was done by Estacio RH et al[22] who reported that most of their patients (30%) were in between 19-35 years of age and Aundhakar S. et al[23] reported 30% cases were in the age group of 21-30 years of age. This may be due to young and middle aged group are being more active outdoors from dawn to dusk.

In our study 51% were P. Falciparum, 37.5% P. Vivax and 11.5% mixed infection by rapid card test (n=400). While in the microscopy out of 240, 48.75% were P. Falciparum, 39.58% were P. Vivax and 11.66% were mixed infection. Kochar DK. et al[24] (2010) reported 49.34% as P. Falciparum, 43.23% as P. Vivax and 7.42% mixed infection while Limaye CS. et al[25] (2012) reported 39% had Falciparum, 31% had Vivax and 30% had mixed infection in Mumbai.

Conclusion & Recommendations:
The study shows that the rapid diagnostic test kit has comparable level of accuracy with microscopy and hence can be used in rapid screening of malaria. RDTs can be useful in areas where specialized laboratories or even microscopy are unavailable and when urgent malaria diagnosis

is needed by a practitioner without the delay and should be correlate clinically.

Table 1: sociodemographic profile of person detected positive by rapid card test and microscopy

	Total No. of positive samples in rapid card test (n= 400)	Total No of positive samples in microscopy (n=240)
Gender		
Male	208 (52.0%)	129 (53.75%)
Female	192 (48.0%)	111 (46.25%)
Age group		
0-20 years	60 (15.0%)	45 (18.75%)
21-40 years	120 (30.0%)	68 (28%)
41-60 years	140 (35.0%)	80 (33.3%)
>60 years	80 (20.0%)	57 (20.22%)

Table 2: Ratio of P. Vivax and P. Falciparum in subjects positive by microscopic examination and rapid card test

Species of plasmodium	Total No. of positive samples in rapid card test (n= 400)	Total No of positive samples in microscopy (n=240)
P. Vivax	150 (37.5%)	95 (39.6%)
P. falciparum	204 (51.0%)	117 (48.75%)
P. vivax+ P. falciparum	46 (11.5%)	28 (11.66%)

REFERENCES

1. Amazu LU, Ebong OO, Azikiwe CCA, Unekwe PC, Simialayi MI, Nwosu PJC, et al. Effects of methanolic seeds extract of Carica papaya on Plasmodium berghei infected mice. Asian Pac J Trop Med. 2009;2(3):1-6.
2. Park K. Park's text book of Preventive and Social medicine.21st edition. Epidemiology of communicable diseases-Malaria,pg.231-243.
3. Malaria: Magnitude of the Problem. National Vector Borne Disease Control Programme (NVBDCP), Ministry of Health & Family Welfare, Government of India. Available from: https://nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=420&lid=3699.
4. Guruprasad BA. Clinical Study of Hepatorenal and Hematological Profile in Malaria.Tumlur.2011.
5. Panikar CKJ. Malaria parasites In :Textbook of medical parasitology.6th ed. New Delhi:Jayapee Brothers medical publishers;2007.p.65-95.
6. Malik S, Khan S, Das A, Samantaray JC. Plasmodium lactate dehydrogenase assay to detect malarial parasites. Natl Med J India. 2004 Sep;17(5):237-9.
7. Krudsood S, Wilairatana P, Mason DP, Treeprasertsuk S, Singhasivanon P, Looareesuwan S. Hidden Plasmodium falciparum infections. Southeast Asian J Trop Med Public Health. 1999;30(4):623-4.
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 Pac J Trop Biomed. 2012;2(4):307-10.
9. Moody A. Rapid diagnostic test for malaria parasites. Clin Microbiol Rev. 2002;15(1):66–78.
10. Bain BJ, Lewis SM, Bates haematological techniques. In : Lewis SM, BB, Bates I, editors. Dacie and Lewis practical haematology.10th ed. New York : Churchill Livingstone;2006.p.25-8.
11. Kakkilaya BS. Malaria Site- All about Malaria. [Monograph on the internet] India; 2009 Available from: URL: http://www.malariasite.com/
12. Chakraborthy P. Plasmodium and Babesia. Textbook of medical parasitology. 1st ed. Kolkata: New Central Book Agency (P) Ltd; 2004:83-104.
13. Gupta P, Gupta P, Rao S, Singh N, Kalita D. Comparison Between Microscopy and Rapid Diagnostic Tests In Diagnosis Of Malaria At A Tertiary Care Medical Institution In Uttarakhand (A 3-year study). Asian J Pharmaceut Clin Res. 2018;11(2):94-6.
14. Bell D, Go R, Miguel C, Walker J, Cacal L, Saul A. Diagnosis of malaria in a remote area of the Philippines: comparison of techniques and their acceptance by health workers and the community. Bull World Health Organ. 2001;79:933-41.
15. WHO. The role of laboratory diagnosis to support malaria disease management: focus on the use of rapid diagnostic tests in areas of high transmission. Geneva: WHO; 2006.
16. Nandwani S, Mathur M, Rawat S. Evaluation of the polymerase chain reaction analysis for diagnosis of falciparum malaria in Delhi India. Indian J Med Microbiol. 2005;23(3):176-8.
17. Jessica M, Gillet P, Cnops L, Bottieau E, Esbroeck MV, Bruggeman C, et al. Evaluation of the rapid diagnostic test SDFK40 (Pf-pLDH/pan-pLDH) for the diagnosis of malaria in a non-endemic setting. Malar J. 2001;10(1):7.
18. 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.
19. Naz R, Khan S, Farooqui MK, Girotra R, Malik AK. Comparison of Microscopic Determination and Rapid Diagnostic Tests (RDTs) in the detection of Plasmodium Infection. Sch J App Med Sci. 2016;4(7):2539-43.
20. Jelia S, Meena S, Arif M, Jain P, Ajmera D, Jatav VS et al. A study of clinical profile and complication of malaria in a tertiary care centre in South-eastern region of Rajasthan, India. Int J Adv Med. 2016 Aug;3(3):614-20.
21. Rajeshwar K, Karibasappa BG. Clinical profile of severe plasmodium vivax malaria in a tertiary centre in JJM Medical College, Davangere. Indian J Basic Applied Med Res. 2015;4(2):133-9. 2015;4(2):133-139.
22. Estacio RH, Edwin ER, CresswellS, Coronel RF, Alora AT. The Quantitative Buffy Coat technique (QBC) in early diagnosis of malaria: The Santotomas University Hospital experience. Phil J Microbiol Infect Dis. 1993;22(2):56-9.
23. Devineni SB, Suneetha O, Harshavardhan N. Study of Platelet Count in Malaria Patients and the Correlation between the Presence and Severity of Platelet Count with Type of Malaria. J Evol Med Dent Sci. 2015;4(67):11734-46.
24. Kochar DK, Das A, Kochar A, Middha S, Acharya J, Tanwar GS, et al. Thrombocytopenia in plasmodium falciparum, plasmodium vivax and mixed infection malaria- a study from Bikaner. Platelets. 2010;21(8):623-7.
25. Limaye CS, Londhey VA, ST Nabar. The Study of Complications of Vivax Malaria in Comparison with Falciparum Malaria in Mumbai. J Asso Physician India. 2012;60:15-7.