A Comparative Study of Diagnostic Methods of Malaria - Microscopy Versus Rapid Diagnostic Test Kits in a Tertiary Care Hospital, Rajasthan

Saumya Singh[1], Neelam Chauhan[2], Jyoti Tomar[3], Aditya Pratap Singh[4]

[1] Department of Microbiology, United Institute of Medical Sciences, Prayagraj, Uttar Pradesh, India. [2] Department of Microbiology, R. N. T Medical College, Udaipur, Rajasthan, India. [3] Department of Microbiology, Pacific Institute of Medical Sciences, Udaipur, Rajasthan, India. [4] Department of Anatomy, United Institute of Medical Sciences, Uttar Pradesh, India.

ABSTRACT

BACKGROUND

Malaria is one of the most prevalent parasitic diseases in the world including India. Majority of malarial cases are found in countries where cost-effectiveness, diagnostic test performance ease, and trained personnel, are the most important considerations. We wanted to compare the two methods of rapid diagnostic tests (RDTs) and microscopy in the diagnosis of malaria parasite infection.

METHODS

We studied 2,723 blood samples of patients who presented with signs and symptoms of malaria from out patient department (OPD) and various wards of Pacific Institute of Medical Science, Udaipur, Rajasthan, from Oct 2018 to Oct 2019. All samples obtained were first tested by RDTs and then the same samples were used to make peripheral blood film (PBF) for microscopy.

RESULTS

During the 1-year period, rapid card test method showed 178 positives for malarial parasite. Of these, 64 cases were positive for *P. vivax* and 108 cases for *P. falciparum*. Peripheral blood smear method showed 160 cases positive for malarial parasite. Of these, 55 cases were positive for *P. vivax* and 99 cases for *P. falciparum* and 6 cases of co-infection. PBF failed to detect 9 cases of *P. vivax* and 9 cases of *P. falciparum* which were positive by rapid card test. So, 18 cases reported PBF negative. Among these patients, there were 83 females and 95 males. Most affected age group was 16 – 30 years followed by 31 – 45 years. Maximum samples were from the month of October 19 with a positivity rate 35.4 %.

CONCLUSIONS

RDTs based on malaria antigen (whole blood) method is as specific and more sensitive than microscopy (which is being considered as the gold standard method). Peripheral blood smear method still remains superior for accurate species differentiation, quantitation of parasite and maintaining a permanent record.

KEYWORDS

Microscopy, Malaria, Rapid Diagnostic Tests

Corresponding Author:
Dr. Aditya Pratap Singh,
Associate Professor,
Department of Anatomy,
United Institute of Medical Sciences,
Vill – Ghoshi, Rawatpur,
Prayagraj - 211012, Uttar Pradesh, India.
E-mail: draditya.singh33@gmail.com

DOI: 10.18410/jebmh/2021/344

How to Cite This Article:
Singh S, Chauhan N, Tomar J, et al. A comparative study of diagnostic methods of malaria - microscopy versus rapid diagnostic test kits in a tertiary care hospital, Rajasthan. J Evid Based Med Healthc 2021;8(22):1826-1830. DOI: 10.18410/jebmh/2021/344

Submission 31-01-2021,
Peer Review 10-02-2021,
Acceptance 06-04-2021,
Published 31-05-2021.

BACKGROUND

Malaria is one of the biggest killer diseases affecting most tropical countries especially India, Africa etc. caused by protozoan parasites of genus plasmodium. The most serious type of malaria is caused by *Plasmodium falciparum*, which can also be fatal sometimes. The other human malaria species, *P. vivax*, *P. malariae, P. ovale,* and sometimes *P. knowlesi* can cause acute, severe illness but mortality rates are low.[1]

The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female Anopheles mosquito inoculates sporozoites into the human host. Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. After this initial replication in the liver (exoerythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect red blood cells. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease. The Male (microgametocytes) and female (macrogametocytes) gametocytes are ingested by an Anopheles mosquito during a blood meal. The parasites multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.[2]

According to the WHO Report estimates, the total cases in India at 1.31 million (0.94 - 1.83 million) and deaths at 23990 (1600 - 46500).[3] India was one of the only two countries to report a malaria reduction burden in 2018. The other nation was Uganda. Between 2017 and 2018, fall in malaria was noticed in India. In the 2016 and 2017 period, India had recorded a 24 percent malaria reduction cases. The states including West Bengal, Jharkhand, Chhattisgarh, Odisha, Madhya Pradesh, Uttar Pradesh and Gujarat reported large reductions in malaria cases, from 14.3 million cases in 2010 to 5.7 million cases in 2018. The rates of reduction were mostly slower in the past 3 years than in preceding years in other nations.[4]

The gold standard for the laboratory diagnosis of malaria parasite is Microscopy with its turned around time much more than that of RDT along with adequate training. RDTs being quick and easy are alternative diagnostic methods. They also require little or no training to perform.[5] HRP-2 and parasite lactate dehydrogenase (pLDH) are two malarial antigens suitable for rapid diagnosis. The detection system for *P. falciparum* malaria is based on the detection of *P. falciparum* specific histidine rich protein-2 (Pf.HRP-2) while *P. vivax* malaria detection is based on presence of *P. vivax* specific pLDH.

Objectives

1. To compare the commonly employed diagnostic techniques used in diagnosis of malaria, i.e., microscopy of smear (PBF) and antigen detection.
2. To compare Giemsa-stained blood film with antigen card test.

METHODS

This retrospective study was conducted in a tertiary care medical institution at Pacific institute of medical sciences (PIMS), Udaipur. Blood samples were collected in Ethylene diamine tetra acetic acid [EDTA] vials from 2723 patients who were clinically suspected for malaria. Thick and thin smear microscopy and RDTs were done on these blood samples collected over 1 year (from Oct. 2018 to Oct 2019). The patient's name, age, sex, details of fever, and other symptoms and clinical examination findings were recorded.

Rapid Diagnostic Test (RDT)

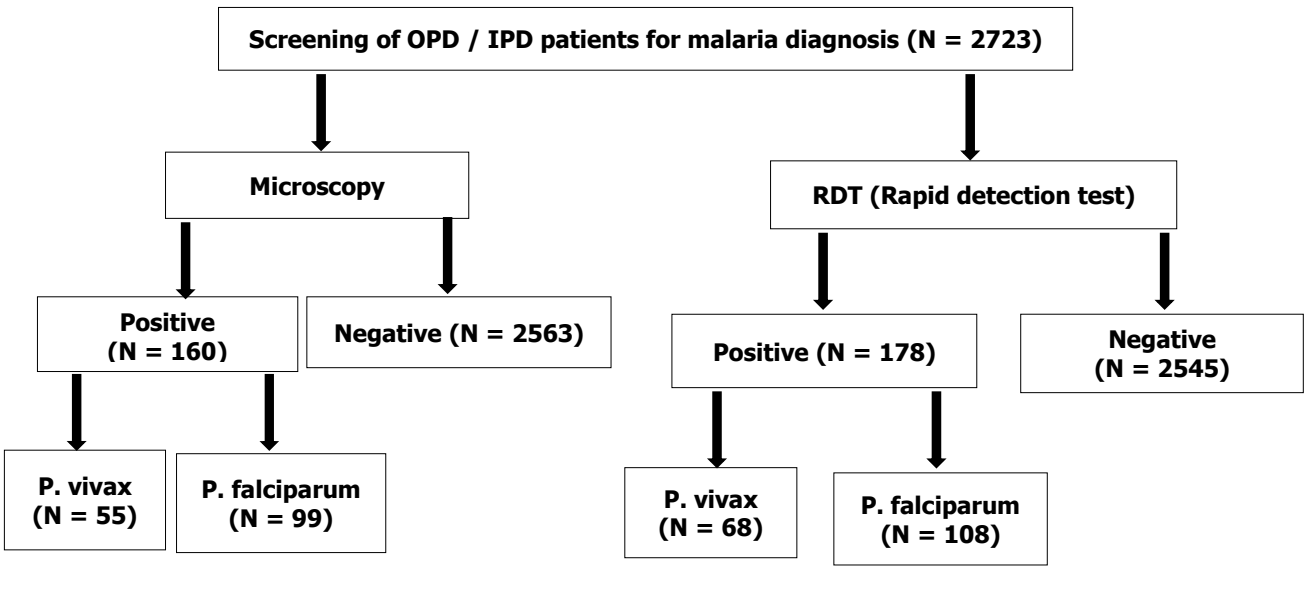
Malaria antigens suitable for rapid diagnostic tests are HRP-2 and pLDH. RDTs capture the parasite antigens from the peripheral blood using either monoclonal or polyclonal antibodies against the parasite antigen targets. RDTs can target the parasite specific lactate dehydrogenase (pLDH) and the histidine-rich protein 2 of *P. falciparum* (PfHRP2). The PfHRP2 test strips have 2 lines, 1 for the control and the other for the PfHRP2 antigen. The pLDH test strips have 3 lines, 1 for control, and the other 2 for *P. falciparum* (PfHRP2 or pLDH specific for *P. falciparum*) and non-falciparum antigens (pan specific pLDH), respectively. Change of colour on the control line is necessary to validate the test and its non-appearance, with or without colour changes on the test lines, invalidates the test. With colour change only on the control line and without colour change on the other lines, the test is interpreted as negative. A pink - purple band will appear under Pf at the test region in falciparum positive samples while under Pan at the test region in non-falciparum positive samples. Mixed infection is depicted by appearance of band under Pf as well as Pan both.

Giemsa Staining Technique

Thin film smear fixed in absolute methanol (methyl alcohol) were processed. Heat fixing was done followed by staining with 10 % Giemsa solution in buffered water, pH 7.2 for 30 min. Once stained, the smears were rinsed with normal water, drained and air dried. Then examination was done by light microscopy under 1000x oil immersion magnification for plasmodium species. A negative malaria blood film was considered after 100 high power fields examination.

Data Analysis

Data analysis was done by using Microsoft excel. Sensitivity & Specificity were done by using the formula TP / TP + FN & TN / TN + FP respectively.



Flow Chart 1. Schematic Diagram of Data Analysis

RESULTS

During the period of 1 year (Oct 18 - Oct 19), a total of 2,723 blood samples were taken from suspected patients of malaria for Peripheral blood smear & Rapid Card Test based on Immunochromatography. Rapid card test method showed 178 positives for malarial parasite. The prevalence rate was 6.53 % by rapid cards. The peripheral blood film result indicated that out of 2723 cases, 160 (5.87 %) cases were positive for malaria and 2563 (94.1 %) cases were negative, that means prevalence rate was 5.87 % by PBF. In total 18 cases, discordance was observed which were negative by microscopy but positive by RDT. PBF failed to detect 9 cases of *P. vivax* and 9 cases of *P. falciparum* which were positive by Rapid card test, so total 18 cases were PBF negative but RDT positive. In none of the cases, microscopy was positive and RDT negative.

Prevalence rate of malaria at our tertiary care was found to be 6.53 %. The Rapid Diagnostic test results indicated a positivity of 6.53 % (178 / 2723) while microscopy indicated the total positivity of 5.87 % (160 / 2723). The result clearly indicated that HRP - 2 antigen detection test had slightly higher sensitivity as compared to microscopic analysis method (Table 1). Thus, RDT's may give false negative results. This result revealed sensitivity & specificity of Microscopy & RDT which was 0.05 %, 0.94 % & 0.06 %, 0.93 % respectively.

Rapid Card Test	Peripheral Blood Smear	Result
178 (6.53 %)	160 (5.87 %)	Positive
2545	2563	Negative
2723	**2723**	**Total**

Table 1. Comparison of Giemsa Stained Blood Film with Antigen Card Test

Sensitivity of card test - 100 %, Specificity - 100 %, According to kit manual we observed that 68 (38.20 %) cases were positive for *P. vivax*, 108 (60.67 %) cases were positive for *P. falciparum* and 2 (1.12 %) cases of co-infection positive by Rapid card tests. In cases of peripheral blood smear, 55 (34.37 %) cases were positive for *P. vivax*, 99 (61.87 %) cases were positive for *P. falciparum*, rest 6 (3.37 %) cases were mixed infection (*P. vivax* and *P. falciparum)* as shown in [Table 2, Graph 1]. That means there was high prevalence of *P. falciparum* species.

Method	Positive for *P. vivax*	Positive for *P. falciparum*	Co-infection of *P. vivax* and *P. falciparum*
Microscopy (N = 160)	55 (34.37 %)	99 (61.87 %)	6 (3.75 %)
Rapid diagnostic test (N = 178)	68 (38.20 %)	108 (60.67 %)	2 (1.12 %)

Table 2. Microscopy and RDT Results for the Two Species of Malaria

Diagnosis Method	Sensitivity / Specificity	Percentage
Microscopy	Sensitivity	0.05 %
	Specificity	0.94 %
RDT	Sensitivity	0.06 %
	Specificity	0.93 %

Table 3. Sensitivity & Specificity of Diagnostic Methods Used for Malaria

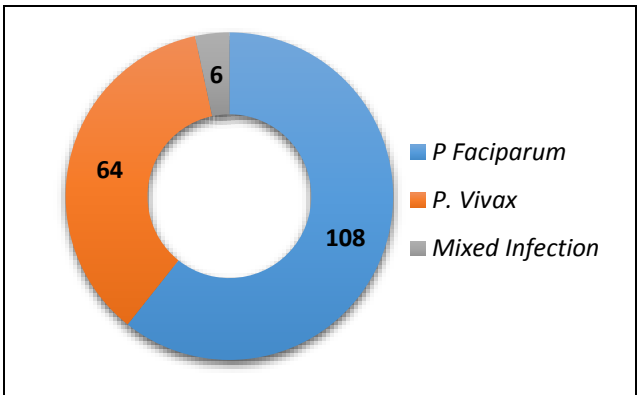
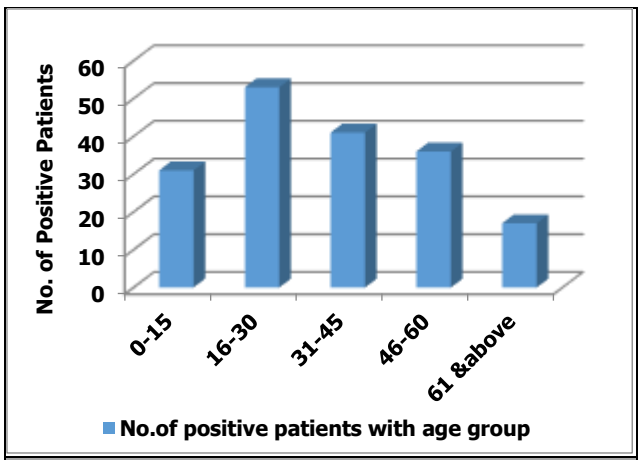


Chart 1. Distribution of Plasmodium Species (N = 178)

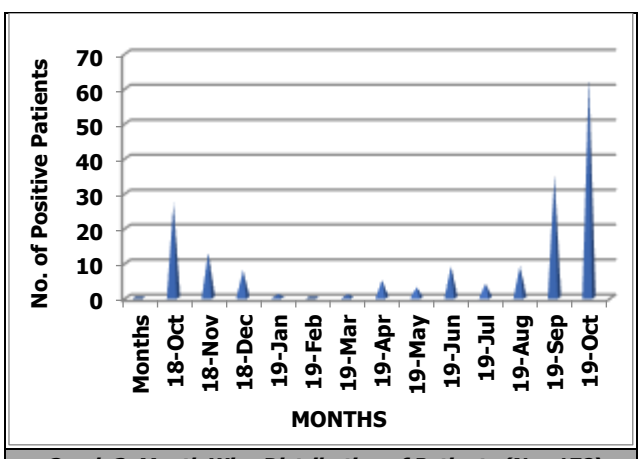
Prevalence of malaria was more in males 95 (53.37 %) as compared to females 83 (46.62 %). Most affected age

group was 16 – 30 years followed by 31 – 45 years age group. [Graph 2]



Graph 2. Age Wise Distribution of Malaria Patients (N = 178)

Maximum samples were from the month of Oct 19 (63) with positivity rate. Positivity rates of remaining months were Sept 19 (35), Oct 18 (27) and Nov.18 (13) as shown in [Graph 3].



Graph 3. Month Wise Distribution of Patients (N = 178)

DISCUSSION

Prevalence rate of malaria at our tertiary care was found to be 6.53 %. Jivabhai et al. from Gujarat and Karlekar et al. from Gadchiroli (Maharashtra) also reported a similar prevalence of 2.10 % and 4.28 %, respectively.[6,7] However, Kumar et al. from Udaipur, Rajasthan (14.4 %), Singh et al. from Mumbai (16.58 %), Sahu et al. from Orissa (16.5 %) Pandey and Manwani from Bilaspur (24.74 %), Baruah et al. (30.2 %) from Nagaland, Das et al. from Assam (49.1 %),[8-13] and Satyanarayana et al. from tribal belt of Andhra Pradesh (69.1 %) reported a much higher prevalence. Some of these studies are hospital based, and others have conducted field survey's.[14]

Hence, variations in prevalence might be due to different study settings, differing ecological conditions, along with socioeconomic condition of patients and local public health practices which determine mosquito breeding and spread.

The Rapid Diagnostic test results indicated a positivity of 6.53 % while microscopy indicated the total positivity of 5.87 %. The result clearly indicated that HRP- 2 antigen detection test had slightly higher sensitivity as compared to microscopic analysis method. Thus, RDT's may give false negative results. This showed that microscopic examination of blood smears remains the "gold standard" for diagnosis of malaria. Microscopy is considered accurate and reliable for diagnosis of malarial parasite.

There is high prevalence of *P. falciparum* species in our study (i.e., 61.87 %). Igbeneghu et al.[15] from Nigeria reported much higher prevalence of *Plasmodium falciparum* 93.3 %, Abdallah et al.[16] from Sudan reported 81.3 % *Plasmodium falciparum* explaining high mortality in these areas, as *Plasmodium falciparum* infection itself has many complications.

However, *P. falciparum* as the predominant species have been reported from studies from Nagaland (76.5 %), Assam (97.1 %), and Orissa (89.1 %).[10,12,13] The difference in prevalence in different areas was due to the regional endemicity of a particular plasmodium species.

Prevalence of malaria was more in males (53.37 %) as compared to females (46.62 %). Most affected age group was 16 - 30 years followed by 31 - 45 years' age group. Our finding correlates with S.R. Karlekar et al.[7] who reported mean age group of 24.8 years and Singh et al.[9] reported 21 - 30 years of age. In India outbreak / epidemic investigations has been done for most of the point prevalence studies. In different paradigms in the country there is limited information on sex and age specific seasonal malarial prevalence. Men shows higher burden than women in all age groups. Movement in wider areas possibly endemic and more chances of exposure to mosquito bites could be the reason for higher prevalence in this age group of men.

The study area is forested with terrain full of high and lowlands along with tropical humid climate. April to mid-May hot dry summer season, mid-May to September Monsoon, October to November Autumn, December to January winter and February to March Spring seasons are experienced.

In Summer maximum temperature rises to around 41°C and the minimum temperature falls to 7° C. The range between 35 and 70 % is relative humidity. Malaria transmission occurs throughout the year but after the monsoon season it peaks. The high prevalence of malaria in this period could be due to collection of water after rainy season and mosquito breeding which continues till December.

Detection and effective treatment is extremely essential for reducing morbidity and mortality due to malaria. Microscopic examination of blood is the most reliable method of diagnosis of malaria. Although rapid diagnostic tests can replace the peripheral blood smear examination being conventional method, it can be just supplemented in absence of Microscopy and Expert opinion. Peripheral blood smear study is simple, least expensive, time consuming thereby delaying diagnosis. New techniques like antigen detection assays are rapid, simple and easy to interpret but blood film can provide more information than card test. At the time of emergency, the rapid card is a valuable adjuvant

for rapid diagnosis while microscopy remains the gold standard for malarial diagnosis.

CONCLUSIONS

Malaria is grossly underdiagnosed using the conventional methods and any rapid technique should be complimentary and not counterproductive. RDTs are rapid, do not require expertise, and are useful in routine diagnosis. However, peripheral blood smear method still remains superior for accurate species differentiation, quantification of parasite and for maintaining a permanent record. The current study confirms that RDTs should be used in conjunction with microscopy to improve the diagnosis of malaria.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

REFERENCES

[1] Tangpukdee N, Duangdee C, Wilairatana P, et al. Malaria diagnosis: a brief review. Korean J Parasitol 2009;47(2):93-102.
[2] Chatterjee KD. Parasitology, Protozoology and Helminthology. 13th edn. New Delhi: CBS Publishers 2009: p. 90-127.
[3] WHO. World Malaria Report, 2017.
[4] WHO. World Malaria Report, 2019.
[5] Naz R, Khan S, Farooqui MK, et al. Comparison of microscopic determination and Rapid Diagnostic Tests (RDTs) in the detection of plasmodium infection. Sch J App Med Sci 2016;4(7D):2539-2543.
[6] Jivabhai HT, Kanubhai VB, Hariharbhai AA, et al. Retrospective study of malaria cases attending at tertiary care level hospital in Rajkot city, Gujarat. Int J Curr Res 2014;6:6273-6276.
[7] Karlekar SR, Deshpande MM, Andrew RJ. Prevalence of asymptomatic plasmodium vivax and plasmodium falciparum infections in tribal population of a village in Gadchiroli district of Maharashtra state, India. Biol Forum Int J 2012;4(1):42-44.
[8] Kumar S, Khan N. Prevalence and detection of malaria at a tertiary care hospital in southern Rajasthan, India. Int J Curr Microbiol App Sci 2016;5(8):663-668.
[9] Singh G, Urhekar AD, Maheshwari U, et al. Prevalence of Malaria in a tertiary hospital in Navi Mumbai, India. J Bacteriol Parasitol 2000;6(2):221-224.
[10] Sahu SS, Gunasekaran K, Vanamail P, et al. Persistent foci of falciparum malaria among tribes over two decades in Koraput district of Odisha state, India. Malar J 2013;12:72.
[11] Pandey S, Manwani VK. A retrospective study of malaria prevalence at Bilaspur District (Chhattisgarh), India. Int J Sci Res 2014;3(3):2277.
[12] Baruah I, Das NG, Das SC. Studies on anopheline fauna and malaria incidence in Dhansiripar PHC of Dimapur, Nagaland. J Vector Borne Dis 2004;41(3-4):67-71.
[13] Das NG, Baruah I, Kamal S, et al. An epidemiological and entomological investigation on malaria outbreak at Tamalpur PHC, Assam. Indian J Malariol 1997;34(3):164-170.
[14] Satyanarayana P, Meka FB, Dulala RK, et al. An epidemiological study on clinical profile of malaria in Rampachodavaram and Maredumilli the tribal belt of east Godavari, Andhra Pradesh, India. Asian Pac J Trop Dis 2014;4(1):S221-225.
[15] Igbeneghu C, Odaibo AB. Plasmodium species among the inhabitants of iwo community, South Western Nigeria. Am Euras J Sci Res 2012;7(3):118-122.
[16] Abdallah TM, Abdeen MT, Ahmed IS, et al. Severe plasmodium falciparum and plasmodium vivax malaria among adults at Kassala Hospital, Eastern Sudan. Malar J 2013;12:148.