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Original Research Article

Comparison of Microscopic Determination and Rapid Diagnostic Tests (RDTs) in the Detection of Plasmodium Infection

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Abstract: Malaria is a protozoan disease caused by the parasites of the genus Plasmodium; Plasmodium vivax, Plasmodium malariae, Plasmodium falciparum, and Plasmodium ovale. Microscopy is the gold standard for the laboratory diagnosis of malaria parasite but its turnaround time is much more than that of RDT and it requires adequate training. RDTs are alternative diagnostic methods because they are quick and easy to carry out. We studied 500 blood samples of patients presented with sign and symptoms of malaria from OPD and various wards of SHKM GMC Nalhar, from June 2015 to May 2016. All of the samples obtained were first tested by RDTs, and then the same samples were used to make peripheral blood film. RDT have more sensitivity than PBF. Specificity and PPV of rapid card test were 93.6%, 87.2% and sensitivity and NPV were 91.3%, 95.7%. In case of outdoor fields activity peripheral regions RDT is a good option. We recommended that RDT in conjunction with microscopy should improve the diagnosis of malaria.
Keywords: RDT, malaria, Peripheral blood film, sensitivity, specificity, PPV, NPV

INTRODUCTION

Malaria is a protozoan disease caused by the parasites of the genus Plasmodium—Plasmodium vivax, Plasmodium malariae, Plasmodium falciparum, and Plasmodium ovale, which are transmitted by the bite of female Anopheles mosquitoes and pose a diagnostic challenge to the clinicians worldwide [1].

The liver cycle in the life cycle of the malaria parasite ends when the mature schizont ruptures and releases the merozoites into the sinusoids of the liver. The merozoites are then discharged into the circulation. Released merozoites can only invade a red blood cell, thus beginning the erythroytic stage. The erythrocytic stage of malaria parasites has several important implications in clinical practice. First, this is the only stage causing the complex and varying spectrum of symptoms (fever, nausea, chills, vomiting, headache, fatigue and muscular aches) characterizing the disease in humans. Secondly, the recognition of parasites in the blood of a patient allows the laboratory diagnosis of the infection and the differentiation of the various species as the causal agent. The merozoites released from the liver recognize, attach, and enter the red blood cells by multiple receptor-ligand interactions in as little as 60 seconds. This quick disappearance from the circulation into the red blood cells minimizes the exposure of the antigens on the surface of the parasites, thereby protecting these parasite forms from the host immune response. This also means that the parasite (merozoites) is usually visible in the red blood cells [2].

Microscopy is the reference/gold standard for the laboratory diagnosis of malaria parasite but its turnaround time is much more than that of RDT and it requires adequate training. RDTs are alternative diagnostic methods because they are quick and easy to carry out. They also require little or no training to perform. RDTs are principally based on the detection of malaria antigens (Histidine Rich Protein (HRP2), parasite Lactate Dehydrogenase (pLDH), Aldolase enzyme) from peripheral blood using monoclonal antibodies prepared against this malaria antigen target

and conjugated to either a liposome containing selenium dye or gold particles in a mobile phase [3].

A second or third capture monoclonal antibody applied to a strip of nitrocellulose acts as the immobile phase. The strip enables the labelled antigen to be captured by the monoclonal anti-body of the mobile phase, thus providing a visible coloured thick line. Incorporation of a labelled goat anti-mouse antibody capture ensures that the system is controlled for migration [4].

The use of symptomatic method has led to an increase in the misdiagnosis and inadvertently misuse of anti-malarial which may eventually contribute to drug resistance. In India mostly malaria treat on basis of symptomatic method antimalarials chloroquin easily available at all quacks. They use chloroquin in every fever case this increase the chloroquin resistance in society underlying missed illness can progress to become complicated due to delay in drug administration. All tests were carried out immediately and examined by well trained and competent laboratory staff. There is urgent need for new, simple, quick, accurate, and cost-effective diagnostic tests for detecting malaria infection, to overcome the deficiencies of light microscopy, numerous new malaria-diagnostic techniques have been developed .The World Health Organization has recommended that management of all malaria cases should be confirmed by quality-assured, parasite-based diagnosis before treatment is started. High sensitivity of malaria diagnosis is important in all settings, and is essential for the most vulnerable population groups [5].

MATERIALS AND METHODS

Study Area

Parasitology laboratory of Microbiology department of SHKM GMC Mewat, Haryana

Samples:

Study was done on 500 blood samples came from OPD and various ward of SHKM GMC Nalhar, from June 2015 to May 2016

Inclusion criteria

- Patients presented with sign and symptoms suggestive of Malaria

Exclusion criteria

- Patients took any anti-malarial for less than 7 days
- HIV positive and Immunological disorder

Clinical Diagnosis

Clinical diagnosis based on fever (temperature > 37.5˚C) and/or history of fever, and other symptoms including; headache, joint pains, body weakness, cough, diarrhoea, loss of appetite/refusal of feeds, abdominal pain, and generalized body weakness was carried out by physicians at the outpatient department of the hospital.

Methodology

All of the samples obtained were first tested by RDTs, and then the same samples were used to make peripheral blood film (thick and thin).

Rapid Diagnostic Test

The blood samples from symptomatic patients were tested using the MERISCREEN Malaria Pf/PAN Ag by Meril Diagnostic. These rapid diagnostic kits are lateral flow immuno-chromatographic antigen detection tests kits in a cassette form. The testing was carried out according to manufacturer's instructions. Negative result is indicated by the presence of a single line, while a positive result is indicated by two bands in the strip.

Light Microscopy

The smears were processed by fixing the thin film in absolute methanol (methyl alcohol), heat fixed and stained with 10% Giemsa solution in buffered water, pH 7.2 for 10-12 min. After staining, the smears were rinsed with normal water, drained and air dried. They were then examined by light microscopy under 1000x oil immersion magnification for malaria parasites, Plasmodium species. A malaria blood film was considered negative after 100 high power fields had been examined.

Cost-effectiveness analysis

For cost effective analysis cost of ICT kits/device and their sensitivity was compared using Microscopy as Gold standard. Sensitivity of a test is defined as the ability to correctively identify the infected individual specificity as the ability to correctively identify the uninfected individual Negative Predictive Value (npv) as the proportion of those with a negative test result who are uninfected and Positive Predictive Value (ppv) as the proportion of those with a positive test result who are actually infected. The sensitivity, specificity, negative predictive value and positive predictive value of rapid test were calculated. These results were then compared with Gold standard ELISA. Sensitivity is the ability of the screening test to give a positive finding when the person tested has the disease. It is expressed as percentage. The data was analyzed using computer statistical package of social sciences (SPSS) Version 17.0. Sensitivity was calculated as true positive / (true positive + false negative) x100; specificity as true negative/ (true negative + false positive) x100 npv as true negative/ (true negative + false negative) x100 ppv as true positive/ (true positive + false positive) x100 [6].

RESULT & DISCUSSION

500 samples were studied out of which 158 were positive for malaria by PBF and 166 were positive for rapid. Results obtained with the rapid card tests

were compared to those obtained with Giemsa-stained PBF from the same sample. Specificity and ppv of rapid card test were 93.6%, 87.2% and sensitivity and npv were 91.3%, 95.7%

Table-1: Evaluation of rapid malaria kit with Microscope

	Microsc positive	Mcroscope negative	Total	Sensi-tivity	Speci-ficity	ppv	Npv	Accu-racy	P value
Rapid reactive	153	13	166 (33.2%)	91.32 %	96.33%	92.39%	95.79%	94.69%	<0.05
Rapid non-reactive	15	319	334 (66.8%)						
	158(31.6%)	332(66.4%)	500						

TP; true positive, FP; false positive, PPV; positive predictive value, TN; true negative, FN; false negative, NPV; negative predictive value (chi sq. 382.08)

In our study 31.6% samples found positive by microscopy and 33.2% were found positive by rapid card test. Other studies also reported high sensitivity ranges from 100% to 96% [7, 8]. Which is higher than the threshold of 95% recommended by the World Health Organization (WHO) some other studies reported from 89% to 100% [9-11]? The specificity in these studies range from 80% to 100%. Also the test results were available faster and it has not detected any false positive cases too, thus it is a reliable test for the diagnosis of the deadly malarial infections. The Positive predictive value (PPV) was 100% by Sandeep *et al.;* while other studies showed a range of 75-95%16-20. The present study too showed a PPV of 92.39%. The negative predictive value was 95.79 % in the present study while some other studies had a range from 91-98.35%. [12-14].

Study of P. falciparum and P. vivax-positive samples from a cohort of malaria-exposed semi-immune individuals found good sensitivity and specificity. However, blood film examination remains the standard method for diagnosing malaria since it detects all Plasmodium spp. and allows visualization of parasite growth stages, which is essential for making therapeutic decisions. In a multicenter trial by using dipsticks for HRP-2 and pLDH investigator found that rapid card test has potential of enhancing the specific accuracy of the diagnosis of P. falciparum malaria if non-specialized laboratories are involved [15]. Various studies indicate that RDTs have shown a comparable level of accuracy to microscopy in clinical settings [16, 17].

However, some investigator reported low efficiency, sensitivity of 65%, specificity of 50%, positive predictive value (PPV) of 56.5%, and negative predictive value (NPV) of 59% [1].

RDTs, however, are sensitive diagnostic tools for malaria. They are also simple to use and provide quick results without the need for good microscopic equipment and electricity, making them a good alternative to microscopy in endemic areas. But there are some limitations of RDT

1. Use of RDT is more cost-effective only in the areas by high-moderate intensity malaria transmission and in situations where health services are inadequate or absent [18]. Some studies reported limited efficacy of RDT in detecting the parasite in low parasitemia [19, 20].
2. RDT Kits need strict storage maintenance according to manufacturers
3. RDT cannot measure parasite density
4. 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.[15, 21, 22]
5. While false negative results can occur due to presence of anti-HRP2 antibodies in humans [23].

Limitations of microscopy

1. Preparing a PBF and staining is much more time consuming than RDT
2. False positive results in microscopy can result from inadequately trained staff that report artifacts as positive result
3. False negative results in microscopy may be due to inadequately trained staff and sequestration of parasite (erythrocytes containing mature parasites clump together in the microvasculature and are, therefore, not seen in the peripheral circulation and blood films, while antigen continues to be released.) [24].

Delay in diagnosis and treatment of malaria can result in severe deterioration of patient conditions, together with the development of a number of life threatening complications. The severe nature of infection, along with its potential for outbreaks, emphasizes the importance of rapid diagnosis to combat the related complications and thereby avoid significant mortality.

CONCLUSION

Our study concluded Peripheral blood film is remains gold standard to detect malaria. Rdt have more sensitivity than PBF. Specificity and ppv of rapid card test were 93.6%, 87.2% and sensitivity and npv were 91.3%, 95.7%. In case of outdoor fields activity peripheral regions rdt is a good option. The current study confirms that RDT in conjunction with microscopy should improve the diagnosis of malaria. However, RDT use should be considered as more cost-effective in the areas characterized by high-moderate intensity malaria transmission and in situations where health services are inadequate or absent. On other hand, RDTs only record the presence or absence of antigens but cannot measure the parasite density. They should, therefore, only be considered to be an extended means of parasite based diagnosis where microscopy is absent due to its varied diagnostic applications and the importance of supportive patient management.

REFERENCES

1. Anagu OL, Ikegbunam MN, Unachukwu CK, Ogwaluonye Uchenna C, Esimone CO; Comparison of Microscopic Determination and Rapid Diagnostic Tests (RDTs) in the Detection of Plasmodium Infection Advances in Microbiology, 2015; 5(8): 604.
2. Chatterjee KD; Parasitology (Protozoology and Helminthology), 13th edn. New Delhi: CBS, 2009; 90–127.
3. 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
4. Piper R, Vanderjagt L, Holbrook J.J, Makler M; Malaria Lactate Dehydrogenase; Target for Diagnosis and Drug Development. Annals of Tropical Medicine & Parasitology, 1996; 90: 433.
5. World Health Organization report from WHO Global Malaria Programme. Good practices for selecting and procuring rapid diagnostic tests for malaria.available:http://www.who.int/malaria/publications/atoz/9789241501125/en/index.html. Accessed on 21 May 2016.
6. Park K; Text book of Preventive and Social Medicine, 2002: XV111 edn; 193-4.
7. Prarthana Karumbaiah K; Efficacy of Parasight F Test in Diagnosis of Falciparum Malaria International Journal of Scientific and Research Publications, 2009.
8. Houzé S, Boutron I, Marmorat A, Dalichampt M, Choquet C, Poilane I *et al.;* Performance of Rapid Diagnostic Tests for imported Malaria in clinical practice: Results of a National Multicenter Study.PLoS ONE 2013; 8(9): e75486.
9. Arora S, Gaiha M, Arora A; Role of the Parasight-F Test in the Diagnosis of Complicated Plasmodium falciparum Malarial Infection The Brazilian Journal of Infectious Diseases 2003;7(5):332-8.
10. Singh N, Shukla M; An assessment of the usefulness of a rapid immuno-chromatographic test, "Determine™ malaria pf" in evaluation of intervention measures in forest villages of central India. BMC Infectious Diseases 2001; 1(1): 1.
11. Tjitra E, Suprianto S, Dyer M, Currie B.J, Anstey N.M; Field Evaluation of the ICT Malaria P.f/P.v Immunochromatographic Test for Detection of Plasmodium falciparum and Plasmodium vivax in Patients with a Presumptive Clinical Diagnosis of Malaria in Eastern Indonesia. J. Clin. Microbiol. August 1999; 37(8): 2412-17.
12. Ben-Edet A.E, Lesi F.E.A, Mafe A.G, Grange A.O; Diagnosis of plasmodium falciparum malaria in children using the immunochromatographic diagnostic technique. Nigerian Journal of Pediatrics 2004; 31(3): 71-8.
13. Rehlis N, Javor P; Interpretation of immunochromatographic tests with HRP2 antigen in children under 5 years in an area of high risk of malaria transmission in Papua New Guinea. Wiad Parazytol. 2004; 50(2):201-8.
14. FOrney JR, Wongsrichhanalai C, Magill AJ, Sirichaisinthop J, Bautista CT, Andersen E.M; Devices for rapid diagnosis of malaria:evaluation of prototype assays that detect plasmodium falciparum hrp2 and a plasmodium vivax-specific antigen. J Clin Microbiol 2003; 41(6):2358-66.
15. Moody A, Hunt-Cooke A, Gabbett E, Chiodini P; Performance of the OptiMAL Malaria Antigen Capture Dipstick for Malaria Diagnosis and Treatment Monitoring at the Hospital for Tropical Diseases, London. British Journal of Haematology, 2000:109; 891-894.
16. Moonasar D, Goga AE, Frean J, Kruger P, Chandramohan D; An exploratory study of factors that affect theperformance and usage of rapid diagnostic tests for malaria in the Limpopo Province,South Africa. Malaria J 2007; 1:1.
17. Moody A; Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev 2002:15; 66-78.
18. Hanscheid T, Grobusch MP; How useful is PCR in the diagnosis of malaria? Trends Parasitol 2002:18; 395-8.
19. Marx A, Pewsner D, Egger M, Nüesch R, Bucher H.C, Genton B *et al.;* Metaanalysis: accuracy of rapid tests for malaria in travellers returning from endemic areas. Ann Int Med 2005:142(10); 836-46.
20. Kurup R, Marks R; A comparison of microscopic examination and rapid diagnostic tests used in Guyana to diagnose malaria report 2012; 2(2): 12-16.
21. Miller R.S, McDaniel P, Wongsrichanalai C; Following the Course of Malaria Treatment by Detecting Parasite Lactate Dehydrogenase Enzyme. British Journal of Haematology, 2001; 113: 558-559.

22. Humar A, Ohrt C, Harrignton M.A, Pillai D, Kain K.C; Parasight F; Test Compared with the Polymerase Chain Reaction and Microscopy for the Diagnosis of Plasmodium falciparum Malaria in Travelers.1997; 56(1):44-8.
23. Biswas S, Tomar D, Rao DN; Investigation of the Kinetics of Histidine-Rich Protein 2 and of the Antibody Responses to This Antigen, in a Group of Malaria Patients from India. Annals of Tropical Medicine & Parasitology, 2005; 99: 553-562.
24. Dondorp AM, Desakorn V, Pongtavornpinyo W, Sahassananda D, Silamut K, Chotivanich K *et al.;* Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. PLoS Med 2005; 2(8):e204.