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A Comparative Study of Peripheral Blood Smear and RDTs in Falciparum Malaria Diagnosis

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A B S T R A C T

The present study was aimed to do comparative evaluation of efficacy of various staining techniques and rapid diagnostic tests used for identifying the *Plasmodium falciparum* parasite, to determine sensitivity and specificity of rapid diagnostic methods and compare the results of rapid diagnostic methods with conventional microscopy. Total of 107 male and female febrile patients of all age, clinically suspected to be positive for malaria, willing to participate and to sign the informed consent form were included in this study. Smears were stained by Leishman, Geimsa and JSB and antigen detection is done b by rapid test. Among the 107 clinically suspected cases, Leishman stain detected 18(16.8%) *P.falciparum*. Geimsa stain detected 16(14.9%) *P.falciparum*. JSB stain detected 15(14%) *P.falciparum*. RDT was positive for *P.falciparum* 18(16.8%) cases. In comparison to Leishman stain as gold standard the sensitivity, specificity, PPV and NPV of Geimsa, JSB and RDTs for *P. falciparum* were 88.8%, 100%, 100%, 97.8%, 83.3%, 100%, 100%, 96.7%, 55.5%, 91%, 55.5% and 91% respectively. Antigen based (HRP-2) RDTs are as specific as conventional microscopy but less sensitive. Their performance is highly affected by parasite density calling for further evaluation before incorporating them as diagnostic tools in peripheral health service. It is also understood that RDTs can be used only in conjunction with microscopy to improve the diagnosis of malaria.

Introduction

Malaria is a disease caused by protozoan of genus Plasmodium and continues to be the main cause of serious illness and death, throughout the world (CDC). The disease is transmitted by bite of blood feeding female anopheline mosquito (Manson's tropical diseases. 20th edition). The word "Malaria" comes from Italian "mal aria" meaning "bad air". Humans can be infected with one (or more) of the following species*: P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* (CDC). Of all the human malarial parasites, *Plasmodium falciparum* is the most pathogenic and frequently fatal if untreated (Nandwani *et al.*,). According to latest estimates of World Health Organisation, there were about 207 million cases of malaria in 2012 in world and an estimated 627000

deaths. In India, a total of 1.82 million cases of malaria and 0.89 million *Plasmodium falciparum* cases with 902 deaths have been reported (World Malaria report, 2013).

In the mid-1970s, malaria re-emerged in India, with 6.4 million new cases in 1976, indicating the need for constant vigilance and prevention. According to the National Vector Borne Disease Control Programme (NVBDCP), of the reported 1.06 million cases in 2012, 50% are due to P. falciparum in India. Also about 95% of the country's population resides in malaria endemic areas, and 80% of malaria reported in the country is confined to regions that have more than 20% of their population residing in tribal, hilly, difficult and inaccessible areas. The most affected states are: Andhra Pradesh, Chhatisgarh, Gujarat, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Odisha, Rajasthan and West Bengal (Gupta *et al.*,)

The increasing incidence of falciparum malaria, the need to identify and treat the additional infective carriers and to reduce the chance of transmission has given an impetus for development of simple and rapid methods for diagnosis. The conventional Leishman stained peripheral blood smear examination remains the gold standard for diagnosis of malaria in developing endemic countries. Conventional light microscopy has advantages that it is sensitive, informative, relatively inexpensive, provides permanent record and can be shared with other disease control programs. However, this technique is time consuming, requires training and may give poor results in cases with low parasitemia and antimalarial treatment (Mendiratta *et al.*,). In addition, in patients with *Plasmodium falciparum* malaria sometimes the parasites can be sequestrated and are not present in peripheral blood. Thus a *Plasmodium falciparum* infection could be missed due to absence of parasites in blood film (Nandwani *et al.*,). In contrast to light microscopy, rapid tests are technically easy to perform. It takes approximately 10 minutes to perform study is to compare microscopic examination of blood film with rapid techniques.

Materials and Methods

A total of 107 blood samples were collected from clinically suspected malaria patients.1 ml venous blood samples were collected in an EDTA vial for antigen detection. On 3 slides both thick and thin blood films were prepared from capillary blood with short distance between films, then air dried and stained with Leishman, JSB, Geimsastain. All blood smears are examined under40X and 100x with oil immersion.

All samples were subjected to 'ag' detection using Para Hit Total. The strip of kit contains Nitrocellulose membrane coated with Anti. HRP II anti body (capture ab) which is specific for *P. falciparum* and anti aldolase antibody which detects the presence of any plasmodium species (*P. falciparum*, vivax, ovale, malariae).When test sample along with reaction buffer flows through Nitrocellular membrane the colloidal gold coupled with Anti-HRP II/Anti aldolase antibody binds to plasmodium ag released from lysed test sample. This 'ag-ab' complex moves through Nitrocellulose membrane and binds to corresponding immobilized 'ab' to HRP II/Anti aldolase leading to formation of magenta red colour band or bands which indicate reactive results. Appearance of 3 magenta red coloured bands, one each at anti falciparum region (Pf), anti malarial ab region (pan) and control region indicates a single infection by *P. falciparum* or mixed infection with another human malarial species (*P. vivax*, *P. ovale*, *P. malariae*). Appearance of 2 magenta red coloured bands (in case of low parasitemia) one each at anti falciparum ab (Pf) and control region indicates *P. falciparum.*

Appearance of 2 magenta red coloured bands one each at pan and control region indicates pan malaria species other than *P. falciparum*. Only one band at control region indicates that sample is non reactive. Test is invalid if no band appears after completion of test.

Results and Discussion

In present study highest number of clinically suspected cases are in the age range of 21-30 years (33.3%) (Table 1) showing male preponderance with M:F ratio of 1.25:1 (Table 2). Clinical picture was depicted in (Table 3 and 4).

Staining and RDTS

Among the 107 cases, 74 were positive for malarial parasite by peripheral blood smear out of these *Pl. vivax* trophozoites were seen in 56 cases, *Pl.falciparum* gametocytes were seen in 7 cases, while ring forms were seen in 11 cases (Table 5).

Among the 107 cases (Table 6), 18 were positive for, *Pl. falciparum* malarial parasite by Leishman stain (Figure 1) 16 were positive for *Pl. falciparum* by Geimsa stain (Figure 2), 15 were positive by JSB (Figure 3) (Table 6). 107 samples were tested by Para HIT for HRP-2(Pf) antigen detection (Figure 4 and Table 7), 18 were positive and 10 among these 18 were also positive by microscopy while 8 were negative. 48 were positive for pLDH (pan) and 1 subject is positive for both HRP-2 and pLDH (Pf/pan). In comparison to Leishman stain as gold standard the sensitivity, specificity, PPV, NPV and of Giemsa, JSB and RDTs for *P. falciparum* were 88.8%, 100%, 100%, 97.8%, 83.3%, 100%, 100%, 96.7%, 55.5%, 91%, 55.5% and 91% respectively. P Value = 0.000001 is significant (calculated by using EPIINFO software).

ParaHIT-Pf/Pan showing

1. unused band
2. only one band at control region-negative
3. two magenta red coloured bands one each at pan and control region-pan malaria species other than *P. falciparum*
4. two magenta red coloured bands one each at anti falciparum ab (Pf) and control region– *P. falciparum*

3 magenta red coloured bands, one each at anti falciparum region (Pf), anti malarial ab region (pan) and control region- a single infection by *P. falciparum* or mixed infection with another human malarial species.

Table.1 Age distribution of *P. falciparum* malaria cases (n=18)

Age	Number of cases	Percentage
0-10	5	27.7
11-20	4	22.2
21-30	6	33.3
31-40	1	5.6
41-50	1	5.6
51-60	1	5.6

Table.2 Sex distribution of *P. falciparum* malaria cases (n=18)

Gender	No of cases	Percentage
Male	10	55.6
Female	8	44.4

Table.3 Symptoms of *P. falciparum* malaria (n=18)

Symptoms	No of cases	Percentage
Fever	18	100
Chills	12	66.7
Body pains	11	61.1
Head ache	10	55.5
Vomiting	9	50
Yellowish discolouration of eyes	6	33.3

Table.4 Signs of *P. falciparum* malaria(n=18)

Signs	No of cases	Percentage
Body temperature > 99^{o} F	18	100
Pallor	9	50
Jaundice	8	44.4
Splenomegaly	4	22
Altered Sensorium	3	16.6
Hepatomegaly	2	11.1

Table.5 Distribution of different forms of parasite by PBS Study

Species forms	No of cases	Percentage
***P. falciparum* gametocyte**	7	6.5
***P. falciparum* ring**	11	10.3
***P. vivax* trophozoites**	56	52.3

Table.6 Results of staining methods for diagnosis of *P. falciparum*

Staining methods	Positive	Negative	Total
JSB	15(14%)	92	107
Leishman	18(16.8%)	89	107
Giemsa	16(15%)	91	107

Table.7 Rapid diagnostics tests: Results of ParaHIT Pf/pan for 107 samples

Pan positive	**48**
Pf positive	18
Pan and Pf positive	1
Negative	40
Total	107

Fig.1 Leishman stain showing female gametocytes of *Plasmodium falciparum* (1000x)

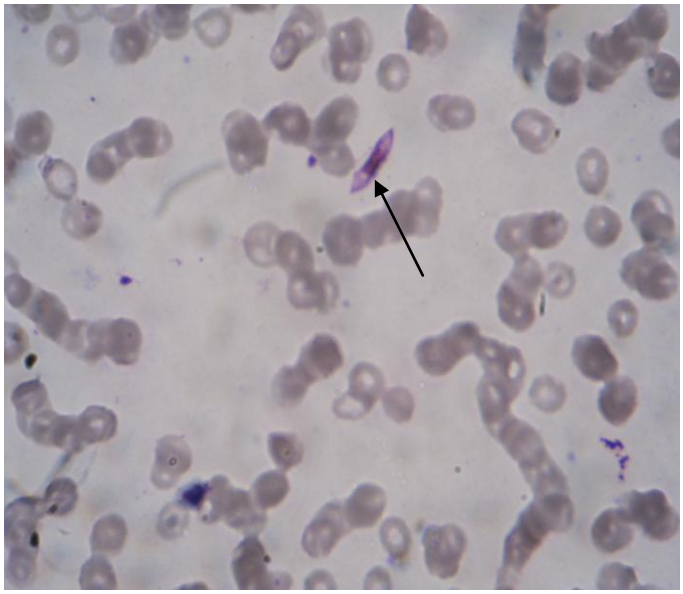


Fig.2 Geimsa stain showing female gametocytes of *Plasmodium falciparum* (1000x)

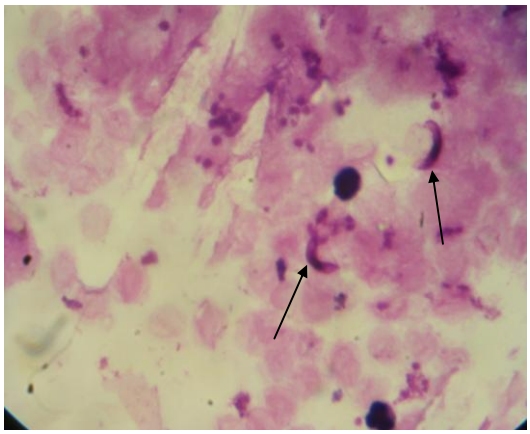


Fig.3 Jaswant Singh Bhattacharya stain showing female gametocytes of *Plasmodium falciparum* (1000x)

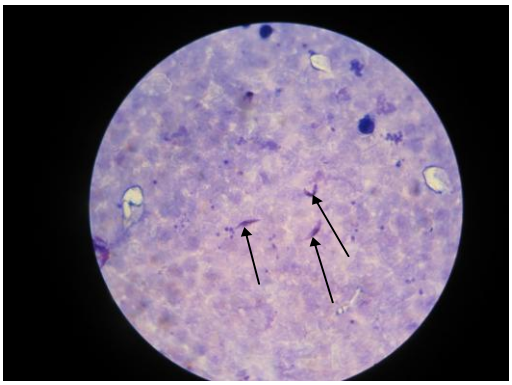
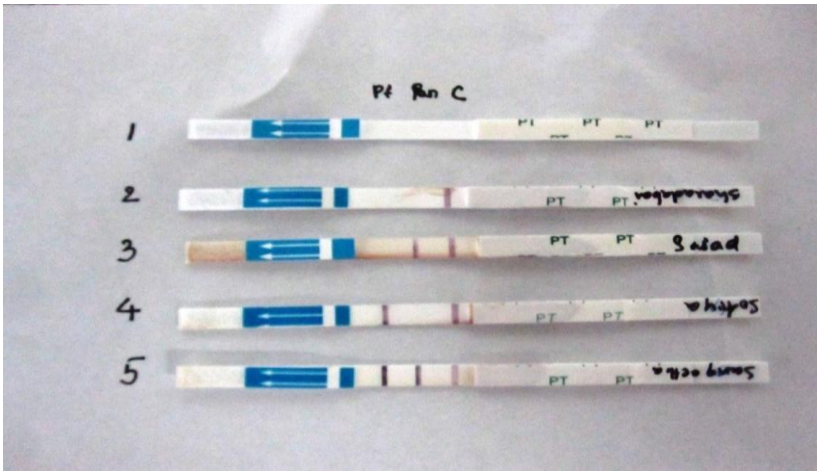


Fig.4 ParaHIT/Pan



In the present study, the thick blood smear positivity was 16.8%, which is comparable with Mendiratta *et al.*, (18.28%) and Parija *et al.*, (19.95%). In study by Nandwani *et al.*, the number of positive cases for *P. falciparum* is 63 out of 310 blood samples (20.3%) while in the current study the positive were 16 out of 107 samples (14.9%) by Giemsa. In study by Singh *et al.*, positive cases for *P. falciparum* were 152 out of 344 blood samples (44.1%), current study shows 14% positivity (15 out of 107 cases) by JSB stain.

In contrast to light microscopy, other diagnostic test kit like the Para HIT test is rapid and technically easy to perform. It takes approximately 10 minutes to perform a single test and one can perform many tests simultaneously. RDT was positive for *P. falciparum* 18 (16.8%) cases.

The value of the Para HIT sensitivity observed in the present study was very less which shows consistent results with other studies conducted in central India and other parts of the world. The specificity appears to be not consistent from various areas ranging from 75% to 100% as our study revealed specificity of 91%. Another study conducted by Kamugisha *et al.*, also showed low sensitivity and specificity of Para HIT, 29.8% and 98.8% respectively.

Microscopy positive para HIT negative

In this study, rapid diagnostic tests were found less sensitive (55.5%) as compare to PBS with good negative predictive value (91%). Microscopy detected high proportion of positive blood slides with low asexual parasite densities below threshold of RDTs. Fever and high parasite density were found to have a strong association with positive RDTs in this study.

Sensitivity of Para HIT in the study has been reported to be lower at low parasitemia. Low sensitivity of malaria rapid test (PfHRP2) has also been reported elsewhere (Azikiwie *et al.*,).

However, in 8 cases the rapid diagnostic test result was false negative, 6 of these shows grade 1 parasitemia. This may be due to insufficient enzyme production which occurs during early malarial infection or the patient blood samples contained parasites at concentration below the RDT's detection level (Chatterji KD. Malarial parasites of man). Occasional false negative results may be caused by heterogeneity of PfHRP2 expression, deletion or mutation of the HRP-2 gene (Kappe *et al.*,). It has been suggested that anti-HRP-2 antibodies in humans may explain why some tests were negative despite significant parasitemia. Presence of an inhibitor in the patient's blood preventing development of the test line is also noted (Ghai Essential Pediatrics, 7th Edn).

The limitation of antigen test is that it cannot distinguish between active infection and recently treated infection which still remains an important advantage of microscopy. In addition, RDT cannot detect the severity of disease and is only useful in diagnosis of malaria. In addition parasite count also cannot be done using RDT which is especially required for *P. falciparum* infection (Kocharekar *et al.*,).

Microscopy negative ParaHIT positive

In present study 8 blood samples in which Para HIT detected *P. falciparum* band were found to be negative in blood smear examination. The high false positivity in falciparum in Para HIT total may be explained by the fact that, HRP-II test detects antigen, chances for positivity could be more than that of blood smear (gold standard considered for diagnosis of malaria in our study). Moreover, in *P. vivax*, *P. ovale* and *P. malariae* infections, the entire asexual cycle of the parasite occurs in peripheral blood and the total number of parasites in the body can be estimated by examination of peripheral smears by light microscopy.

However, in P. falciparum, the total burden of parasites cannot be estimated since they disappear from the peripheral blood after 24-26 hrs of asexual development as a result of adherence to infected erythrocytes to the endothelium of venule and capillaries in the vital organs. Thus, if peripheral smear is examined after this stage, it may not detect parasite but the HRP-II test is not likely to miss the diagnosis. This could be another reason for discrepancy in both the tests. Occasionally persistence of antigens due to sequestration and incomplete treatment, delayed clearance of circulating antigens and cross-reactivity with rheumatoid factor in blood generates a false positive test line (Malik *et al.*,) Cross-reactivity with heterophile antibodies may also occur (Suthar *et al.*,)

HRP-II has been shown to persist and is detectable after clinical symptoms of malaria have disappeared and the parasites have apparently been cleared from the host (Kamugushi *et al.*,). Humar *et al.*, detected circulating HRP-II antigen in 68% of treated patients on day seven and in 27% on day twenty-eight. The persistence of HRP-II is still unclear (Humar *et al.*,).

Generally, RDTs achieve a sensitivity of >90% at high parasite densities >1000 parasites/µl and, the sensitivity decreases markedly below that level of parasite density (Goodman *et al.*,). Further, RDTs have been reported to give false negative results even at higher levels of parasitaemia. Therefore, in cases of suspected severe malaria or complex health emergencies, a positive result may be confirmatory but a negative result may not rule out malaria, and should always be confirmed by microscopy (Reyburn *et al.*,).

It is concluded that, the present study re-emphasizes the importance of conventional microscopy and the need for proper training in staining and interpretation methodology for specific diagnosis of falciparum malaria. Better analysis, better comparison and more sample size is necessary to say Leishman should be the gold standard. Antigen based (HRP-2) RDTs are as specific as conventional microscopy but less sensitive. Their performance is highly affected by parasite density calling for further evaluation before incorporating them as diagnostic tools in peripheral health service. It is also understood that RDTs can be used only in conjunction with microscopy to improve the diagnosis of malaria.

References

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): 307-310.

Centers for Disease Control and Prevention [Internet]. Atlanta [cited July 25 2014]. Available from www.cdc.gov/malaria

Chatterji KD. Malarial parasites of man. Parasitology, 12th edition: Chatterjee Medical Publishers;1980: 71-100 p. 308-310.

Kamugisha, E, H Mazigo, M Manyama, P Rambau, M Mirambo, JB Kataraihya, S Mshana. Low sensitivity but high specificity of ParaHIT-f in diagnosing malaria among children attending outpatient department in Butimba District Hospital, Mwanza, Tanzania. Tanzania Journal of Health Research. April 2009; 11(2): 97-99.

Goodman CA, Mutemi WM, Baya EK, Willetts , Marsh, V. (2006) The cost effectiveness of improving malaria home management: shopkeeper training in rural Kenya. Health Policy Planning July 2006; 21(4): 275-288

Gupta I, Chowdhury S. Economic burden of malaria in India: the need for effective spending. WHO South-East Asia J Public Health 2014; 3(1): 95–102.

Ghai OP, Infections And Infestations, Malaria. In: Ghai Essential Pediatrics, 7th Edn. Ghai O Paul VK, Bagga A, Delhi, Dr. O.P Ghai, CBS Publishers & distributors; 2009; p227-237.

Humar A, Ohrt C, Harrington MA, Pillai D, Kain KC. Parasight F test compared with the polymerase chain reaction and microscopy for the diagnosis of *Plasmodium falciparum* malaria in travelers. American Journal of Tropical Medicine and Hygiene Jan 1997; 56(1): 44–48

Kappe SH, Duffy PE. Malaria liver stage culture: *in vitro* veritas? Am J Trop Med Hyg. 2006 May;74(5):706-707

Kocharekar MM, Sarkar SS, Dasgupta D. Comparative Study of Modified Quantitative Buffy Coat and Two Rapid Tests in Comparison with Peripheral Blood Smear in Malaria Diagnosis in Mumbai, India. Journal of Parasitology Research 2014; Article ID 194651, Published online Mar 27, 2014.

http://dx.doi.org/10.1155/2014/194651

Malik S, Khan S, Das A, Samantaray JC. Plasmodium lactate dehydrogenase assay to detect malarial parasites. Natl Med J India. Sep 2004; 17(5): 237-9.

Mendiratta D, Bhutada K, Narang R, Narang P. Evaluation of different methods for diagnosis of P. falciparum malaria. Indian J Med Microbiol. Jan 2006; 24(1):49-51

Parija S, Dhodapkar R, Elangovan S, Chaya D. A comparative study of blood smear, QBC and antigen detection for diagnosis of malaria. Indian J Pathol Microbiol April 2009; 52(2): 200-202.

Reyburn H, Mbakilwa H, Mwangi R, Mwerinde O, Olomi R, Drakeley C, Whitty, C J. Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomised trial. BMJ. Feb 2007; 334(7590): 403

Singh N, Mishra AK, Shukla MM, Chand SK, Bharti PK. Diagnostic and prognostic utility of an inexpensive rapid onsite malaria diagnostic test (ParaHIT f) among ethnic tribal population in areas of high, low and no transmission in central India. BMC Infectious Disease June 2005; 5: 50.

SaNandwani, M Mathur, S Rawat. Evaluation of the Polymerase Chain Reaction analysis for diagnosis of Falciparum Malaria in Delhi, India. Indian Journal of Medical Microbiology (2005) 23 (3):176-178.

Suthar MN, Mevada AK, Pandya NH, Desai KS, Patel V, Goswami T, A comparative study of blood smear, QBC and antigen based rapid diagnostic test for diagnosis of malaria. International Journal of Biomedical And Advance Research 2013; 04 (04). DOI:10.7439/ijbar.

White NJ. Malaria. In: Cook, GC, ed. Manson's tropical diseases. 20th edition. London: W. B. Saunders; 1996. p.1085 -1164

World Malaria report 2013 [Internet]. World Health Organization. Available from www.who.int/malaria

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