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Original Research Article

Correlation and comparative evaluation of Thick smear, thin smear and Antigen detection test in the diagnosis of Malaria.

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Abstract: Malaria presents a diagnostic challenge in most tropical countries like India. Microscopy remains the gold standard for diagnosing malaria, but it is labour intensive and depends upon the skill of the examiner. Malaria rapid diagnostic tests (RDT's) have been developed as an easy, convenient alternative to microscopy. The aim of this study was to correlate and compare the conventional diagnostic methods like thick smear, thin smear with immunological methods in the diagnosis of malaria. The present study was conducted in the department of pathology at Dr R.N Cooper hospital for a period of 2 years. A total of 6366 blood samples referred for malaria testing were included in the study. All the samples were subjected to three 3 different techniques like thin smear stained by fields stain, thick smear stained by JSB stain and antigen detection test. Among the 6366 samples tested 300 samples (4.7%) samples were positive for malaria parasite. Of the positive samples 298(99.33%) samples were positive by thick smear, 208(69.6%) samples were positive by thin smear and 279(93%) were positive by antigen detection test. The sensitivity of thick smear thin smear and antigen detection was 99.33%, 69.8% and 91.03% respectively. Mixed infections were easily detected by all the three methods. In conclusion we suggest the combined use of antigen detection and smear examination to improve the sensitivity in detecting malaria. Antigen detection can be used as an initial screening test followed by smear examination for confirmation of species, diagnosis of mixed infections and for calculation of parasitic index.
Keywords:plasmodium species, diagnosis, thick smear, thin smear, antigen detection test, sensitivity

INTRODUCTION:

Malaria presents a diagnostic challenge to the medical community worldwide the estimated cases of malaria worldwide in 2015 were 214 million with 4.38 lakh deaths [1]. Most cases were in the African region (88%) followed by the South East Asian region (10%). Since 2010 WHO has recommended that all persons with suspected malaria in all settings should undergo malaria diagnostic testing by either microscopy or Rapid diagnostic tests (RDT) [2]. Parasitic confirmation of malaria ensures that treatment is given only to those that are infected with malaria. India undertakes a high number of diagnostic tests with >1 million tests done in 2014 which comprised 29% of the global number of tests performed. The proportion of Vivax and Falciparum malaria is almost equal in India with regional variations [3]. Parasitic confirmation of malaria ensures that treatment is given only to those that are infected with malaria. .The earliest symptoms of malaria are very nonspecific and variable which poses difficulty in clinical diagnosis. This may lead to overtreatment of malaria in endemic areas and missing the diagnosis in low transmission areas. Plasmodium falciparum malaria may present as a medical emergency and requires accurate diagnosis and appropriate treatment.

The diagnostic tests available for malaria range from conventional thick and thin smear, to recent ones like antigen detecting tests detecting parasitic antigens like histidine rich protein-2(HRP 2) plasmodium lactate dehydrogenase (pLDH), pan specific aldolase, fluorescent staining (Quantitative buffy coat)) and molecular methods like PCR [4, 5].Each of these techniques has advantages and disadvantages in terms of cost, ease of performance, sensitivity and technical complexity. The commonly employed method comprises microscopic examination of Romanowsky stained blood films. Since its introduction in 1903 microscopy has been regarded as a gold standard in the

diagnosis of malaria. This procedure is cheap and simple but it is labour intensive and requires skilled personnel [6].Thin smear examination is not very sensitive especially when parasitemia is low. In recent years quick and new techniques for malaria diagnosis have been developed like RDT [7].All these tests vary in their sensitivity, specificity, positive and negative predictive value and time consumption .This study was done to compare the efficacy of various methods for diagnosis of malaria and to find the optimum approach to detect maximum malaria cases in a peripheral hospital of Mumbai. With the spread of parasite resistance to antimalarial drugs early and accurate detection has become important.

MATERIAL AND METHODS:

The present study was a prospective observational study carried out in the department of pathology at Dr.R.N.Cooper Hospital Mumbai for a period of 2 years from 1st June 2009 till 31st May 2011.Institutional ethics committee permission was obtained. Study population included all indoor and outpatients referred to pathology department for the detection of malaria parasite. All patients who tested positive by thick smear, thin smear or RDT were included in the study. Blood was collected in EDTA bulbs from in patients and by finger pricks using a lancet in outpatients. Thick smear and thin smears were prepared and simultaneously blood was tested by the rapid diagnostic test. The thick smear of correct thickness is the one through which newsprint is barely visible. Thick smear was stained by Jaswant Singh Bhattacharya (JSB) stain. After drying for 30 minutes dehemoglobinization was done using distilled water. After dehemoglobinisation, the thick smear was immersed in JSB II stain two to three times. Smear was washed by dipping in buffer water for 2 to 3 times. Then the thick film was dipped In JSB I stain for 40 -60 seconds. Then the smear was washed with buffer water .Thin smear was stained by using field stain. Smears were examined under oil immersion microscopy for 100 fields for 5 minutes. For the RDT the Accucare malaria antigen test was used. It contains a strip coated with 2 monoclonal antibodies one specific to the falciparum histidine rich protein (pfHRPII) and the other is pan specific lactate dehydrogenase (pLDH) which detects vivax and other species. Samples were subjected to antigen detection as per kit instruction.5micoliter of whole blood was added to the sample well and 2 drops of assay buffer into the buffer well. The test result was read after 20 minutes.

RESULTS:

Out of the 6366 samples, 300(4.7%) samples were found to be positive for malaria parasite. Among the 300 cases there were 197(66%) cases of P.vivax, 76(25%) cases of Plasmodium falciparum and 27 cases (9%) had a mixed infection of both P.vivax and P.falciparum. The age and sex distribution of the cases is given in (Table-1). Among the 300 positive samples 298 were positive by thick smear, 208 by thin smear and 277 by the rapid test. Comparision of malaria detection by thick smear, thin smear and antigen detection test is shown in (Table 2).Out of the 197 vivax cases 196 were positive by thick smear, 137 by thin smear and 183 by the kit method. One case negative on thick smear was positive on kit.19 cases were negative on kit were positive on thick smear but only 8 out of them were positive also on thin smear.59 cases of vivax were negative on thin smear. Out of 76 cases of falciparum 75 were positive by thick smear 45 by thin smear and 68 by kit method. One case of falciparum negative on thick smear was positive on kit. Out of 9 negative falciparum cases by kit 7 were negative by thin smear also. Out of 31 falciparum cases negative on thin smear 24 were positive by kit and 7 were negative by both kit and thin smear. All 27 cases of mixed infections were positive by thick smear while 26 cases were positive by thin smear and kit method.

Table-1: Age and sex distribution of cases of vivax,falciparum and mixed infections.

AGE GROUP	**0-25years**		**26-50years**		**>50years**	
SEX	**M**	**F**	**M**	**F**	**M**	**F**
plasmodium vivax (PV)	51	37	67	29	10	03
Plasmodium falciparum (PF)	23	14	21	08	09	1
mixed infection(PV+PF)	06	03	10	04	03	1
TOTAL	80	54	98	41	22	05
TOTAL	134		139		27	

Table 2: Comparison of thick smear, thin smear and antigen detection in diagnosis of malaria.

SPECIES	Thick smear	Thin smear	Antigen detection
PV	196	137	183
PF	75	45	70
PV,PF	27	26	26
TOTAL	298	208	279
SENSITIVITY	99.33%	69%	93%

Table 3: Combined improved sensitivity of thin smear plus RDT versus thin smear and RDT

Diagnostic technique	Thin smear	Rapid diagnostic test	Thin smear+RDT
sensitivity	69.8%	92.95%	93.62%
specificity	100%	99.97%	99.97%
Positive predictive value	100%	99.28%	99.29%
Negative predictive value	98.54%	99.66%	99.69%

Table: 4 Comparison of sensitivity of Antigen detection test depending upon the species.

Species	Present study	Chayani *et al.;*[17]	Palmer *et al.;*[18]	Farcas *et al.;*[19]	Singh *et al.;*[14]
P. falciparum	90.2%	88.4%	94%	95.5%	94.7%
P. vivax	90.31%	96.8%	88%	87%	84.2%

Data analysis

In this study thick smear was used as a gold standard. The sensitivity specificity positive predictive value and negative predictive value of thin smear was 69.8%,100%,100% and 98.4% while that of RDT was 92.95%,99.97%,99.28% and 99.96%.When both thin smear and rapid tests were done the sensitivity increased from 69.8% and 92.95% to 93.62%(Table-3).The sensitivity of thin smear for detection of vivax infection was 69.9% and rapid test was 90.31%.The sensitivity of thin smear for detection of falciparum infection was 69.61% and for rapid test was 90.2%.

DISCUSSION:

Diagnosis of malaria involves identification of malaria parasite or its antigen/products in the blood of the patient. Rapid detection and effective treatment is a prerequisite for reducing the morbidity and mortality in the treatment of malaria cases. It is necessary to separate malarial illness from other febrile illness which can mimic it clinically. The use of easy rapid convenient tests for the detection of malaria is needed. In our study 65.66% cases of vivax, 25.33% 0f falciparum and 9% cases of mixed infections were found which was similar to a study done by Jadhav *et al* .;[8] in Navi Mumbai. The proportion of vivax and falciparum in India is 50% each but varies in different parts of India.

JSB or Giemsa stained thick smears are considered to be a gold standard in the diagnosis of malaria [9, 10]. Jaswant Singh Bhattacharya stain is a standard method used by the laboratories under the national malaria eradication programme in India .However it is time consuming, labour intensive and requires expertise of the examiner. The results also depend on the quality of the microscope, technique of staining, quality of blood film and motivation of the microscopist. It can miss low levels of parasitemia and falciparum infection when the parasite is sequestered and not in the peripheral blood. It is a cheap cost effective method where parasitic load can be quantitated. In our study the sensitivity was 99.33% for thick smear and the specificity was 100% which was similar to a study done by Binesh *et al.;*[11] who reported a sensitivity of 97.77% and specificity of 100%.A lower sensitivity and specificity of 85% and 86.79% has been reported by Bhandari *et al*[12]. This could be because we used standard method of preparation and staining smears and all smears were examined by a trained pathologist for adequate time before rendering any smear as negative. In our study the sensitivity was high and hence it was used as a gold standard to compare the other methods in our study we compared the thin smear and RDT tests with the thick smear in vivax falciparum and mixed infections.

Thin smear technique had a sensitivity of only 69.8% though specificity was 100%. Parija *et al.;*[13] found the sensitivity of thin smears to be 54.8% while Panigrahi *et al.;*[14] found it to be 66.12% which is similar to our study. The sensitivity of thin smear for vivax and falciparum infections was 69.9% and 60%.The sensitivity of thin smear was low for both vivax and falciparum malaria and using only this technique one may miss the diagnosis in many cases. However it is 100%specific, has high positive predictive value, can help in species identification and in calculation of the parasitic index. An idea about haematological parameters especially low platelets can also be made.

RDT are immunochromatography based tests based on the capture of parasite antigen from the peripheral blood using monoclonal or polyclonal antibodies prepared against a malaria antigen target. Histidine rich protein 2 detects only falciparum and persists in the blood even after treatment. Parasite lactate dehydrogenase (pLDH) is produced by all viable malarial parasites and differentiates between Parasite falciparum from other infections. The sensitivity of RDT is high and it takes 15 minutes to complete the test. It is a convenient, simple method which does not require skilled operator and is easy to interpret. It can be done in field setting where other infrastructure is not available. However kits require strict temperature

control of 4 degrees which may be disrupted while transportation[14]. Parasite index cannot be calculated and thrombocytopenia cannot be assessed. Few cases can remain positive after treatment as body can take longer to clear the HRP2 antigen[15]. Humar *et al.;*[16] found HRP2 antigen in 68% cases of treated patients on day 7 and in 27% cases on day 28.In our study 2 cases detected by antigen detection test were negative by thick smear. Another disadvantage of antigen detection test is that it cannot diagnose relapse in plasmodium vivax cases. The sensitivity and specificity of RDT in the present study was 91.03% and 99.98%.Binesh et *al.;*[8] in their study on RDT found sensitivity to be 97.10% and specificity to be 95.42% which is similar to our study. Bhandari *et al.;*[9]reported a lower sensitivity of 86.79%.The sensitivity of rapid test was 90.31% for vivax infections and 90.2% for faciparum infections.(Table-4) gives the comparison of sensitivity of antigen detection test of present study with other studies[17,18,19]. A combined approach of testing both by thin smear and rapid detection was done and it improved the sensitivity in detection of both vivax and falciparum infections. One case of falciparum negative on thick but positive on kit could be due to sequestration of parasites. Cases positive on thick but negative on thin smear or RDT could be due to low level of parasite density and low levels of antigen and enzymes which cannot be detected.

CONCLUSION:

In conclusion taking all factors into consideration we suggest using a combination of antigen detection and smear should be done to detect the maximum number of cases and combining the advantages of both methods. This will help in the early diagnosis of malaria along with calculation of the parasitic index. The antigen detection can be used as a primary screening tool followed by microscopy in all positive cases.

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