COMPARISON OF IMMUNOCHROMATOGRAPHIC PARAHIT TEST WITH CONVENTIONAL MICROSCOPY FOR THE DIAGNOSIS OF MALARIA.

Das Munmun[1]
Paul Suhrita, [2]
B.Anuradha[3]
Sharma Neelam[4]

[1] Demonstrator, Dept. of Pharmacology, Medical College, Kolkata
[2]Associate Professor, Dept. of Pharmacology, Medical College, Kolkata
[3]Associate Professor, Dept. of microbiology, Mamata Medical College, Khammam
[4] Professor, Dept. of microbiology, Mamata Medical College, Khammam

Summary

To compare the efficacy of immunochromatographic test HRP-II and aldolase (ParaHIT test) with Geimsa stained smear microscopy for detection of malaria parasites (MP).
Paired blood samples (800) were obtained from suspected cases of malaria. Of each pair, one sample was tested with conventional microscopy for the diagnosis of malaria while the other was tested with immunochromatographic test HRP-II and aldolase (ParaHIT test)**.**
MP visualized in 160(20%) cases with light microscopy, 96 out of these showed Plasmodium vivax (Pv) and 52 showed Plasmodium falciparum (Pf). ParaHIT test revealed 144(18%) positive for MP (84positive for Pv and 44 for Pf). Both the methods detected 12 cases each having mixed infection with Pv and Pf. Thus ParaHIT test had sensitivity and specificity of 91.3% and 99.44% for Pv and 92.31% and 100% for Pf respectively when compared to conventional microscopy.
ParaHIT test showed very good correlation with microscopy in the diagnosis of Plasmodium vivax and Plasmodium falciparum malaria. Though this cannot replace conventional microscopy and involves more expenditure, it has the advantage of being rapid, simple and effective in the diagnosis of malaria.

Key Words: *immunochromatographic ParaHIT test, malaria,*

Introduction

Malaria is a parasitic infection of global importance and it remains to be one of the most significant causes of morbidity and mortality in human's worldwide.[1] Efforts to control malaria demands prompt and accurate detection of parasite for effective treatment. Under ideal circumstances, the clinical suspicion of malaria would be confirmed by a laboratory test that is simple to perform, rapid, sensitive, specific and inexpensive. Microscopic examination of stained blood films remains the standard diagnostic method.[2] Microscopy has the advantages of being informative, sensitive, and inexpensive and provides a permanent record.[3] However the examination of blood films requires technical expertise and the availability of a good quality microscope. It is also time consuming and has limited sensitivity when parasitaemia is low.[4]

Besides, majority of malaria cases occur in rural areas of India where there is little or no access to reference laboratories and in many of these areas, facilities for microscopy is not available. In such a scenario, alternative non-microscopic rapid diagnostic test (RDT) for malaria would be highly beneficial.

A rapid immunochromatographic dipstick test based on the detection of P. falciparum specific Histidine Rich Protein II (HRP II) and a pan malarial species specific enzyme Aldolase, produced by the respective parasites and released in to the blood as described by Dobeli H**,** [5] and marketed as ParaHIT [TM] Total (Span Diagnostics, Surat; India) is widely used these days. The present study was carried out to compare the efficacy of this RDT with standard microscopy method.

Patients and methods

The present study was done in Mamata General Hospital, Khammam, Andhra Pradesh, India from May 2008 to April 2009 over a 12 month period. Eight hundred blood samples were obtained from patients attending Medicine out patients department and emergency of Mamata General Hospital, Khammam, who were suspected cases of Malaria. Written informed consent was obtained from each of the subjects entering the study in their own language. About 50% of the patients had symptoms compatible with malaria like high fever with rigor, chill and body ache; whereas rest had non-specific symptoms such as malaise, anorexia, irregular fever, bone and joint pain etc. The patients who had received treatment for malaria during the preceding four weeks were excluded from the study.

Paired 1.0 ml whole blood samples were collected from each patient in sterile tubes containing potassium EDTA. Thin and thick smear blood films were made immediately from each sample after blood collection. Geimsa staining was done and smears were examined under the microscope at 100 X magnification for the presence of MP. Thin smears were examined for at least 15 minutes and minimum 200 fields of thick smears were examined before considering negative.[6] The other sample was tested with ParaHit [TM] according to manufacturer's instructions wherein blood was aspirated into a heparinized glass capillary tube to a specified mark and then transferred on to the test strip. This strip was placed into the reaction tube containing 200 microlitres of reaction buffer. The dipstick was removed after 15 minutes and the results were interpreted according to the appearance of magenta red colored bands. Microscopic examination and antigen detection assay was performed by two different independent microbiologists and the study was blinded by not sharing the results between them until all samples had been processed.

Results

Blood samples (800) were tested for MP by the ParaHIT test and results were compared with those obtained from examination of thin and thick blood film smears by microscopy. The microscopy results indicated that only 160 (20%) patients were infected with malaria. P. vivax was detected in 60% (96 of 160) and P. falciparum in 32.5% (52 of 120) cases. The ParaHIT test results showed that 144 (18%) samples were positive for malaria parasite and 656 (82%) were malaria negative. Infection with P. vivax accounted for 58.3% (84of 144) while that with P. falciparum was present in 33.33% (48 of 144) cases. Blood smear examination for microscopy as well as test with ParaHIT revealed 12 patients with mixed infection of P. vivax and P. falciparum each. The microscopic examination identified two samples to be P. vivax positive that were not detected by ParaHIT test (false negative), and one sample tested positive by ParaHIT test which was not so by microscopy (false positive). Similar false negative and false positive figures for P. falciparum were 1 and 0 respectively.

Table 1

Comparison of ParaHIT test results with peripheral blood smear examination for malaria parasite detection

	Geimsa staining		By ParaHIT			
N=200	+ive	--ive	+ve	--ive	False +ive	False --ive
All sp.	40	160	36	163	1	3
Pv	24	176	21	178	1	2
Pf	13	187	12	188	0	1
Mixed (Pf, Pv)	3		3			

There was 100 % agreement between blood film and ParaHIT results for remaining 21 samples containing P.vivax. ParaHIT test had the sensitivity of 92.31% for P. falciparum when compared to traditional blood film microscopy. For P. vivax infection, the sensitivity was 87.5%. Further, the test had 100% specificity for both malarial species. Positive and negative predictive values were 100% and 98.2% respectively for P. vivax, and 100% and 99.39% for P. falciparum.

Table 2

Comparison of the sensitivity, specificity, negative and positive predictive values of the ParaHIT test with Geimsa Staining method.

	Sensitivity	Specificity	PPV	NPV
All species	92.31 %	99.39 %	97.56 %	98.19 %
Pv	91.30 %	99.44 %	96.00 %	98.89 %
Pf	92.31 %	100 %	100 %	99.41%

PPV: Positive Predictive Value; NPV: Negative Predictive Value

Table 3

Comparison of the sensitivity and specificity of the present study with other authors for P. falciparum and P. vivax by ParaHIT test

	Price RN et al		Van Den Broek et al		Present study	
	Pf	Pv	Pf	Pv	Pf	Pv
Sensitivity	95.5%	75%	90.8%	81.4%	92.31%	91.30%
Specificity	89.8%	94.8%	90.6%	99.4%	100%	99.44%
PPV	88.1%	50%	66.3%	98.7%	100%	96%
NPV	96.2%	98.2%	98%	90.5%	99.41%	98.89%

Discussion

This study was done to compare the performance of ParaHIT immunochromatographic test in the diagnosis of malaria with traditional microscopy. Peripheral blood smear examination is a gold standard for the diagnosis of malaria but this procedure, although inexpensive and reliable, yet is time consuming and requires considerable training to obtain the necessary skills.[2] Examination of the study samples obtained from suspected malaria cases revealed that 20% blood films were positive for MP while ParaHIT identified 18% positive cases.

Overall sensitivity of ParaHIT test as compared to blood film microscopy was 92.31% and sensitivity of ParaHIT for P. falciparum and P. vivax was 92.31% and 91.30% respectively. It had 100% specificity for P. falciparum and 99.44 % for P. vivax when compared to conventional microscopy (Table 2). These figures for sensitivity and specificity in our study is comparable with Van Den Broek et al [7] who evaluated immunochromatographic test using HRP-II and pan-malarial antigen among 3 rapid tests ; and Price R N et al[8] who conducted a study using HRP-II and aldolase immunochromatographic test, as in the present study.

However some malaria infections detected by microscopy were not detected by ParaHIT test. This difference in test results can be explained on the fact that these blood samples contained parasites at a concentration below the ParaHIT detection level.[9] Two cases of P. vivax observed in the blood film but not by ParaHIT test perhaps had dead parasites in the samples that had not yet cleared from the host blood [10] since the test detects HRP-II and aldolase which is produced by living parasites only.[2] Other explanation for not detecting the parasite may be due to insufficient enzyme production which occurs during early malaria infection. [11]

The sensitivity of ParaHIT test is very close to peripheral smear examination of blood film. It has many advantages such as easier to perform, is rapid and requires little training to interpret the results. Therefore ParaHIT test can be used with or without traditional blood film examination for detection of both P. vivax and P. falciparum malaria. ParaHIT test has the added advantage that it detects all four plasmodium species and can be used to follow the efficacy of drug therapy administered, since it detects the enzymes produced only by living parasites.

Microscopy remains the GOLD standard for detecting malaria parasites. ParaHIT test shows excellent correlation with microscopy in the diagnosis of P. vivax and P. falciparum malaria. Although the cost is more, this test has an advantage of being simple, rapid and effective in the diagnosis of malaria, especially where technicians well trained in microscopy are not available or the work load is too high.

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