



COMPARATIVE EVALUATION STUDY OF IMMUNOCHROMATOGRAPHIC TEST AND MICROSCOPIC DIAGNOSTIC TEST IN DIAGNOSIS OF MALARIA

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ABSTRACT

Introduction: The commonly employed method for diagnosis of malaria involves the Immunochromatographic test and microscopic diagnostic test in diagnosis of malaria.

Objectives: Detection and identification of Malarial parasite Microscopically in blood films. Detection and identification of Malarial Antigen by Immunochromatographic Rapid Diagnostic test. Comparison of results of Microscopic and Immunochromatographic Rapid Diagnostic test.

Materials and Methods: A total 82 whole blood samples were examined by preparing thin smear blood films and staining with J.S.B (Jaswant Singh Bhattacharjii) stain. All the blood samples were also subjected to Immunochromatographic diagnostic test for detection of Antigen. Results of both J.S.B stained smears and Immunochromatographic test were compared.

Results: Out of 82 blood samples processed, 6 (7.31%) samples were Microscopic positive for malarial parasite and 5 were Immunochromatographic test positive for malarial antigen. Out of 6 samples 5 positive by microscopy were positive for *P.vivax* and only 1 sample was positive for *P.falciparum*. out of 5 samples 4 positive by Immunochromatographic were positive for *P.vivax* and only 1 sample was positive for *P.falciparum*.

Conclusion: Immunochromatographic test are rapid, do not require expertise and are useful in Routine diagnosis. The sensitivity of antigen detection test is lower (97.4%) specificity (100%). When compared to microscopy, microscopy is simple, economical, sensitive and specific, hence still remains the gold standard method for malaria diagnosis.

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INTRODUCTION

Malaria is caused by protozoan parasite of the genus *Plasmodium*.⁽¹⁾ The name malaria is derived from the Italian mal-aria or bad air.⁽²⁾ It is wide spread in the tropical and subtropical regions due to significant amount of rain fall, consistent high temperatures and warm humidity along stagnant water.⁽³⁾ Malarial parasite of the genus *Plasmodium* is transmitted through the bite of infected mosquito's. Female anopheles mosquitoes transmit *Plasmodium* species that commonly cause illness in humans: *P.falciparum*, *P.vivax*, *P.ovale* and *P. malaria* mixed infections with multiple species are possible and occur in areas where more than one species is in circulation. *P.falciparum* and *P.vivax* is the cause of morbidity worldwide. *P.falciparum* the most pathogenic malaria species. Rarely human can be infected with *P.knowlesi*.⁽⁴⁾ Common symptoms and signs are: fever, chills, sweating, headache, nausea, and vomiting, body aches, malaise, weakness and splenomegaly. In *falciparum* malaria, additional finding are mild jaundice, hepatomegaly and tachynea. If *falciparum* malaria is not treated properly, following complications may occur cerebral malaria, severe anemia, hemoglobinuria, pulmonary edema,

thrombocytopenia, cardiovascular collapse, shock, kidney failure, hyperparasitemia, metabolic acidosis and hypoglycemia.⁽⁵⁾ Microscopy diagnosis is performed by manual visual examination of blood smears. It is an ability to differentiate between non-parasitic stained components: red blood cells, white blood cells, platelets. During the lifecycle in peripheral blood and the different species observable in the four different life cycle stages which are generally morphologically distinguishable: ring, trophozoites, schizont, gametocyte. The species show different change in the infected cells and presents as some dots: Schuffner's dots, Maurer's dots and the presence and absence of malarial pigment.⁽⁶⁾ Another dipstick assay detects plasmodium specific lactate dehydrogenase. It can detect plasmodium species by detecting antigenic differences between various p-LDH isoenzymes. p-LDH antigen in lysed whole blood. p-LDH is released from live malarial parasites and differentiation of plasmodium species is based on antigen differences between its forms. pLDH and pan specific pLDH monoclonal antibody that recognize all other plasmodium species : *P.vivax*, *P. malariae*, *P. ovale*.⁽⁷⁾

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Objectives

Detection and identification of Malarial parasite Microscopically in blood films. Detection and identification of Malarial Antigen by Immunochromatographic Rapid Diagnostic test. Comparison of results of Microscopic and Immunochromatographic Rapid Diagnostic test.

MATERIALS AND METHODS

The present study was conducted in the Parasitology laboratory of Microbiology Department of AIMSR.(Bathinda) on OPD/IPD blood samples suspected of malaria of all age group coming to AIMSR. This work was started after the approval from AIMSR Research Committee & Institutional Ethics Committee. A total of 82 whole blood samples were examined by preparing thin smear blood films and staining with J.S.B (Jaswant Singh Bhattacharji) stain. They were examined for malarial parasite by microscopy. A thin blood smear examined for 15 minutes. All the blood samples were also subjected to Immunochromatographic test for detection of Antigen. Results of both J.S.B stained smears and Immunochromatographic test were compared. Microscopic diagnosis of malaria: Thin blood smears were prepared as per the gold standard methods. The smears were approximately 80-100 fields were examined over 8-10 minutes. Antigen detection by ICTs: Antigen was detected by ICTs method with PAN+PF card malaria antigen test kit manufactured by J.mitra Co. Pvt.Ltd.(8) Test procedure was done as per the instructions given in the kit manual.

RESULTS

A total of 82 blood samples suspected to be of malaria cases were received from Various departments (Medicine, Pediatrics, OBG, Emergency) in the Parasitology laboratory of Microbiology department, AIMSR over a period of six months. Out of 82 blood samples received, 6(7.31%) samples were microscopy positive for malarial parasite and these samples were subjected to rapid diagnosis, 5 (6.09%) were ICTs positive for malaria Antigen.

Table 1 Percentage and positivity rate by microscopy

Method	Positive samples	Percentages
Microscopic	6/82	7.31%
ICTs test	5/82	6.09%

6 (7.31%) out of 5 samples were positive by Microscopy were positive for P.vivax and only 1 were positive for P.falciparum. 5 out of 4 samples were positive by ICTs were positive for P.vivax and only 1 positive for P.falciparum.

Table 2 Microscopy and RDT results for the two species of malaria

Method	Positive for P.vivax	Positive for P.falciparum
Microscopy	83.34%	16.66%
ICTs	80%	20%

DISCUSSION

In our study the microscopy results of blood smears indicated a positivity of (7.31%). Our study compares well with that of Muhammad *et al* (9), Dhodpkar *et al* (10), Pawandee *et al* (11), Mannur S *et al* (12). Higher prevalence of (73.6%) Azike BH(13) *et al*. In our study the Immunochromatographic antigen detection assay results indicated a positivity of (6.09%) define well with that of Mannur S *et al* (12), Bankole HS *et al* (14). higher prevalence of Jamil r *et al* (15). In present

study, prevalence of P.vivax detected by microscopy was (83.34%) and that of P.falciparum (16.66%). The positivity of P.vivax in our study; (83.34%) stands well with that of Pawandee *et al* (11)(96.77%). Positivity of P.falciparum in the present study was (18.18%) which is comparable to that of Bankole HS *et al* (14) (45.45%).

The ICTs antigen detection assay results indicated positivity of (6.09%) microscopy indicated the total positivity of (7.31%). The results clearly indicated HRP-2 antigen detection test had slightly lower sensitivity as compared to microscopic analysis method. 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. The ICTs antigen detection assay results indicated positivity of (6.09%) microscopy indicated the total positivity of (7.31%). The results clearly indicated HRP-2 antigen detection test had slightly lower sensitivity as compared to microscopic analysis method. 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.

CONCLUSIONS

In present study a total of 82 samples were received. 6 samples were positive for malarial parasite infection by Microscopic method and only 5 positive by Immunochromatographic method. Out of 6 patients, were male and 2 were female. 4 patients were from OPD and 2 were from IPD. Male are more susceptible to malarial parasitic infection as compares to females. Out of 6 patients, highest being (3) from medicine and lowest from (1) each OBG, Emergency and Pediatrics departments. Out of 6 patients positive in malarial parasite infection diagnosis by microscopic method. 4 patients were males and 2 were females. Out of 5 patients positive by Immunochromatographic test. 3 patients were male and 2 were females. ICTs are rapid, do not require expertise and are useful in routine diagnosis. The sensitivity of antigen detection test in lower (97.4%), specificity 100% when compared to microscopy. Microscopy is simple, economical, sensitive and specific, hence still remains the gold standard method for malaria diagnosis. This method has the advantage of high sensitivity, quantifiable results and accurate speciation, though it is fairly time consuming.

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