Original Research Article

A comparative study of malaria antigen test with peripheral blood smear in diagnosis of malaria

Gunvanti Rathod[1], Anita[2*], Pankajkumar Keshri[3], Pragnesh Parmar[4], Santosh Kumar[5]

[1]Additional Professor, Pathology and Lab Medicine, AIIMS, Bibinagar, Hyderabad, Telangana, India
[2]Senior Resident, Microbiology Department, IGIMS, Patna, Bihar, India
[3]Asociate Professor, Dept. of Physiology, Lady Hardinge Medical College, New Delhi, India
[4]Additional Professor and HOD, Forensic Medicine and Toxicology Department, AIIMS, Bibinagar, Hyderabad, Telangana, India
[5]Assistant Professor, Trauma and Emergency Medicine, IGIMS, Patna, Bihar, India
[*]Corresponding author email: **dranita2000@gmail.com**

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Abstract

Background: Malaria is a common parasitic disease with remarkable morbidity and mortality all over the world. The number of cases of malaria is significantly noted each year. The causative organism of Malaria is protozoa of genus *Plasmodium*. It is a highly devastating parasite and causing marked morbidity and mortality. In all over the world, total four species of plasmodium cause malaria in humans. If we want to reduce the burden of malaria from society, we have to work towards rapid detection and effective treatment for malaria. The study was aimed to compare microscopic examination of blood films with newly develop Immunochromatography card test which can be the alternative for the microscopy and diagnosis can be made at the earliest.

Materials and methods: This was a prospective study conducted at tertiary care hospital and research institute, Gujarat, India from January 2015 to January 2016. Total 350 blood samples were collected in sterile EDTA vacutainer from patients belonging to all age groups presenting with fever, chills and rigors, from various inpatient and outpatient departments. Thick and thin smears were prepared and stained with Leishman's stain and examined for malarial parasites using light microscopy. All the samples were subjected to malaria antigen card test according to the manufacturer's instructions.

Results: Total of 70 samples was positive by thick smear and thin smear and 53 samples were positive by the antigen detection test. Total incidence of malaria was found to be 19.95%. Out of 70 positive patients 44 cases were of P. vivax positive and 26 cases were P. falci positive. Total 48 males and 22 females were positive for Malaria infection.

Conclusion: We can conclude that the card test has got a number of advantages though one needs to keep in mind the cost of the test which may not be affordable by many. The high cost of the test may prevent its regular and routine use in many of the laboratories.

Key words

Malaria, Hemolysis, Thick and thin smear, Malaria antigen card test.

Introduction

Parasitic diseases are major burden for human being. Malaria is a common parasitic disease with remarkable morbidity and mortality all over the world. The number of cases of malaria is significantly noted each year. The causative organism of Malaria is protozoa of genus *Plasmodium*. It is a highly devastating parasite and causing hemolysis. In all over the world, total four species of plasmodium cause malaria in humans. *Plasmodium* falciparum, *plasmodium*vivax, *plasmodium* malaria, *plasmodium* ovale are the different species of Plasmodium. Majority of morbidity and mortality are caused by two species of Plasmodium, P. falciparum and P. vivax. They are responsible for many complications too. Among these, infections because of P. Falciparum are if left untreated might cause multi organ failure and even death [1]. The diagnosis of malaria is based upon the presence of fever alone in many developing countries like India. Because of this there is development of overuse of anti-malarial drugs. I*deally* the clinical suspicion of Malaria would be confirmed by a laboratory test that is simple to perform, rapid, sensitive and specific. The most common test for malaria diagnosis remain the microscopic examination of Giemsa or Fields – Stained blood smears. However, the examination of blood films requires technical expertise and the availability of a good quality microscope. The microscopy is also time consuming and has limited sensitivity when parasitemia is low [2-4]. In many developing countries the facility of referral lab is not available where the diagnosis of malaria can be made at the earliest. Keeping all these in a mind this study was done to compare microscopic examination of blood films with newly developed Immunochromatography card test which can be the alternative for the microscopy.

Materials and methods

This was a prospective study conducted at tertiary care hospital and research institute, Gujarat, India, from January 2015 to January 2016. Total 350 blood samples were collected in sterile EDTA vacutainer from patients belonging to all age groups presenting with fever, chills and rigors, from various inpatient and outpatient departments. Patients without fever and not having any other clinical features of malaria were excluded for the study. Informed consent from the patients was obtained, prior to collection of blood samples and declaration of health records. Thick and thin smears were prepared and stained with Leishman's stain and examined for malarial parasites using light microscopy. All the samples were subjected to malaria antigen card test according to the manufacturer's instructions. The Malaria Ag P.f/P.v test is a rapid, qualitative and differential test for the detection of histidine-rich protein II (HRP-II) antigen of Plasmodium falciparum and common Plasmodium lactate dehydrogenase (pLDH) of Plasmodium species in whole human blood. The test was done using anticoagulated blood. The test is highly sensitive and specific for the diagnosis of Plasmodium Falciparum, Plasmodium Vivax, Plasmodium Ovale and Plasmodium Malarial Infection. Appearance of three colored bands, one each in

the anti falciparum antibody region, anti malarial antibody region and control region indicates that the sample is reactive or positive for Plasmodium falciparum and mixed infection with another malarial species. (Plasmodium vivax is most commonly encountered in India). Appearance of only one colored band in the control region indicates that the sample is non-reactive or negative for Plasmodium species.

Results

A total of 350 samples were observed over the period of study. Of these a total of 70 samples were positive by thick smear and thin smear and 53 samples were positive by the antigen detection test (**Table – 1**). Total incidence of malaria was found to be 19.95% .Out of 70 positive patients 44 cases were of P. Vivax positive and 26 cases were P. falciparum positive. Total 48 males and 22 females were positive for Malaria infection (**Table – 2**).

Discussion

Many of the infectious disease are creating morbidity and mortality in the society. The reappearance of malaria leads to develop not only preventive measures, but also rapid diagnostic techniques. There are multiple factors which are responsible for the reemergence of malaria, including (i) Insecticide resistance in the Anopheles Mosquito. (ii) Social instability resulting in movements of unexposed non immune individuals in areas where malaria is endemic, and (iii) The failure to develop an effective malaria vaccine. If we want to reduce the burden of malaria from society, we have to work towards rapid detection and effective treatment for malaria. Leishman's or Giemsa stained thick smears are considered to be the 'Gold standard' in diagnosis. However, the interpretation of thick smears is laborious and results depend on the quality of microscope, staining, technique with which blood film is prepared and also the concentration and motivation of microscopist [5, 6]. Newer techniques like QBC and Antigen detection assays are rapid, simple and easy to interpret. In the present study, we compared Antigen detection card methods available for rapid diagnosis with Leishman-stained thick and thin smears. The sensitivity of Leisman-stained thin smear was found to be lowest (54.8%); however, this method had a high specificity and positive predictive value (100%) (**Table – 1**).

Table - 1: Sensitivity, specificity, positive predictive value and negative predictive values of thin and thick smear and antigen detection methods for diagnosis of malaria.

	Sensitivity (%)	**Specificity (%)**	**Positive predictive value (PPV) (%)**	**Negative predictive value (NPV) (%)**
Thin and thick smear	54.8	100	100	89
Antigen detection	75	100	100	94.2

Table - 2: Demographic details of malaria cases.

Gender	**No. of P. vivax cases**	**No. of P. falciparum cases**	**Total**
Male	30	18	48
Female	14	8	22
Total	44	26	70

Malarigen for detection of malaria antigen had a sensitivity, specificity and PPV of 75%, 100% and 100%, respectively. This test was based on detection of pLDH and aldolase with the help of monoclonal antibodies. The values obtained for this kit-based procedure were much lower than those observed for other tests based on similar principle [7, 8]. This low sensitivity could be attributed to low parasitemia levels as observed by Iqbal, et al. [9] who observed 75% sensitivity at parasitemia< 100/µl. The specificity was comparable to other observers using the tests

based on similar principle [7, 8]. However, the test was found to be user friendly and interpretation was more objective as compared to smear.

Conclusion

We can conclude that the card test has got a number of advantages though one needs to keep in mind the cost of the test which may not be affordable by many. The high cost of the test may prevent its regular and routine use in many of the laboratories. However, it was a valuable adjunct at the time of emergency for rapid diagnosis, although microscopy remains the mainstay for the diagnosis of malaria for routine use in countries like India.

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