

Comparison between Different Staining Techniques And Rapid Card Test For Diagnosis Of Malaria At A Tertiary Care Centre.

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Abstract

Background: Malaria is a potentially life threatening parasitic disease caused by parasite known as Plasmodium. There are five species of Plasmodium- Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax and Plasmodium knowlesi. It is transmitted by the bite of Anopheles mosquito. Malaria is a major health problem in India. 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.

Material And Methods: A total of 50 acute pyrexia cases of both sexes of all age groups were included in the study. After taking informed consent 2ml of blood in EDTA tube was collected from each patient, under aseptic conditions and labelled appropriately. Thick and a thin blood smears were made from the each sample and then stained with Jaswant Singh-Bhattacharji stain and Leishman stain and visualized under 40x, 100x. Rapid antigen detection was carried out with a drop of blood.

Results: Among all different methods used in this study Leishman staining has shown highest sensitivity and antigen detection kit showed least sensitivity in the diagnosis of malaria.

Conclusion: The knowledge of different diagnostic modalities help in choosing appropriate test for early diagnosis and proper clinical management. Leishman stain is cheap, easily available and easy to perform which provides better contrast and is highly sensitive

Key Word: Malaria, leishman stain, JSB stain, rapid card test

Date of Submission: 14-03-2021

Date of Acceptance: 28-03-2021

I. Introduction

Malaria is a potentially life threatening parasitic disease caused by parasite known as Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax. It is transmitted by the bite of Anopheles mosquito. Malaria is a major health problem in India. In 2020 total malaria cases recorded in India are 0.15 million of which Plasmodium falciparum cases are 0.09 million and death due to malaria are 55. [1] According to WHO malaria report 2020 each year more than 4,00,000 people die of malaria, a preventable and treatable disease. An estimated two thirds of deaths are among children under 5 years [2]. 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 persons on antimalarial treatment. [3][4]. India being an endemic country for malaria with huge number of cases, low cost traditional methodologies like peripheral smear examinations are still widely practised over the newer methodologies. [5] The detection of malarial antigens by variety of Rapid diagnostic test available is of great value especially in severe and complicated malaria wherein blood smears may be negative [6]. Hence a rapid, reliable and effective species-specific diagnostic tool is essential for early treatment to prevent complications of Malaria

II. Material And Methods

The present cross sectional comparative study was carried out at Sir Ronald Ross Institute of Tropical and Communicable Diseases, Nallakunta, Hyderabad, Telangana from September 2019 to November 2019 after obtaining institutional ethical committee approval. A total of 50 acute pyrexia cases of both sexes of all age groups were included in the study after taking informed consent.

Inclusion criteria:

- Patient of all ages of both sexes
- Patients with clinical features suggestive of Malaria like fever with chills and rigors, fatigue, abdominal discomfort.
- Patients willing to give consent

Exculsion criteria:

- Patients on anti-malarial drugs
- Patients with previous history of Malaria

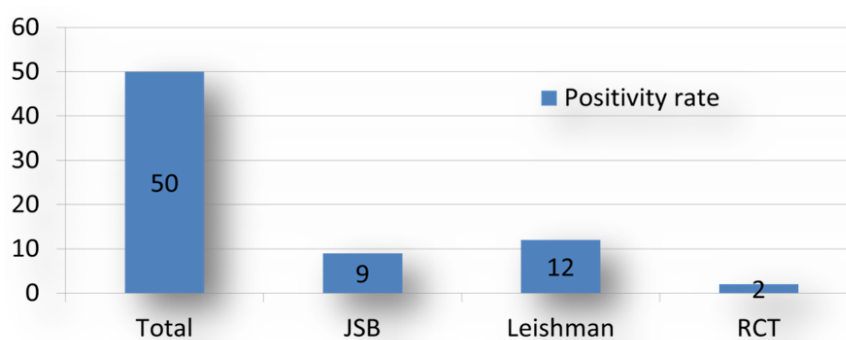
After obtaining written informed consent from each patient 2ml of blood in EDTA tube was collected., under aseptic conditions and labelled appropriately.

Thick and a thin blood smears were made from the respective sample and then stained with JSB stain and Leishman stain and visualized under 40x,100x .

Rapid antigen detection was carried out with a drop of blood using MALARIGEN rapid card test-qualitative detection of malaria parasite(Pf &Pv)antigen in human blood.

III. Results

Out of 50 smears stained by Jaswant Singh–Bhattacharji stain, 9(18%) were positive for malaria of which 5(10%) were P.falciparum & 4(8%) were P.vivax. By Leishman staining method 12(24%) were positive for malaria of which 8(10%) were P falciparum & 4(8%) were P vivax. Rapid card test detected only 2 positive cases. This study shows Leishman stain is more sensitive over the other methods



IV. Discussion

Malaria is a mosquito-borne disease and they breed predominantly in stagnant water. The increasing burden of the disease, the emergence of resistance to antimalarials, and availability of expensive artemisinin-combination therapies, especially in highly endemic regions, are increasing the need for rapid accurate diagnosis of patients with suspected malaria(7)Malaria Control Programmes have emerged and awareness among people in protecting themselves from mosquito-exposure has increased which has lead to the reduction in the incidence of malaria(8). Microscopic examination of properly prepared and stained blood smears by well-trained microbiologist is accurate and reproducible and allows for the identification of the plasmodium species, differentiating between stages and density of infection. However, it is not always feasible to sustain accurate microscopy especially in the remote areas.(9). RDTs detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies (immunochromatographic lateral-flowstrip technology); they do not require laboratory equipment, are easy to perform and provide results within half an hour. Despite their ability to discriminate between different species of malaria, the dipstick methods are poor at detecting mixed infections when one species is present at a significantly lower parasitemia than the other.(7)

In the present study of comparison between different staining technique and rapid card test for malaria detection ,blood sample was collected from 50 acute febril patients.Out of the 50 smear stained with 24% were positive for malaria parasite with Leishman stain and 18% were positive with JSB stain. Rapid card test was positive only in 4%cases ,correlated with previous studies[table1]

Table no 1

	Leishman stain	JSB stain	RCT
Swathi et al,2019	16.8%	14%	-
Vijaysathiskumar et al,2015	16%	-	20%
Yasmeen khatiba et al,2016		-	4.3%
Present study ,2019	24%	18%	4%

V. Conclusion

The knowledge of different diagnostic modalities help in choosing appropriate test for early diagnosis and proper clinical management. Leishman is cheap,easily available and easy to do to perform which provides better contrast and has good sensitivity for malaria parasite

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Dr. Syeda Hafsa Fatima. "Comparison between Different Staining Techniques And Rapid Card Test For Diagnosis Of Malaria At A Tertiary Care Centre." *IIOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 20(03), 2021, pp. 09-11.