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A comparative study of peripheral blood smear and rapid diagnostic test for diagnosis of malaria in Jharkhand, India.

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Abstract: ***Malaria is a disease that can cause fatal complication if the diagnosis and treatment are delayed. So, quick detection and early treatment of malaria are the best policies of disease management. Blood sample (fresh blood from finger prick) was collected from total number of 2500 patients present with classical symptoms of malaria. PBS$_S$ were stained with Leishman's stain. Antigen detection tests were done using commercially available kits. Out-of 2500 patients 421 cases were positive by Peripheral blood smear and 427 were positive by card test. Peripheral blood smear has superior for species identification but the RDT$_S$ are easy to use, interpret and simple to test, therefore the test can be used as an epidemiological tool where the microscope and Leishman's stain are unavailable.***

Keywords: Peripheral Blood Smears, Rapid Diagnostic Tests, Plasmodium falciparum, Plasmodium vivax, Positive Predictive value, Negative Predictive value**.**

1. Introduction

Malaria is a major public health problem in India. Jharkhand, Orissa, West Bengal, Chhattisgarh and Karnataka contribute the most number of cases of malaria in India. Malaria is a serious disease caused by the parasites called plasmodia. There are four identified species of malaria parasites causing human malaria. But in our state (Jharkhand) two species are very common one is Plasmodium vivax and another is Plasmodium falciparum. The infection is transmitted between humans by the female anophales mosquito.

Malaria occurred more commonly in rural areas. The higher prevalence at rural areas may be due to higher transmission, less availability of preventive measures and limited access to anti malarial drugs. In humans, the parasites (called sporozoites) migrate to liver where they mature and release another form, the merozoites.

The disease now occurs in more than 90 countries world-wide, and it's estimated that there are over 600 million clinical cases and 3.1 million malaria-caused death per year. Still the malaria is diagnosed by detecting the parasite in the peripheral blood smear. Blood will be put on-to a microscope slide and stained by Leishman's stain, so that the parasites will be visible under a microscope.

Conventional peripheral blood smear examination for demonstration of malaria parasites remains the gold standard for diagnosing malaria. However this technique is time consuming and requires skilled – personnel. Many newer malaria detecting tests have been purposed to replaced the conventional microscopic method. For that reason these study was done to compare the peripheral blood smear test with malaria antigen card test (company SDBIOLIN antigen card).

This card contains one monoclonal antibodies (test line p.f) that are specific to the HRP-II (histidine-rich protein II) of p.falciparum and the other polyclonal antibodies (test line pan) that are pan specific to the lactate dehydrogenase of plasmodium species (P.vivax, P.malariae, P.ovale).

2. Materials and Methods

Material consisted of Leishman's stain, microscopic slides, lancets, light microscope with good 40X and 100X objectives, RDTs kits from S D BIOLINE malaria antigen p.f/pan.

The study was carried-out during January, 2014 to December, 2015. The study group constituted of 2500 patients presenting with fever, chills, rigor and other suggestive symptoms of malaria attending various outpatient and inpatient departments of Mahatma Gandhi Memorial Medical College and Hospital, Jamshedpur, Jharkhand. Thick and thin films were made from finger prick blood and stained Leishman's stain and examined for oil immersion fields for malaria parasites by light microscopy.

All blood films with visual malaria parasites were simply presented as positive while those of without visible malaria parasites were simply taken as negative. All the samples were also tested by using rapid malaria antigen card test (company S D BIOLINE) according to the manufacturer's instruction.
S D BIOLINE malarial antigen p.f/pan card test is one step immunochromatographic rapid, qualitative and differential card test for the detection of HRP-II (histidine-rich protein II) specific to p.falciparum and PLDH (Plasmodium Lactate Dehydrogenase) pan specific to other plasmodium species in human blood sample. The test results were read in 10-20 minutes.

Outcome of peripheral blood smear and antigen card test were compared in all patients for their sensitivity and specificity.

3. RESULT AND DISCUSSION

A total of 2500 blood samples were investigated for malarial parasites. The blood film result indicated that out of 2500 cases 421 (16.84%) cases were positive for malaria and 2079 (83%) cases were negative. Of these 383 (90.97%) cases were positive for P. vivax, 33 (7.83%) cases were positive for P. falciparum rest 5 (1.18%) cases were mixed infection (P.vivax and P. falciparum). SD BIBOLINE malaria antigen card test could detect 427 positive cases of malaria as shown in table-1.

Table 1: Comparison of Leishman stained blood film with Antigen Card Test.

Peripheral Blood Smear	Antigen Card Test	RESULT
421	427	Positive
2079	2073	Negative
2500	2500	Total

In the present study we assessed the use of malaria antigen card test for diagnosis of malaria, here we observe 06 cases of malaria were positive by card test and negative by peripheral blood smear examination. Detection and effective treatment is extremely essential for reducing morbidity and mortality due to malaria. Microscopic examination of blood is the most reliable method of diagnosis of malaria. Peripheral blood smear study is simple, list expansive, time consuming and therefore delay in diagnosis. New techniques like antigen detection assays are rapid, simple and easy to interpret but blood film can provide more information than card test.

In the present evaluation we observe 100% sensitivity, 99.5% specificity, 98.59% PPV and 100% NPV in antigen card test comparing with the peripheral blood smear. Leishman stained thin blood smear detects malaria parasites only when there are 50 parasites per ml of blood. How-ever, we compare thin smear with antigen card test 421 cases were positive by both the methods, while thin smear missed to rule out 06 cases which was positive by antigen card test (Table-1).

Although, stained smear is useful to keep a permanent record of the smear, its low cost and species identification without much difficulty in the most of the cases. Rapid diagnostic test is recommended by WHO when reliable microscopy is unavailable. Malaria antigen test is the most rapid test and require minimum training for performing the test especially in non-endemic zone.

4. CONCLUSION

The study highlights that the rapid diagnostic test for diagnosis of malaria is as reliable as microscopy but only the antigen based method is suitable in endemic zone. The explorations show that malaria antigen test is simple, reliable and rapid test with some limitations like high cost, unable to detect the number of parasites and differentiation between the plasmodium species. Even than the test can be a promising alternative to microscopy in places where the facility for microscopy are poor. Hence, it is sensible to consider future use of rapid card test as an epidemiological tool for rapid screening of malaria.

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