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Malarial Parasite Identification, Comparison of Different Techniques

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Abstract

Traditionally thick and thin Giemsa staining was used for the identification of Plasmodium vivax. Examination of Quantitative buffy coat after staining with acridine orange is also used now days. Rapid Diagnostic test (RDT) a rapid color immunochromatography assay for qualitative determination of malaria parasite is the most convenient method for the identification of different species of Plasmodium. The present study has been carried out to find out the accuracy and authenticity of different techniques for the identification of malarial parasites. The investigations were carried out in 60 patients suffering from malaria caused by Plasmodium vivax. Out of the 60 Patients, 50 patients were positive by the Giemsa staining, 41 patients showed positive result for Florescence Microscopy (QBC) and 58 patients gave positive result for Rapid Diagnostic Test.

Keywords- *Plasmodium vivax, Giemsa stain, Fluorescence Microscopy (QBC), Rapid Diagnostic Test (RDT).*

Introduction

Malaria is a major health problem in India accounting for sizable morbidity, mortality and economic loss. A person suffering from malaria always gets fever but all fevers are not due to malaria. Apart from preventive measures early diagnosis and complete treatment are the important modalities. The world now has the means to rapidly diagnose malaria and treat it efficiently ("Dr. Robert Neumann, director of the WHO Global malaria program 09 March 2010). The diagnosis of malaria is usually based on a history or presence of fever

and dependent upon the demonstration of malarial parasite in the peripheral blood films. The presumptive treatment of fever result in our administration of antimalarial drugs thus reliable diagnosis of malaria is necessary.

The aim of this endeavour is to find out the accuracy and authenticity of current method of detecting the presence and identification of the malaria parasite. Malaria is curable if effective treatment is started early. Delay in treatment may lead to serious consequences including death. An effective treatment is also important for controlling the malaria.

The present study is carried out in 60 patients suffering from malaria caused by *Plasmodium vivax*. Meerut region is dominated by *vivax* species of *Plasmodium*. While studying the changing pattern in the symptoms of *vivax* malaria it was found that some patients (recommended by doctors) were not positive for all the three methods used for the diagnosis of malaria parasite. The present study is focused on the specificity and sensitivity of the different techniques used for the detection of malaria parasite.

In the present study blood sample was taken from the patient by the trained pathologist recommended by the doctors and with the verbal resume of the patient at two multispecialty hospital of Meerut. A questionnaire was prepared for the symptoms beside the confirmation of the suspected species at their laboratory. The pathological confirmation of the malaria parasite was done at own research lab.

Materials and Methods

RDT (Rapid diagnostic test)

The suspected malaria patients were diagnosed by Rapid diagnostic test (Moody 2002 and Aslan *et.al*.2001) for malaria. It is a rapid colored immunochromatography assay for qualitative determination of antibodies of malarial parasites (p. f. /p. v.) in human blood. Nitro cellulose membrane of the test device is immobilized with highly specific recombinant antigens (Merozoite Surface Protien: MSP) of Pf at test line zone 1 and recombinant antigen (MSP) of Pv at test line zone 2. Human blood containing malarial antibodies is allowed to react with recombinant Pf/ Pv antigens coupled gold conjugate followed by reaction with recombinant antigens immobilized at test line zones. Appearance of pink /purple visible line in result window at test line zone (1&2) in addition to pink /Purple line "C" at control line zone, indicates positive test result. Appearance of pink/ purple line at control line "C" validates the procedure. Absence of any band in the test regions suggests a negative result. Appearance of pink/ purple line at "C" and 1 indicates *Plasmodium falciparum* infection and appearance of

pink/ purple line at "C" and 2 indicates *Plasmodium vivax* infection while the appearance of pink/ purple line at "C" 1and 2 indicates mixed infection with *Plasmodium vivax* and *Plasmodium falciparum* (Warhurst and Williams, 1996).

GIEMSA STAIN

Giemsa stain is used to differentiate nuclear and cytoplasmic morphology of platelets, RBCs, WBCs, and parasites. The most important dependable stain for blood parasites, particularly in thick films, is Giemsa stain containing azure B. Liquid stock is available commercially. The stain must be diluted for use with water buffered to pH6.8 or7.0 to7.2. The specimen usually consists of fresh whole blood collected by finger puncture or of whole blood containing EDTA (0.020g/10 ml of blood) that was collected by venipuncture and is less than 1h old. The slide may be a thin blood film that has been fixed in absolute methanol and allowed to dry, a thick blood film that has been allowed to dry thoroughly and is not fixed.

FLUORESCENT MICROSCOPY -

The antigen positive patients were also examined by fluorescent microscopy. The whole blood was collected by venipuncture or fingertip into the collection tube containing the anticoagulant (EDTA).The blood is filled into Microcapillary which is coated with Acridine orange. The fluorescence dye Acridine orange has an affinity for the nucleic acid in the parasite nucleus. The malaria parasite picks up fluorescent stain into their nucleus and cytoplasm, so that its morphological characteristics can be examined by fluorescent microscopy when excited by UV light at appropriate wavelength. The centrifugal quantitative buffy coat or QBC combines an acridine orange coated capillary tube and an internal float is formed. The float occupies the area midpoint between red cells and plasma and settle at different levels in the capillaries (Nandwani *et al.* 2003).

Discussion

Rapid diagnosis is prerequisite for institution of effective treatment and reducing the mortality and morbidity of malaria. This study was taken up to compare the efficacy of various rapid methods. Accuracy of clinical diagnosis varies with the level of endemicity, malaria season and age group. No single clinical algorithm is a universal predictor (Dicko *et. al.* 2005 and Mwangi *et.al.* 2005)

Above three methods were used for the identification of the species of malaria parasite. The present study was based on 60 severe malaria patients who were admitted to a multispeciality hospital at Meerut from April to September 2013 (summer season). (Table -1)

In1904, Gustav Giemsa introduced a mixture of methylene blue and eosin stains- (Fleischer 2004). Microscopic examination of Giemsa-stained blood smears has subsequently become the gold standard of malaria diagnosis.In the present study 50 out of 60 patients showed positive test results for Giemsa stain. This might have been due to low parasitemia. Several studies have shown that the ability to diagnose malaria by blood film examination alone is about 75% (Maline *et.al.*1998, Lee *et al* 1999.) The other reason for negative results may be due to washing of the slide during staining and there were insufficient malaria parasite for interpretation.

All malaria countries share a common need for reliable laboratory-diagnostic services to ensure early and rational treatment, reliable epidemiologic information and epidemic preparedness. Giemsa microscopy is regarded as the most suitable diagnostic instrument for malaria control because it is inexpensive to perform, able to differentiate malaria species, and quantify parasites. So microscopy has become more feasible in remote areas. However, microscopy requires well-trained, competent microscopists and rigorous maintenance of functional infrastructures plus effective quality control (QC) and quality assurance (QA). Poor microscopy has long been recognized in practice and is a function of multiple factors, including training and skills maintenance, slide preparation techniques, workload, condition of the microscope, and quality of essential laboratory supplies. Even among local laboratories with similar equipment and equal training and among reputed experts, abilities vary significantly. (Durrheim *et al.*1997 and Maguire *et al* 2006). This variability combined with the risk of untreated malaria in the face of safe, inexpensive therapy in the past led clinicians to treat febrile patients without regard to the laboratory results.(Othnigue *et al* 2006 and Zurovac *et. al* 2006). A well-trained, proficient microscopist should be able to recognize the *Plasmodium* species correctly in thick blood films at relatively low parasite density. Sometimes it may be necessary to check the thin film for morphologic, differential-diagnostic details such as erythrocyte size, shape, and crenation, characteristic dots in the erythrocyte stroma, pigment structure and color, as well as schizonts.

In the present study concentration of malarial parasite infected red blood cells by centrifugation coupled with staining with acridine orange and fluorescence microscopy (Quantitative Buffy Coat) system was reported to be positive in 41 out of 60 patients. There was difficulty in recognizing parasites using this

technique because other structures that fluorescence eg. Howell jolly bodies may have been mistaken for parasites (Lema *et.al* 1999).

Rapid diagnostic test seems to be most reliable and easy technique for the presence and identification of the malaria parasite. In the present study 58 patient out of 60 patients showed positive results by this technique. It can even be done by antimalarial personal without any laboratory.

Rapid diagnostic test is a device that detects malaria antigen in a small amount of blood, usually 5–15 iL, by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip. The result, usually a colored test line, is obtained in 5–20 min. RDTs require no capital investment or electricity, are simple to perform and are easy to interpret. Accuracy of RDTs is considered to be a useful diagnostic; RDTs must achieve greater than 95% sensitivity. (World Health Organization. 2010)

In developed countries, RDTs can be useful in screening febrile returnees from endemic areas. (Moody *et.al*.2000, Marx *et.al*.2005 and Jelinek *et.al* 2001). Self-use by travelers, however, produces variable outcomes (Jelinek *et.al* 1999 and Trachsler *et.al* 1999). RDTs are also recommended in situations exceeding microscopy capability, such as in an outbreak or in occupationally exposed groups (WHO 2004). As RDTs improve, including insensitivity for *Plasmodium vivax* and inability to measure parasitemia levels, at least semi-quantitatively, the scope of RDT applications will expand. Current RDTs are not intended to replace microscopy. Successful implementation of RDTs requires complex planning. Use of RDTs at peripheral levels such as by health workers, in informal health sectors and for self-diagnosis/self-treatment is a challenge (Bell *et.al* 2006). Implementation requires new local-level algorithms for actions to be taken based on RDT results. Quality RDT is a valuable complement to microscopy because it helps to expand the coverage of parasite-based diagnosis to the periphery and minimize exclusively clinical diagnosis.

Table No. -1 List of patients observed:

S.No	Age/Yrs	Sex	Giemsa stain	RDT	Fluorescence Microscopy
1.	70	F	Positive	Positive	Negative
2	15	M	Positive	Positive	Positive
3	24	M	Negative	Positive	Positive
4.	16	M	Positive	Positive	Negative
5.	19	M	Positive	Positive	Positive
6.	65	M	Positive	Positive	Positive
7.	20	F	Positive	Positive	Negative
8.	21	F	Negative	Positive	Positive
9.	40	M	Positive	Negative	Positive
10.	60	M	Positive	Positive	Negative
11.	20	M	Positive	Positive	Positive
12.	25	M	Positive	Positive	Positive
13.	3	F	Negative	Positive	Negative
14.	54	M	Positive	Positive	Positive
15.	22	M	Positive	Positive	Positive
16.	75	F	Positive	Positive	Negative
17.	13	F	Positive	Positive	Positive
18.	25	M	Positive	Positive	Positive
19.	40	M	Positive	Positive	Negative
20.	21	M	Negative	Positive	Positive
21.	48	M	Positive	Positive	Negative
22.	58	M	Positive	Positive	Positive
23.	25	F	Positive	Positive	Positive
24.	30	M	Positive	Positive	Negative
25.	58	F	Negative	Positive	Positive
26.	22	M	Positive	Positive	Positive
27.	35	M	Positive	Positive	Positive
28.	32	F	Positive	Positive	Negative
29.	20	M	Negative	Positive	Positive
30.	26	F	Positive	Positive	Positive
31	26	F	Positive	Positive	Positive
32	30	M	Positive	Positive	Positive
33	18	M	Positive	Positive	Positive
33	15	M	Negative	Positive	Positive
34	55	M	Positive	Positive	Negative
35	32	F	Positive	Positive	Positive
36	21	M	Positive	Positive	Negative
37	30	M	Positive	Positive	Positive
38	41	F	Positive	Positive	Negative
39	12	M	Negative	Positive	Positive
40	28	M	Positive	Positive	Negative
41	43	M	Positive	Positive	Positive

42	18	M	Positive	Positive	Positive
43	62	F	Positive	Positive	Negative
44	50	F	Positive	Positive	Positive
45	36	M	Negative	Positive	Positive
46	22	M	Positive	Negative	Negative
57	10	F	Positive	Positive	Positive
48	45	M	Positive	Positive	Positive
49	45	M	Positive	Positive	Negative
50	22	M	Positive	Positive	Positive
51	16	F	Negative	Positive	Positive
52	23	M	Positive	Positive	Negative
53	16	M	Positive	Positive	Positive
54	25	M	Positive	Positive	Negative
55	36	M	Positive	Positive	Positive
56	20	M	Negative	Positive	Positive
57	15	F	Positive	Positive	Positive
58	34	F	Positive	Positive	Negative
59	62	M	Positive	Positive	Positive
60	70	M	Positive	Positive	Positive

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