Section: Neurosurgery

Original Research Article

COMPARISION OF UTILITY OF IMMUNOCHROMATOGRAPHY TEST WITH BLOOD FILM MICROSCOPY FOR RAPID DIAGNOSIS OF MALARIA.

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Abstract

Background: Malaria caused by the protozoan parasite plasmodium species, presents a diagnostic challenge to laboratories in most countries. In India, about 70% of the infections are reported to be due to P.vivax; 25-30% due to P.falciparum that is commonly associated with complications. Microscopy & RDT are two important laboratory methods widely used in early diagnosis & treatment of malaria which currently play a significant role in malaria control. This study was aimed to compare peripheral smear and immunochromatography test for diagnosis of malaria. **Aims And Objectives**: 1. To detect malarial infection by RDT {rapid diagnostic tests} based on principle of Immunochromatography and Peripheral Blood Film Microscopy. 2.To compare the sensitivity and specificity of RDT with gold standard Blood film method. **Materials and Methods:** 1. BLOOD FILM MICROSCOPY: Thick and thin blood smears were prepared and stained with Giemsa stain. Thick smear were used for initial screening and quantification in positive smears while thin smears were used for further species identification. 2. **Immunochromatography Test:** Rapid test was performed using Advantage mal card (J.mitra & co pvt.ltd - NewDelhi). It detects PAN pLDH and P.falciparum specific pLDH. Results: Out of the 75 cases tested, 8% (6 out of 75) of the cases were positive by thick blood smear examination, of which all smears showed positivity for Plasmodium vivax. The Advantage Mal malaria card gave positive results for panspecific pLDH for 5 samples. The sensitivity, specificity, positive predictive value, negative predictive value of the rapid test is found to be 83%,100%, 100%, 98.5% respectively. **Conclusion:** In places where facilities are not available rapid diagnostic test devices can be used. Microscopy is simple, rapid, sensitive and specific, hence still remains the gold standard method for malaria diagnosis.

INTRODUCTION

Malaria caused by the protozoan parasite Plasmodium species, if left untreated can be fatal. Malaria presents a diagnostic challenge to laboratories in most countries. The common symptoms of malaria are intermittent high- fever with chills & rigors, headache, fatigue, abdominal discomfort, and muscle aches often misdiagnosed and it is one of the multiple differential diagnosis of fever of unknown origin. Hence the need to develop specific laboratory methods for identification of malarial parasites is utmost essential.[1]

P.vivax has the widest geographic distribution throughout the world. In India, about 70% of the infections are reported to be due to P.vivax; 25-30% due toP.falciparum that is commonly associated with complications and 4-8% due to mixed infection. P.malariae has a restricted distribution and is said to be responsible for less than 1% of the infections in India.[1]

The urgency and importance of obtaining results quickly from the examination of blood samples from patients with suspected acute malaria aid the clinical diagnosis and initiation of appropriate therapy. For the purposes laboratory methods that require more than 1 hr to provide a clear diagnosis of malaria are not considered rapid tests, although they may be considered reference procedures. Rapid diagnosis is prerequisite for effective treatment and reducing mortality and morbidity of malaria.

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Laboratory confirmation of malaria infection requires the availability of a rapid, sensitive, and specific test at an affordable cost. Diagnosis involves identification of malaria parasite or detection of its antigens/products such as P. falciparum–specific, histidine-rich protein 2 (PfHRP2), lactate dehydrogenase, or aldolase antigens.

Recently, many new rapid diagnostic tests like Quantitative Buffy Coat (QBC) examination and rapid antigen detection methods are being widely used but conventional method by smear microscopy remains the gold standard against which all other tests have been evaluated. Microscopy & RDT are two important laboratory methods widely used in early diagnosis & treatment of malaria which currently play a significant role in malaria control.

This study was aimed to compare peripheral smear and Immunochromatographic test for diagnosis of malaria.

Aim and Objectives

1. To detect malarial infection by RDT {Rapid diagnostic test} based on principle of Immunochromatography and Peripheral Blood Film Microscopy.
2. To compare the sensitivity and specificity of RDT with gold standard Blood film method.

MATERIALS AND METHODS

Sample Collection: After getting informed consent from the patients, 2ml venous blood was collected from each patient in EDTA coated test tubes for RDT and preparation of thick & thin blood film for smear microscopy.

Study Design: This prospective study was conducted at The Institute of Microbiology in association with The Institute of Internal Medicine, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai – 3 during the study period July 2016 to December 2016.

Sample Size: 75 patients.

Inclusion Criteria: Outpatients / inpatients aged ≥18years of Madras Medical College & Rajiv Gandhi Government General Hospital with clinical diagnosis of malaria are included in this study.

Exclusion Criteria: Patients who had Antimalarial treatment for past two weeks.

Conventional Blood Film Microscopy

Peripheral smear preparation

Thick and thin blood smears were prepared and stained with Giemsa stain. Thick blood film was stained as an unfixed preparation using diluted Giemsa stain. The thin blood film was methanol fixed and stained with diluted Giemsa using buffered water at pH 7.2 to emphasize the parasite inclusions in the RBC. After staining, the smears were examined at 100X magnification.[1,2,3]

Atleast100-200 fields, each containing 20 WBCs were examined before thick smear was reported as negative for malaria. Thick smear were used for initial screening and quantification (done for P. falciparum infection) in positive smears while thin smears were used for further species identification. The species identification of plasmodium was based on following observation: [4,5,6,7,8]

P.vivax: Parasitic appearance: Trophozoites (ring occupies 1/3rd of RBC) , schizonts (Large, completely fills the enlarged RBC. 12 - 24merozoites) & gametocytes (male- Round, pale blue cytoplasm; female- large, dark blue cytoplasm with pigments).

RBC changes: RBC's enlarged. Pale red Schuffner's dots increase in number as the parasite matures.

P.falciparum: Parasitic appearance: Early Trophozoites (ring occupies 1/6th of RBC – multiple rings, Accole, double dot forms seen) & Gametocytes (male: Banana-shaped, light blue; female: darker blue; blue-black pigment granules in cytoplasm)

RBC changes: Infects young & old RBC; RBC's normal in size; Maurer's cleft seen.

P.malariae: Parasite appearance: Trophozoites (band forms seen in late trophozoites), gametocytes & schizonts similar to P.vivax seen.

RBC changes: Infects old RBC's & they are normal in size. Ziemann's dots seen.

P.ovale: Parasitic appearance: Trophozoites, gametocytes & schizonts similar to P.vivax seen.

RBC changes: Infects young RBC's that become enlarged, oval with fimbriated margin. James's dots seen.

RAPID DIAGNOSTIC TEST BASED ON IMMUNOCHROMATOGRAPHY:

Rapid test was performed using Advantage mal card (J.mitra & co pvt.ltd - NewDelhi). It detects PAN pLDH and P.falciparum specific pLDH. ADVANTAGE MAL CARD is a visual, rapid and sensitive immunoassay for the qualitative diagnosis of P.falciparum and other Plasmodium Species (P.vivax/ P.malariae/ P.ovale/ P.falciparum) based on pLDH antigen in human whole blood.

- Produced only by viable parasites
- Used to diagnose infection & monitor response to treatment.

Principle

ADVANTAGE MAL CARD is an immunoassay based on the "Sandwich principle". The conjugate contains colloidal gold conjugated to monoclonal anti-pan specific pLDH (plasmodium lactate dehydrogenase) antibody. The test uses monoclonal anti-Pf pLDH antibody (test line F) & monoclonal anti-Pan specific pLDH antibody (test line P) immobilized on a nitrocellulose strip.

Procedure

1. The test sample is added to the device. On addition of assay buffer, the red blood cells get lysed.
2. If the sample contains P.falciparum or P.vivax/P.malariae/P.ovale, the colloidal gold conjugate complexes the P.f specific pLDH/Pan

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specific pLDH in the lysed sample. This complex migrates through the nitrocellulose strip by capillary action.

3. When the complex meets the line of the corresponding immobilized antibody, the complex is trapped forming a purplish pink band which confirms a reactive test result. Absence of a coloured band in the test region indicates a negative test result.
4. To serve as a procedural control an additional line of anti-mouse antibody has been immobilized on the strip as control.

Interpretation

- Appearance of three purplish pink coloured lines one each in P.f. region (F), Pan region (P) & Control region (C) indicates that the sample is reactive for P.falciparum and/or P. vivax / P. malariae / P. ovale.
- Appearance of two purplish pink coloured line one each at P & C region only indicates that the sample is reactive for P. vivax / P. malariae / P. ovale only.
- Depending on the concentration of pLDH positive results may be observed within 60 seconds. However, to confirm a negative result the test result should be read only at 20 minutes.

RESULTS

Maximum numbers of cases were seen between the age group of 21-40 years. The male to female ratio was 1.5:1. Fever with chills was the most common symptom (100%) followed by body ache, headache, abdominal pain, nausea, vomiting.

Table 1: Distribution of Symptoms: (N=75)

S.NO	SYMPTOMS	NO. OF PATENTS
1	Fever with chills	45
2	Fever with chills with Headache & myalgia	26
3	Fever with chills & abdominal pain, nausea,vomiting	4

Of the 75 samples tested, 8% (6 / 75) of the samples were positive by thick blood smear examination, of which all respective thin smears were positive for Plasmodium vivax. Out of 75 samples tested by ICT with Advantage Mal card 6% (5/75) were positive for panspecific pLDH.

Table 2: Distribution of smear and RDT positivity among samples. (n=75)

TOTAL SAMPLES n=75	NO OF THICK SMEAR POSITIVE (n=6)	NO OF THIN SMEAR POSITIVE (n=6)	RDT (n=5)
75	8%	8%	6%

Table 3: Concordance of RDT Method with Blood film (Thick Smear):

TEST	SMEAR POSITIVE	SMEAR NEGATIVE	TOTAL
RDT POSITIVE	5(a)	-(b)	5
RDT NEGATIVE	1(c)	69(d)	70
TOTAL	6	69	75

Concordance = 98.7%

Table 4: Comparison of Thin blood smear and RDT taking Thick blood smear examination as Gold standard:

TESTS	SENSITIVITY	SPECIFICITY	PPV	NPV
Thin blood smear	100%	100%	100%	100%
RDT	83.3%	100%	100%	98.5%

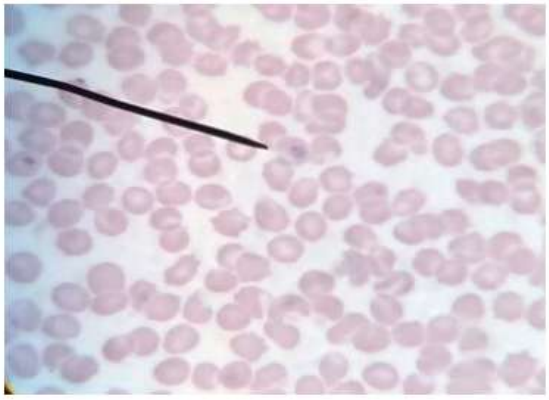

Figure 1: Giemsa Thin Blood smear Microscopy showing Ring form of P.vivax

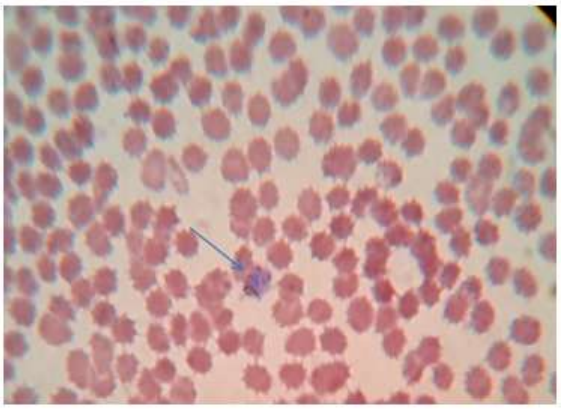

Figure 2: Giemsa Thin Blood Smear showing Schizont of P.vivax

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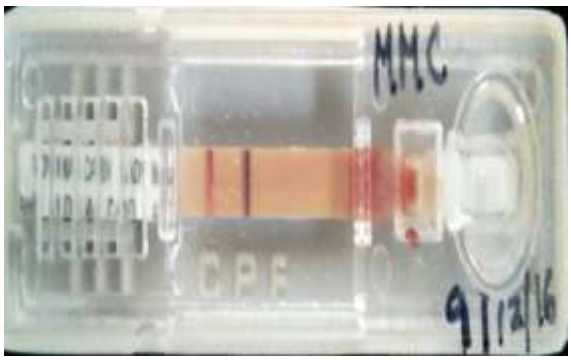

Figure 3: RDT showing positivity for pLDH

DISCUSSION

Malaria is a Parasitic infection of global importance and is a Major public health problem in India that accounts for Morbidity, Mortality and Economic loss. A key to effective management of malaria is prompt and accurate diagnosis. During the last decade, several new rapid diagnostic techniques have been developed and evaluated widely.[1,2,3]

The Present study aimed at prompt and accurate diagnosis of malaria parasite that helps in early start of appropriate Antimalarial drug to prevent the complications.

Microscopic analysis of appropriately stained thick and thin blood smears have been the standard diagnostic technique for identifying malaria for more than a century. This technique is capable of accurate and reliable diagnosis when performed by skilled Microscopist using defined protocols.[1,9] It is relatively inexpensive, sensitivity of thick and thin blood smears for detecting parasites are 5-10 parasites/µl and 200 parasites/ µl respectively. The infecting species and their relative densities can also be found by gold standard smear examination. However, it has limitations like time consuming, labour intensive and need for a skilled technician. other disadvantage with smear microscopy was in patients with P.falciparum malaria, sometimes the parasites can be sequestered and are not present in the peripheral blood. The average time taken for performance of smear microscopy was noted which was 90- 100min and that of the ICT was 1-20min.[4,5,6]

In the present study, the thick blood smear positivity was 8% among the 75 patients which is comparable to study done by Bhat Sandhya, Sastry Apurba S et al. where smear positivity was around 13% (1) All 6 samples were found to be positive for p.vivax infection.(table 1)

pLDH assay based on principle of immunochromatography detects all four Plasmodium species and can also be used to monitor the efficacy of drug therapy since it detects the enzyme(pLDH) which is only produced by viable parasites, so that parasitological clearance coincides with clinical improvement.[1] Disadvantages of this test- Its high cost, detects but cannot speciate the non falciparum malaria infection & inability to quantify the parasitic load and severity of the infection and low sensitivity when level of parasitaemia is <100 parasites/ µl.[7,8] But the advantage is the rapidity of test that is the time taken for performing RDT was 1minute when compared to 1hour for smear Microscopy.

In present study, out of 75 samples 5 were detected to be positive for pLDH. The sensitivity, specificity, positive predictive value, negative predictive value of the rapid test is found to be 83%,100%, 100%, 98.5% respectively (table 2). A low level of parasite load could be a reason for a negative RDT in 1sample that is positive for malarial infection by peripheral Blood smear Examination. The results of present study regarding sensitivity, sensitivity are consistent with study by Anthony Moody where sensitivity varies between 80-100%.[9,10,11] The concordance of RDT with Blood film method was 98.7%.

CONCLUSION

There are many considerations to be taken into account when reviewing the methods for laboratory diagnosis of malaria like sensitivity, rapidity, availability. Rapid antigen detection kits are useful in places where facilities are not available & in endemic areas but it does not exclude the necessity of reviewing correctly stained thick and thin blood films as the standard procedure when malaria is suspected. The lower sensitivity (83%) of RDT observed in our study implies that a negative RDT does not necessarily indicate an absence of Malarial infection. Microscopy is simple, rapid, sensitive and specific, hence still remains the gold standard method for malaria diagnosis. As the peripheral Blood film method is time consuming, labour intensive and needs skilled Microscopist, Immunochromatographic dipstick assays offer the possibility of more rapid, nonmicroscopic method for Malaria diagnosis, thereby saving on training and time, thereby making it more suitable for point of care testing.

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