ORIGINAL ARTICLE

COMPARISON OF MICROSCOPIC METHOD AND IMMUNO-CHROMATOGRAPHIC TECHNIQUE IN DETECTING PLASMODIUM SPECIES

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ABSTRACT

Background and Objective: Light microscopy of thick and thin films is a cost effective gold standard for diagnosis of malaria yet it is time consuming and requires expertise. Immunochromatographic Technique (ICT) has been claimed an alternative to light microscopy in detecting Plasmodium species. This study was conducted to evaluate sensitivity and specificity of rapid malaria test in reference to light microscopy of the smears.

Methods: This study was conducted out in Mansoorah Hospital Lahore between July and November 2015. Seventy patients with history suggestive of malaria were subjected to both tests i.e. light microscopy and Immunochromatographic Technique (ICT) malaria P.f./Pan Rapid Test Device for comparison of two methods.

Results: The blood film results indicated that 32 (45.71%) patients were infected with malaria and the rest 38 (54.28%) were malaria negative. Among the positive patients P.falciparum was detected in 10 cases (31.2%) and non-falciparum plasmodium species were found in rest of the 22 cases (68.75%). The ICT malaria P.f./Pan Test results showed that 30 (42.85%) of the patient samples were positive for malaria parasites and rest 40 (57.14%) were negative for malarial parasite. Infection with P. falciparum accounted for 10 cases (33.34%) and non-falciparum plasmodium species cases accounted for rest of the 20 (66.67%) cases. Thus, ICT malaria showed 93.75% sensitivity and 95.00% specificity for detection of malarial parasites.

Conclusions: Immunochromatographic technique provides sensitive, specific, user – friendly and practical alternative to slide microscopy for diagnosis of malaria without adding cost and effort.

Key words: Malaria, plasmodium falciparum, plasmodium vivax, plasmodium ovale, plasmodium malariae, Immunochromatographic technique, thick and thin films, light microscopy, diagnostic test, sensitivity, specificity

INTRODUCTION

Malaria is one of the commonest parasitic diseases. Worldwide it infects about 200 million people and about 2 million people are killed each year due to malaria.[1]

A rapid and accurate diagnosis is the first step in the effective management of malaria. Several laboratory procedures like routine light microscopy, immunological methods for antigen detection, polymerase chain reaction, specie specific DNA probe method and ribosomal RNA method have been developed for the diagnosis of malaria.[2] Out of these the most widely used method for demonstration of plasmodium species is by thick and thin films. This method is considered gold standard because it is relatively simple and cheap.[3-5] But the drawbacks of routine microscopy are it is time consuming, require considerable expertise and its reliability is questionable when the levels of parasitemia are low.[1,6]

Immunochromatographic technique (ICT) is one of the newly developed techniques for the rapid diagnosis of malaria.[1] This method is based on malarial antigen detection that is released from parasitized cells. This is a new technique developed for situations where reliable microscopy is not available.[7] Malarial antigens detected by ICT are histidine rich proteins-2 (HRP-2), plasmodium LDH and plasmodium aldolase.[8,9] Dipstick format kits are commercially available for detection of malarial antigens with excellent sensitivity and specificity.[1]

This study was conducted to evaluate sensitivity and specificity of rapid malaria test in reference to the conventional light microscopy.

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PATIENTS AND METHODS

This study was conducted out in Mansoorah Hospital Lahore between July and November 2015. Seventy patients of all ages and sexes with history of high grade fever associated with chills and rigors, and other non-specific symptoms like generalized body aches, fatigue and abdominal discomfort were included in the study. Patients who received any antimalarial drug or have any other known cause of fever and critically ill patients were excluded from the study. Venous blood was collected from each patient into a sterile vial containing anticoagulant EDTA.

Microscopy

Thick and thin smear blood films were prepared and stained with Giemsa's technique. All slides were examined for malaria parasites independently by two microscopists by light microscope. A thin blood smear was examined for 15 minutes and for a thick blood smear, 200 fields were visualized. The results were recorded.

ICT Malaria

Blood samples were tested with ICT malaria P.f./Pan Rapid Test Device manufactured by ABON PLUS Biopharm (Hangzhou) Co. Ltd. It is a qualitative membrane bound assay for the detection of plasmodium falciparum, plasmodium vivax, plasmodium ovale and plasmodium malariae. The membrane is precoated with anti-HRP 2 specific antibodies for plasmodium falciparum and plasmodium specific anti aldolase antibodies for the detection of other three species of plasmodium. Test was performed as per manufacturer's instructions.

Interpretation of the assay test results was done as below:

(i) Test was considered P. falciparum positive if one line appear in the control region and one in the plasmodium falciparum specific region.
(ii) Test was considered as non-falciparum plasmodium specie specific positive if one line appear in the control region and one in the pan malarial region
(iii) Test was considered to be mixed infection if one line appear in the control region, one in the plasmodium falciparum region and one in the pan malarial region.
(iv) Test was considered to be negative if one line appear only in the control region.

RESULTS

A total of 70 blood samples were tested for malarial parasites by the ICT malaria P.f/Pan test device and the light microscopy and the results were compared. The blood film results indicated that 32 (45.71%) patients were infected with malaria and the rest 38 (54.28%) were malaria negative. Among the positive patients P. falciparum was detected in 10 cases (31.2%) and non-falciparum plasmodium species were found in rest of the 22 cases (68.75%). Mixed infection is found in none of them.

Correspondingly, the ICT malaria P.f./Pan Test results showed that 30 (42.85%) of the patient samples were positive for malaria parasites and rest 40 (57.14%) were negative for malarial parasite. Infection with P. falciparum accounted for 10 cases (33.34%) and non-falciparum plasmodium species cases accounted for rest of the 20 (66.67%) cases. Mixed infection is found in none of the case again.

Table 1: *Rate of detection of malarial parasite by different methods.*

Test	*Results*	
	Positive	*Negative*
Peripheral blood film (n = 70)	32 (45.71%)	38 (54.28%)
ICT method (n = 70)	30 (42.85%)	40 (57.14%)

Table 2: *Evaluation of ICT as Diagnostic Test for Malarial Parasite.*

Test (ICT)	*Malarial Parasite*		*Total*
	Present	*Absent*	
Positive	30 = a	02 = c	32 = a + c
Negative	02 = b	38 = d	40 = b + d
Total	32 = a + b	40 = c + d	72

a = True positive, b = False negative, c = False positive
d = True negative

$$\text{Sensitivity} = \frac{a}{a+b} \qquad \text{Specificity} = \frac{d}{c+d}$$

Considering microscopy as gold standard for the diagnosis of malarial parasite, current study finds sensitivity and specificity of ICT malaria as high as 93.75 (95% confidence interval: 79.19 – 99.23) % and 95.00 (95% confidence interval: 83.08 – 99.39) % respectively.

DISCUSSION

Although the gold standard for diagnosis of malaria remains microscopy yet there are a number of constraints such as lack of trained microscopists, time consumed for microscopy. An easily performed, rapid, and accurate test for the detection of plasmodial infections is needed especially in countries like Pakistan with endemic malaria, huge population and scarcity of trained microscopists. Immunochromatographic technique (ICT) provides an opportunity to diagnose mala-

ria earlier in the course of disease, and facilitate an appropriate therapy in malarial patients, thereby reducing mortality.[10-13]

Current study was performed to compare ICT with microscopic method of malarial parasite detection and to determine diagnostic accuracy (sensitivity and specificity) of ICT method whereby considering microscopy as gold standard. The results showed sensitivity of 93.75 (95% confidence interval: 79.19 – 99.23) % and specificity of 95.00 (95% confidence interval: 83.08 – 99.39) %. These findings are consistent with a number of similar studies conducted within and outside Pakistan. Findings from a few pertinent studies are summarized below. Jan Mohammad et al.[14] showed that ICT yielded a very high sensitivity (96.1%) and Specificity (95.7%) for Malaria. The false positive rates and false negative rates were also very low, being 4.3% and 3.9% respectively. According to Zareen Fasih et al.[15] ICT method was found to have highly specific (reaching 91%) and 85% sensitive in detection of Malaria in children. Positive Predictive Value (PPV) of ICT is 68%. Negative Predictive Value (NPV) of ICT is 96%. Jahan Zeb and coworkers[16] used two rapid test techniques i.e ICT and OptiMAL devices showing 100% sensitivity and specificity for P.falciparum and 75 to 87.5% sensitivity and 100% specificity for P.vivax. Sheikh S et al, showed that rapid diagnostic tests (RDTs) had same sensitivity and specificity compared with routine microscopy. The sensitivity, specificity positive predictive value and negative predictive value of RDT was found to be 95%, 91.6%, 0.55% and 99.3% respectively. Mahadev Harani[1] also found similar results i.e. in their study, for P.falciparum the ICT was 97.0% sensitive, 98.3% specific, with positive predictive value (PPV) of 78.0% and a negative predictive value (NPV) of 99.8%. For P.vivax the sensitivity was only 89.7%, specificity 97.9%, PPV was 70.3% and NPV 99.4%.[17] Batwala V et al, found that the sensitivity of presumptive diagnosis based on axillary temperature, health care centre (HC) microscopy and rapid diagnostic test were: 42.6%, 85.1% and 97.9% respectively. The corresponding specificity rates were found to be 73.1%, 93.7% and 74.7% respectively, thus found that the malarial antigen based tests demonstrated a superior sensitivity compared to microscopy.[18]

Considering consistent findings of all these studies in favour of rapid, easily performed, sensitive and specific ICT test and taking in account the prevalence and complexity of malarial disease, and rapidly spreading drug resistance to antimalarial medication in Pakistan[19-21], it would be beneficial to look at ICT as an accepted standard to diagnose malarial parasite.

It is ***concluded*** that immunochromatographic technique provides sensitive, specific, user – friendly and practical alternative to slide microscopy for diagnosis of malaria without adding cost and effort.

Conflict of Interest: None.

Author's Contributions

R. J. performed light microscopy and ICT method and participated in article writing and statistical analysis of the data. R. S. reviewed the article and gave expert suggestions. T. M. and A. W. participated in data collection and sample collection of the patients. All authors contributed in this study and preparation of manuscript.

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