Original Article

Immunochromatographic Methods in Malaria Diagnosis

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Abstract

Background: Malaria remains a major cause of morbidity and mortality in India. This study was carried out to evaluate the use of parasite lactate dehydrogenase (pLDH) test in diagnosis of malaria.
Methods: Blood slides of 400 patients who presented with fever including 104 patients with clinical features suggestive of malaria were studied. The results were compared with microscopy and another immunochromatography test (ICT) based on detection of histidine rich protein-2 antigen [Pfhrp-2] secreted by *Plasmodium falciparum*.
Result: In this study the sensitivity and specificity for detection of *Plasmodium vivax* was 100% while for *Plasmodium falciparum* the values were 96% and 100% respectively.
Conclusion: ICT is useful for diagnosis of malaria caused by *Plasmodium falciparum* in field but microscopy of a well-prepared blood smear must not be omitted in a laboratory setting.

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Key Words: Malaria; Immunochromatography

Introduction

Malaria is estimated to kill between 1.5 and 2.7 million people each year with an average of one death every 12 seconds [1]. The morbidity due to malaria is estimated at around 300-500 million every year [2] and the clinical diagnosis is at best 50% accurate [3]. Light microscopy is the gold standard but it requires considerable expertise and time for examination especially in patients with low parasitemia [1]. Sometimes in *Plasmodium falciparum* malaria, the parasites may be sequestered and are not present in peripheral blood. The enzyme parasite lactate dehydorgenase (pLDH) is a unique enzyme of glycolytic pathway present in all malarial parasites infecting man and can be differentiated from host LDH (hLDH) with 3-acetylpyridine adenine dinucleotide (APAD) an analogue of nicotine adenine dinucleotide (NAD) [4]. Table 1 shows the biochemistry of cofactor binding to the hLDH and pLDH enzymes. Although the Michaelis-Menten's constant (K_m) is similar, the K_{cat} (turnover number) of the pLDH in the presence of APAD is much greater than the human enzyme with the same cofactor. In this study we have compared immunochromatography test (ICT) based on pLDH enzyme secreted by *P falciparum* for diagnosis of malaria in 104 patients with results of direct microscopy and with another ICT based upon detection of histidine rich protein-2 antigen (Pfhrp-2) secreted by *P falciparum* for malaria diagnosis.

Material and Methods

This prospective study was undertaken to study the accuracy of ICT and direct microscopy for the diagnosis of malaria. This study was performed during the four month period, from July to October 2003 and 1343 blood slides from 400 fever cases were examined. Of these 400 cases, 104 patients presented with clinical features suggestive of malaria with or without splenomegaly, systemic complications or central nervous system manifestations. Fifty age and sex matched controls out of the non-malaria cases were included

Table 1

Biochemistry of cofactor binding to hLDH and pLDH at pH 9.2 and 25°C

Cofactor	hLDH isozyme A4		hLDH isozyme B4		Recombinant pLDH	
	K_{cat} (per min)	K_m (μM)	K_{cat} (per min)	K_m (μM)	K_{cat} (per min)	K_m (μM)
NAD^+	961,000	93	630,000	37	241,000	210
$APAD^+$	19,000	56	4,600	37	2,280,000	120

+Nicotinamide adenine dinucleotide (NAD), +3 acetylpyridine adenine dinucleotide (APAD)

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in this study. Giemsa stained thick and thin films were made for all patients as per World Health Organization [5] recommendation and examined three times followed by ICT.

Fresh blood was collected in ethylenediamine tetraacetic acid (EDTA) and the test was carried out as per recommendations of the manufacturers. A thick smear was considered negative if after 10 minutes of searching no parasites were detected [6]. The Diamed OptiMAL® Rapid Malaria Test kits [48-test pack size] marketed by Diamed, Cressier, Switzerland was used in this study. This assay uses two monoclonal antibodies, one specific for *P falciparum* and the other recognizing all four *Plasmodium* species infecting man. For ICT 10 ml of whole blood sample was mixed with 30 ml of lysing buffer containing conjugated monoclonal antibody. This mixture was allowed to soak into the OptiMAL® dipstick. After eight minutes 100 ml of clearing buffer was added. If *P falciparum* is present in the sample, 3 lines appear. Two lines indicate infection by other species – commonest being *P vivax* in India. Four samples, which were negative for ICT using Diamed OptiMAL® kit, were tested using Parachek kit manufactured by Orchid Diagnostics, Australia and the results were concordant. All patients were followed up for clinical response to antimalarials. Parachek Pf is based on detection of histidine rich protein-2 antigen (HRP-2) specific to *P falciparum* by immunochromatographic technique. It is released in large amount during rupture of schizonts and is considered pathognomonic of trophozoite and merozoite stages of falciparum infection. In the test procedure, specimen of blood is reacted with the antibodies of HRP-2 antigen and chromogenic substance tagged to the antibodies makes this reaction visible. Non-development of specific colour band denotes absence of HRP-2 antigen and excludes *P falciparum* infection.

Results

During the study period 1343 slides from 400 fever cases were received, of which 104 smears were diagnosed as malaria based on the results of microscopy or ICT. A total of 104 cases were diagnosed as malaria, of which 73 were caused by *P falciparum*, 25 by *P vivax* and six had mixed infection. Eightysix cases were positive by both microscopy and ICT. Fourteen patients were positive on ICT but negative on smear. Four patients were negative on ICT using both OptiMAL® and Parachek kits but showed *P falciparum* on microscopy. The smear contained ring forms and the parasite density was < 60 parasites/ ml. Six patients who were positive on smear for only *P vivax* showed similar results on ICT. Two of them had acute renal failure and four developed icterus with serum bilirubin in the range of 3.5-8 mg/dl. The smears were negative for parasites. In this study the sensitivity and specificity for detection of *P vivax* was 100% while the values for *P falciparum* were 96% and 100% respectively (Table 2).

Table 2

Malarial parasite detection by blood films and by OptiMAL

	OptiMAL Result		Blood film Result	
Total	Positive	Negative	Positive	Negative
P vivax	25	0	25	0
P falciparum	69	4	59	14
Mixed infection	6	0	6	0

Discussion

Conventional microscopy has undergone very little improvement since its development in the early 1900s [1]. Both thick and thin smears have to be examined, as some authors [6] report that thin blood smears are only one tenth as sensitive as examination of thick smears. In armed forces, we found that microscopy is adequate for diagnosis of malaria in > 95% of patients presenting with fever and many technicians tend to miss *P falciparum* parasites with monocular microscopes. Therefore, the kit will be extremely useful in remote areas. Identification of *P vivax* is never a problem for most of the laboratory technicians and pathologists.

OptiMAL® and Parachek kits are commonly available rapid immunochromatography based tests for rapid diagnosis of malaria with high sensitivity and specificity especially in cases of low parasitemia. The advantages of OptiMAL® include the ability to detect both common species of plasmodium and its use for monitoring antimalarial treatment. This kit cannot discern between mixed infections and only *P falciparum* parasitemia, as both show as three lines, and once again microscopy provides the answer. Parasite level in peripheral blood has been shown to correlate with pLDH levels [7] and the kit may be used to identify resistant cases which cannot be done with Parachek kits as the Pfhrp-2 persists for 10 days or more [8, 9]. In this study the sensitivity and specificity for detection of *P vivax* was 100% while for *P falciparum* the values were 96% and 100% respectively. In an earlier study [9], the authors analysed ICT based on Pfhrp-2 and found it a useful adjunct to blood smear examination. Gokhale[10], has evaluated the Parachek Pf kit and found it particularly useful for diagnosis of falciparum infection in far flung places. We have compared the results of this study with other studies (Table 3). The advantages of ICT include its high sensitivity, no specialized training and shorter time (10-15 minutes) to complete the test. The reported sensitivity of ICT kits in samples with parasite density of less than 50 parasites /ml is in the range of 50-70%, while for 50-100 parasites / ml it is > 90 %. The sensitivity and specificity in the present study was higher than reported by Cook et al [11]. Palmer et al [12], found 100% specificity with sensitivity of 94% for *P vivax* and 88% for *P falciparum*. The high cost of the ICT (Rs 200/ per test) precludes their routine use but they are extremely useful for smear negative patients and in patients with altered sensorium for exclusion of cerebral malaria, shock and in pregnant females with malaria. In a large study, 20% of pregnant ladies had placental

Table 3

A comparison of the sensitivity and specificity of various studies

Authors	Sensitivity (%)	Specificity (%)
Cooke *et al* [11]	91.3	92
Gupta *et al* [9]	87.5	100
Palmer *et al* [12]	94 (*P vivax*)	100
	88 (*P falciparum*)	99.9
This study	100 (*P vivax*)	100
	96 (*P falciparum*)	100

malaria, but the peripheral blood smear was negative [13]. In a study which compared quantitative buffy coat (QBC) method and Pfhrp-2 based detection kit *Para*Sight® (Becton Dickinson) for falciparum malaria, authors report high rate of false positive results and low positive predictive value of QBC as compared to ICT [14].

Significant haemolysis and acute renal shut down are not known with vivax infections, but we had six patients of confirmed *P vivax* infection with complications. The possibility of a concomitant *P falciparum* infection with low parasite density being missed, cannot be entirely ruled out. Gupta et al [9], have also reported one smear positive case with mixed infection by *P falciparum* and *P vivax,* which gave a negative result with ICT. Other studies [14-16], also show excellent results with OptiMal® for diagnosis and treatment of malaria with a sensitivity of 88 - 98% and specificity exceeding 95%.

We recommend the use of microscopy as first line of investigation for all patients and ICT for patients with a negative smear in whom a strong suspicion of malaria exists. ICT may also be used as an initial investigation in critically ill patients who are suspected to have malaria where the parasites may be sequestrated. Polymerase chain reaction based diagnosis of malaria although gives far better results is confined to few centres in India [17] .

Conflicts of Interest

None identified

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