IMMUNOCHROMATOGRAPHIC TEST : A NEW DIMENSION IN DIAGNOSIS OF PLASMODIUM FALCIPARUM MALARIA

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Abstract

75 patients with clinical features suggestive of malaria were studied to evaluate the efficacy of immunochromatographic test (ICT), which detects histidine rich protein-2 antigen secreted by *Plasmodium falciparum* (Pfhrp-2), as against direct microscopy. There were 40 cases of *P falciparum* malaria, 14 cases of *P vivax* malaria and 21 cases of non-malarial fevers. Direct microscopy could detect 27(67.5%) *P falciparum* cases but failed to detect 13 cases (32.5%) whereas ICT could detect 35(87.5%) *P falciparum* cases out of 40 but failed to detect 5(12.5%) cases. All the *P vivax* cases and non-malarial fever cases were negative for ICT. The sensitivity and specificity of ICT is 87.5% and 100% respectively where as the positive predictive value and the negative predictive value of the test is 100% and 87.5% respectively. It is concluded that ICT test is a good adjunct to blood smear studies in fever cases with neurological and multiorgan dysfunction and in antenatal ladies.

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Introduction

Early diagnosis of *Plasmodium falciparum* infection remains one of the most important challenges in our country, particularly in the endemic zones. Though detection of *P falciparum* by microscopy still remains the gold standard, it may not be detected in the peripheral blood [1]. Identification of malarial parasite species is extremely important because *P falciparum* produces overwhelming parasitaemia by invading red cells of all ages, development of drug resistance and fatal complications compared to other species [2]. *P falciparum* synthesizes histidine rich protein-2 (HRP-2) and releases this protein from the infected erythrocytes into blood stream [3]. This protein is secreted as a water soluble antigen and is present during acute infections [4]. Studies have been conducted to detect the persistence of *P falciparum* antigen after a full course of antimalarial treatment [5]. We have undertaken a study to compare the diagnostic efficacy of direct microscopy with immuno chromatographic test which detects *P falciparum* HRP-2 (pfHRP-2), in a tertiary care Naval hospital.

Material and Methods

This prospective study was undertaken in INHS Asvini, Mumbai to compare the accuracy of the two readily available methods for diagnosis of *P falciparum* infection, (i) direct microscopy & (ii) detection of pfHRP-2 by an immunochromatography test (ICT). A total of 75 patients were studied who had clinical features suggestive of malaria with or without splenomegaly, systemic complications or central nervous system involvement. The thick and thin Giemsa stained blood smears were screened three times followed by an ICT [6,7]. Fresh blood from the patients was collected in EDTA/heparin and the test carried out as per the recommendations of the manufacturers. The kits from ICT Malaria Plasmodium falciparum, ICT diagnostics, Australia; and Paracheck, Orchid diagnostics, Orchid Biomedical System were used. The test in short uses two antibodies specific for pfHRP-2 antigen. One of the antibodies is attached to a visible colloidal gold impregnated into sample pad, while the second antibody is immobilised as a line across the test strip. 10 µL of whole blood is added to the sample pad where lysis occurs and pfHRP-2 antigen if present, binds to the colloidal gold labelled antibody. When a given reagent is added to the sample pad, blood and labelled antibody migrate towards test strip crossing the second antibody line. Appearance of a pink line indicates binding of pfHRP-2 to second antibody and thus presence of *P falciparum* antigen. The patients were followed for the response to antimalarials and the presence of the antigen was checked for at intervals of 5,10 and 15 days. 21 age and sex matched nonmalarial fever cases were selected as controls. All cases were tested for Rheumatoid factor and found to be negative [8].

Results

A total of 75 patients who presented with fever and other signs and symptoms suggestive of malaria were studied. The diagnosis of malaria was confirmed in 54 (Table-1). Out of 40 cases diagnosed as *P falciparum* malaria, 22 were positive by both direct microscopy and ICT. 27(67.5%) cases were *P falciparum positive*

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by direct microscopy. ICT was positive in 35(87.5%). *P vivax* was present in 14 cases (Table-2). In none of the 21 nonmalarial fevers and *P vivax* cases ICT was positive, accounting for 100% specificity. But it failed to detect 5 smear positive cases accounting for 87.5% sensitivity. The positive predictive value of the test is 100% and the negative predictive value is 87.5%.

TABLE 1
Clinical presentation and distribution of cases

Clinical presentation	(n=75)	P falciparum No (%)	P vivax No (%)	Nonmalarial cases No (%)
Fever with chills	(75)	40 (100)	14 (100)	21 (100)
Splenomegaly	(26)	18 (45)	5 (36)	3 (14)
Hepatomegaly	(10)	5 (12.5)	3 (21)	2 (9.5)
Septicaemia, shock	(6)	6 (15)	-	-
DIC / thrombocytopenia	(6)	6 (15)	-	-
CNS manifestation	(8)	8 (20)	-	-

TABLE 2
Comparision of blood smear and ICT

Case type (Total No.)		Blood smear MT(+)	Blood smear MT(-)	ICT test Positive	ICT test Negative
P falciparum	(40)	27 (67.5%)	13 (32.5%)	35 (87.5%)	5 (12.5%)
P vivax	(14)	0	14	0	14
Controls	(21)	0	21	0	21
Total	75	27	48	35	40

TABLE 3
Comparision of sensitivity and specificity among various studies

Authors	Sensitivity	Specificity
Mishra *et al*	97	100
Kumar *et al*	100	98.7
Kar	100	100
Kharakurwa	93	92* 72** 85***
Present study	87.5	100

* - Hypoendemic; ** - Mesoendemic; *** - Hyperendemic

Discussion

In this study the specificity and sensitivity of ICT test was found to be 100% and 87.5% respectively. Different studies in India have specificity and sensitivity ranging from 97-100% (Table-3). Our findings are more close to study conducted by Mishra et al [5]. 93% sensitivity and variable specificity of 85%, 72% and 92% was recorded in hyperendemic, mesoendemic and hypoendemic zones respectively in a study in Zimbawe [11]. The findings of present study is comparable to that of hyperendemic zone in Zimbawe study. The ICT test in our study had a positive predictive value of 100% and negative predictive value of 87.5% as against 98.4% and 100% respectively reported by Kumar et al [10].

Though smear positivity is considered as gold standard, it is well documented that in some cases the parasite is localised only in the internal organs. A large study in South-Africa revealed that as many as 20% pregnant ladies had placental malaria but failed detection in the peripheral blood smear [1]. 1 of our patients had *P falciparum* malaria during her third trimester resulting in still birth. Histopathological examination of placenta revealed heavily parasitized erythrocytes. In 8 of our patients there was enough clinical evidence of *P falciparum* malaria, such as fever with chills and rigor, hepatosplenomegaly, central nervous symptoms and multiple organ dysfunction, but their peripheral blood smears were repeatedly negative for *P falciparum* though ICT test was positive. 6 of them responded to antimalarial treatment but 2 ended in fatality due to thrombocytopenia and disseminated intravascular coagulation.

1 of our cases was smear positive for both *P falciparum* and *P vivax* but the ICT test was negative. Though there is no plausible explanation in literature, there could be a masking of antigen due to the presence of both types of parasites. This is in contrast to the findings of Kumar et al [10].

The pfHRP-2 antigen has been shown to persist upto 15 days after initiation of antimalarial therapy [5]. Kharakurwa et al reported gradual decline of antigen after starting chloroquine treatment and it almost disappeared by 10^{th} day [11]. In our cases who were followed after treatment the antigen was detected by ICT upto 15 days. This has two implications :

a. If the patient is treated successfully, persistence of the antigen for 15 days does not necessarily mean treatment failure.

b. Treatment history with antimalarials is important before planning fresh treatment.

Usually the antigen becomes negative after this time and if positive indicates fresh infection [12]. 1 of the *P falciparum* cases in our study was also HIV positive and pfHRP-2 could be detected upto 30 days. Deficiency in cell mediated immunity (CMI) could be the reason for the persistence of antigen. 2 other HIV positive cases with heavy *P vivax* parasitaemia were found to be ICT negative.

The ICT test was helpful in the diagnosis of *P falciparum* infection in cases of fever with shock, seizure or abnormal behaviour where the parasitaemia may be either low or absent as the parasites may be sequestrated in the internal organs. In the latter situation

antigen can be detected by ICT and treatment instituted successfully in most of the cases.

We conclude that ICT for *P falciparum* has specificity of 100% with sensitivity varying between 87-100% and is a good adjunct to blood smear studies. It is particularly useful in cases of fever with neurological and multiorgan dysfunction as well as in antenatal cases.

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