Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2015; 3(2D):821-824 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2015.v03i02.057

Research Article

Diagnosis of Malaria by OptiMal Immuno-Chromatographic Antigen and Microscopy: A Comparison

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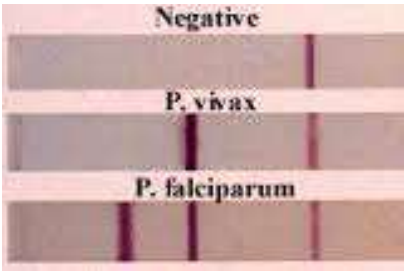
Abstract: The objective of the study was to compare OptiMAL test method with conventional microscopy for the detection of Malaria. 150 patients below the age of 15 years who were clinically suspected to be suffering from Malaria from July'14 - Feb'15 were studied. OptiMAL test and Light microscopic examination of thick and thin blood films were done and the results compared. Out of 150 suspected cases of malaria 80 patients (46 falc, 34 viv) were positive with microscopy while only 76 patients (43 falc, 33 viv) were positive with OptiMAL. The sensitivity and specificity for *Plasmodium falciparum* was 93.47%, and 98.57%, and for *Plasmodium vivax* 97.05% and 100%. OptiMal test is simple, effective and rapid diagnostic test which can be used with or without traditional blood film examination for detection of both *P. vivax* and *P. falciparum*.
Keywords: OptiMAL test method, Conventional microscopy, *Plasmodium falciparum*, *Plasmodium vivax*.

INTRODUCTION

Rapid accurate diagnosis is necessary for effective management of malaria and prevention of rampant drug resistance [1-3]. Modern methods include fluorescent microscopy, PCR, molecular methods and laser desorption mass spectrometry, flow cytometry and serology antibody detection and were limited due to need for complex apparatus, unavailability, high cost and lack of specificity and sensitivity [1, 4]. Light microscopic examination, an old gold standard technique can provide good sensitivity and specificity [5, 6] but It is laborious, requires trained personnel, time taking resulting in delay of treatment, sometimes difficult to detect *P. falciparum* if sequestered and may not be available in rural areas [5]. Diagnosis by clinical syndrome alone is not reliable needs proper rapid diagnosis close to sensitivity of microscopy. Thus the evolution of rapid diagnostic tests which include older ones using histidine-rich protein 2 antigen (HRP-2) secreted by *P. falciparum* alone such as Para Sight F test, ICT Malaria PF test to more recent OptiMal which detects parasite specific lactate dehydrogenase (pLDH), a specific glycolytic enzyme secreted by viable parasites of all plasmodia species [1, 4]. Many studies have shown that rapid diagnostic test detects malaria in 10-15 minutes, can be done at bed side, is simple, doesn't require skilled personnel and is highly specific. The only limitation is lesser sensitivity when the parasite density is <100parasites/ul [1, 4]. PLDH persists for only 7-10 days compared to HRP-2 which persists for prolonged periods of 7-28 days. Hence the OptiMAL has also been used to monitor treatment outcome [3, 4]. The OptiMAL test has been shown to be better than the HRP-2 tests as it detects all species of plasmodia and can be used for monitoring treatment [3, 4].

MATERIALS AND METHODS

This study was conducted during July '14 to Feb '15, on a series of 150 patients under the age of 15 yrs. These patients were clinically suspected of having malaria when fever with at least two findings of pallor, splenomegaly, jaundice and convulsions, were present. Blood samples were collected in potassium EDTA and thick and thin smear blood films made. They were stained with Giemsa stain and examined for malarial parasite by light microscopy. AII these blood samples were also tested with the OptiMAL immunochromatographic test for pLDH. The pLDH first binds to a gold labeled antibody particle and this complex migrates to the test strip which is captured by an immobilized second antibody forming an Ab-Ag-Ab at reaction site. This test was interpreted as follows:



- When one control and two test bands appeared, the test was considered positive for *P. falciparum*.
- When one control and one test band appeared, the test was considered positive for *P. vivax*.
- When only control band and no test band appeared, the test was considered negative.

RESULTS

All 150 patients were tested for malarial parasite by microscopy and OptiMAL method. With microscopy 80 patients (53.33%) were positive (46 falc. and 34 viv). Correspondingly, in the OptiMal test, 76 patients (50.66%) were positive (43 falc. and 33 viv.) (Table 1).

In 80 proved cases of malaria 24 were in 1-5 years age, 25 were in 6-10 years of age and 31 were in 11-15 years of age. In our study there were 46(57.5%) males and 34(42.5%) were females (Table 2).

All patients with malaria had fever (100%). 58 patients (72.5%) had Splenomegaly, 45 (56.25%) had pallor, 22 (27.5%) had jaundice and 10 (12.5%) had convulsions (Table 4).

The blood film examination identified three *P. falciparum* and one *P. vivax* which were missed by optiMAL method and the reason could be low parasite density. OptiMAL had sensitivities of 93.47% and 97.05% when compared to blood film for identification of *P. falciparum* and *P. vivax* infections. OptiMAL method detected one patient with *P. falciparum* which was missed by microscopy and the reason could be sequestration of the parasites. OptiMAL had specificity of 98.57% and 100% when compared to blood film for identification of *P. falciparum* and *P. vivax* (Table 5).

Table 1: Malarial parasite detection by microscopy and optimal

Findings	Microscopy	OptiMAL
Total Positive	80	76
Plasmodium falciparum	46	43
Plasmodium vivax	34	33
Negative	70	73

Table 2: Age distribution among malaria positive cases

Age in Years	No.	%
1-5	24	30.0
6-10	25	31.25
11-15	31	38.75
Total	80	100.0

Table 3: Sex distribution among the proved malarial cases

Sex	No.	%
Male	46	57.5
Female	34	42.5
Total	80	100.0

Table 4: Clinical Findings in Malaria positive cases

Clinical finding	No.	%
Fever	80	100
Splenomegaly	58	72.5
Pallor	45	56.25
Jaundice	22	27.5
Convulsions	10	12.5

Table 5: Performance comparison of OptiMal method with Microscopy

Optimal vs Microsc	*Plasmodium falciparum*	*Plasmodium vivax*
True positive	43	33
False positive	1	0
False negative	3	1
Sensivity (%)	93.47	97.05
Specificity (%)	98.57	100

DISCUSSION

Rapid accurate diagnosis of malaria is necessary for proper management, control of malaria and prevention of resistance, resulting in development of various methods to replace conventional microscopy [1-3]. The most promising new malarial diagnostics are the serological dip stick tests [5]. HRP 2 antigen detection tests detects only *P. falciparum* only and can't be used for monitoring treatment response where as OptiMAL detects all species of plasmodia species and can be used for monitoring treatment outcome [3, 4]. So this study was done to compare OptiMAL with conventional microscopy.

In our study of 80 proved malaria cases 46 were males and 34 were females. There were 24 patients in group of 1-5 years, 25 patients in 6-11 years and 31 patients in 11-15 years. All patients had fever, 72.5% had splenomegaly, 56.25% had pallor, 27.5% had jaundice and 12.5% had convulsions.

Out of 150 clinically suspected cases of malaria, microscopy showed 80 positive cases of malaria (46pf, 34pv), where as OptiMal test was positive for 76 cases (43pf, 33pv). Three cases of *P. falciparum* and one case of *P. vivax* were not detected by this method and the probable cause could be due to insufficient enzyme production at lower parasitemia below the OptiMAL test detection level (<100parasites/ul) [1, 4, 5]. The other possible cause is, these patients may have received antimalarial drugs for fever prior to testing [5]. Optimal detects pLDH which is produced only by living parasites, the blood samples judged negative may have been dead parasites and not yet cleared from the host detected positive by microscopy [1, 4, 5].

One blood sample in which OptiMAL detected P.falciparum was found to be negative by microscopy may be due to the fact that P.falciparum can sometimes be sequestered and may not be present in circulating blood [5].

Our study showed 93.47% and 97.05% sensitivity for *P. falciparum* and *P. vivax* respectively, 98.57% and 100% specificity for *P. falciparum* and *P. vivax* respectively, correlating with many studies who had sensitivity of > 85% and specificity of >95% [5, 7-10, 11] except by Mason *et al*. [12] study who had very low sensitivity of 47% may be due to field study.

Table 6: Comparsion of our study wioth other similar studies

Study	P.F Sensitivity %	P.F Specificity %	P.V Sensivity %	P.V Specificity %
Present study	93.47	98.57	97.05	100
Chayan [3]	88.4	96	96.8	99.2
Palmer [7]	88	99.9	94	100
Iqbal [8]	87	99	79	97
Mishra [9]	96	100	100	100
JC Mouatcho [10]	93.2	98.5	78.9-98.8	98.5
Mason [11]	42.6	97	47	96.9
Ferro [12]	90.6	98.6	96.5	97.6

CONCLUSION

The results of this study further substantiated that OptiMAL test is an effective, simple and sensitive diagnostic test for both *P. falciparum* and *P. vivax* in countries where both species are present. The sensitivity of this test is close to microscopic examination of blood smear and does not require skilled personnel, detects all Plasmodium species and can be used to monitor the efficacy of drug therapy since it detects pLDH produced only by living parasites. Though the cost of OptiMAL test is high when compared to microscopy which may prevent its routine use, it is an useful adjunct in diagnosing early in emergency situations, so that treatment is not delayed in places where experienced microscopy is not available and in hospitals where work load is high that could delay the results.

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