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Original Research Article

Diagnosis of malaria by the optimal method in children with routine microscopy and its comparison

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ABSTRACT

Background: The term malaria (meaning bad air of the marsh and swampland) first originated in the 17th century. Malaria is one of the most serious medical conditions, Malaria causes symptoms which usually include fever, fatigue, vomiting, and headaches. It may cause yellow skin, seizures, coma, or death in extreme cases. The population of tribal areas of Andhra Pradesh, Tamilnadu, Karnataka, Chhattisgarh, Gujarat, Bihar, Orissa, Northeastern states are contributing 50% of cases of Plasmodium falciparum.
Methods: All the clinically suspected cases of Malaria, 'The optiMAL' test was done at the bedside and simultaneously thick and thin smears are prepared and sent for microscopic examination. Study was carried out at Narayana Medical College, Nellore, Andhra Pradesh, India. The total number of patients in our study was 150. 1-14 years of age were included in the present study after applying the inclusion and exclusion criteria.
Results: The 'OptiMAL'test method had excellent sensitivity and specificity (100%) for detecting plasmodium vivax, very good sensitivity, and specificity (98.57%, 100%) for detecting plasmodium falciparum. The optimal test had a positive predictive value of 100%, the negative predictive value of 98.61% with p-value <0.001.
Conclusions: Our study has shown that the 'OptiMAL' test is an easy and successful diagnostic test that can be performed at the bedside for malaria diagnosis. This is very similar to traditional microscopy and do not need highly qualified workers to conduct experiments or interpret.

Keywords: Children, Malaria, Microscopy, Optimal method, Sensitivity, Specificity

INTRODUCTION

The term malaria (meaning bad air of the marsh and swampland) first originated in the 17th century. Malaria is one of the most serious medical conditions, Malaria causes symptoms which usually include fever, fatigue, vomiting, and headaches. It may cause yellow skin, seizures, coma, or death in extreme cases.[1] The population of tribal areas of Andhra Pradesh, Tamilnadu, Karnataka, Chhattisgarh, Gujarat, Bihar, Orissa, Northeastern states are contributing 50% of cases of Plasmodium falciparum.[2] Among all the tropical infectious and noninfectious diseases, Malaria is considered the most important as it kills more peoples. *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malaria*, and *Plasmodium ovale* are the malarial parasites of man. Among the above, *P. falciparum* is more dangerous as it invades red blood cells of all stages and leads to more complications.[3]

Generally, malaria spreads among humans by the bite of an infected female anopheles mosquito. Rarely Malaria can be transmitted by blood transfusion, transplacentally (congenital Malaria), or through the contaminated needles. Malaria is treated successfully and can be cured if diagnosed early. So early diagnosis of the disease is critical because of its complications in curtailing the mortality and morbidity. Malaria has classic clinical

symptoms and must be suspected of infectious fever in all cases.[4]

Microscopic blood smear analysis is commonly used as a routine procedure for detecting malaria parasites and remains the gold standard for diagnosis.[5] Nonetheless, and this solution includes an integrated health system, facilities with working microscopes, qualified technicians, regular provision of reagents, monitoring and quality control.[6]

Rapid antigen detection of Parasite lactate dehydrogenase (pLDH) released from the parasitized Red Blood Cells offers a simple and efficient method for detecting malarial cases by even untrained personnel in 10 minutes.[7]

Early detection of malarial parasites will help to decrease the morbidity and mortality and in the development of complications of Malaria.8 So the study to assess 'optiMAL'-IT test as an aid in the rapid diagnosis of Malaria at the bedside in an endemic area.

METHODS

All the clinically suspected cases of malaria, the 'optiMAL' test was conducted on the bedside & thick and thin smears are prepared and submitted for microscopic analysis at the same time. The study conducted in Narayana Medical College, Nellore, during the period of 1st November 2014 to 1st November 2016.

Pediatric patients who were suspected of having malaria, that is, admitted to the PICU / Pediatric unit. Fever with two or more scientific findings splenomegaly, pallor, convulsions, and jaundice are included from the study. Patients with infective hepatitis and other known causes of anemia are excluded from the study. In the study, the total number of patients was 150, and the age group was 1year to 14 years.

Microscopic examination

Jaswant Singh–Bhattacharji (JSB) stain is the regular stain used for peripheral smear staining for the diagnosis of Malaria. This has two stains Jaswant Singh–Bhattacharji (JSB) - 1, Jaswant Singh–Bhattacharji (JSB) - 2, and buffered water to wash the stain. All fever cases with suspicion of malaria blood smears were submitted along with other investigations for JSB staining. Thick and thin smears were prepared and air-dried. Thin smear would take 1-2 minutes to dry and thick smear around 20 minutes. The thick smear is dehaemoglobinised by dipping in water. Thick smears were dipped in Jaswant Singh-Bhattacharji (JSB) - 2 stain for 5 minutes after air drying, and washed with buffer water later. The slides were dipped for 1 minute after drying in Jaswant Singh-Bhattacharji (JSB) - 1 stain and washed with buffer water. Then the water is drained and dried. These smears were examined under the compound microscope. 100X oil immersion objective was used with a 10X eyepiece.

A total of 200 leucocytes were counted in 100 areas, and parasite presence or absence was observed. If smears are positive for malaria, grading according to the plus method was given. The microscopic analysis took <5 minutes, and after having counted at least 200 leucocytes, smears were declared negative after 5 minutes of examination.

After analyzing the thick smear, thin smears were only tested if the slides were positive for Malaria. The thin smears were initially fixed with methanol and then stained with JSB-2 and JSB-1, respectively, as mentioned above. Slides were washed, air-dried, and then examined under a microscope. Testing was done with a 10X eyepiece with a 100X oil immersion objective. Thin smear testing was performed to identify the Malaria species.

The time needed for the whole procedure was around an hour. Repeat smear tests were performed twice at fever levels for all the smear-negative cases.

'*OptiMAL' test*

The Optimal rapid malaria test is an immunochromatographic test that can be performed using a drop of a finger prick. This test detects the presence of Parasite lactate dehydrogenase (pLDH) antigen in the blood. pLDH is released from live malarial parasites and differentiation of Plasmodium species is based on antigen differences between its isoforms. The optimal dipstick contains two lines or reaction zones apart from a control antibody reaction zone at the top of the stripe.

The first line the sample encounters contains an antibody unique to plasmodium falciparum Parasite lactate dehydrogenase (pLDH). The second test line is composed of a pan-specific monoclonal antibody of Parasite lactate dehydrogenase (pLDH) that recognizes all species of Plasmodium.

Test procedure: To properly conjugate, two drops of reagent A (buffer solution) were added and four drops of reagent B (cleaning solution) were added to the wash well provided on a well-configured plate. Ten microliters of blood were added to the conjugate well and mixed gently. Dipstick was well situated upright for ten minutes at the conjugate and stand.

The dipstick was then moved to wash well and left there until the bands (5-10 minutes) were clear. Interpretation of the findings was carried out immediately after the clearing stage was completed as follows *Plasmodium falciparum* - One control band and two test bands visible *Plasmodium vivax* and other plasmodium species- One control and one test bands visible Negative test only one

control band at top of strip visible. The time taken was 15-20 minutes for the whole process.

All positive cases of malaria were treated, depending on the severity. Cerebral malaria cases were treated with intravenous Quinine with cardiac and glucose monitoring with supportive therapy IV quinine was changed to oral after the patient is stabilized for a total of 7 days. For non-responsive cases, intravenous Artesunate was used for 5-7 days. Uncomplicated cases of Malaria were treated with oral chloroquine for three days.

Smears were prepared every 24 hours to detect the rate of parasitemia. The child was declared cured when smears were negative for ring forms. Follow up smears were repeated on day 7, day 14, and day28 for any persistent parasitemia.

Comparitive study

The second part of the study was comparing the 'OptiMAL' method with conventional microscopy in terms of sensitivity, specificity, positive predictive value and negative predictive value. After the completion of 150 cases, the results were compared with the chi-square test and tabulated with the graph for analysis.

RESULTS

Table 1 shows out of 150 clinically suspected cases of malaria 52 (35%) patients were in 1 to 5-year age group, 56 (37%) were in 6 to 10 years, and 42 (28%) were in 11 to 15 years.

Table 1: Age distribution among clinically suspected cases of malaria.

Age in years	No. of patients	Percentage
1-5	52	35%
6-10	56	37%
11-15	42	28%
Total	150	100%

Figure 1 shows among the clinically suspected cases of malaria male and female distribution was almost equal

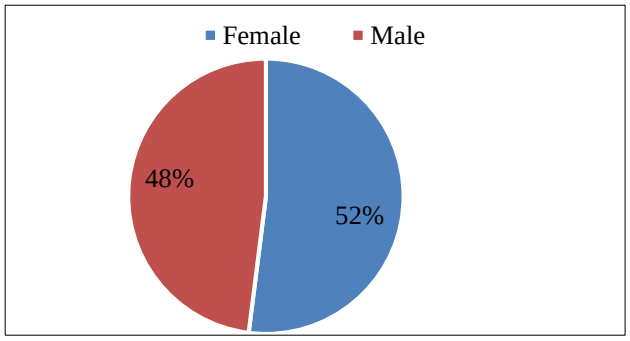

Figure 1: Gender distribution among children with suspected malaria.

Table 2 shows presenting symptoms, fever 150 (100%), Splenomegaly 86 (57%), Pallor 79 (53%), Convulsion 53 (35%), Jaundice 25 (17%). All clinically suspected cases of malaria had a fever; the majority of them had splenomegaly, pallor, and convulsions. Very few of them presented with jaundice

Table 2: Clinical findings in children with suspected malaria.

Presenting symptoms	Number (n-150)	Percentage
Fever	150	100%
Splenomegaly	86	57%
Pallor	79	53%
Convulsion	53	35%
Jaundice	25	17%

Table 3 shows in the study of 150 clinically suspected cases, 70 (46.7%) cases were positive for P. falciparum by microscopy, and 69 (46%) were positive by the Optimal method. 9 (6%) cases were positive for *P.vivax* by both the methods.

Table 3: Diagnosis of malaria by conventional microscopy and optimal method.

Findings	Microscopy (n=150)		Optimal method (n=150)	
	No.	Percentage	No.	Percentage
Negative for malaria	71	47.3%	72	48%
Plasmodium falciparum	70	46.7%	69	46%
Plasmodium vivax	9	6%	9	6%
Total	150	100%	150	100%

Table 4 shows In 79 proved cases of malaria, 25 (32%) were in 1 to 5-year age, 26 (33%) were in 6 to 10 years age, and 28 (35%) were in 11 to 15-year age.

Table 4: Age distribution among positive malaria cases.

Age in years	Number	Percentage (%)
1-5	25	32%
6-10	26	33%
11-15	28	35%
Total	79	100%

Table 5 shows in our study 44 (56%) of males and 35 (44%) of females were positive for malaria.

Table 6 shows presenting symptoms, fever 79 (100%), Splenomegaly 68 (86%), Convulsion 37 (47%), Pallor 55 (70%), Jaundice 15 (19%). Table 7 shows that the 'OptiMAL'test method had excellent sensitivity and

specificity (100%) for detecting *Plasmodium vivax*, very good sensitivity and specificity (98.57%, 100%) for detecting plasmodium falciparum. The optimal test had a positive predictive value of 100%, the negative predictive value of 98.61% with p-value <0.001.

Table 5: Sex distribution among the proved malaria cases.

Gender	Number	Percentage (%)
Male	44	56%
Female	35	44%
Total	79	100%

Table 6: Clinical findings in malaria positive cases.

Presenting symptoms	Number (n-79)	Percentage (%)
Fever	79	100%
Splenomegaly	68	86%
Convulsion	37	47%
Pallor	55	70%
Jaundice	15	19%

Table 7: Performance of optimal method in comparison with conventional microscopy in the diagnosis of malaria.

Optimal vs. Microscopy	Malaria species		Total
	Plasmodium falciparum	*Plasmodium vivax*	
True positive (n)	69	9	78
False Positive (n)	0	0	0
False negative(n)	1	0	1
True negative (n)	71	71	71
Sensitivity (%)	98.57	100	98.73
Specificity (%)	100	100	100
Positive Predictive Value (PPV) (%)	100	100	100
Negative Predictive Value (NPV) (%)	98.61	100	98.61
p value	<0.001***	<0.001***	<0.001***

DISCUSSION

Malaria's resurgence has sparked interest in developing not only prevention steps but fast diagnostic techniques, too. Several methods to supplement and replace the traditional microscopic method were developed. Serological dipstick tests are the most promising diagnostic. Among them is' OptiMAL'test. Authors used this method and compared it to the traditional smear test for malaria diagnosis.

In our study of 150 clinically suspected cases of malaria. Microscopy showed 79 positive cases for malaria (70 *Plasmodium falciparum*, and 9 *Plasmodium vivax*), whereas 'OptiMAL'test was positive for 78 cases (69 *Plasmodium falciparum*, 9 *Plasmodium vivax*). One case of Plasmodium falciparum was not detected by this method; the probable cause could be due to insufficient enzyme production at lower parasitemia below the 'OptiMAL'test detection level (<100 parasites/ microliter). The other possible cause that can be explained is that the child has already received the anti-malarial drug. Optimal detects Parasite lactate dehydrogenase (pLDH), which is produced only by living parasites, the blood samples judged negative by Optimal may have been dead parasites and not yet cleared from the host.

In our study among the 79 proved malaria cases, 44 were males, and 35 were females. All the patients had fever 79 (100%), spleenomegaly 68 (86%), convulsion 37 (47%), pallor 55 (70%), jaundice 15 (19%).

This study showed 98.57%, 100% sensitivity for plasmodium falciparum and plasmodium vivax, respectively. 100% specificity for both plasmodium falciparum and plasmodium vivax. The positive and negative predictive values were 100% and 98.61%, respectively, with a p-value <0.001.

This findings of the study were similar to the study from Palmer CJ, which showed 98% sensitivity, 100% specificity, the positive predictive value of 100%, and a negative predictive value of 99%.[9]

A study was done by Singh N, which showed 100% sensitivity, 97% specificity, a positive predictive value of 98%, and negative predictive value of 100%.[10]

In a prospective study done by Azazy AA, of 434 stained films, 161 were positive for *P.falciparum*. One hundred sixty-one positive blood samples on light microscopy examination 152 were found positive by the dipstick antigen test with 94% sensitivity and 100% specificity.[11]

In a study done by Gonul A, of 190 malaria suspected cases, 81 were positive for malaria by microscopy, and 76 were positive by optimal test. There was no false positivity observed with the Optimal method. They concluded that this rapid malaria test has a lower level of sensitivity than the classical thick blood film test for malaria, but these methods have equal specificity.[12]

So, the results of this study further substantiated that the Optimal test is an effective and sensitive tool in the diagnosis of Malaria. It is a useful adjunct in diagnosing

early so that treatment is not delayed in places where experienced microscopist is not available and in hospitals where workload is high, which may delay the results.

CONCLUSION

The 'OptiMAL' test is an easy and successful diagnostic test that can be performed at the bedside for malaria diagnosis. It has a sensitivity of 98.57% and 100% for *Plasmodium falciparum* and *Plasmodium vivax*, the specificity of 100% for both *Plasmodium falciparum* and *Plasmodium vivax*, this test showed positive and negative predictive values of 100% and 98.61% respectively which were very close to conventional microscopy and does not require highly qualified personnel to perform or interpret results.

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