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# Diagnostic utility of rapid strip test (optimal test) versus conventional microscopy in children with suspected malaria

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***Abstract:***
***Background:****Malaria is one of the major health problem in India with high mortality and morbidity. Conventional microscopy is the gold standard for the diagnosis of malaria but this approach requires an organized health system, infrastructure with functioning microscopes, trained technicians, regular provision of reagents, supervision, and quality control which is impractical especially in rural areas and in emergency situation. Rapid diagnosis of Malaria by OptiMAL method is an immunochromatographic test which can be done at bedside and does not require expertise personnel to interpret the results*
***Objective:****To assess OptiMAL test as an aid in rapid diagnosis of malaria at bedside in an endemic region.*
***Method:****A total of 100 clinically suspected cases admitted to the paediatric ward in Niloufer hospital, Hyderabad were taken for this study and tested for Malaria by both microscopy and strip test(OptiMAL) methods at bed side. Thick and thin smears are prepared and sent for microscopic examination by JSB staining. Results were compared at the end of the study.*
***Results:****A total of 100 clinically suspected cases of Malaria by both microscopy and strip test methods of which 53 were positive by microscopy and 52 were positive by strip test method. Strip test had sensitivity of 97.3% for Plasmodium falciparum, 94.7% for Plasmodium vivax and specificity of 98.4% for Plasmodium falciparum, 100% for Plasmodium vivax. Positive predictive value of 97.29% for Plasmodium falciparum, 100% for Plasmodium vivax and negative predictive value of 98.4% for Plasmodium falciparum,98.78%for Plasmodium vivax.*
***Conclusions:****In our study OptiMAL test showed excellent correlation with Microscopy in diagnosis of Malaria. Though it is expensive, it has an advantage of being simple, rapid and effective in diagnosis of Malaria, especially in areas where well trained microbiologist is not available and where the work load delays the results.*
***Keywords:****Malaria, Strip test, Conventional microscopy*

## I. Introduction

Malaria is one of the biggest medical problems with 1600 million people exposed to the risk of the disease and 300 - 500 million cases occurring annually. The estimated deaths, each year are between 1 - 2 million cases, the majority being children under 5years and almost all the deaths are attributed to P. falciparum. India is amongst the worst affected countries.[1,2] In 2003 there were 1.64 million cases reported in India of which 0.74 million were due to P. Early diagnosis of malaria is important in curtailing the mortality and morbidity due to its complications. Malaria has classical clinical features and has to be suspected in all cases of fever in endemic areas.[3,4] Malaria caused by P. falciparum is a medical emergency. Accurate and timely detection of species is imperative in order to provide appropriate and supportive therapy.[4] Cerebral malaria has high case fatality rate when managed under routine hospital conditions in developing countries (30 -40%).[5]

Microscopic examination of the blood smears is widely used as a routine method for the detection of malarial parasites and remains the gold standard for diagnosis.[6] However this approach requires an organized health system, infrastructure, with functioning microscopes, trained technicians, and regular provision of reagents, supervision, and quality control.[7] Besides these, majority of malaria cases occur in rural areas where there is little or no access to reference laboratories.[8]

Rapid antigen detection of pLDH released from the parasitized RBCs offers simple and efficient method for detecting malarial cases by even untrained personnel in 10 minutes. Early detection of malarial parasites will help greatly in preventing further morbidity and development of complications of malaria. To the

best of our knowledge there are few studies done in India about this procedure and none have been done in this region.

**Objective**
To assess OptiMAL test as an aid in rapid diagnosis of malaria at bedside in an endemic region.

**Methodology**
For all the clinically suspected cases of malaria, OptiMAL test was done at bed side and simultaneously thick and thin smears are prepared and sent for microscopic examination. Results were compared at the end of the study.

**Study design**
**Study place:** Niloufer Hospital, Hyderabad
**Study period:**One year from September 2011 to September 2012.

**Patient selection:** Paediatric patients admitted to Paediatric ward/PICU, who were suspected to have malaria i.e. Fever with two or more of following clinical findings: chills and rigors, splenomegaly, pallor, convulsions, bleeding manifestations and jaundice. Patients with other known causes of anemia are excluded from the study. The total number of subjects in our study was 100 and age group was l year to 12 years.

**Microscopic examination**: J S B Stain is the regular stain used for peripheral smear staining for diagnosis of malaria in our hospital under NMEP. This has two stains JSB-1, JSB-2 and buffered water to wash the stain. In all cases of fever with suspicion of malaria, blood smears were sent for JSB staining along with other investigations. Thick and thin smears were prepared and air dried. Thick smear is dehaemoglobinized by dipping in water. After air drying, thick smears were dipped in JSB-2 stain for 5 minutes and later washed with buffer water. After drying, the slides were dipped in JSB-1 stain for 1 minute and washed with buffer water. Then water is drained and dried. These smears were examined under compound microscope. 100 X oil immersion objectives were used with 10X eye piece.A minimum of 200 leucocytes in 100 fields was counted and presence or absence of parasite was detected. If smears are positive for malaria grading was given according to the plus system. The microscopic examination required <5minutes and smears were declared negative after 5 minutes of examination, after counting at least 200 leucocytes. Thin smears were examined only if the slides were positive for malaria after examining the thick smear. The thin smears were also staine with JSB-2 and JSB-1 respectively as mentioned above. Examination was done with 100X oil immersion objective with l0X eye piece. Thin smear examination was done to identify the species of malaria. The time taken for the entire procedure was about one hour. For all the smear negative cases repeat smear examinations were done twice at fever spikes. The cost of examination of each smear is 20 rupees.

**OptiMAL test:** The OptiMAL rapid malaria test is a immunochromatographic test that can be performed with a drop of finger prick or EDTA blood. This test detects the presence of pLDH antigen in blood. pLDH is released from live malarial parasites and differentiation of plasmodium species is based on antigen differences between its iso forms. Aside from a control antibody reaction zone at the top of the test strip, the OptiMAL dip stick contains two test lines or reaction zones. The first line encountered by the sample comprises of an antibody that is specific for P.falciparum pLDH. The second test line is composed of a pan specific pLDH monoclonal antibody that recognizes all the plasmodium species.

**Test procedure:** Two drops of reagent A (buffer solution) were added to conjugate well and four drops of reagent B (cleaning solution) were added in to the wash well provided on a configured well plate. 10 microletre of blood was added to the conjugate well and mixed gently. Dip stick was placed vertically in to the conjugate well and allowed to stand for 10 minutes. The dip stick was then transferred to wash well and left there until the bands are clearly visible (5-10 min). The interpretation of results was performed immediately after completion of the clearing step as follows. P.falciparum - One control band and two test bands visible P.vivax& other plasmodium species- One control and one test bands visible Negative test- only one control band at top of strip visible

The time taken for the entire procedure was 15-20 minutes, the cost of the test is 150 rupees. All positive cases of malaria by either of the methods were treated according to WHO guidelines, depending on the severity.

**Comparitive study:**

Second part of the study was to compare OptiMAL method with conventional microscopy with respect to sensitivity, specificity, positive predictive value and negative predictive value.

After the completion of 100 cases, the results were compared with chi-square test and tabulated with graph for analysis.

## II. Results

A total of 100 blood samples were tested for malaria parasites by the strip test and results were compared to those obtained from examination of thin and thick smear blood film.The Blood film results indicated that 52 patients were infected with malaria and rest 48 were malaria negative. Among the positive patients, plasmodium vivax was detected in 19 cases and plasmodium falciparum in 37 cases.Correspondingly the strip test result indicated that 51 of the patients samples were positive for malaria parasites and 49 were malaria negative. Among the positive patients P. vivax was detected in 18 cases and P. falcipuram in 37 cases.Both methods identified four patients with a mixed infection of P. vivax and P. falciparum.The blood film examination identified one P. vivax positive sample that was not detected by the strip test, however there was 100 % agreement between blood film results and strip test results for the other 18 samples containing P. vivax.One case of P. falciparum detected by blood film was not detected by strip test and one case of P. falciparum detected by strip test was not detected by blood film.Comparision of strip test with peripheral smear for both P. vivax and P. falciparum were shown n Table 1and 2. Clinical findings in Malaria suspected and malaria positive cases were shown in Graphs 1 and 2. Sex distribution among proved malaria cases was shown in Graph 3, whereas case distribution among the malaria suspects was described in Table 3 and Graph 4. Details of clinical features like splenomegaly, chills and rigors, pallor, jaundice, thrombocytopenia, bleeding manifestations and convulsions in malaria positive cases were explained in Graphs 5,6,7,8,9,10 and 11. Microscopic findings in correlation with splenomegaly for both P.falciparum and P.vivax was described in Table 4 and 5. Microscopic findings in correlation with pallor for both P.falciparum and P.vivax was described in Table 6 and 7.

**Table 1: Comparison of Strip Test with peripheral smear for P. vivax**

| Strip Test results | Blood Film Result | | Total |
|---|---|---|---|
| | Yes | No | |
| Yes | 18 (a) | O (b) | 18 |
| \\\\\\NO | 1 (c) | 81 (d) | 82 |
| Total | 19 | 81 | 100 |

$$\text{Sensitivity} = \frac{a}{a+c} X100 = \frac{18}{19} X100 = 94.7\ \%$$

$$\text{Specificity} = \frac{d}{b+d} X100 = \frac{81}{81} X100 = 100\ \%$$

$$\text{Positive Predictive value} = \frac{a}{a+b} X100 = \frac{18}{18} X100 = 100\ \%$$

$$\text{Negative Predictive value} = \frac{d}{c+d} X100 = \frac{81}{82} X100 = 98.78\ \%$$

$$\%\ \text{of False} - \text{ve} = \frac{c}{a+c} X100 = \frac{1}{19} X100 = 5.2\ \%$$

$$\%\ \text{of False} + \text{ve} = \frac{b}{b+d} X100 = \frac{0}{81} X100 = 0\ \%$$

**Table 2: Comparison of Strip Test with peripheral smear for P. falciparum**

| Strip Test results | Blood Film Result | | Total |
|---|---|---|---|
| | Yes | No | |
| Yes | 36 (a) | 1 (b) | 37 |
| NO | 1 (c) | 62 (d) | 63 |
| Total | 37 | 63 | 100 |

$$\text{Sensitivity} = \frac{a}{a+c} X100 = \frac{36}{37} X100 = 97.3\ \%$$

$$\text{Specificity} = \frac{d}{b+d} X100 = \frac{62}{63} X100 = 98.412\ \%$$

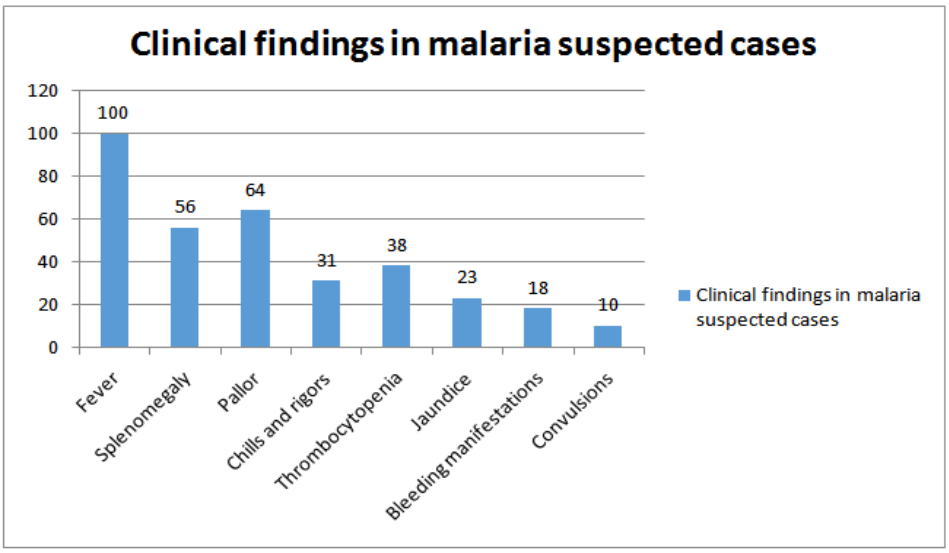
$$\text{Positive Predictive value} = \frac{a}{a+b} X100 = \frac{36}{37} X100 = 97.29\ \%$$

$$\text{Negative Predictive value} = \frac{d}{c+d} X100 = \frac{62}{63} X100 = 98.4\ \%$$

$$\%\ \text{of False} - \text{ve} = \frac{c}{a+c} X100 = \frac{1}{37} X100 = 2.7\ \%$$

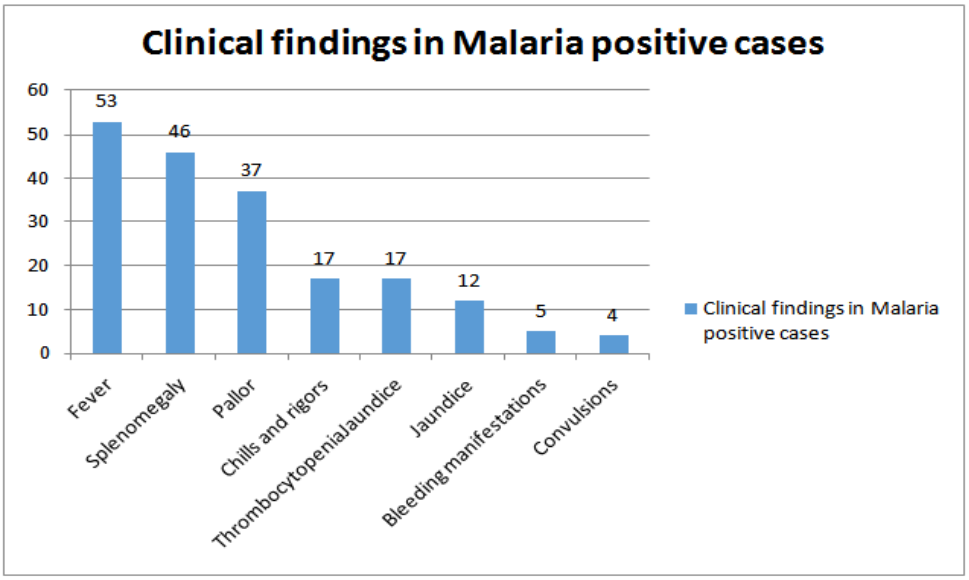
$$\%\ \text{of False} + \text{ve} = \frac{b}{b+d} X100 = \frac{1}{63} X100 = 1.58\ \%$$

**Graph 1: Clinical findings in Malaria Suspected Cases**



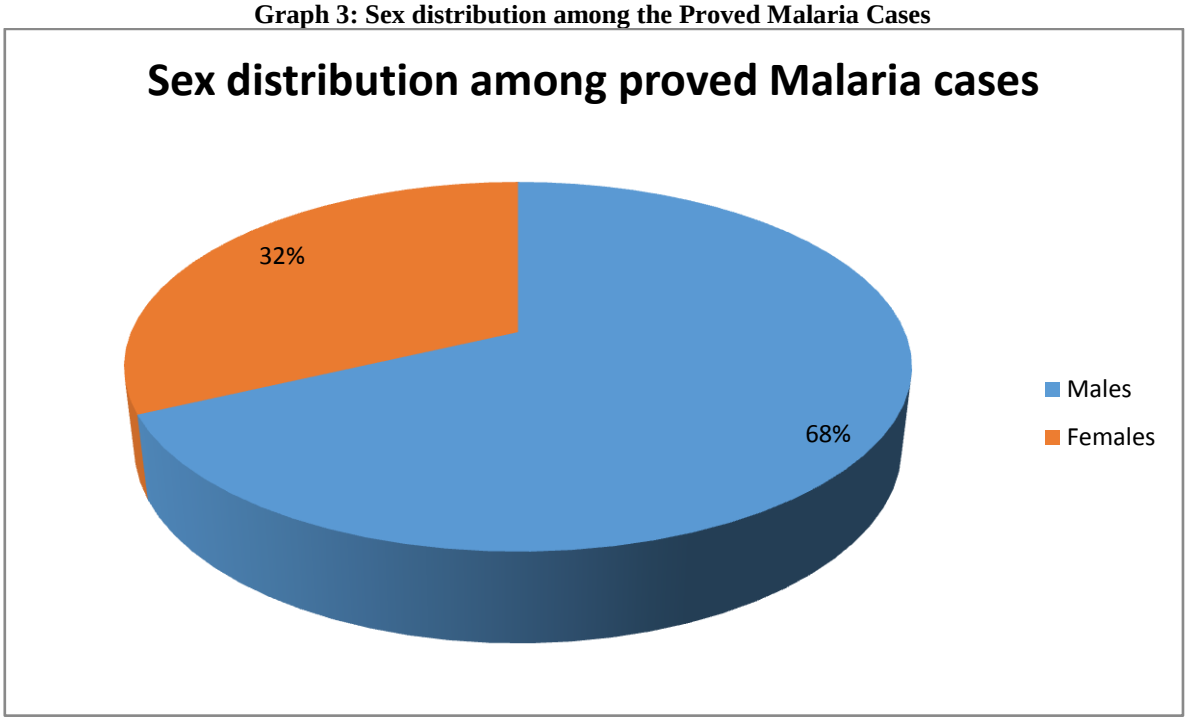

All clinically suspected cases of malaria had fever and majority of them had Splenomegaly and Pallor.

**Graph 2: Clinical findings in Malaria Positive Cases**




All Malaria positive cases had fever, majority them had Splenomegaly and Pallor.

**Graph 3: Sex distribution among the Proved Malaria Cases**

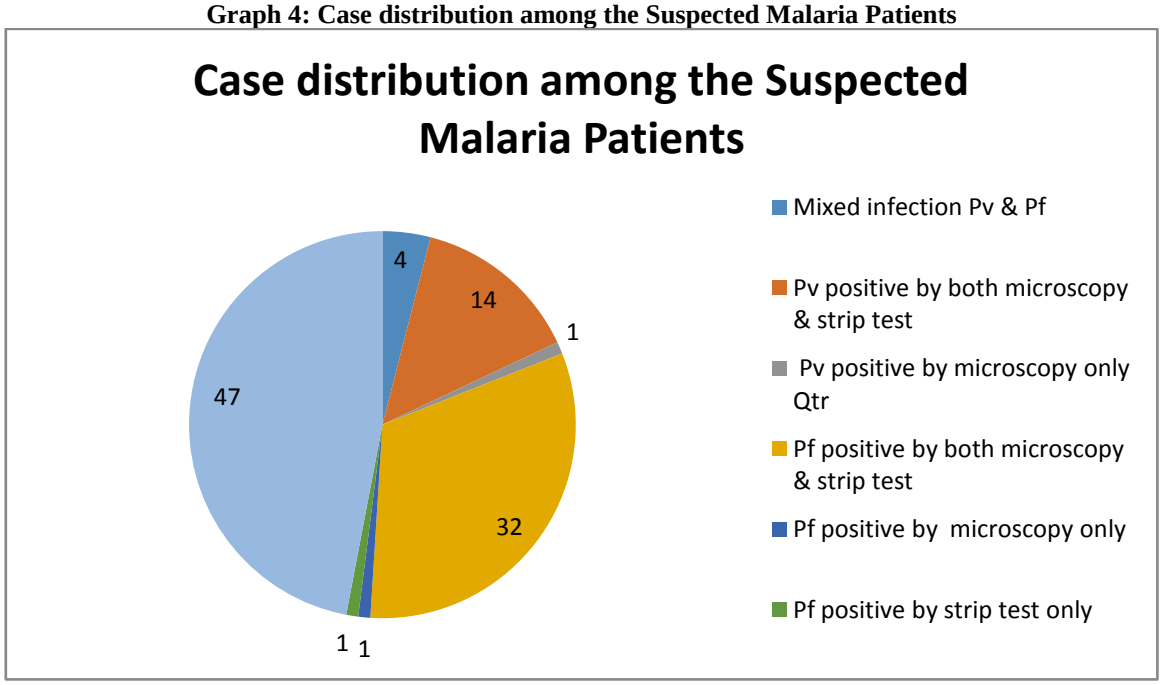



In our study 68% of Males and 32% of Female were Positive for Malaria

**Table 3: Case distribution among the Suspected Malaria Patients**

| | |
|---|---|
| Total | 100 |
| Mixed infection Pv & Pf | 4 |
| Pv positive by both microscopy & strip test | 14 |
| Pv positive by microscopy only | 1 |
| Pf positive by both microscopy & strip test | 32 |
| Pf positive by microscopy only | 1 |
| Pf positive by strip test only | 1 |
| Malaria negative | 47 |

**Graph 4: Case distribution among the Suspected Malaria Patients**



**Graph 5: Splenomegaly in Malaria Positive Cases**

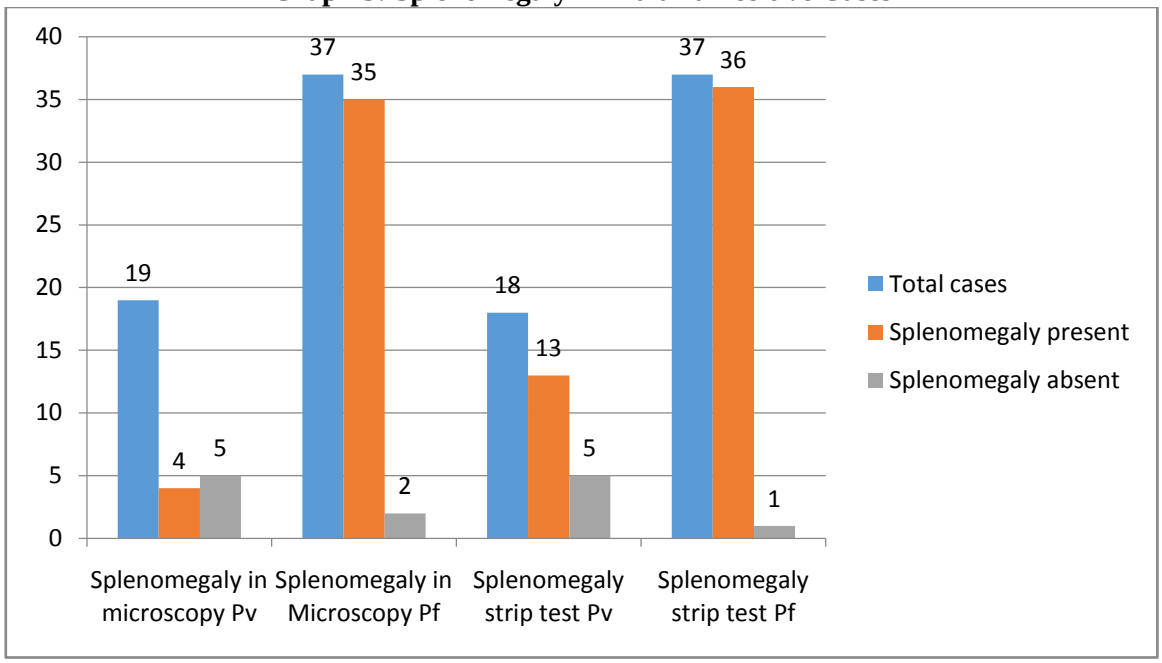



**Table 4: Splenomegaly – Microscopy P. vivax**

| | | | MICROSCOPY Pv | | |
|---|---|---|---|---|---|
| | | | Yes | No | Total |
| SPLENOMEGALY | Yes | Count | 14 | 42 | 56 |
| | | % Within Splenomegaly | 25.0% | 75.0% | 100.0% |
| | | % Within Microscopy Pv | 73.7% | 51.9% | 56.0% |
| | | % Of Total | 14.0% | 42.0% | 56.0% |
| | No | Count | 5 | 39 | 44 |
| | | % Within Splenomegaly | 11.4% | 88.6% | 100.0% |
| | | % Within Microscopy Pv | 26.3% | 48.1% | 44.0% |
| | | % Of Total | 5.0% | 39.0% | 44.0% |
| Total | | Count | 19 | 81 | 100 |
| | | % Within Splenomegaly | 19.0% | 81.0% | 100.0% |
| | | % Within Microscopy Pv | 100.0% | 100.0% | 100.0% |
| | | % Of Total | 19.0% | 81.0% | 100.0% |

**Chi-Square Tests**

| | Value | Df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|---|---|---|---|---|---|
| Pearson Chi-Square | 2.977[a] | 1 | .084 | | |

Association of Splenomegaly in P. vivax Positive cases has not shown significant p –value.

**Table 5:Splenomegaly – Microscopy P. falciparum**

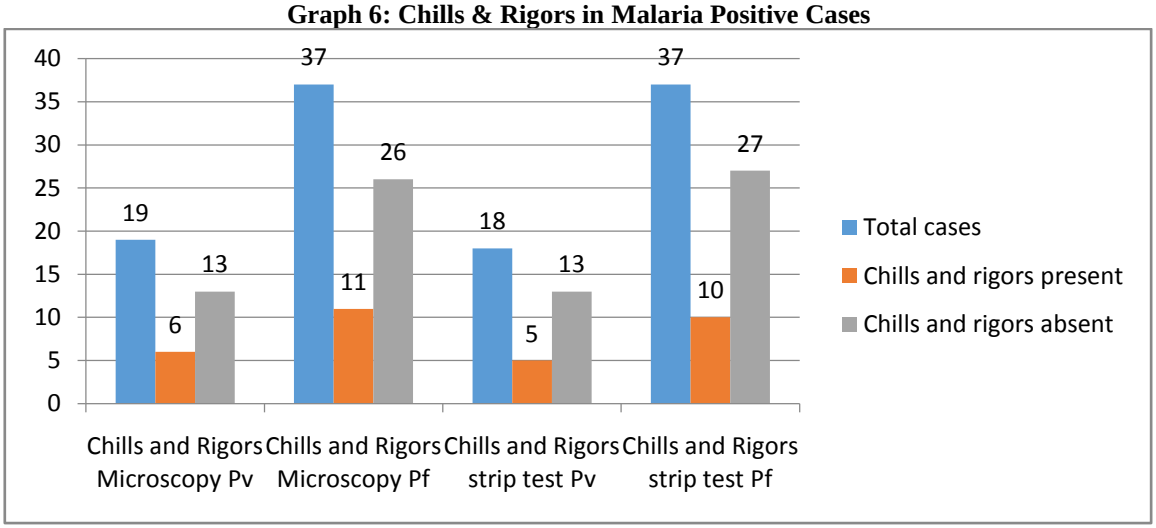
| | | | MICROSCOPY Pf | | |
|---|---|---|---|---|---|
| | | | Yes | No | Total |
| SPLENOMEGALY | Yes | Count | 35 | 21 | 56 |
| | | % Within Splenomegaly | 62.5% | 37.5% | 100.0% |
| | | % Within Microscopy Pf | 94.6% | 33.3% | 56.0% |
| | | % Of Total | 35.0% | 21.0% | 56.0% |
| | No | Count | 2 | 42 | 44 |
| | | % Within Splenomegaly | 4.5% | 95.5% | 100.0% |
| | | % Within Microscopy Pf | 5.4% | 66.7% | 44.0% |
| | | % Of Total | 2.0% | 42.0% | 44.0% |
| Total | | Count | 37 | 63 | 100 |
| | | % Within Sple2megaly | 37.0% | 63.0% | 100.0% |
| | | % Within Microscopy Pf | 100.0% | 100.0% | 100.0% |
| | | % Of Total | 37.0% | 63.0% | 100.0% |

**Chi-Square Tests**

| | Value | Df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|---|---|---|---|---|---|
| Pearson Chi-Square | 35.504[a] | 1 | .000 | | |

Association of Splenomegaly in P.falciparumpositive cases has shown significant p -value is < 0.01

**Graph 6: Chills & Rigors in Malaria Positive Cases**




**Graph 7: Pallor in Malaria Positive Cases**

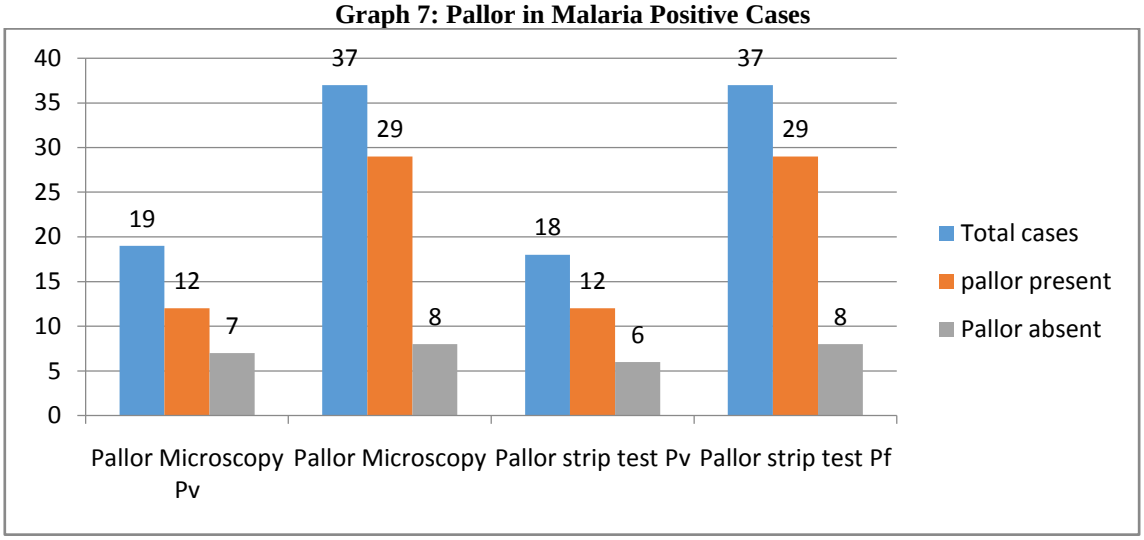



**Table 6: Pallor – Microscopy P. vivax**

| | | | MICROSCOPY Pv | | Total |
|---|---|---|---|---|---|
| | | | Yes | No | |
| PALLOR | Yes | Count | 12 | 52 | 64 |
| | | % within PALLOR | 18.8% | 81.3% | 100.0% |
| | | % within MICROSCOPY P | 63.2% | 64.2% | 64.0% |
| | | % of Total | 12.0% | 52.0% | 64.0% |
| | No | Count | 7 | 29 | 36 |
| | | % within PALLOR | 19.4% | 80.6% | 100.0% |
| | | % within MICROSCOPY Pv | 36.8% | 35.8% | 36.0% |
| | | % of Total | 7.0% | 29.0% | 36.0% |
| Total | | Count | 19 | 81 | 100 |
| | | % within PALLOR | 19.0% | 81.0% | 100.0% |
| | | % within MICROSCOPY Pv | 100.0% | 100.0% | 100.0% |
| | | % of Total | 19.0% | 81.0% | 100.0% |

| Chi-Square Tests | | | | | |
|---|---|---|---|---|---|
| | Value | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
| Pearson Chi-Square | .007[a] | 1 | .932 | | |

Association of Splenomegaly in P. vivax Positive cases has not shown significant p value.

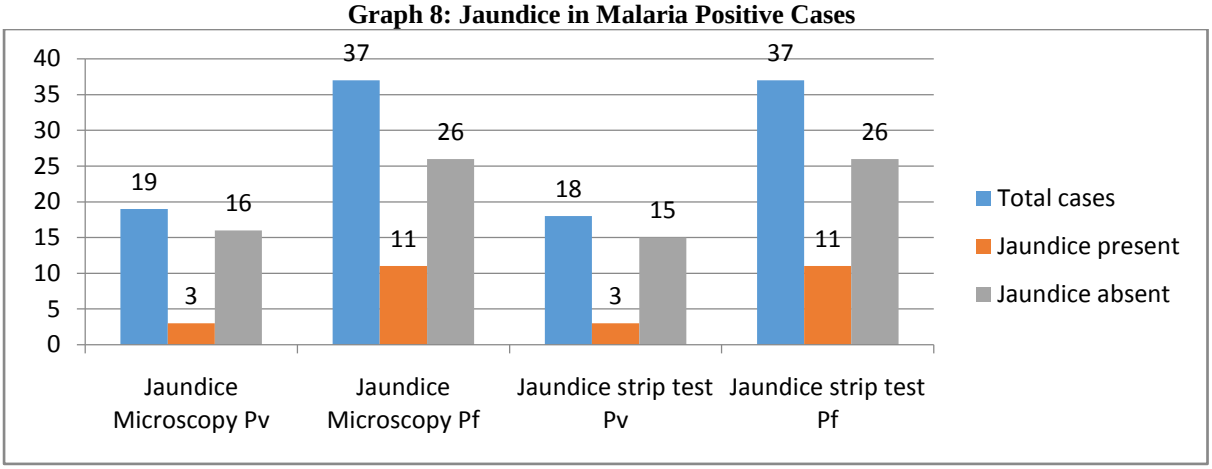
**Table 7: Pallor – Microscopy P. falciparum**

| | | | MICROSCOPY Pf | | |
|---|---|---|---|---|---|
| | | | Yes | No | Total |
| PALLOR | Yes | Count | 29 | 35 | 64 |
| | | % within PALLOR | 45.3% | 54.7% | 100.0% |
| | | % within MICROSCOPY Pf | 78.4% | 55.6% | 64.0% |
| | | % of Total | 29.0% | 35.0% | 64.0% |
| | No | Count | 8 | 28 | 36 |
| | | % within PALLOR | 22.2% | 77.8% | 100.0% |
| | | % within MICROSCOPY Pf | 21.6% | 44.4% | 36.0% |
| | | % of Total | 8.0% | 28.0% | 36.0% |
| Total | | Count | 37 | 63 | 100 |
| | | % within PALLOR | 37.0% | 63.0% | 100.0% |
| | | % within MICROSCOPY Pf | 100.0% | 100.0% | 100.0% |
| | | % of Total | 37.0% | 63.0% | 100.0% |

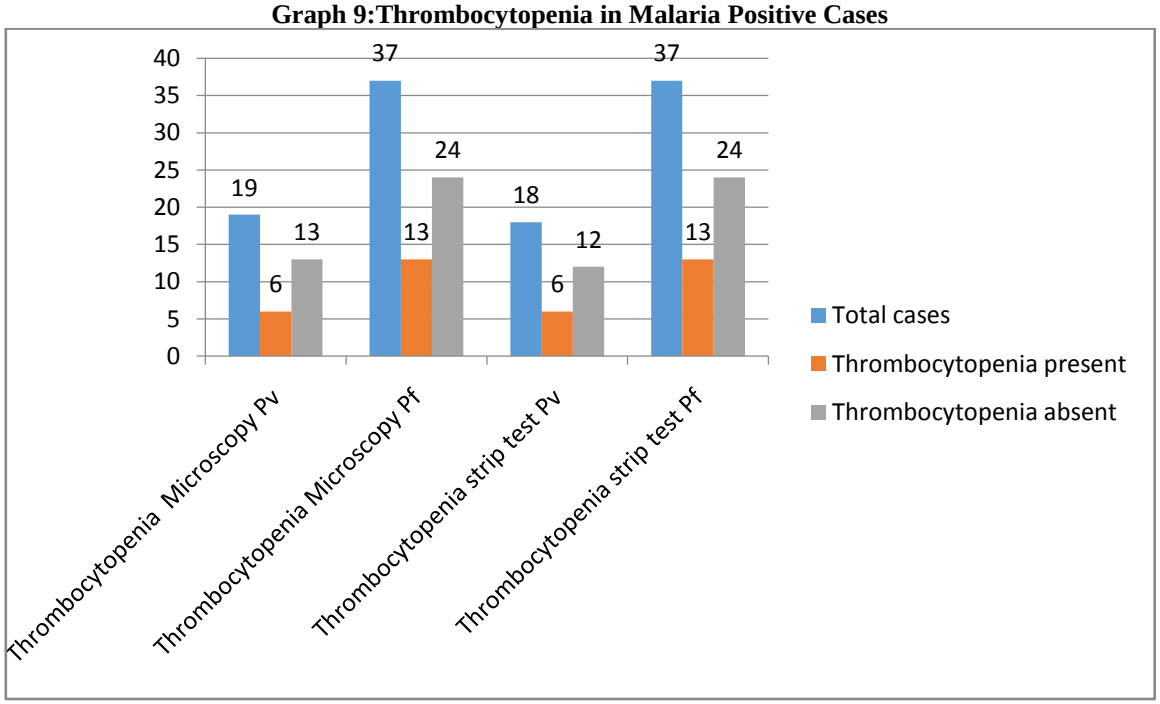
| Chi-Square Tests | | | | | |
|---|---|---|---|---|---|
| | Value | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
| Pearson Chi-Square | 5.270[a] | 1 | .022 | | |

Association of splenomegaly in P. falciparumpositive cases has shown significant p value is < 0.05.

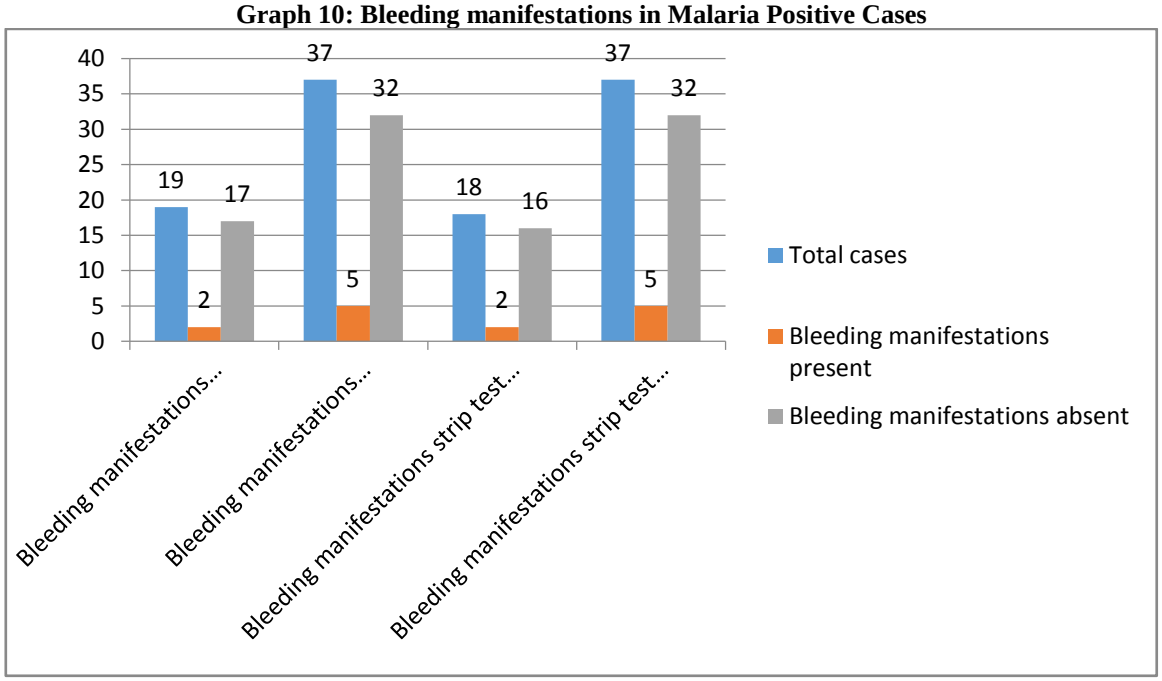
**Graph 8: Jaundice in Malaria Positive Cases**



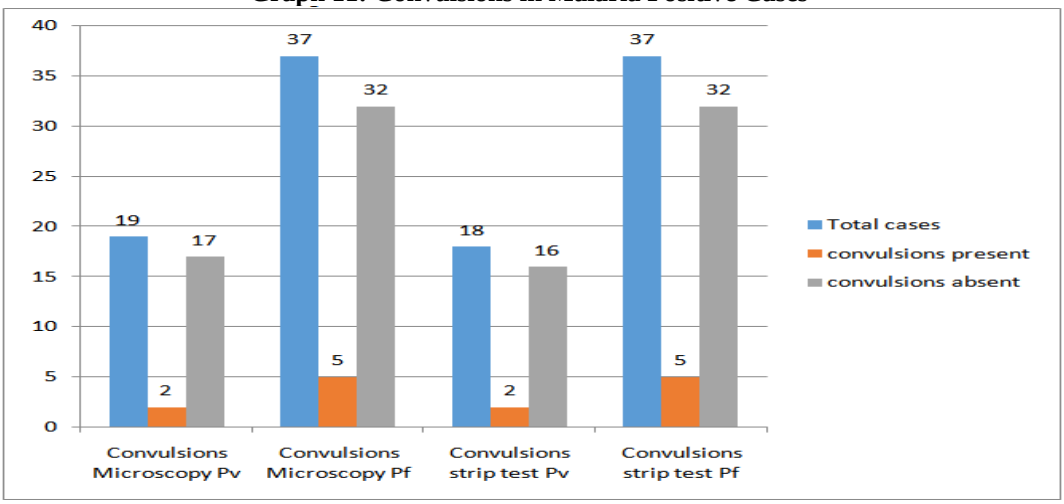

**Graph 9:Thrombocytopenia in Malaria Positive Cases**



**Graph 10: Bleeding manifestations in Malaria Positive Cases**




**Graph 11: Convulsions in Malaria Positive Cases**




## III. Discussion

The Resurgence of malaria has renewed interest in developing not only preventive measures, but also rapid diagnostic techniques. Several methods have been developed to supplement and replace the conventional microscopic method. The most promising new malaria diagnostics are the serological dipstick tests OptiMAL is one amongst them. We employed this test and compared it with conventional smear examination for diagnosis of P. falciparum and P. vivax infection.

The antigen detection test identified 51 cases malaria positive while the blood film identified 52 cases as malaria positive. Some malaria infections detected by blood film were not detected by the strip test. This may be explained by the fact that increased awareness of malaria among the general public has led to a rampant misuse of anti-malarial drugs in inadequate doses empirically for any fever. Since strip test detects pLDH which is produced only by living parasites, the blood samples judged negative by strip test may have been dead parasites and not yet cleared from the host.One case of P. vivax and P. falciparum detected by blood film were not detected by strip test. This may be due to insufficient enzyme production which occurs during early malarial infection or the patient blood samples contained parasites at concentration below the strip test's detection level. One blood sample in which strip test detected P. falciparum was found to be negative in blood smear examination. This may be explained by the fact that P. falciparum can sometimes sequester and may not be present in circulating blood.

In our study strip test has shown sensitivity of 97.3% and 94.7%, specificity of 98.4% and 100% for P. falciparum and P. vivax respectively. This test showed positive predictive values of 97.29% and 100%,negative predictive values of 98.4% and 98.78% for P. falciparum and P. vivax respectively, which were very close to conventional microscopy.This evaluation has shown that strip test is a simple, sensitive and effective diagnostic test for P. falciparum and P. vivax in countries where both species are present and where many patients need a fever screen. The sensitivity of this test is very close to microscopic examination of blood smears but does not require highly skilled personnel to perform or interpret results. The test has the added advantage that it can detect all four Plasmodium species and can be used to follow the efficacy of drug- therapy since it, detects an enzyme produced only by living parasites. Although it has got a number of advantages one needs to keep in mind the cost of the test which may not be affordable by many. The high cost of the test may prevent its regular and routine use in many of the laboratories. However, it is a valuable adjunct at the time of emergency for rapid diagnosis, although microscopy remains the mainstay for the diagnosis of malaria for routine use in countries like India. Simailar studies were reported in the literature.[8-16]Our study results were similar to the Study done by Carol J Palmer, J. Alfrendo Bonilla et al, which showed 98%sensitivity, 100% specificity, positive predictive value of 100% and negative predictive value of 99%.[16] Study done by Singh N, Valecha et al, which showed 100% sensitivity, 97% specificity, positive predictive value of 98% and negative predictivevalue of

100%.[12] Therefore the results of this study further substantiated that strip test is an effective and sensitive tool in the diagnosis of Malaria. The cost of strip test is high when compared to microscopy which may prevent its routine use. But it is a useful adjunct in early diagnosis, so that treatment is not delayed in places where experienced microbiologist is not available and in hospitals where work load is high, which may delay the results.

## IV. Conclusions

Our study has shown that strip test is a simple and effective diagnostic test which can be done at bed side for diagnosing malaria. It has a sensitivity of 97.3% and 94.7%, specificity of 98.4% and 100% for P. falciparum and P.vivax respectively. This test showed positive predictive values of 97.29% and 100%,negative predictive values of 98.4% and 98.78% for P.falciparum and P.vivax respectively, which were very close to conventional microscopy and does not require highly skilled personnel to perform or interpret results. Though the cost of the test may prevent its routine use, if complications associated with malaria are considered, it may be a better option in emergency situations, areas where work load is high which delays results and in places where experienced microbiologist is not available.

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