STUDY OF THREE MALARIA RAPID DETECTION TESTS AND ITS CORRELATION WITH PARASITIC INDEX FOR P.FALCIPARUM AND P.VIVAX

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ABSTRACT **OBJECTIVE:** To determine the sensitivity and specificity of three Malaria rapid antigen detection tests(RDTs) .To study the sensitivity of the RDTs in relation to parasitic index. **MATERIALS AND METHODS:** The study was conducted at a tertiary care hospital. Peripheral smear were prepared and stained.Parasite index was calculated. Three rapid antigen detection tests ; Optimal – IT, Paramax-3 and QDx malaria PAN/Pf were tested. **RESULTS:** Sensitivity for Optimal – IT was highest (98.47%). QDx malaria PAN/Pf gave highest specificity (97%). All the three RDTs gave sensitivity of 100% at a parasitic index of more than 100 parasites per µl. Sensitivity of Optimal – IT and QDx malaria PAN/Pf for P falciparum and P vivax was 100% and 88.88% respectively at a parasitic index of 51-100 parasites/ µl. **CONCLUSION:** Malaria RDTs are a good diagnostic tool in health care set ups where quick results are desired and expert microscopy is not available.

KEYWORDS : Malaria, Rapid Antigen Detection Tests, Optimal – IT, Malaria Diagnosis, Malaria Parasitic Index

INTRODUCTION

Malaria has been known since ancient times and has been diagnosed based on patient's signs and symptoms. Today with increasing chloroquine resistance and widespread use of expensive artemisinin combination therapies, an accurate and rapid diagnosis is essential. Clinical features of malaria have been described from Hippocrates to Osler.¹ In 1891, Romanowky introduced staining methods for these parasites.^{2,3} Today more than a century later, microscopic detection and identification of Plasmodium species in Giemsa stained blood films remains the gold standard for laboratory diagnosis. Methods using advances in technology have been introduced as alternatives to light microscopy.⁴ Becton and Dickenson developed a fluorescent staining technique using a capillary tube called as Quantitative Buffy Coat test in 1991-92.² Polymerase chain reaction (PCR) based methods have been used since early 1990's for the detection of Plasmodium parasites in human patients.⁵ Many non-microscopic, rapid dip stick tests based on the detection of various antigens of malaria parasites were introduced in the early 1990s.⁶ P.falciparum Histidine Rich Protein II (1987), parasite aldolase (1992) and parasite Lactate dehydrogenase (1998) are the target antigens used for such tests.²

Several commercial kits are available detecting different malaria antigens like the Histidine rich protein 2 (HRP-2), parasite lactate dehydrogenase (pLDH) and aldolase. These tests can be performed in 15-20 minutes. Reporting the test results of the Rapid detection tests (RDT's) also does not require the expertise as it is for microscopy. There are several Rapid antigen detection tests available. The choice of an appropriate assay would help in accurate and rapid malaria diagnosis.

MATERIAL AND METHODS

The present study was carried out at a tertiary care hospital after approval by the Ethical committee of the parent college.

Two hundred blood samples were obtained from patients of all ages with clinical suspicion of malaria. Patients who came for follow-up visits of an earlier episode of malaria or within 4 weeks post treatment were excluded from the study. Patients with history of treatment with antimalarials were excluded from the study.

From each case 2 ml. of blood was collected into an ethylenediamine tetra acetic acid bulb(EDTA) and one thick and thin blood film each was prepared on different slides and stained with Giemsa stain as per standard protocol. A thick smear was considered negative if no parasites were seen in at least 200 fields.⁷

For examination of parasite density Giemsa stained thick blood films were scored by counting the total number of asexual parasites per 200

white bloodcells (WBCs) and the parasite index was calculated using the following formula.⁸

$$\frac{\text{Parasites counted} \times 8000}{\text{microlitre of blood}} = \text{parasite count per 200}$$

Thin films were examined for at least 100 fields in not more than 10 minutes.³ Speciation of the parasites was done by examining stained thin films. Three malaria rapid antigen detection kits (RDTs) were used and compared with peripheral smear. The three RDTs were :

Optimal - IT (DiaMed, Switzerland), Paramax -3 (Zephyr Biomedicals, India), and QDx MALARIA PAN / Pf (Piramal Healthcare, India).

Optimal - IT (DiaMed, Switzerland)

DiaMed Optimal – It is an immunochromatographic test, using monoclonal antibodies against the metabolic enzyme pLDH of Plasmodium spp. These monoclonal antibodies are classified in two groups; one specific for Plasmodium falciparum and the other is a pan - specific monoclonal antibody which reacts with all four species of Plasmodium spp. which can occur in human beings: P.falciparum, P.vivax, P.ovale, P.malariae

Paramax -3 (Zephyr Biomedicals, India)

It is a rapid, qualitative, two site sandwich immunoassay utilizing whole blood for the detection of P.falciparum specific histidine rich protein-2 (Pf. HRP-2), P.vivax specific pLDH and pan malaria specific pLDH.

QDx MALARIA PAN / Pf (Piramal Healthcare, India)

QDx Malaria PAN / Pf is a qualitative, sandwich immunoassay for the detection and differentiation of P.falciparum specific histidine rich protein-2 (Pf. HRP-2) and pan malaria specific pLDH.

Rapid diagnostic tests were read by the same bacteriologist and confirmed by a second independent reader when needed, all according to the manufacturer's instructions.

Statistical analysis: The performance of RDTs was expressed by calculating the sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV), for P. vivax and P. falciparum separately, taking microscopy results as the “gold standard”. The sensitivity and specificity of the gold standard was taken as 95%. The sample size of the study was 200. Based on this, standard error was calculated to be 1.54. The normal range of sensitivity and specificity is 95% 1.54% ie 93.46%-96.54%. Data were analyzed in SPSS. Proportions were compared using the Chi – square

test.

RESULTS

Of the 200 samples processed in the laboratory, 132 had positive blood films. Ninety four of the 132 infecting species were identified as *P.vivax*, 33 as *P.falciparum*, and 5 as mixed infections.

To assess the performance of OptiMAL-IT and QDx MALARIA PAN / Pf for diagnosis of *P.vivax*, cases with mixed infections with *P.falciparum* were excluded, because the pan -malaria antigen turns up positive due to *P.falciparum* infection. For performance of *P.falciparum* detection, mixed infections with *P.falciparum* were included. One slide which had only gametocytes was not included in further analysis.

TABLE 1

Based on the above observations OptiMAL-IT had a sensitivity and specificity of 98.47% and 95.58% respectively. The Positive predictive value (PPV) and Negative predictive value (NPV) was 97.72% and 97.01% respectively.

Sensitivity and specificity obtained for Paramax-3 was 95.41 % and 94.11% respectively. It had a PPV of 96.89% and NPV of 91.42 %.

The sensitivity and specificity recorded for QDxMALARIA PAN/Pf was 96.94% and 97.05% respectively. The PPV and NPV was 98.44% and 94.28% respectively.

The confidence interval calculated for the sensitivity and specificity of Giemsa stained peripheral blood smear which was the gold standard was found to be 93.46% - 96.54%. The sensitivity and specificity obtained for QDxMALARIA PAN/Pf was higher than this confidence interval, thus giving better results than microscopy. The sensitivity for OptiMAL-IT was also higher than the normal range for microscopy. The specificity for OptiMAL-IT and sensitivity and specificity for Paramax-3 is within the confidence interval giving comparable results with microscopy.

TABLE 2

Sensitivity of OptiMAL-IT for *P.falciparum* was 97.29%. The specificity recorded was 99.38%. The PPV and NPV obtained was 97.29 % and 99.38% respectively.

Based on the above observations Paramax-3 had a sensitivity and specificity of 94.59% and 98.76 % respectively for *P.falciparum*. The PPV and NPV was 94.59 % and 98.76 % respectively.

QDxMALARIA PAN/Pf recorded a sensitivity and specificity of 97.29 % and 99.38% respectively when tested for *P.falciparum*. The PPV observed was 97.29% while the NPV was 99.38%.

The sensitivity and specificity for OptiMAL-IT and QDxMALARIA PAN/Pf for *P.falciparum* was higher than the upper limit of confidence interval (93.46%- 96.54%). The specificity for Paramax-3 was also high while its sensitivity was within the range. Thus rapid tests gave either better or comparable results for *P.falciparum* when compared with microscopy.

TABLE 3

Sensitivity of OptiMAL-IT for *P.vivax* was 98.93%. The specificity recorded was 98.00%. The PPV and NPV obtained was 97.89 % and 98.98% respectively.

Paramax-3 had a sensitivity and specificity of 95.95% and 98.00 % respectively for *P.vivax*. The PPV and NPV was 97.93% and 96.07% respectively.

QDxMALARIA PAN/Pf recorded a sensitivity and specificity of 96.80 % and 99.00% respectively when tested for *P.vivax*. The PPV observed was 98.91% while the NPV was 97.05%.

The sensitivity and specificity for OptiMAL-IT and QDxMALARIA PAN/Pf for *P.vivax* was higher than the upper limit of confidence interval (93.46%- 96.54%). The specificity for Paramax-3 was also high while its sensitivity was within the range. Thus OptiMAL-IT and QDxMALARIA PAN/Pf for *P.vivax* gave better results when compared with microscopy. Paramax-3 also gave comparable results with microscopy.

TABLE 4

Percentage sensitivity for rapid tests with relation to parasitic index was lower at an index of 0-50 parasites / μ l for *P.falciparum* cases. Percentage sensitivity for Paramax-3 at parasitic index 50-100 parasites / μ l was lower than other rapid tests. Overall percentage sensitivity was higher for OptiMAL-IT and QDxMALARIA PAN/Pf as compared to Paramax-3 for *P.falciparum* cases.

TABLE 5

The overall percentage sensitivity with relation to parasitic index was highest for OptiMAL-IT for *P.vivax* cases. OptiMAL-IT gave a sensitivity of 100% at a parasitic index of 0-50 parasites/ μ l but one case was found to be negative with a parasitic index of 51 -100 parasites/ μ l.

DISCUSSION

The aim of the study was to compare three rapid antigen detection tests with peripheral blood smear for the diagnosis of malaria. The number of cases were limited to only symptomatic indoor patients with clinical suspicion of malaria. Sub-clinical infection or normal controls were not included in the study, hence the number of samples with low parasitaemia (<100 per cumm) and gametocytes only, were few in the study.

It was found that rapid tests yielded comparable results with microscopy.

OptiMAL-IT a pLDH based assay had an overall sensitivity of 98.47% and a specificity of 95.58 %. The positive predictive value (PPV) was 97.72% and negative predictive value (NPV) was 97.01%. It had a sensitivity of 97.29 % and specificity of 99.38 % for *P.falciparum* and sensitivity of 98.93% and specificity of 98% for *P.vivax*. QDx MALARIA PAN / Pf gave similar results as OptiMAL-IT. It had an overall sensitivity of 96.94 % and a specificity of 97.05 %. The PPV was 98.44 % and NPV of 94.28 %. It had a sensitivity of 97.29 % and specificity of 99.38 % for *P.falciparum* and sensitivity of 96.80 % and specificity of 99% for *P.vivax*. The results obtained for Paramax -3 were slightly lower than the above two tests. It showed an overall sensitivity of 95.41 % and a specificity of 94.11 %. The PPV was 96.89 % and NPV of 91.42 %. It had a sensitivity of 94.59 % and specificity of 98.76 % for *P.falciparum* and sensitivity of 95.95 % and specificity of 98% for *P.vivax*.

The overall sensitivity and specificity obtained for QDxMALARIA PAN/Pf was higher than the confidence interval calculated for comparing rapid tests with microscopy. Thus it gave better results than microscopy. The sensitivity for OptiMAL-IT was also higher than the normal range for microscopy. The specificity for OptiMAL-IT and sensitivity and specificity for Paramax-3 was within the confidence interval giving comparable results with microscopy.

Sensitivity of rapid tests with relation to parasite index was comparable with microscopy at a parasitic index of >100 parasites/ μ l. However the sensitivity dropped when the parasitic index was below 100 parasites / μ l.

Several authors who have tested OptiMAL-IT for malarial diagnosis from different countries have given more or less similar results. They have reported a sensitivity and specificity in the range of 80-100%.⁹ Palmer et al in Honduras has reported a sensitivity of 94% and specificity of 100% for *P.vivax* and sensitivity of 88% and specificity of 99% for *P. falciparum*.¹⁰ From hospital of tropical diseases UK, Moody et al while conducting their study in sub-Saharan Africa, Asia and South America have reported a sensitivity of 95.3% and specificity of 100% for *P. falciparum* and sensitivity of 96% and a specificity of 100% for *Plasmodium vivax*.¹¹ John et al obtained a sensitivity of 94% for *P. falciparum* and 98.2% for *P.vivax* in a trial in Southern India.¹² The sensitivity and specificity of the present study falls in the range given above and is comparable to the various studies. Therefore the results of this study further substantiates that OptiMAL-IT is an effective and sensitive tool in the diagnosis of malaria.

There are no published reports for Paramax-3 and QDx MALARIA PAN / Pf except for the technical support sheet by Malaria Research Centre (ICMR) for testing Paramax.¹³ There are several studies on other HRP2 based assays like ICT P.f/P.v, NOW-Malaria-ICT, Paracheck-Pf, ParaSight-F, and Parascreen pan/pf etc. Paramax and Parascreen malaria diagnostic tests manufactured by M/s Zephyr Biomedicals, Goa were tested at Malaria Research Centre (ICMR),

Goa, India in 2004. Both tests gave a 100% sensitivity, specificity, NPV, and PPV for both *P. falciparum* and *P. vivax*.

The parasitaemia range for *P. falciparum* and *P. vivax* was 400-22,720 and 520-33600 parasites/μl of blood respectively.¹³ In the present study Paramax -3 was 100 % sensitive at a parasitic index of >100 parasites/μl.

Among the various HRP2 based tests for malaria, there is a wide range of sensitivity and specificity seen. In the present study the rapid tests showed a sensitivity and specificity at the higher range. The sensitivity and specificity for Paramax -3 was slightly lower than OptiMAL-IT and QDx MALARIA PAN / Pf. However it can identify the infection caused by *P. falciparum* and *P. vivax* separately. It can also diagnose mixed infection. The identity of the *Plasmodium* species helps clinicians to anticipate complications and treat the patients promptly and accurately.

In spite of over hundred published RDT trial reports, comparative assessment is difficult because i) trials do not share common guidelines; ii) clinical and epidemiological characteristics of the study populations, especially parasitemia level vary; iii) reference standards are different; even among those using Giemsa microscopy, reading rules and microscopist's skill vary; and iv) products of different lots may differ in quality or be damaged by extreme temperature or humidity during transportation and storage.⁹

HRP-2 based tests commonly give *P. falciparum* sensitivity of > 90 % in clinical cases. For pLDH assays, results varied among studies and product lots and variable field stability of the test could not be ruled out. Sensitivity for *P. falciparum* is excellent (>95%) in some studies and poorer (80 %) in others. Recent studies suggest that the tests were less sensitive for non - *P. falciparum* than for *P. falciparum*. Extremely low sensitivity had been reported earlier for both HRP-2 and pLDH tests and batch specific problems were suspected. Overall RDT specificity is commonly above 85%, approaching 100% when used in some groups of returning non immune travellers.¹⁴

In the present study OptiMAL-IT missed two cases which were positive on microscopy. Paramax-3 missed 6 cases while QDx MALARIA PAN / Pf missed 4 cases of malaria. The reason for these false negative results could be that rapid tests are not sensitive below a parasitic index of 100 parasite/ul.¹⁵ This fact is also reflected in the present study, as, all the cases which were missed had a parasitic index below 100 parasite/ul on the peripheral blood smear. Besides, occasional false negative results may be caused by deletion or mutation of the hrp-2 gene. Secondly, It has been suggested that anti HRP-2 antibodies in humans may explain why some tests were negative. Presence of an inhibitor in the patient's blood preventing development of control line has also been noted.¹⁴ pLDH is produced by living parasites. It is possible that some patients might have already taken antimalarials and not disclosed it which could also account for negative results of the RDTs.¹⁶

The rapid tests also gave some false positive results. OptiMAL -IT gave 3 false positive results while Paramax-3 and QDx MALARIA PAN / Pf gave 4 and 2 false positive results respectively. These may be due to a number of factors. Cross reactivity with rheumatoid factor in blood generates a false positive test line, but replacement of IgG with IgM in recent products reduces this problem. Cross reactivity with heterophile antibodies may also occur.¹⁴ HRP -2 has been shown to persist and is detectable after the clinical symptoms of malaria have disappeared and the parasites have apparently been cleared from the host. Low level parasitemia seen in areas of endemic infection because of constant exposure to the malarial parasites may also result in positive results with doubtful clinical significance.⁸ Furthermore, in some infections parasites may have been sequestered and would not be detected on peripheral blood smear examination.¹⁷

It was observed that five cases of mixed malarial infection were seen on microscopy. OptiMAL-IT and QDx MALARIA PAN / Pf reported these cases as *P. falciparum* due to the configuration of the test. Paramax-3 identified these cases as mixed infections with 100% sensitivity and specificity. As the management of mixed infections includes *P. vivax* treatment, the format used in Paramax-3 is advantageous. One case in this study showed only the gametocytes of

P. falciparum in the blood smear and no ring forms were seen. The rapid tests reported it as *P. falciparum* infestation. Such results can lead to unnecessary medication.

The commercially available malaria rapid detection tests in kit form are a good alternative to microscopic diagnosis for malaria especially where quick results are required or in health care facilities where there is a lack of manpower and equipments as these tests do not require extensive training or equipments to perform or to interpret the results. Besides, the tests can be performed in 15 to 20 minutes.

CONCLUSIONS

Peripheral blood smear examination remains the gold standard for diagnosis of malaria while Rapid antigen detection tests are a good alternative but cannot be a substitute. Rapid antigen detection tests have maximum utility in emergency laboratories, intensive care setups and the casualty where quick results are desired. They can also be used for malaria diagnosis where facilities for expert microscopy are not available

Table 1: Results Of Malaria Rapid Detection Tests For Malaria Positive Cases (n=199)

Results of RDTs	Results of Microscopy		Total
	Positive	Negative	
OptiMAL-IT results (n=199)			
Positive	129	3	132
Negative	2	65	67
Paramax-3 results(n=199)			
Positive	125	4	129
Negative	6	64	70
QDxMALARIAPAN/Pf (n=199)			
Positive	127	2	129
Negative	4	66	70

Table 2: Results Of Rapid Tests For P.falciparum

Results of RDTs <i>P. falciparum</i> (n=199)	Results of Microscopy		Total
	Positive	Negative	
OptiMAL-IT <i>P. falciparum</i>			
Positive	36	1	37
Negative	1	161	162
Paramax-3			
Positive	35	2	37
Negative	2	160	162
QDxMALARIAPAN/Pf			
Positive	36	1	37
Negative	1	161	162

Table 3: Results Of Rapid Tests For P.vivax

Results of RDTs <i>P. vivax</i> (n=194)	Results of Microscopy		Total
	Positive	Negative	
OptiMAL-IT			
Positive	93	2	95
Negative	1	98	99
Paramax-3			
Positive	95	2	97
Negative	4	98	102
QDxMALARIAPAN/Pf			
Positive	91	1	92
Negative	3	99	102

Table 4: Percentage Sensitivity Of Rapid Tests With Relation To Parasitic Index For P.falciparum (n=32)*

<i>P. falciparum</i> (n=32)* Parasites/μl	Microscopy Results	OptiMAL-IT positive (% Sensitivity)	Paramax-3 positive (% Sensitivity)	QDxMALARIA PAN/Pf positive (% Sensitivity)
0-50	3	2(66.66)	2(66.66)	2(66.66)
51-100	3	3(100)	2(66.66)	3(100)
101-500	3	3(100)	3(100)	3(100)
501-5,000	7	7(100)	7(100)	7(100)
5,001-50,000	14	14(100)	14(100)	14(100)
>50,000	2	2(100)	2(100)	2(100)
Overall	32	31(96.87)	30(93.75)	31(96.87)

* False positive cases and a case with only gametocytes was not included

Table 5: Percentage Sensitivity Of Rapid Tests With Relation To Parasitic Index For P.vivax (n=94)*

P.vivax (n=94)* Parasites/µl	Giemsa positive	OptiMAL-IT positive (% Sensitivity)	Paramax-3 positive (%Sensitivity)	QDxMALARIA PAN/Pf positive (% Sensitivity)
0-50	9	9 (100.00)	7 (77.77)	7 (77.77)
51-100	9	8 (88.88)	7 (77.77)	8 (88.88)
101-500	16	16 (100)	16 (100)	16(100)
501-5,000	17	17 (100)	17 (100)	17 (100)
5,001-50,000	41	41 (100)	41 (100)	41 (100)
>50,000	2	2 (100)	2 (100)	2 (100)
Overall	94	93 (98.93)	90 (95.74)	91 (96.80)

* False positive cases were not included.

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