Evaluation of Rapid Diagnostic Tests for Early Diagnosis of Malaria in Rural India to Prevent Deadly Complications

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ABSTRACT

BACKGROUND

As per World Malaria Report, 2018, There were an estimated 219 million cases and 435000 related deaths in 2017, with about 9.5 million malaria cases and 16,733 related deaths in 2017 in India. The most severe clinical form of malaria is caused by *P. falciparum* infection. The disease is usually fatal in non-immunes. The complications of falciparum malaria are cerebral malaria, pulmonary oedema, septicaemia and shock. If these complications are not diagnosed early and are not treated adequately, the patient becomes fatal. To prevent these complications, we need early diagnosis. Clinical diagnosis of malaria requires microscopic detection of the parasite. Diagnosis of malaria by microscopy is the gold standard but requires expertise and is time consuming. Recently, new rapid malaria detection tests based on detection of an enzyme produced by live parasites, parasite lactate dehydrogenase (pLDH) and Histidine Rich Proteins (HRPII) have been developed. It is simple, easy to use and does not require expertise. In the present study, the Rapid Diagnostic Tests are evaluated for the effectiveness for early diagnosis of Malaria in Rural India to prevent deadly complications.

METHODS

The study, conducted in a hospital, included 733 patients with fever of 3 days duration after obtaining consent from the subjects and approval from the ethical committee. 733 whole blood samples were collected and tested for Smear Microscopy for Malarial parasites and were also tested with Rapid Diagnostic Tests. The study was spread over 10 months from June, 2018 to March, 2019 and results were sent for analysis. Rapid Diagnostic Test (RDT) was compared with 'Gold Standard' technique i.e.; Microscopy- Smear Examination with the help of X² values, sensitivity, specificity, positive predictive values, negative predictive values, percentage of false negatives, percentage of false positives, positive likelihood ratio(LR+), and negative likelihood ratio (LR-) values.

RESULTS

The results of Rapid Diagnostic Test (RDT) were compared with smear microscopy and it was found that RDT is as effective as Smear Microscopy in diagnosing Malaria with 97.81% sensitivity, 97.82% specificity, 97.20% Positive predictive value and 98.30% Negative predictive value. In diagnosing the species of Malaria also, RDT is found to be as effective as Smear Microscopy in differentiating Falciparum Malaria with 97.98% sensitivity value, 98.79% specificity value, 97.49% positive predictive value, 99.03% negative predictive value. RDT is found to be as effective as Smear Microscopy in differentiating Vivax Malaria with 97.54% sensitivity, 99.03% specificity, 96.75% positive predictive value and 99.27% negative predictive value.

CONCLUSIONS

The present study concludes Rapid Diagnostic Tests are effective screening tests as shown by the high sensitivity, specificity and predictive values, high Likelihood positive(LR+) and low Likelihood Negative(LR-) values.

KEY WORDS

Smear for Malarial Parasite, QBC Test, Rapid Diagnostic Test, Falciparum Malaria, Vivax Malaria, Cerebral Malaria

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BACKGROUND

As per World Malaria Report 2018, there were an estimated 219 million cases and 435 000 related deaths in 2017,¹ with about 9.5 million malaria cases and 16,733 related deaths in 2017 in India.² In India, malaria is more prevalent in Orissa accounting for 25% followed by Chhattisgarh (13%), West Bengal(11%), North Eastern States (10%), Jharkhand(7%) and Karnataka (7%).³ The most severe clinical form of malaria is caused by *P. falciparum* infection.⁴ The disease is usually fatal in non-immunes. Falciparum malaria may be fatal if treatment is delayed beyond 24 h after the onset of clinical symptoms.⁵ The complications of falciparum malaria are cerebral malaria, pulmonary oedema, septicaemia and shock.⁶ If these complications are not treated adequately the patient becomes fatal. To prevent these complications, we need early diagnosis. Delayed diagnosis and comatose condition were the main determinants of death. Clinical diagnosis of malaria requires microscopically observation of the parasite. Diagnosis of malaria by microscopy is a gold standard but requires expertise, consumes time. Recently, a new rapid malaria detection tests based on detection of an enzyme produced by live parasites, parasite lactate dehydrogenase (pLDH) and Histidine Rich proteins (HRPII). In the present study, the Rapid Diagnostic Tests are evaluated for the effectiveness for early diagnosis of Malaria in Rural India to prevent deadly complications.

The authors wanted to evaluate the effectiveness of Rapid Diagnostic Test (RDT) in early diagnosis of Malaria in Rural India to prevent deadly complications of Malaria.

METHODS

Research Design

The Study was hospital based cross sectional study conducted in a hospital included 733 patients with fever of 3 days duration. Fever with chills is included and the subjects with known history of HIV and Tuberculosis are excluded. After obtaining consent, 733 whole blood samples were collected and tested for Smear Microscopy for Malarial parasites and also tested with Rapid Diagnostic Tests. The results of RDT are compared with the results of Gold Standard technique i.e., peripheral smear for Malarial parasites with the help of χ^2 values and sensitivity, specificity, positive predictive values and negative predictive values, Percentage of False Negatives, Percentage of False Positives, Positive Likelihood Ratio(LR+), Negative Likelihood Ratio(LR-) values. The study included 733 patients with fever of several days' duration. Consent was taken for the study and sample collection. Ethical committee approval was obtained. The study was spread over 10 months from June, 2018 to March, 2019 and results were analysed.

Statistical Techniques

High sensitive test is one that correctly identifies patients with a disease, also called true positive rate. High specificity test is one that correctly identifies patients without disease, also called true negative rate. Positive Predictive Value indicates the probability of a disease in persons who have a

positive test result. Negative Predictive Value indicates the probability of not having the disease in persons who have a negative test result. Chi-square is applied to test the goodness of fit to verify the distribution of observed data with assumed theoretical and expected frequencies. A null hypothesis is established and verified with Chi-square test.

RESULTS

Comparison of Smear Microscopy and Rapid Diagnostic Test in the Diagnosis of Malaria

In Diagnosis of Malaria, Rapid Diagnostic Test has shown Sensitivity value as 97.81%, Specificity value as 97.82%, Positive predictive value as 97.20% and Negative predictive value as 98.30%. Percentage of False Negatives as 2.19, Percentage of False Positives as 2.18, Positive Likelihood Ratio (LR+) 44.89, Negative Likelihood Ratio (LR-) 0.02. At Degrees of freedom 1 and at 0.05 significance level, the table value is 3.841. The calculated value is 0.02 which is less than the table value. Hence the null hypothesis, 'There is no difference in the diagnosis of malaria between smear microscopy and Rapid Diagnostic Test' is retained. Comparison data shows Rapid Diagnostic Test is as effective as Smear for MP in diagnosis of Malaria.

Blood Smear RDT	Positive	Negative	Total
Positive	313	9	322
Negative	7	404	411
Total	320	413	733
Sensitivity			97.81
Specificity			97.82
Positive Predictive Value			97.20
Negative Predictive Value			98.30
Percentage of False Negatives			2.19
Percentage of False Positives			2.18
Positive Likelihood Ratio (LR+)			44.89
Negative Likelihood Ratio (LR-)			0.02

Table 1. Calculation of Sensitivity and Specificity of Rapid Diagnostic Test in the Diagnosis of Malaria

Test	Positive					Negative				
	O	E	(O-E)	(O-E) ²	(O-E) ² /E	O	E	(O-E)	(O-E) ²	(O-E) ² /E
Blood Smear	320	320	0	0	0.00	413	413	0	0	0.00
RDT Test	322	320	2	4	0.013	411	413	-2	4	0.010
$\Sigma \chi^2 =$										0.02

Table 2. Chi-Square Estimation between Smear Microscopy and Rapid Diagnostic Test in the Diagnosis of Malaria

Comparison of Smear Microscopy and Rapid Diagnostic Test in the Diagnosis of Falciparum Malaria

In diagnosis of Falciparum Malaria, Rapid Diagnostic Test has shown Sensitivity value as 97.98%, Specificity value as 98.79%, Positive predictive value as 97.49% and Negative predictive value as 99.03%. Percentage of False Negatives as 2.02, Percentage of False Positives as 1.21, Positive Likelihood Ratio (LR+) 80.93, Negative Likelihood Ratio (LR-) 0.02. At Degrees of freedom 1 and at 0.05 significance level, the table value is 3.841. The calculated value is 0.01 is less than the table value. Hence the null hypothesis, 'There is no difference in the diagnosis of Falciparum malaria between smear microscopy and Rapid Diagnostic Test' is retained.

Comparison data shows Rapid Diagnostic Test is as effective as Smear for MP in diagnosis of Falciparum Malaria.

Blood Smear	Positive	Negative	Total
RDT			
Positive	194	5	199
Negative	4	408	412
Total	198	413	611
Sensitivity			97.98
Specificity			98.79
Positive Predictive Value			97.49
Negative Predictive Value			99.03
Percentage of False Negatives			2.02
Percentage of False Positives			1.21
Positive Likelihood Ratio (LR+)			80.93
Negative Likelihood Ratio (LR-)			0.02

Table 3. Calculation of Sensitivity and Specificity of Rapid Diagnostic Test in the Diagnosis of Falciparum Malaria

Test	Positive					Negative				
	O	E	(O-E)	(O-E) ²	(O-E) ² /E	O	E	(O-E)	(O-E) ²	(O-E) ² /E
Blood Smear	198	198	0	0	0.00	413	413	0	0	0.00
RDT Test	199	198	1	1	0.005	412	413	-1	1	0.002
$\Sigma x^2 =$										0.01

Table 4. Chi-Square Estimation between Smear Microscopy and Rapid Diagnostic Test in Diagnosis of Falciparum Malaria

Comparison of Smear Microscopy and Rapid Diagnostic Test in the Diagnosis of Vivax Malaria

In diagnosis of Vivax malaria, Rapid Diagnostic Test has shown Sensitivity value as 97.54%, Specificity value as 99.03%, Positive predictive value as 96.75% and Negative predictive value as 99.27%. Percentage of False Negatives as 2.46, Percentage of False Positives as 0.97, Positive Likelihood Ratio (LR+) 100.71, Negative Likelihood Ratio (LR-) 0.02. At Degrees of freedom 1 and at 0.05 significance level, the table value is 3.841. The calculated value is 0.01 is less than the table value. Hence the null hypothesis, 'There is no difference in the diagnosis of Vivax malaria between smear microscopy and Rapid Diagnostic Test' is retained. Comparison data shows Rapid Diagnostic Test is as effective as Smear for MP in diagnosis of Vivax Malaria.

Blood Smear	Positive	Negative	Total
RDT			
Positive	119	4	123
Negative	3	409	412
Total	122	413	535
Sensitivity			97.54
Specificity			99.03
Positive Predictive Value			96.75
Negative Predictive Value			99.27
Percentage of False Negatives			2.46
Percentage of False Positives			0.97
Positive Likelihood Ratio (LR+)			100.71
Negative Likelihood Ratio (LR-)			0.02

Table 5. Calculation of Sensitivity and Specificity of Rapid Diagnostic Test in Diagnosis of Vivax Malaria

z	Positive					Negative				
	O	E	(O-E)	(O-E) ²	(O-E) ² /E	O	E	(O-E)	(O-E) ²	(O-E) ² /E
Blood Smear	122	122	0	0	0.00	413	413	0	0	0.00
RDT Test	123	122	1	1	0.008	412	413	-1	1	0.002
$\Sigma x^2 =$										0.01

Table 6. Chi-Square Estimation between Smear Microscopy and Rapid Diagnostic Test in Diagnosis of Vivax Malaria

DISCUSSION

The present study compared Rapid Diagnostic Tests with Smear Examination as gold standard and concluded RDT with high sensitivity 97.81%, high specificity 97.82%, Positive predictive value as 97.20% and Negative predictive value as 98.30%. Percentage of False Negatives as 2.19, Percentage of False Positives as 2.18, Positive Likelihood Ratio (LR+) 44.89, Negative Likelihood Ratio (LR-) 0.02. In diagnosis of Falciparum malaria also, Rapid Diagnostic Test has shown High Sensitivity value as 97.98%, High Specificity value as 98.79%, Positive predictive value as 97.49% and Negative predictive value as 99.03%. Percentage of False Negatives as 2.02, Percentage of False Positives as 1.21, Positive Likelihood Ratio (LR+) 80.93, Negative Likelihood Ratio (LR-) 0.02. In differentiating Vivax malaria, Rapid Diagnostic Test has shown High Sensitivity value as 97.54%, High Specificity value as 99.03%, Positive predictive value as 96.75% and Negative predictive value as 99.27%. Percentage of False Negatives as 2.46, Percentage of False Positives as 0.97, Positive Likelihood Ratio (LR+) 100.71, Negative Likelihood Ratio (LR-) 0.02.

Sensitivity refers to the test's ability to correctly detect ill patients who do have the condition. A high sensitivity test is reliable, since it rarely misdiagnoses those who have the disease. A negative test result would definitively rule out presence of the disease in a patient. Specificity relates to the test's ability to correctly reject healthy patients without a condition. The test with high specificity rarely gives positive results in healthy patients. A test with a higher specificity has a lower type I error rate. Likelihood Ratio (LR+) Values greater than 1 increase the probability of disease. Likelihood Ratio (LR-) Values between 0 and 1 decrease the probability of disease. As Rapid Diagnostic Test has shown high Sensitivity and high specificity, high Likelihood Ratio (LR+) and Likelihood Ratio (LR-) values less than 0 in diagnosis of malaria and differentiating the malarial species, it can be considered as good screening test for the diagnosis of malaria and also malarial species differentiation.

At Degrees of freedom 1 and at 0.05 significance level, Chi-Square estimations are less than the table value 3.841. Hence the hypotheses 'There is no difference in the diagnosis of malaria between smear microscopy and Rapid Diagnostic Test', 'There is no difference in the diagnosis of Falciparum malaria between smear microscopy and Rapid Diagnostic Test' and 'There is no difference in the diagnosis of Vivax malaria between smear microscopy and Rapid Diagnostic Test' are retained. Hence it is concluded that Rapid Diagnostic Tests is as effective as Smear for Malarial parasite examination which is gold standard in diagnosis of malaria and differentiating the malarial species.

The data of the present study coincides with the previous studies. Parija SC et al⁷ in their study compared thick smear as gold standard with RDT shown sensitivity of 75%. Bhat Sandhya K et al⁸ in their study, compared RDT with thick smear as gold standard and shown RDT sensitivity, specificity of 95.45%, 100%, respectively. Bhavnaben A. Vora et al⁹ in their comparative study, Peripheral smear had 85.5% sensitive and 100% specific compared to RDT which was 100 % sensitive and specific whereas centrifuged buffy coat was 92.7% sensitive and 99.3% specific. Samina Naz Mukry et al¹⁰

concluded in their study that RDT with a sensitivity of 91.52%. Manali M. Kocharekar et al¹¹ studied the efficacy of RDT and concluded the sensitivity of Parahit total, Advantage mal card, and modified QBC for *P. falciparum* detection was 70.3%, 95%, and 98%, and specificity was 98%, 98%, and 96%, respectively. Saha S et al¹² concluded in their study that the sensitivity and specificity of microscopy was 63%, 99.4% respectively. Sensitivity and specificity of RDT was 94% and 99% respectively. RDT had the highest LR for positive test result 175 and the lowest LR for negative test result 0.058. SS Sahu et al¹³ concluded in their study to improve the efficiency (Sensitivity) of the kit so that the true falciparum infections will not be missed especially in areas where *P. falciparum* has been the predominant species causing cerebral malaria with the results of the sensitivity of ParaHIT-f was 63.6% and specificity was 98.9%. With positive and negative predictive values (PPV and NPV) of 92.6% and 93.0% respectively. NitiN Joseph et al¹⁴ stressed in their study that Diagnostic Antigen detecting MRDT showed good performance as a screening test and hence can be recommended for wide-scale usage at these settings with sensitivity of 98.6%, specificity of 86.9%, Positive Predictive Value (PPV) of 94.1% and Negative Predictive Value (NPV) of 96.6%. The study by Falade et al¹⁵ concluded that

RDTs are useful in guiding malaria management and were successfully used for diagnosis by trained CHWs. Sensitivity and specificity of SD-Bioline RDT were 94.3% and 41.6% respectively, while the negative and positive predictive values were 86.1% and 65.6%, respectively. Gupta et al¹⁶ concluded that the RDT based on malaria antigen method is as specific and sensitive as the traditional PBS microscopy with Sensitivity and specificity of RDT were 91.8% and 93.8%, respectively. Positive predictive value and negative predictive value were 97.8% and 98.9% respectively. Batwala et al¹⁷ compared the RDT with smear for microscopy and concluded with the results of the sensitivity of RDT and Microscopy 91.0%, 46% respectively. The specificity of RDT and Microscopy were 86.3%, 93.4% respectively. Ayogu E E¹⁸ concluded that Rapid diagnostic tests could be useful in areas with high parasite density as an alternative to smear microscopy with the sensitivity and specificity PPV and NPV of RDT 82.2%, 100.0%, 100.0%, and 34.3%, respectively. Oyetunde T et al¹⁹ in their study considering microscopy as the gold standard, RDT exhibited high specificity 87.1% and low sensitivity 42.5% with positive predictive and negative predictive values of 86.6 and 43.5%, respectively. Jaswinder Sharma et al²⁰ concluded in their study that the sensitivity of RDT is 97.4%, specificity 100% when compared to microscopy.

CONCLUSIONS

The Rapid Diagnostic Tests are effective screening tests. Comparison with Smear Microscopy has shown that Rapid Diagnostic Tests have high sensitivity, specificity and predictive values, high Likelihood positive (LR+) and low Likelihood Negative (LR-) values. The rapid test is simple, cheap and reliable tool to diagnose malaria early to prevent

deadly complications, especially in rural and tribal areas where facilities are insufficient.

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