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Original Research Article

A Comparative evaluation of diagnostic performance of Immunochromatographic Rapid Card Test for malarial antigen against Peripheral smear Microscopy

Abhishek Mehta[*1], Vijay Prakash Singh[1] and Amod Borle[2]

[1]*Department of Microbiology, KD Medical College, Hospital & Research Center, Mathura, India*
[2]*Department of Community Medicine(PSM), KD Medical College, Hospital & Research Center, Mathura, India*

***Correspondence Info:**
Dr. Abhishek Mehta
Assistant Professor,
Department of Microbiology,
KD Medical College, Hospital & Research Center, Mathura, India
E-mail: abhishekmehta623@gmail.com

Abstract

Background: Malaria poses a major public health problem in India and has emerged as a biggest health burden in terms of morbidity and mortality. Rapid detection of malarial parasite can ensure the prompt timely and efficient management of clinical cases and control of the disease.

Objectives: To evaluate the diagnostic performance of Immunochromatographic Rapid Card Test (ICT) for malarial antigen against Peripheral smear microscopy for diagnosing Malaria.

Materials and Methods: This Cross sectional study was conducted in Dept. of Microbiology of a Rural tertiary care teaching hospital of Western Uttar Pradesh from January 2016 to August 2016. Eight Hundred thirty seven patients with clinical suspicion of malaria whose samples were sent on advice by clinicians to the Microbiology laboratory for the peripheral smear microscopy and Rapid card test for malarial antigen were included in this study after obtaining clearance from Institutional Ethics committee.

Results: Out of the 837 blood samples tested, 106(12.66%) samples tested positive for malaria by Peripheral smear microscopy and 109(13%) by ICT Rapid card tests. The sensitivity of ICT for non-falciparum malaria (82.65%) came out to be lower than that for falciparum malaria (87.5%). The specificity of ICT for non falciparum malaria (98.9%) was almost same as for falciparum malaria (99.3%).The accuracy of ICT for falciparum malaria (99.16%) was slightly higher than for non-falciparum malaria (97%).

Conclusion: This study suggests that ICT Rapid card test couldn't replace PS microscopy completely, but can be used as an alternative or complimentary to microscopy, particularly in resource constrained remote rural areas of India.

Keywords: Malaria, Peripheral smear microscopy, Immunochromatographic Rapid Card test (ICT), Plasmodium falciparum, Non-falciparum Plasmodium species, Diagnostic performance.

1. Introduction

Malaria is the most important protozoal disease endemic in 108 countries with a population of more than 2.4 billions at risk and killing 10-30 lacs humans every year. Throughout the world the annual incidence of malaria is around 300-500 million cases [1,2].

Malaria poses a major public health problem in India and has emerged as a biggest health burden in terms of morbidity and mortality. Malaria is caused by a protozoan parasite of genus Plasmodium and transmitted by female anopheline mosquitoes. Rapid detection of malarial parasite can ensure the prompt timely and efficient management of clinical cases and control of the disease [3,4].

With the diverse presentations of the disease, clinical diagnosis alone is not reliable and requires confirmation by Laboratory investigations [1].

Nowadays many diagnostic modalities for malaria are available ranging from conventional Peripheral smear microscopy to Quantitative Buffy coat examination (QBC), Rapid Immunochromatographic tests and molecular diagnostics like PCR. But still the old time tested conventional method of Peripheral smear microscopy is considered as the Gold standard against which all the other tests are evaluated[1,3].

In India Peripheral smear microscopy still exists as the most commonly employed method for diagnosing malaria. Though it is cost effective but cumbersome, difficult, labour intensive, time consuming and requires technical expertise, adequate quality control, laboratory set up and resources [5].

So, as an alternative non-microscopic immunochromatographic rapid antigen detection tests are available as rapid cards/dipsticks/cassettes and is based on detection of *P. falciparum* specific antigen Histidine Rich Protein-2 enzyme(HRP-2) and Pan malarial antigens (Pan Lactate dehydrogenases-pLDH / Pan aldolase enzymes). These tests are relatively easier, simpler and give quicker results [1].

In the light of above facts present study was undertaken to evaluate the diagnostic performance of Immunochromatographic Rapid antigen detection card tests against the Peripheral smear microscopy.

2. Materials and Methods

This Non-randomized Descriptive and Cross Sectional study was conducted in Department of Microbiology of a Rural tertiary care teaching hospital of North India from January 2016 to August 2016.

Eight Hundred thirty seven patients with clinical suspicion of malaria whose samples were sent on advice by clinicians to the Microbiology laboratory for the peripheral smear microscopy and Rapid card test for malaria antigen were included in this study after obtaining clearance from Institutional Ethics committee.

The venous blood samples were collected from antecubital fossa into EDTA tubes for microscopy and rapid card tests. Thick and thin blood smears were prepared and stained with Giemsa following standard procedure and examined microscopically for different stages of malarial parasite under 100x objectives. Thick smears were considered negative only when no malarial parasites were seen in 100 consecutive high power fields. The microscopists were kept totally unaware about the results of rapid card tests .The results of rapid card test was read by person who was blinded to microscopy results[6].

All the samples were also tested by Malaria antigen rapid card test as per manufacturer's instructions. This test is a immunochromatographic test detecting Pan specific pLDH and *P. falciparum* specific Pf-HRP-2 enzymes.

Four µl. of whole anticoagulated blood is taken from EDTA vial by sample dropper and added onto the sample pad in the Sample well 'S'. Thereafter 3 drops of assay buffer added into buffer well 'B'. The result was read after 20min.

Appearance of 3bands one each in *P. falciparum* region (F), Pan region (P) and Control region(C) indicates that the sample is reactive for *P.falciparum* and/or *P.vivax/P.malariae/P.ovale*.

Appearance of 2 bands one each at P & C region indicates that the sample is reactive for *P.vivax/P.malariae/P.ovale* only.

Appearance of 2 bands one each at F & C region indicates that the sample is reactive for *P.falciparum* only. Appearance of only 1 band at Control(C) region indicates that the sample is non-reactive for all 4 Plasmodium species.

If no band appears in control (C) region after completion of test; the test is considered invalid and repeated using new card.

2.1 Statistical Analysis

After compiling the data of tests conducted during the study period; Sensitivity, Specificity, Positive predictive value, Negative predictive value and Accuracy of the ICT Rapid Card tests were estimated using Peripheral smear microscopy as gold standard. The variables measured were the number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN).

Sensitivity was calculated as TP/ (TP+FN), Specificity as TN/(TN+FP), the positive predictive value (PPV) as TP/(TP+FP), the negative predictive value (NPV) as TN/(FN+TN), Test accuracy as (TP +TN)/Total no. of tests, Positive Likelihood ratio as Sensitivity/(1-specificity) and Negative Likelihood ratio as (1-sensitivity)/specificity.

3. Observations and Results

Out of the 837 blood samples tested by Peripheral smear microscopy, 106(12.66%) samples tested positive for malaria out of which 98(92.45%) for *P.vivax* and 8(7.5%) for *P.falciparum*.

Out of 837 blood samples tested by Rapid card tests 109(13%) samples tested positive for malaria out of which 96(88.1%) for *P.vivax* and 13(12%) for *P.falciparum*.

The Diagnostic performance of ICT Rapid Card test as compared to Peripheral smear microscopy is depicted in Tables 1 to 6.

The overall sensitivity and specificity of ICT in diagnosing malaria came out to be 84% and 98% respectively with a PPV of 87.225%, NPV of 97.68% and Accuracy of 96.4%.

Out of 98 malaria cases found to be caused by non-falciparum Plasmodium spp.(diagnosed by PS microscopy) 81 cases could be detected by ICT but 17 cases were missed (False negatives).

Out of 8 malaria cases found to be caused by *P.falciparum*, ICT was able to detect 7cases while missing 1 case (False negative).

88 cases tested positive by both ICT as well as PS microscopy.

Six cases of *P.falciparum* and 8cases of Non-falciparum Plasmodium spp. as diagnosed by ICT only were found to be False positives.

The sensitivity of ICT for non-falciparum malaria came out to be 82.65% which was lower than that for falciparum malaria (87.5%).

The specificity of ICT for non falciparum malaria (98.9%) was almost same as for falciparum malaria (99.3%). The accuracy of ICT for falciparum malaria (99.16%) was slightly higher than for non-falciparum malaria (97%).

Table 1: The Diagnostic performance of ICT Rapid Card test as compared to Peripheral smear microscopy

Test	Peripheral Smear		
ICT	**PS +ve**	**PS-ve**	**Total**
Positive	89	13	102
Negative	17	718	735
Total	106	731	837

Table 2: Diagnostic test characteristics of ICT Rapid Card tests compared to Peripheral smear microscopy

Indices	Estimated value	95% Confidence Interval	
		Lower limit	**Upper limit**
Sensitivity	0.84	0.75	0.90
Specificity	0.98	0.97	0.99
PPV	0.87	0.79	0.93
NPV	0.98	0.96	0.986
LR^+	47.21	27.37	81.43
LR^-	0.16	0.105	0.25
Accuracy	96.416		

Table 3: Diagnostic performance of ICT Rapid Card tests compared to PS microscopy for detecting *P. falciparum*

Test	Peripheral Smear		
ICT	**PS +VE**	**PS-VE**	**Total**
Positive	7	6	13
Negative	1	823	824
Total	8	829	837

Table-4: Diagnostic test characteristics of ICT Rapid Card tests compared to Peripheral smear microscopy for *P. falciparum*

Indices	Estimated value	95% Confidence Interval	
		Lower limit	**Upper limit**
Sensitivity	0.87	0.467	0.99
Specificity	0.99	0.98	0.997
PPV	0.54	0.26	0.796
NPV	0.999	0.99	0.9999
LR^+	120.896	52.23	279.806
LR^-	0.126	0.02	0.79
Accuracy	99.16		

Table 5: Diagnostic performance of ICT Rapid Card tests compared to PS microscopy for detecting Non falciparum Plasmodium spp.

Test	Peripheral Smear		
ICT	PS +VE	PS-VE	Total
Positive	81	8	89
Negative	17	731	748
Total	98	739	837

Table 6: Diagnostic performance of ICT Rapid Card tests compared to PS microscopy for detecting Non *P.falciparum* species

Indices	Estimated value	95% Confidence Interval	
		Lower limit	**Upper limit**
Sensitivity	0.83	0.73	0.89
Specificity	0.99	0.98	0.99
PPV	0.91	0.826	0.96
NPV	0.98	0.96	0.986
LR^+	76.35	38.1	153
LR^-	0.175	0.11	0.27
Accuracy	97.013		

4. Discussion

With a number of tests introduced over the years as a development in malaria diagnostics, the good old method of peripheral smear microscopy is still recommended as the Gold standard against which the diagnostic performance of all other tests have been evaluated[1-6].

Though it is cost effective but requires technical expertise, adequate quality control, Laboratory set up and is cumbersome, difficult, labour intensive and time consuming [6].

In recent years Immunochromatographic Rapid Card test for detecting malarial antigens has been recognised as a more rapid, easier, simpler non-microscopic diagnostic modality for malaria. Ease of performance, simpler result interpretation, low capital involvement, non-requirement of electricity and other resources, no need of much technical expertise and quicker results are its advantages over microscopy [7].

But on the other hand, these tests are expensive, may be associated with discrepancies in the results as observed in several studies. False positives are reported due to persistent antigenemia or due to self medication with antimalarial drugs during an attack of fever [8].

False negatives reported due to a number of reasons like cross reactions with auto-antibodies like Rheumatoid Factor or with heterophile antibodies, Immune complex formation in severe malaria, prozone phenomenon etc[9].

This study has shown that ICT Rapid card tests exhibited considerably good specificity(98%) with slightly higher specificity for *P.falciparum* (99.16%) as compared to non-falciparum species(97%).The sensitivity of ICT was however lower(82.65%) for non-falciparum as compared to falciparum malaria(87.5%). The accuracy of the ICT was also higher for falciparum malaria.

Our results are in accordance with a number of similar studies conducted over number of years [1,2,5,6,8] The high positive diagnostic and low negative diagnostic likelihood ratios reported in our study indicates that the ICT rapid card test presents a good likelihood of presenting a positive test in malaria infected cases as compared to the uninfected individuals. So, ICT Rapid card test can serve as a useful diagnostic tool in early prompt diagnosis of malaria.

This study does not suggest that ICT Rapid card test for malarial antigens can completely replace PS microscopy as a diagnostic tool at present, but can be used as an alternative or complimentary to microscopy, particularly in remote rural areas with frequent prolonged power cuts, lack of skilled technicians, laboratory facilities and other resources. However Peripheral smear microscopy will remain as the Gold standard in expert hands.

Conflict of Interest: None declared.

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