ORIGINAL RESEARCH

Comparison of Rapid Antigen Detection, ELISA and PCR Methods for diagnosis of Malaria

Dr. Rohit Pandey
Assistant Professor, Department of Microbiology, Krishna Mohan Medical College & Hospital, Mathura, Uttar Pradesh, India

Corresponding Author
Dr. Rohit Pandey
Assistant Professor, Department of Microbiology, Krishna Mohan Medical College & Hospital, Mathura, Uttar Pradesh, India

Received: 25 March, 2022 Accepted: 28 April, 2022 Published: 08 May, 2022

ABSTRACT
Background: Malaria, caused by four species of the genus Plasmodium, is an endemic tropical disease found in 109 countries. The present study was conducted to compare Rapid Antigen Detection, ELISA and PCR Methods for diagnosis of Malaria. **Materials & Methods:** 80 cases of malaria of both genders were selected. Blood samples were collected from all patients. The blood samples were screened by rapid malaria antigen detection method (Histidine Rich Protein-2 and Lactate dehydrogenase), solid phase ELISA (Lactate dehydrogenase) and conventional PCR for 18s rRNA. **Results:** From 80 samples collected, 50 were males and 30 were females. Among the 15 samples positive, 12 (80%) were males and 3 (20%) were females. RDT and ELISA was positive in 15 cases, out of 12 were Plasmodium vivax and 3 were identified as Plasmodium falciparum. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of RDT found to be 75%, 100%, 100% and 94% and for ELISA was 70%, 100%, 100% and 94.5%. **Conclusion:** The sensitivity of PCR was greater; it could identify more positive casesthan the antigen detection method, and ELISA. Thus, using two tests in combinationantigen detection along with PCR—will enhance the identification of malaria-positive cases.

Keywords: Malaria, Plasmodium, Rapid malaria antigen

INTRODUCTION
Malaria, caused by four species of the genus Plasmodium, is an endemic tropical disease found in 109 countries. Common vectors, Anopheles mosquitoes are found in humid regions where the temperature ranges from 20°C to 30°C. About 80 species of these mosquitoes transmit malaria.[1] In the Indian subcontinent, Anopheles stephensi is the main vector that breeds in stagnant water. The rise in malaria-related deaths has been an issue for hundreds of years; The Indian government initiated the National Malaria Control Program in 1953, which turned out to be very successful. At first, microscopy was considered the gold standard for diagnosing malaria; alternative techniques such as malaria antigen detection, fluorescent microscopy, antibody detection, and molecular amplification have become more important.[2]

Assessing more recent diagnostic methods that utilize the most sensitive approach is beneficial for establishing improved point-of-care diagnoses, particularly concerning antigenic variants of Plasmodium species and hypnozoites of Plasmodium vivax.[3] In low transmission areas selected in this study, active case detection and strategies to standardize various diagnostic methods are crucial.[4] During periods of high transmission, microscopy and rapid tests may be more suitable, while in cases of asymptomatic infections linked to residual immunity, molecular methods such as PCR are particularly important.[5] When it comes to identifying active infections, the significance of antibody detection via ELISA is limited. However, it may serve as a surrogate method for diagnosing relapses caused by Plasmodium vivax and for excluding liver stages of the parasite.[6]The present study was conducted to compare Rapid Antigen Detection, ELISA and PCR Methods for diagnosis of Malaria.

MATERIALS & METHODS
The study was carried out on 80 cases of malaria of both genders. All gave their written consent to participate in the study.

Data such as name, age, gender etc. was recorded. Blood samples were collected from all patients. The blood samples were screened by rapid malaria antigen

detection method (Histidine Rich Protein-2 and Lactate dehydrogenase), solid phase ELISA (Lactate dehydrogenase) and conventional PCR for 18s rRNA.Results thus obtained were subjected to statistical analysis. P value < 0.05 was considered significant.

RESULTS

Table I Gender wise distribution of Plasmodium species

Gender	Plasmodium vivax positive	Plasmodium falciparum positive	Positive %
Male (12)	10	2	80%
Female (3)	3	0	20%
Total (15)	13	2	100%

Table I shows that from 80 samples collected, 50 were males and 30 were females. Among the 15 samples positive, 12 (80%) were males and 3 (20%) were females.

Table II Plasmodium species positive by different methods

Diagnostic method	Genus Plasmodium	Plasmodium vivax	Plasmodium falciparum
RDT	15	12	3
ELISAGenus specific	15	12	3
PCRGenus specific	20	-	-

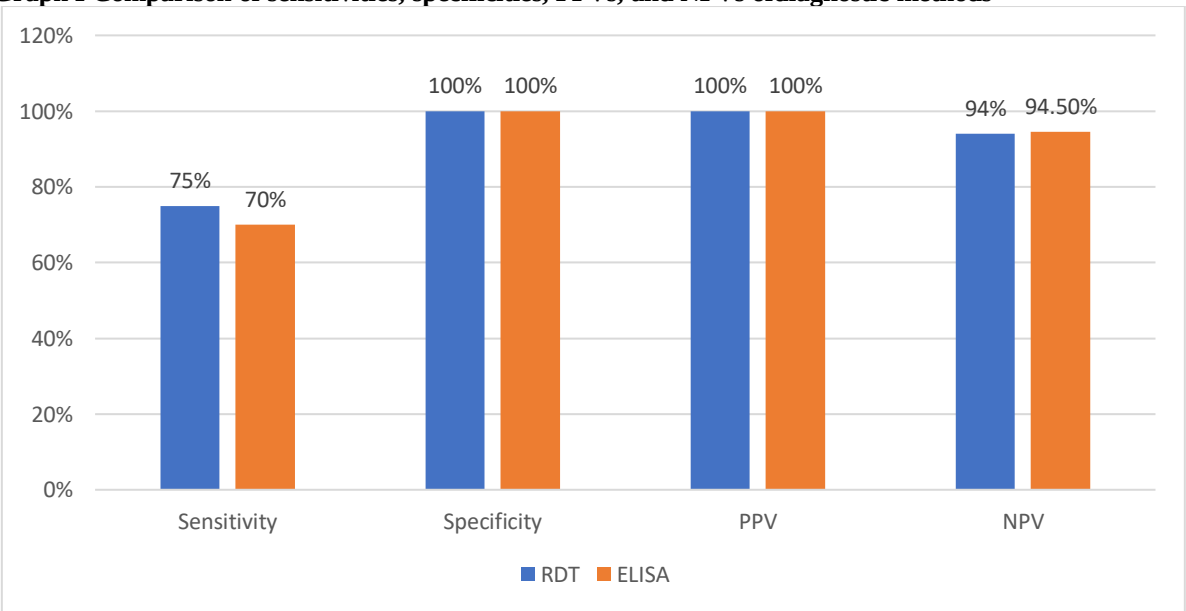
Table II shows that RDT and ELISA was positive in 15 cases, out of 12 were Plasmodium vivax and 3 were identified as Plasmodium falciparum.

Table III Comparison of sensitivities, specificities, PPVs, and NPVs ofdiagnostic methods

Test	Sensitivity	Specificity	PPV	NPV
RDT	75%	100%	100%	94%
ELISA	70%	100%	100%	94.5%

Table III, graph I shows that sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of RDT found to be 75%, 100%, 100% and 94% and for ELISA was 70%, 100%, 100% and 94.5%.

Graph I Comparison of sensitivities, specificities, PPVs, and NPVs ofdiagnostic methods



DISCUSSION

Advanced techniques such as PCR or ELISA may help to augment the sensitivity of microscopy, which is the gold standard test for the diagnosis of malaria.[7] Even though low parasite densities are reliably detected with HRP-2 ELISA, the biggest challenge is to detect Plasmodium falciparum parasitemia when it is superimposed by Plasmodium vivax, in which case PCR or ELISA are essential for accurate diagnosis.[8] PCR provides the added advantage of species differentiation, whereas it is not possible with ELISA, which is based on antibodies directed against a single antigen.[9]The present study was conducted to compare Rapid Antigen Detection, ELISA and PCR Methods for diagnosis of Malaria.

We found that from 80 samples collected, 50 were males and 30 were females. Among the 15 samples positive, 12 (80%) were males and 3 (20%) were

females.Jaya Singhet al[10]evaluated the blood samples collected from suspected patients of malaria by various diagnostic methods. PCR was found to be the most sensitive method from this study. The specificity and positive predictive values were 100% for microscopy, rapid antigen detection method and ELISA. The negative predictive values were 94.4% for microscopy and rapid tests, followed by 93.5% for ELISA. The sensitivity was 75% for microscopy and rapid tests and 70% for ELISA

We found that RDT and ELISA was positive in 15 cases, out of 12 were Plasmodium vivax and 3 were identified as Plasmodium falciparum.Farcas GA et al[11] in their study a real-time PCR assay based on detection of the K76T mutation in PfCRT (K76T) of P. falciparum was developed on a LightCycler platform (Roche). The performance characteristics of the real-time assay were compared with those of the nested PCR-restriction fragment-length polymorphism (RFLP) and the sequence analyses of samples obtained from 200 febrile returned travelers, who included 125 infected with P. falciparum (48 of whom were infected CQ-susceptible [K76] and 77 of whom were CQ-resistant [T76] P. falciparum), 22 infected with Plasmodium vivax, 10 infected with Plasmodium ovale, 3 infected with Plasmodium malariae malaria, and 40 infected with other febrile syndromes. All patient samples were coded, and all analyses were performed blindly.

We observed that sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of RDT found to be 75%, 100%, 100% and 94% and for ELISA was 70%, 100%, 100% and 94.5%. Tham JM et al[12]evaluated samples from 52 patients. Plasmodium infections were identified with a genus-specific primer set, and species differentiation between Plasmodium falciparum and Plasmodium vivax was analyzed by multiplex PCR. The PCR test with any of the three primer sets was able to detect as few as four parasites per microliter by gel electrophoresis or by nonisotopic paper hybridization chromatography. The diagnoses obtained by PCR correlated closely with those obtained by Giemsa staining except for two samples observed to have mixed P. falciparum-P. vivax infections. These were initially missed by microscopic analysis. In comparison with antigen-capture assays for P. falciparum, the PCR assays were able to detect three infections that were missed by the ParaSight-F test. The PCR test was negative for nine ParaSight-F-positive samples and one ICT Malaria Pf-positive sample, and these were confirmed to be false-positive results. The PCR thus gave no false-negative or false-positive results. Patients undergoing antimalarial therapy were also monitored by the PCR assay. Four of seven patients who were PCR positive for P. vivax at the time of discharge were later readmitted to the hospital with a recurrence of P. vivax infection.

The shortcoming of the study is small sample size.

CONCLUSION

Authors found that the sensitivity of PCR was greater; it could identify more positive casesthan the antigen detection method, and ELISA. Thus, using two tests in combinationantigen detection along with PCR—will enhance the identification of malaria-positive cases.

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