Journal of Cardiovascular Disease Research
ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 08, 2024

Original Research Article

Utility of Malaria Rapid Kit in the Diagnosis of Malarial Parasite in A Tertiary Care Hospital-An Observational Study

Dr. Beulah Priscilla Maddirala[1], Dr. Kollabathula Arpitha[2], Dr. Raviteja Kakarla[3], Dr. Srikanth Reddy Kamireddy[4]

[1]Assistant Professor, Department of Pathology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India.
[2]Assistant Professor, Department of Pathology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India.
[3]Assistant Professor, Department of Neurology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India.
[4]Assistant Professor, Department of Pathology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India.

Corresponding Author
Dr. Srikanth Reddy Kamireddy, Assistant Professor, Department of Pathology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India.

Received: 07-06-2024 / Revised: 17-06-2024 / Accepted: 25-07-2024

ABSTRACT

BACKGROUND
Malaria is one of the tropical infectious diseases with 249 million cases occurring in 2022. The World Health Organization-South East Asia Region (WHO-SEARO) accounted for about two percent of the total malaria burden. Early diagnosis is the mainstay of effective management of any disease and its importance is well recognized in malaria.

METHODS
An observational cross-sectional study conducted from April 2023 to May 2024 on 100 febrile patients who were sent to Clinical Pathology department for screening of malarial parasites at Government General Hospital, Kakinada. The main objective of the study was to analyse the utility of Malaria rapid diagnostic tests (MRDT) in the diagnosis of malaria and to compare both MRDTs and peripheral blood smear microscopy in the diagnosis of malaria.

RESULTS
After taking a consent form, blood samples were collected and screened for malaria parasites microscopically and by using MRDTs. Data collection forms were filled with relevant information and obtained results for MRDTs and for peripheral blood smear were recorded. The collected data were statistically analyzed using MS Excel sheet. The age group range from 2yr to 83yr. In this study peripheral blood smear microscopy was considered as a gold standard method. The sensitivity and specificity of MRDT antigens were calculated and found to be 96.6% and 60% respectively. The negative predictive value was found to be 92.85% where positive predictive value was 73.3%.

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CONCLUSION
MRDTs should be used along with microscopy to avert complications associated with delayed diagnosis and similar studies are required to identify high sensitivity and specificity for the diagnosis of malaria.

KEYWORDS: Plasmodium, Malaria Rapid diagnostic kit, Malaria control program,

INTRODUCTION

Malaria is one of the tropical infectious diseases with 249 million cases occurring in 2022.[1] The World Health Organization-South East Asia Region (WHO-SEARO) accounted for about two per cent of the total malaria burden.[2] India account for 66% of cases in this region. Timely diagnosis is the mainstay of effective management of any disease and its importance is well recognized in malaria control programme. When the diagnosis for malaria remains accurate, there is a potential to save about 100,000 lives annually and to deter 400 million unnecessary treatments.[3] Fever with chills and rigors being the most common clinical presentation of malaria, can mimic many other diseases including various viral infections. Clinical diagnosis alone does not suffice disease recognition but the laboratory detection plays an important role.

The 'gold standard' method to diagnose malaria is thick and thin peripheral blood smear examination under a microscope.[4] This method is widely used but with certain limitations, like round the clock availability for an expert opinion on species identification and its limited threshold concentration of detection of parasites per microliter of blood compared to that of other techniques.[5] The other commonly used methods are malaria rapid diagnostic test (MRDT) kits and polymerase chain reaction (PCR).[6] The PCR is a sensitive technique compared to microscopy. Owing to its high cost as well as the requirement of a laboratory to be equipped with the instrument and availability of trained expertise make its use unrealistic for a resource-limited settings.[7] MRDTs require one drop of blood for malaria detection based on immunochromatographic technique (the malaria-specific antigen). Results can be read in 12-15 minutes and thus is being extensively used for malaria detection worldwide.[7,8,9]

MRDTs can be performed by individuals receiving a short training with an ease to perform and to interpret the results.[10] Moreover, it does not require expertise as is a pre-requisite for microscopic diagnosis and also does not require electricity or any equipment. MRDT are commercially available kits. These tests are based on the detection of antigens released from parasitized red blood cells. In the case of *P. falciparum*, these RDTs are based on detection of the *P. falciparum* histidine rich protein 2 (HRP2) or of the Plasmodium specific lactate dehydrogenase (pLDH). Species specific pLDH isoforms have been used to develop a test for *P. vivax*. Recently another rapid test Combo Malaria Antigen (pLDH/HRP2) card test was developed in India for differential diagnosis between *P. falciparum* and the other plasmodium species.[11]

To determine the usefulness of new rapid test in low endemic area where both *P. falciparum* and *P. vivax* are prevalent, the diagnostic capacity of Malaria Antigen (pLDH/HRP 2) card test (Oscar Medicare Pvt, Ltd, New Delhi, India) was compared with that of expert microscopy, the gold standard method.

METHODS

Study Area

This study was conducted in Government General Hospital, Kakinada, Andhra Pradesh, South India.

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Study Design and Period
A cross-sectional observational study over a period of one year (April 2023 to May 2024) was conducted among patients attending tertiary care hospital from in and around Kakinada.

Study population and Sample Size
100 samples that tested for Malaria were used. The samples were taken from patients who attended Government General Hospital, Kakinada between April 2023 to May 2024.

Inclusion and Exclusion Criteria
All samples from consented patients presenting with clinical suspicion of malaria based on fever were included in the study.
Samples from patients on antimalarial treatment, inadequate samples, improperly labelled samples and clotted samples were excluded from the study

Ethical Consideration
The study was approved by the Institutional Ethical Committee, Rangaraya Medical College, Kakinada. The objectives and procedure were carefully controlled according to set Standard Operating Procedures (SOPs). Written consent was sought for from study participants or study participant's caretaker for minors.

Sample Collection and Processing
After filling the consent form, blood sample was collected from the patient by using syringes and 2ml whole blood collected in EDTA vial. 5µl of whole blood was added into MRDT, then 4 drops of buffer added into MRDT according to the manufacture's protocol and test to detect malaria parasite antigen (pLDH & HRP-II) detection method. The results were read after 15 min. Similarly, peripheral blood smears were made on a clean slide and allowed to air dry before being sent to the clinical laboratory. Leishman stain was used to stain thin and thick smear for 15 minutes and tested with light microscopy with good 100X objectives. The results from both MRDT and microscopy were reported qualitatively (Positive or Negative). MRDT results and smear results were recorded and tabulated in excel sheet.

Data Collection and Analysis
Collected data was checked and analyzed using MS Excel 2013 software. The validity of diagnostic test was used in calculation and then the measurements were reported in number and percentage.

RESULTS
Among the total of 100 participants; 42 (42%) females and 58 (58%) males. The patients age group ranges from 2yr to 83yr. The demographic details of the study subjects are shown in table 1.

Age (Years)	Male	Female	Total
1-20	16	15	31(31%)
21-40	12	11	23 (23%
41-60	22	12	34(34%)
61-80	6	3	9(9%)
81-90	2	1	3(3%)
	58(58%)	42(42%)	100

Table 1: Demographic details of the patients

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Diagnosis of malaria by RDT and peripheral blood smear microscopy are presented in table 2. True positive and true negative results were 58% and 28% respectively while false positive and false negative results were 11% and 4% respectively.

RDT	Peripheral smear	% of patients	
Positive	Positive	True positive	58%
Negative	Negative	True negative	28%
Positive	Negative	False positive	11%
Negative	Positive	False negative	4%

Table 2: Malaria diagnosis by RDT and peripheral smear (n=100)

Sensitivity of RDTs in Diagnosis of Malaria:
Peripheral blood smear microscopic examination was considered as a standard method. The sensitivity of RDTs HRP2 in diagnosis of malaria is 95.1% table 3. Therefore, 58% of the patients who tested positive with both methods (peripheral bold smear microscopy and RDTs) were considered true positives. Patients who tested negative with MRDT and positive to peripheral blood smear microscopy were 3% and were considered false negative. This high sensitivity (96.6%) may be due to factors relating to how health community workers transported the MRDTs, thus being damaged by extreme temperature or humidity during transportation, and storage.

Variables and formula	Values
True positive (TP)	58%
False negative (FN)	3%
Sensitivity=TP/(TP+FN) x 100	**95.1%**

Table 3: Sensitivity of RDTs (pLDH & HRP-II) in the diagnosis of Malaria

Specificity of MRDTs in Diagnosis of Malaria:
The specificity of MRDTs (pLDH & HRP-II) in this study was calculated in table 4 and result was 71.79% in diagnosis of malaria. True negative was 28% (patients who were tested negative by both MRDT and peripheral blood smear microscopy), whereas the false positive was 11%, (patients who were tested positive with MRDTs but tested negative with peripheral blood smear microscopy).

Variables and formula	Values
True negative (TN)	28%
False positive (FP)	11%
Specificity =TN/(TN+FP) x 100	**71.79%**

Table 4: Specificity of RDTs (pLDH & HRP-II) in the diagnosis of Malaria

Predictive Values of MRDTs in Diagnosis of Malaria
Predictive values of MRDT (pLDH & HRP-II) were tabulated in Table 5. Negative predictive value was found to be 90.32% whereas positive predictive value was 84.05%.

Variables & Formula	Values
True negative	28%
False negative	3%
True positive	58%

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False positive	11%
PPV= TP/(TP+FP) X 100	**84.05%**
NPV= TN/(TN+FN) X 100	**90.32%**

Table 5: Positive and Negative Predictive Values of RDTs (pLDH & HRP-II) in the diagnosis of Malaria

DISCUSSION

Malaria is one of the most common vectors borne infectious disease in the world. The prevalence is more in tropical and subtropical countries like India. The causative agent of malaria is by genus *Plasmodium*, a protozoal parasite. In India, *Plasmodium vivax* followed by *Plasmodium falciparum* are common subspecies. *P. falciparum* is a more serious and sometimes fatal one compared to other human malaria species like *P. vivax*, *P. ovale*, *P. malariae*, and sometimes *P. knowlesi*. For the effective treatment of Malaria, prompt and accurate diagnosis is required. Malaria always presents a diagnostic challenge in most tropical countries like India.

Microscopy remains the gold standard investigation for diagnosing malaria, but it requires more labour work and depends upon the skill of the examiner. MRDTs have been developed as an easy, convenient alternative to microscopy.[12] MRDTs are known to capture at least 2 target antigens: *Plasmodium vivax* lactate dehydrogenase (LDH) and *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2). HRP-2 antigens are the most sensitive for parasite detection and are heat-stable under field conditions compared to the other antigen tests.[13] However, HRP-2 antigen have limitations, as its performance has shown to be affected by product quality and parasite-related factors such as pfhrp2/3 gene deletion, non-*P.falciparum* species and prozone effects that may lead to false-negative MRDTs.[14,15] Microscopy is the most widely tool used to diagnose malaria at peripheral levels.

Present study was done to compare this MRDTs usefulness with that of peripheral smear which is gold standard method. This study shows male (58%) are more commonly affected than females (42%) similar to the study done by Joseph et al.,[16] but in study Izere *et.al*[17] female preponderance was seen. Common age group in the present study was 5th - 6th decade followed by 1st and 2nd decade of age. In this study, MRDTs have high sensitivity and low specificity which was similar to the study done by Izere C *et.al*[17] however other studies showed high sensitivity and high specificity. The possible reasons for this low specificity can be due to transportation of MRDTs, sample collection, storage and humidity could be the factors. The negative predictive values of RDTs were high compared to the positive predictive values and were in contradiction to the study conducted in Egypt.[17] These results indicate that if you tested negative for Malaria by MRDT (pLDH or HRP-2), you would have 90.32% chances of not having the disease. When you tested positive for Malaria with MRDT (pLDH or HRP-2), you would have a chance of 84.05 % of truly having the disease.[18]

CONCLUSION

This research on the correlation of malaria rapid test and peripheral blood smear microscopy shows MRDT antigen kits were good as screening test. It is recommended to confirm MRDTs results with peripheral smear microscopy before administering treatment and also monitoring on procuring of MRDTs, transportation of kits and storage should be taken into consideration. Further studies should determine the use of MRDTs in combination with microscopy.

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