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COMPARATIVE EVALUATION OF PLASMODIUM LACTATE DEHYDROGENASE BASED RAPID IMMUNOCHROMATOGRAPHIC TEST ASSAY AND ROUTINE MICROSCOPIC TEST IN DIAGNOSIS OF MALARIA AMONG PATIENTS ATTENDING IN A RURAL TEACHING HOSPITAL, SANGAREDDY

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ABSTRACT

Objectives: The present study was aimed to evaluate routine microscopic examination and plasmodium lactate dehydrogenase (pLDH) based immuno chromatographic assay for the rapid detection of malaria.

Methods: This study was carried out at the Department of Research, MNR medical collage & Hospital, sangareddy, Telangana from 2012 to 2015. Thick and thin blood smears were stained by giemsa staining followed by microscopy. All the specimens were tested by rapid test kit and confirmed by Western blot method.

Results: In our study out of 1870 clinically suspected cases, 295 (15.78%) were positive for P. falciparum by microscopy and 296 (15.82%) were positive by ICT method. 72 (3.85%) cases were positive for P. vivax by both the methods.

Conclusion: Although microscopy is gold standard method to detect malaria parasites from blood smear, it requires well experienced person and well established laboratory. On other hand rapid ICT kit is a very simple, inexpensive, user-friendly, point of care and effective diagnostic assay that can be done at the bedside for detecting malarial parasites.

Key Words: Immunochromatgraphic test (ICT), *Plasmodium Lactate Dehydrogenase* (pLDH), *Plasmodium falciparum* (pf), *Plasmodium vivax* (pv), Positive Predictive Value (PPV), Negative Predictive Value (NPV).

INTRODUCTION

Globally malaria has become one of the major health problems. According to WHO malaria report, an estimated of 3 to 5 billion cases are annually reported and 90% of the cases are reporting in Africa alone. [1] Worldwide Malaria is present in more than 100 countries. Most of them are developing or under developing countries. So, it has a great impact on their economy. In India, according to National Vector Borne Disease Control Programme (NVBDCP) around 0.85 million confirmed cases are reported annually, of which 40-50% is due to *plasmodium falciparum*. [2]

Malaria is an acute parasitic disease caused by *Plasmodium falciparum* or *Plasmodium vivax* in India. It is one of the most endemic diseases, especially countries like India and African tropical countries. In the past malaria is widely spread among mankind. But now it is mostly ignored as a simple disease. The main clinical presentation is fever with chills; however, nausea and headache can also occur. The

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majority affected being children under 5 years and almost all the deaths are attributed to *Plasmodium falciparum* (PF). [2,3]

In rural areas, clinical diagnosis is widely used for detecting malaria, where laboratory facility does not exist. [4] According to WHO 2011 guidelines, clinical diagnosis of malaria based on signs and symptoms alone is not recommended. It has low specificity and increased the chance of misdiagnosis of the patient and leads to misuse of drugs. [5,6] A laboratory diagnosis of malaria is one of the possibility in the management of a patient presenting with fever. Microscopic examination of the Giemsa-stained blood smears is widely used as a routine method for the detection of malaria parasites and remains the gold standard for diagnosis. [7] But, it is not 100% sensitive and specific. [8] Expert microscopy gives information about parasite stage and parasitemia. However, maintaining a high standard of microscopy requires trained technicians, supervision, quality control, and regular provision of reagents and also it will take 60 minutes to prepare a blood smear slide, which is a time consuming process. [9,10]

In India, a majority of malaria cases occur in rural areas. Where there is a little or no access to reference laboratories. So, WHO recommended another diagnostic method called as Rapid diagnostic tests (RDTs) that detect malarial parasitic proteins by Immunochromatography have been used as a complementary detection method for malaria diagnosis. [11] RDTs detect a variety of proteins, including *P. falciparum* Histidine-rich protein 2 (PfHRP2) and plasmodium lactate dehydrogenase (pLDH), both specific to *P. falciparum*, and also Plasmodium LDH (pLDH) and aldolase, enzymes shared by the 5 human-pathogenic Plasmodium species. [12]

The main goal of this study was to evaluate, routine microscopic examination and pLDH based immunochromatgraphic assay for the rapid diagnosis of malaria.

METHODS AND MATERIALS

This study was carried out at the Department of Research, MNR medical collage & Hospital, sangareddy, Telangana from 2012 to 2015. 3 ml volume of venous blood was drawn with aseptic precautions and collected into a sterile Ethylene diamine tetra acetic acid (EDTA) collection tubes for microscopy and immunochromatgraphic testing. The thick and thin blood films were made shortly after being drawn to prevent alteration in the morphology of malarial parasites. The total number of subjects in our study was 1870 numbers and at an age group was from 10 years to > 61 years. The subjects were selected from medical ward, who were suspected to have malaria i.e., fever with two or more of following clinical findings splenomegaly, pallor, convulsions and jaundice. Patient with infective Hepatitis and other known causes of anaemia are excluded from the study. For all the clinically suspected cases of rapid malaria plasmodium lactate dehydrogenase (pLDH) based immuno chromatography test (ICT) assay (Genomix Malaria Pf/Pv antigen Rapid Detection kit, Genomix Molecular Diagnostics (P) Ltd. Hyderabad, India), was done at bed side and simultaneously thick and thin smears are prepared and sent for microscopic examination.

The study was to compare immuno chromatography test (ICT) method with conventional microscope with respect to the Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Efficiency.

RESULTS

In our study out of 1870 clinically suspected cases of malaria, 165 (8.82%) patients were belongs to 10 to 20 years, 465 (25.02%) were in 21 -30 years, 512 (27.37%) were in 31-40 years, 370 (19.78%) were in 41-50 years, 248 (13.26%) were in 51-60 years and 110 (5.88%) were belongs to > 61 years as shown in the table 1. In our study, among malaria suspected cases 1089 (58%) are males and 781 (42%) are females as shown in the figure 1. All clinically suspected cases of malaria had fever, majority of them had splenomegaly, pallor and convulsions. Very few of them presented with jaundice as shown in table 2. In our study out of 1870 clinically suspected cases, 295 (15.78%) were positive for P. falciparum by microscopy and 296 (15.82%) were positive by ICT method, 72 (3.85%) cases were positive for P. vivax by both the methods as shown in table 3. Among 368 positive cases of malaria, 73 (19.83%) were in 10- 20 age group, 112 (30.44%) were in 21-30, 96 (26.09%) were in 31-40 year, 48 (13.04%) were in 41- 50 year, 22 (5.97%) were in 51-60 year and 17 (4.62%) were in >61 age group as shown in table 4. In our study, among malaria positive cases 219 (60%) are males and 149 (40%) are females as shown in the figure 2. All the malaria proved cases had fever 368 (100%), 318 (86.4%) had splenomegaly, 251 (68.20%) had pallor, 159 (43.20%) had convulsions and 69 (18.75%) had jaundice as shown in the table 5.

DISCUSSION

The resurgence of malaria has renewed interest in developing not only preventive measures, but also rapid diagnostic techniques. Several methods have been developed to supplement and replace the conventional microscopic method. The most promising new malaria diagnostics are the serological rapid immuno chromatography test (ICT) kit. Genomix Malaria (Pf/Pv) Antigen Rapid Detection kit is one amongst them. We employed the test and compared it with conventional smear examination for diagnosis of malaria.

In our study, among 1870 clinically suspected cases of malaria, microscopy showed 367 positive cases, in that 295 (15.78 %) cases were shown positive for P. falciparum and 72(3.85 %) for P. vivax. Whereas Genomix Malaria rapid ICT kit showed 368 positive cases in that, 296 (15.82%) cases positive for P. falciparum and 72 (3.85%) cases for P. vivax. One case of P. falciparum was not detected by microscopic method. The probable cause could be misdiagnosis of a specimen; due to the lack of technical experience in handling and observing the slides and also might be the low levels of parasitemia count in the specimen. In general, the highly qualified microscopist can detect up to 200 p/µl of malaria parasites easily, whereas the ICT can detect up to 100 p/µl. [13,14]

In continuation to the Microscopy and lateral flow results, the specific sample which showed false negative (pf) in microscopy and positive (pf) in lateral flow was further confirmed by using the western blot analysis. The pLDH anti-sera immunoreactivity signal was clearly formed at near to 37 kd position on nitrocellulose membrane (the size of pLDH in *plasmodium falciparum* is 33 kd [15] in western blot stating that the specific sample was positive for malarial parasites as shown in figure 3.

In our study among the 368 proved malaria cases 219 (60%) were male and 149 (40%) were females. All the patients had fever 368 (100%), splenomegaly 318(86.4%), pallor 251(68.20%), convulsions 159 (43.20%), and jaundice 69(18.75%). The rapid ICT method had excellent sensitivity and specificity (100%) for detecting *P. vivax* and *P. falciparum*. Rapid ICT had positive predictive valve of 100%, negative predictive value of 100% and efficiency of 100%.Whereas microscopic method is showing sensitivity (99.72%), specificity (100%), positive predictive valve of 100%, negative predictive value of 99.93% and efficiency of 99.94% shown in table 6 & 7.

The results of the present study were similar to the study done by Diarra et al. in 2012 at Burkina Faso [16]. This study revealed that ICT had a high level of sensitivity and specificity compared with microscopy which is considered as the gold standard method for malaria diagnosis. Therefore, the results of this study further substantiated that ICT is an effective and sensitive tool in the diagnosis of malaria.

Compared to microscopy the ICT kits are simple, rapid, inexpensive, point of care, easy to use diagnostic test kits for disease diagnosis. Worldwide, from the past few years, the ICT kits played a major role in the diagnosis and became backbone for most of the commercial diagnostic assays.

CONCLUSION

The present study reveals that ICT is a very simple, inexpensive, user-friendly, point of care and effective diagnostic assay that can be done at the bedside for diagnosing malaria. It has sensitivity, specificity, PPV, NPV, and efficiency were more or less similar to conventional microscopy and do not require highly skilled personnel to perform or interpret the results.

Early diagnosis and treatment are imperative in preventing the complications. Microscopy is the gold standard for malaria parasites. But it is laborious and requires experts to interpret the results. Rapid immunochromatgraphic test that detects pLDH produced by malaria parasite in the blood which can be performed at bedside in 10-15 minutes.

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Ethical Clearance: The study was approved by the Institutional Human Ethical Committee.

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Conflict of interest: None

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Table 1: Age distribution among clinically suspected cases of malaria

Age distribution among clinically suspected cases of malaria		
Age (in years)	**No of cases**	**Percentage**
10-20	165	8.82%
21-30	465	25.02%
31-40	512	27.37%
41-50	370	19.78%
51 -60	248	13.26%
>61	110	5.88%
Total	1870	100%
Mean ± SD	311 ± 163	

Table 2: Clinical findings among patients with suspected malaria.

Presenting symptoms	Number (n=1870)	Percentage (%)
Fever	1870	100%
Splenomegaly	1420	75.93%
Pallor	1270	67.91%
Convulsion	820	43.85%
jaundice	240	12.83%

Table 3: Diagnosis of malaria by conventional microscopy and ICT assay

Findings	Microscopy (n=1870)		ICT assay (n=1870)	
	Number of patients	Percentage	Number of patients	Percentage
Negative for malaria	1503	80.37%	1502	80.32%
P.falciparum	295	15.78%	296	15.82%
P.vivax	72	3.85%	72	3.85%
Total	1870	100%	1870	100%

Table 4: Age distribution among malaria positive cases.

Age in years	Number of positives	Percentage (%)
10-20	73	19.83%
21-30	112	30.44%
31-40	96	26.09%
41-50	48	13.04%
51 -60	22	5.97%
>61	17	4.62%
Total	368	100%
Mean ± SD	61.33 ± 38.97	

Table 5: Clinical findings in malaria positive cases

Presenting symptoms	Number(n=368)	Percentage (%)
Fever	368	100%
Splenomegaly	318	86.4%
Convulsions	159	43.20%
Pallor	251	68.20%
Jaundice	69	18.75%

Table 6: Evaluation of Malaria rapid ICT kit with Microscope.

	Evaluation of Malaria rapid ICT kit with Microscope						
	Total samples	Reactive	Non- Reactive	True Positive (TP)	True Negative (TN)	False Positive (FP)	False Negative (FN)
Rapid ICT assay	1870	368	1502	368	1502	00	00
Microscope	1870	367	1503	367	1503	00	01

Table 7: Comparison of parameters between rapid ICT and Microscope

Comparison of parameters between rapid ICT and Microscope		
Parameters	Rapid ICT	Microscope
Sensitivity %	100%	99.72%
Specificity %	100%	100%
PPV	100%	100%
NPV	100%	99.93%
Efficiency	100%	99.94%

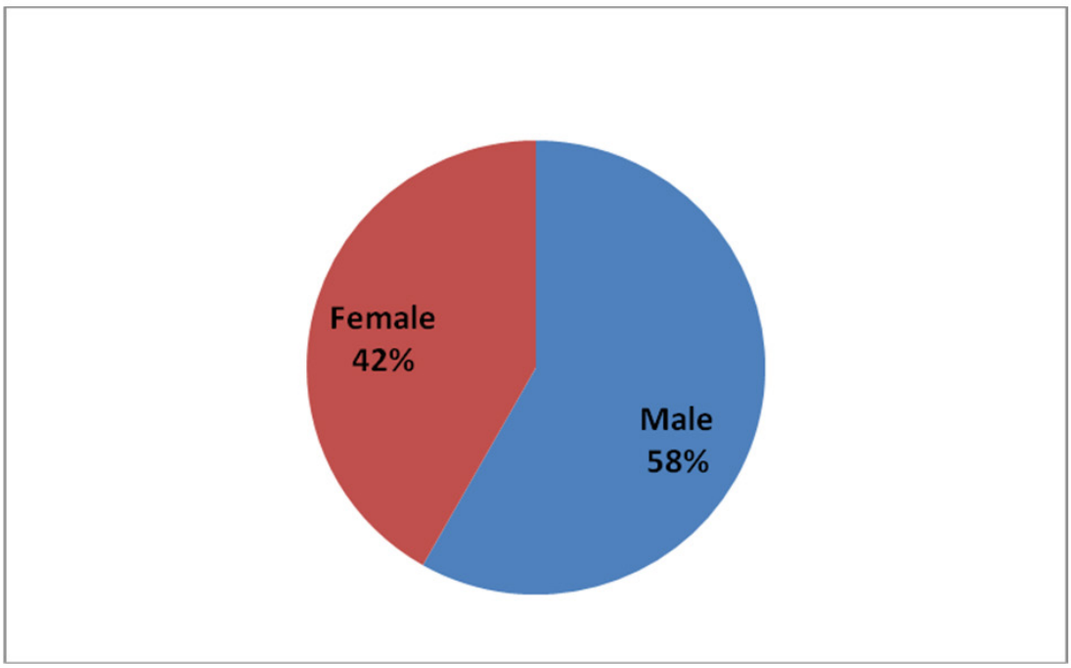

Figure 1: Gender wise distribution of suspected cases of Malaria

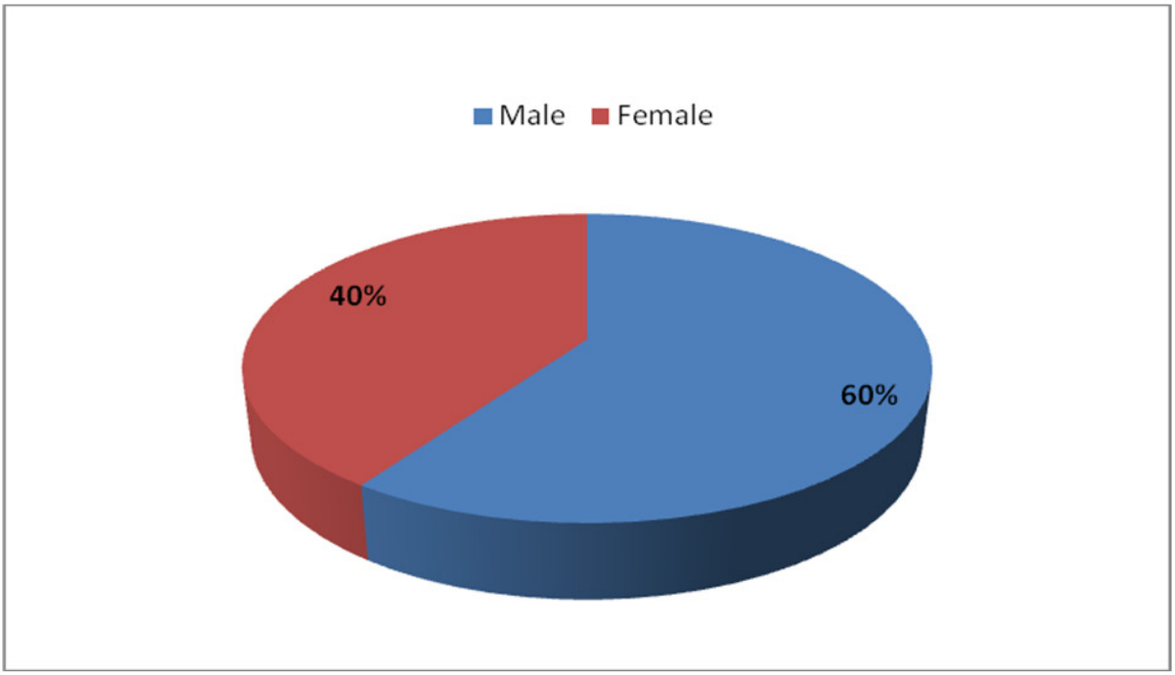

Figure 2: Gender wise distribution of proved malaria cases

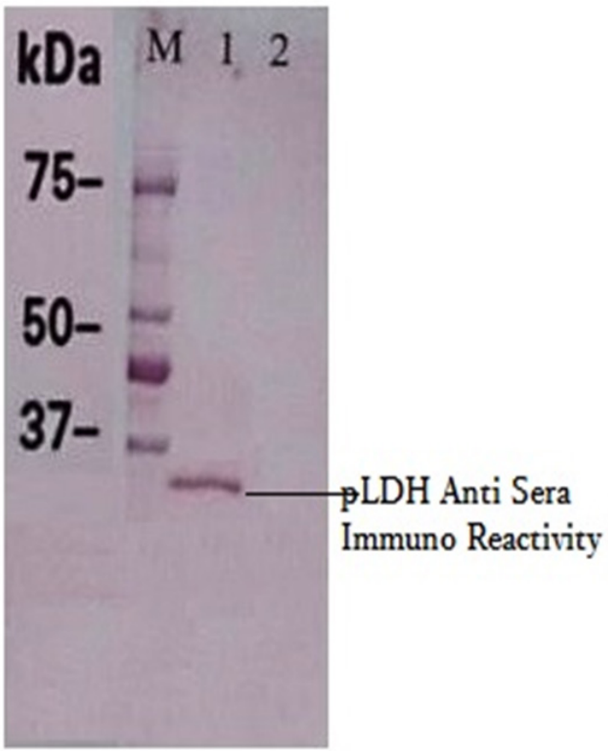

Figure 3: Westron Image of malaria positive sample showing pLDH band near to the 37 kd protein position. The gel was loaded with Marker-M, Malaria Positive sample- 1 and Malaria Negative Sample- 2.