Utility of Rapid Diagnostic Tests for Detection of Malarial Antigens and Their Comparison with Peripheral Blood Smear Examination

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ABSTRACT

BACKGROUND

Malaria, sometimes called the “king of diseases” is caused by protozoan parasites of the genus *Plasmodium*. The disease is transmitted by nine Anopheline species out of which six are primary vectors. This hospital based descriptive study aimed to determine the diagnostic accuracy of rapid antigen detection tests for diagnosis of malaria by immunochromatography (ICT) as compared to microscopic examination for detection of malarial parasites by Leishman, Field’s and Giemsa stains.

METHODS

This study was undertaken in the Department of Microbiology and samples were collected from December 2017 to November 2018. Patients with fever without any history of treatment with antimalarials were included in this study. A total of 200 blood samples were collected and subjected to microscopy and ICT by three different commercially available kits. Stains used for staining thick and thin smears were acquired from HiMedia (Mumbai).

RESULTS

Out of the 200 patients, 128 (64.0%) were male and 72 (36.0%) were females. Maximum number of cases were from the Department of Medicine 57.0% followed by the Department of Paediatrics 32.5%. Majority of the cases were seen in the post monsoon season 31.5% followed by summer and monsoon season 29.0% and 55/200 respectively. Least number of cases were seen in winter 12.0%. ICT was positive in 26% of cases and 24% were positive on microscopy. *Plasmodium vivax* infection was seen in 60%, *Plasmodium falciparum* was seen in 33 % and 7% had mixed infection. Positivity of Giemsa, Leishman and Field’s stain was found to be 24%, 22.5% and 15.6% respectively. Positivity of Parahit total, Paramax 3 and Is It Medsource was found to be 26%, 24% and 14.5% respectively.

CONCLUSIONS

Malaria is still a major public health concern. In this study, *Plasmodium vivax* was more common. Sensitivity of ICT was found to be better than microscopy. This study gives a basic insight about malaria and the problems associated with its diagnosis in a rural setup.

KEY WORDS

Malaria, ICT, Microscopy

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BACKGROUND

Malaria, sometimes called the “king of diseases” is caused by protozoan parasites of the genus *Plasmodium*. The most serious and sometimes the most fatal type of malaria is caused by *Plasmodium falciparum*. The other species are *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and sometimes *Plasmodium knowlesi* can cause acute, severe illness but mortality rates are low¹. The disease is transmitted by nine Anopheline species out of which the six primary vectors.

As per world Malaria report 2016, India contributes for 89% of the total malaria incidence in the South-East Asia Region.² In India, maximum malaria cases are reported from Odisha state. Although Odisha has a population of 36.7 million (3.5%), it contributes 25% of total cases reported in India. Similarly, in the other states inhabited by ethnic tribes mainly in the forest ecosystems, meso-to hyper-endemic conditions of malaria exist with the preponderance of *Plasmodium falciparum* to the extent of 90% or even more.³ Recently, it has been suggested that the malaria incidence is between 9 to 50 times greater than what is being reported, with a 13-fold underestimation of malaria-related mortality.⁴ Such claims reinforce the need for robust and comprehensive epidemiological surveillance studies across the country to determine the actual burden.⁴ Clinical diagnosis of malaria has poor accuracy and results in inappropriate management of febrile illness and wastage due to indiscriminate use of anti-malarials. Presumptive treatment is not recommended routinely. Treatment is recommended only after confirmation of suspected malaria case is done through prompt quality assured diagnostic testing in all settings.²

In the laboratory, malaria is diagnosed using different techniques, e.g. conventional microscopic diagnosis by staining thin and thick peripheral blood smears and other techniques like quantitative buffy coat (QBC) method, rapid diagnostic test and molecular diagnostic techniques such as polymerase chain reaction (PCR).¹

Currently, 86 malaria Rapid Diagnostic Test (RDTs) are available from 28 different manufactures. RDTs are all based on the same principle and detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies; they do not require laboratory equipment. Most products targets a *Plasmodium falciparum* specific protein, e.g. histidine-rich protein 2 or parasitic lactate dehydrogenase (pLDH). Recently, a new RDT method has been developed for detecting *Plasmodium knowlesi*.¹

Malaria as a disease has always been a problem as far as diagnosis is concerned which could be attributed to a number of factors. Conventionally the mainstay of diagnosis was microscopy which suffered in accuracy due to various reasons like timing of blood collection, improper smear making and staining techniques as well as the experience of the person examining the smears. As a result of this many cases went undetected or were unnecessarily treated with antimalarials based on clinical suspicion. The advent of RDT mitigated this problem to a certain extent but there were pitfalls of this method too. The present study was therefore undertaken to determine the utility and efficacy of RTD as compared to peripheral blood smear examination.

METHODS

Ethical clearance for the study was obtained from the Institutional Ethical Committee as per guidelines laid down by ICMR. After obtaining proper informed consent, a total of 200 blood samples were collected from patients either venepuncture and subjected to microscopy and ICT by three different commercially available kits. 3 Thick and 3 thin smears were made and stained as per standard protocol.^{5,6} Stains were acquired from HiMedia Laboratories (Mumbai) viz Giemsa stain, Leishman stain, Field's stain A and B. A good spreader from a slide which has ground glass polished sides was taken.

Thin blood films were examined under the oil immersion objective (1000X). The presence of different stages of the parasite was looked for and noted down, additional findings like size of RBCs, presence of malarial pigment and presence of Schuffner's dots or Maurer's clefts were looked for.

The number of parasitized RBC were seen in 10,000 RBCs. The approximate number of parasites/oil immersion field (1000 X) was noted and at least 100 fields were examined.

Thick smear: The number of parasites present was counted until 200 WBC have been seen and then it was multiplied by 40 which gave the parasite count per microliter of blood.⁷

Commercial kits for rapid antigen detection of HRP2 and pLDH were as follows-

- 1 ParaHit Total, Arkray Health Pvt. Ltd, Surat, India.
- 2 Paramax 3, Zephyr Biomedicals, Goa, India.
- 3 Is It Medsource, Medsource Ozone Biomedicals Pvt. Ltd. Haryana, India.

Rapid antigen detection tests were performed as per the instructions of the manufacturer.

Statistical Analysis

Statistical analysis was carried out using online software www.physics.csbsju.edu/cgi-bin/stats/contingency accessed on 16.10.2019. P value was calculated using Chi-Square test, P value <0.05 was considered to be significant and p value <0.001 was considered to be highly significant.

RESULTS

Out of a total of 200 patients 128 (64.0%) were males and 72 (36.0%) were females. The male to female ratio was 1.8:1. This finding was found to be statistically highly significant (P=0.001). Maximum number of cases were from OPD 87 (43.5%) followed by Paediatrics 59 (29.5%) and Medicine 27 (13.5). Table 1. Majority of patients belonged to the age group 0-10 years 56/200 (28%) followed by 21-30 years 33/200 (16.5%), least number of cases was found in 71-80 years age group 1/200 (0.5%). Table 2

Seasonal variation in the number of cases reporting with fever was less evident in the post monsoon season 63/200 (31.5%) summer season 58/200 (29.0%) and monsoon season 55/200 (27.5%). However there was a lot of variation between the number of cases seen post monsoon season and

winter season 24 (12.0%). This finding was found to be statistically highly significant ($P=0.000$).

Malaria could be diagnosed by microscopy in 48/200 (24.0%) patients out of which *Plasmodium vivax* 29/48 (60.4%) was the commonest infecting species followed by *Plasmodium falciparum* 15 (31.3%) This finding was however, not found to be statistically significant. Table 3 Giemsa stain was the most sensitive 48/200 (24.0%) followed by Leishman stain 45/200 (22.5%). Field's stain was found to be least sensitive 26/200 (15.6%). The differences in the positivity by these three staining techniques was not found to be statistically significant ($P=0.235$)

Out of the three RDT kits Parahit total was found to be most sensitive with 55/200 (27.5%) cases being positive followed by Paramax 3 50/200 (25.0%) and Is It 33/197 (16.8%). The differences in the positivity rates was not statistically significant ($P=0.105$) Table 4. The sensitivity, specificity, positive and negative predictive values of the three commercial RDT kits were calculated taking Giemsa stain positivity as the gold standard. Table 5. Parahit Total was found to be most sensitive of the RDT kits being 96.0% followed by Paramax 3 91.8% and Is It Medsorce 56.5 %. Specificity of the kits were as follows Paramax 3 96.6% followed by Parahit Total 95.3 % and Is It Medsorce 96.0%

Paramax 3 had highest PPV being 90.0 % as compared to Parahit Total 87.3%. NPV was highest with Parahit Total 98.6 % followed by Paramax 3 97.3 %. Is It Medsorce had the lowest PPV and NPV being 81.3% and 87.9% respectively.

Department	Male (%)*	Female (%)*	No. of Positive Cases (%)	Total (%)
OPD	62 (71.3)	25 (28.7)	8 (09.2)	87(43.5)
Medicine	12 (44.5)	15 (55.5)	14 (51.9)	27(13.5)
Paediatrics	36 (61.1)	23 (38.9)	24 (40.7)	59 (29.5)
Gynae	00 (00.0)	08 (100.0)	01 (12.5)	08 (04.0)
Surgery	05 (100.0)	00 (00.0)	01 (125)	05 (02.5)
Orthopaedics	06 (100.0)	00 (00.0)	00 (00.0)	06 (03.0)
ENT	02 (100.0)	00 (00.0)	00 (00.0)	02 (01.0)
Dermatology	05 (83.3)	01 (16.7)	00(00.0)	06 (03.0)
Total	128 (64.0)†	72 (36.0)†	48 (24)	200 (100.0)

Table 1. Department Wise Distribution of Cases (n=200)

*Percentages calculated horizontally
† p value= 0.001

Age	Male (%)*	Female (%)*	No. of Cases	No. of Positive Cases
0-10	29 (51.8)	27 (48.2)	56 (28.0)	8 (16.7)
11-20	12 (48.0)	13 (52.0)	25 (12.5)	16 (33.3)
21-30	17 (51.5)	16 (48.5)	33 (16.5)	17(35.4)
31-40	16 (72.7)	06 (27.3)	22 (11.0)	4 (08.3)
41-50	26 (83.9)	05 (16.1)	31 (15.5)	2 (04.2)
51-60	17 (85.0)	03 (15.0)	20 (10.0)	1 (02.1)
61-70	08 (80.0)	02 (20.0)	10 (5.0)	0 (00.1)
71-80	01 (100.0)	0 (00.0)	01 (0.5)	0 (00.0)
81-90	02 (100.0)	0 (0.00)	02 (1.0)	0 (00.0)
Total	128 (64.0)	72 (36.0)	200.0	48

Table 2-Distribution of Case and Number of Positive Cases According to Age and Sex

Malaria Forms	No. of Patients (%)
<i>Plasmodium vivax</i>	29 (60.4)*
<i>Plasmodium falciparum</i>	15 (31.3)*
Mixed infection	04 (08.3)
Total	48 (100)

Table 3. Different Malarial Species Found (n=48)

*p value=0.079

PPV Positive Predictive Value; NPV Negative Predictive Value

ICT Kit	Positive (%)	Negative (%)
Parahit Total (n=200)	55 (27.5)	145 (72.5)
Paramax 3 (n=200)	50 (25.0)	150 (75.0)
Is It (n=197)	33 (16.8)	164 (83.3)

Table 4. Positivity of Various Immunochromatographic Kits

p=0.10

KIT	Sensitivity	Specificity	PPV	NPV
Parahit Total	96.0 %	95.3 %	87.3%	98.6%
Paramax 3	91.8 %	96.6 %	90.0%	97.3%
Is It Medsorce	56.5 %	96.0 %	81.3%	87.9%

Table 5. Sensitivity, Specificity, PPV and NPV of RDT KITS

PPV Positive Predictive Value; NPV Negative Predictive Value

Stains Name	No. of Positives by Smear	No. of Positives by Parahit Total (%)	No. of Positives by Paramax 3 (%)	No. of Positives by is it Medsorce (%)
Giemsa (n=48)	Thick 23 Thin 25			
Field (n=45)	Thick 20 Thin 06	55 (27.5)	50 (25.0)	33 (16.8)
Leishman (n=26)	Thick 28 Thin 17			

Table 6. Number of Positives by Direct Smear and Positives by Paramax 3, Parahit Total, and Medsorce

DISCUSSION

In the present study there was predominance of male patients, the male to female ratio was 1.8:1, this finding was found to be statistically significant ($P=0.001$). Other studies have reported a similar ratio of 1.4:1 (Mundkar and Bhaktvatsalam 2019). Overall it was seen that majority of patients belonged to the age group 0-10 years 56/200 (28.0%) followed by 21-30 years 33/200 (16.5%). Mundkar and Bhaktvatsalam in 2019 also reported similar findings in their study conducted in Telangana.

Maximum number of cases were seen in the post monsoon season 63 (31.5%) followed by summer and monsoon season 58 (29.0%) and 55 (27.5%) respectively. Least number of cases were seen in winter 24 (12.0%) which is similar to reports made by Panchal et al 2016⁸. In their series of patients maximum number of cases were seen in the post monsoon season viz August, September and October. Maximum number of patients were found to be infected with *Plasmodium vivax* 29/48 (60.4%) followed by *Plasmodium falciparum* 15 (31.3%). This finding was however not found to be statistically significant ($P=0.079$). Mixed infection was seen in 04 (8.3%) In a study conducted by Naveen et al in 2012, similar findings were reported by the authors. Mundkar and Bhaktvatsalam in 2019⁹ however, reported a slightly higher rate of infection by *Plasmodium vivax* 64.6%.

In this study, fever was the most common presentation. Various other studies also reported fever as the most common symptom. (Bartoloni and Zammarchi 2012; Ravishanker et al 2015; Mundkar and Bhaktvatsalam 2019). Out of 48 confirmed cases 48 (100%) came with fever followed by chills and rigor 38 (79.2%) and headache 31 (64.6%), convulsions and altered sensorium was seen only in severe cases 8 (17.8%) each. Trampuz et al in 2003 also reported that in their patients fever was seen in > 92% of cases, chills and rigor was seen in 79% and headache in 70% of cases, these findings are similar to that of the present study.

Maximum number of patients reporting to various departments had pallor 164/200 (82.0%), followed by icterus 39/200 (19.5%). Hepatomegaly was seen in 35 (17.5%) and 6 (3.0%) patients were in coma. Meena et al 2017¹⁰ reported pallor in 69.0% and icterus in 25.4% of patients, findings which are different from the present study.

This could be due to the fact that our study was conducted in a rural setup where the general health status of the population may not be as good as people in an urban area. ALT was increased in 61/200 (30.5%) and AST in 60/200 (30.0%). Increased bilirubin levels were seen in 59/200 (29.5%) of cases. Least number of patients had Acidosis 12/200 (6.0%). Meena et al in ¹⁰ reported deranged hepatic function in 25.4% of cases which is much less as compared to the present study. Hypoglycaemia was seen in 13.0% of patients in our study whereas other authors have reported hypoglycaemia in 10.9% (Meena et al 2017) and 4.0% (Ravishanker et al 2015). The findings of the first study is more or less similar to that of the present study.

CONCLUSIONS

Occurrence of malaria in our region is fairly high with approximately one fourth cases reporting to the hospital with fever were diagnosed as malaria both by microscopy and RDT. Thick and thin smear examination by Giemsa stain was considered as the gold standard and as compared to this method, Parahit Total had the highest sensitivity and Paramax 3 had the highest specificity. Likewise, Paramax 3 had the highest PPV and Parahit Total had the highest NPV. Similar findings were reported by Mendiratta et al in 2006.¹¹ In another similar study, the sensitivity and specificity of RDT was reported as 94% and 99% respectively which was even better than PCR for which sensitivity and specificity was 90% and 95% respectively.¹² The Is It kit proved to be poor in terms of sensitivity, specificity, PPV and NPV and as per the findings of this study its use cannot be advocated. The use of RDTs can be recommended as a point-of-care testing or in areas where proper microscopy facilities are not available. However, taking into consideration the pitfalls of microscopy, even in settings where microscopy facilities are available, the RDTs should be performed in conjunction with microscopy. Performing these tests can prevent presumptive or empirical treatment which is much less cost effective. The other advantages of these RDTs is that they can be performed very easily with minimum infrastructure and they are excellent for point-of-care testing which prevents delay in treatment which is a significant determinant of death due to malaria. Microscopy on the other hand has the potential for being considered as a point-of-care test but is known to have poor sensitivity especially in the hands of inexperienced persons. Presumptive diagnosis and empirical treatment is also not advisable in the present

scenario with increase in drug resistance, wherein a patient needs to be treated with expensive antimalarials like artemether and artemisinin.

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