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Comparative Study of Different Diagnostic Modalities for the Detection of Malaria Parasite at a Tertiary Care Centre Kanpur

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ABSTRACT: Globally, the incidence of malaria continues to increase and India contributes to one third of cases of malaria globally with the highest incidence reported by *Plasmodium vivax* species. Comparative study of different diagnostic modalities for the detection of malaria parasite at a tertiary care centre Kanpur. The study was carried out in the Department of Microbiology and Central Research Lab of RMCH & RC, Kanpur, for a period of 1 year March 2021 to March 2022. A total of 151 whole blood samples were collected & examined by Peripheral blood smear (PBS) stained with Giemsa stain, rapid diagnostic test (RDT) for detection of antigen and CBS (Centrifuged buffy coat smear) tests respectively. The Ethical Clearance was taken from the Ethical Committee of the institution. Out of the total 151 Malaria suspected cases, 10 positive cases of *Plasmodium vivax* species were detected by RDT, CBS and 6 by PBS tests. 7 (70%) were male and 3 (30%) were female, the maximum number of cases were in 41-50 years followed by 1-30 years. The comparative performance of RDT, CBC and PBS were analysed, the RDT and CBS showed a sensitivity of (80%), specificity (98.58%) PPV (80%), NPV (98.58%) and accuracy (97.35%), the sensitivity of PBS is 60%, specificity 98.58%, PPV(70%), NPV(98.58%) and accuracy (96.02%) respectively. In our study the sensitivity, specificity of PBS is lesser than RDT and CBS, hence owing to the high number of false negatives in microscopy, it is necessary to reinforce training in microscopy, the "Gold Standard" in endemic areas, especially for confirmation of clinical diagnosis.

Keywords: RDT, CBS, sensitivity and specificity.

INTRODUCTION

Globally infections due to malaria contributes to increased morbidity and mortality among Vectorborne diseases, especially in tropical and subtropical countries. Among the vectorborne infections, malaria contributes to a high rate of morbidity and mortality (Githeko *et al.*, 2000; World Malaria Report 2016). The incidence of malaria continues to increase worldwide (World Malaria Report 2017) and India contributes to one third of cases of malaria globally with the highest incidence reported by *Plasmodium vivax* species (Siwal *et al.*, 2018). Malaria caused by *Plasmodium vivax* is a significant public health issue and is more prevalent than *Plasmodium falciparum* outside Africa, where *P. falciparum* is the predominant species (World malaria report 2015). WHO estimates, malaria kills more than 600,000 people every year, mostof them children under 5 years of age. Malaria transmission occurs in 97 countries, where about 3.4 billion people at risk of illness. Sub-Saharan Africa is the most vulnerable community where the burden is around 90% of annual global malaria deaths. (World Health Organization 2014). The complications due to *Plasmodium falciparum* can lead to increased morbidity and mortality if not treated early (Nandwani *et al.*, 2005). In a study conducted at Nigeria, malaria occurred more in out patients 50% compared to in patients about 40% of hospital admissions and mortalities was seen in 30% of cases (Ughasoro *et al.*, 2013) out of about 30 million children under the age of five years (Roll Back Malaria 2012). Moreover, it was found that 11% of maternal mortality and about N132 billion is lost to the disease annually (Federal Ministry of Health 2008). The different diagnostic modalities are thick & thin film microscopy (TFM), Rapid diagnostic tests (RDT) & Centrifuge quantitative buffy coat smear (CBS) are used for detection of microscopic parasitaemia and PCR techniques (Akhtar *et al*., 2010). Detection of antigens like Aldolase, lactate

dehydrogenase and HRP II are used in immunochromatograpgy tests done by Rapid card where the results are observed within 5-20 minutes (Moody, 2002). Accurate diagnosis of malaria is important for effective disease management & control. The drug of choices for treating *Plasmodium vivax* is Chloroquine and primaquine. Resistance to anti-malarial drugs is a major hurdle for malaria control strategies. The major concern in treating *P. falciparum* is the development of drug resistance to all the currently used anti-malarial drugs (amodiaquine, chloroquine, mefloquine, quinine, and sulfadoxine-pyrimethamine) and more recently, resistance to artemisinin derivatives (World Health Organization 2010). Although *P. falciparum* resistance to chloroquine (CQ) was reported in the 1950s, the resistance to *P. vivax* resistance to CQ was reported only in 1989 from Papua New Guinea (Rieckmann *et al.*, 1989**).** The great difficulty in understanding the mechanisms of drug resistance in *P. vivax* have not been extensively studied due to the non-availability of continuous in vitro culture system (Plowe, 2003).

MATERIAL AND METHODS

The was a prospective observational study carried out in the Department of Microbiology and Central Research Lab of RMCH & RC, Kanpur for a period of 1 year March 2022 to February 2023. All patients from both IPD & OPD, clinically suspected for Malaria infection, presenting at Rama Medical College, Hospital & Research Centre hospital during the study period were included. Patients already on treatment with anti-malarial drugs in the past two weeks were excluded. Ethical clearance was obtained from the Institutional Ethical Committee.

Sample collection: After taking a proper history of the patients name, age, gender details and clinical examination findings a 3-4 ml blood sample were collected from anti-cubital vein of all patients by taking sterile precaution.

Microscopic examination: A peripheral blood (thick & thin) smear was prepared and stained with a Romanovsky stain (most often Giemsa), and examined with a 100x oil immersion objective. The presence of malarial parasite was done by screening the thin smear and species were identified by thin smear (Mohanty *et al.*, 2015).

Centrifuge buffy coat smear: Centrifuged buffy coat smear (CBCs) was prepared as described (Mohanty *et al.*, 2015). Briefly this consisted of centrifuged the 2 ml of EDTA blood in a wide bore 4 ml of tube at 2000-3000rpm for 15 minutes. The supernatant plasma was separated and layer of buffy coat and equal thickness of red blood cells layer just below was picked up to prepare smears. The CBCs was examined for 200 oil immersion field being reported negative.

Rapid card test: Antigen detection was performed using a commercially available antigen detection kit. Detect *P. falciparum* HRP-2 and *P. vivax* p LDH malaria antigen in human blood as per manufacturer's instructions. (SD Bioline)

RESULTS

A total of 151 clinical cases suspected for Malaria were studied.

10 positive cases of *Plasmodium* species (Table 1) were detected by RDT and CBS, 6 by PBS tests. Out of which all were of *Plasmodium vivax* species.

Table 1: Total Number of Positive Cases.

Sr. No.	Total No. of samples (n=151)	Percentage
Plasmodium vivax Positive	10	6.62%
Plasmodium spp. Negative	141	93.37%

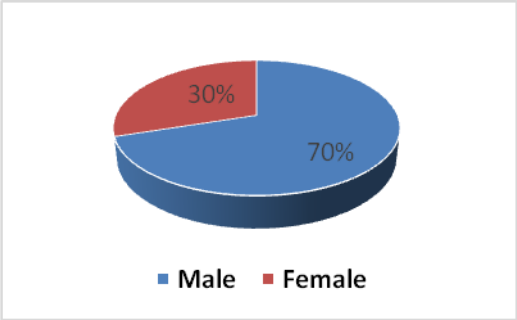

Fig. 1. Gender wise distribution of the Positive Cases (n=10).

Table 2: Age wise distribution of the Positive Cases (n=10).

S. No.	Age	No. of Isolates	Percentage
1.	1-10	2	20%
2.	11-20	2	20%
3.	21-30	2	20%
4.	31-40	1	10%%
5.	41-50	3	30%
6.	51-60	-	-
7.	61-70 and Above	-	-

In this study, 7 (70%) were male and 3 (30%) were female, the maximum number of cases were in 41-50 years followed by 1-10, 11-20 & 21-30 years. There was no positive case reported in the 51-60 and 61-70 age groups (Fig. 1 and Table 2).

The comparative performance of RDT, CBC and PBS were analysed, the RDT and CBS showed a sensitivity of (80%), specificity (98.58%) PPV (80%), NPV (98.58%) and accuracy (97.35%), the sensitivity of PBS is 60%, specificity 98.58%, PPV (70%), NPV (98.58%) and accuracy (96.02%) respectively.

Table 3: Shows comparison of three phenotypic methods.

Technique used	Total no. of samples examine	No. of Positive	% Positive	No. of Negative	% Negative
Peripheral Blood smear	151	6	3.97	145	96.02
Rapid card test	151	10	6.62	141	93.37
Centrifuge buffy coat smear	151	10	6.62	141	93.37

Table 4: The comparative performance characteristics of Microscopic thick/thin test, RDT & Centrifuge Buffy coat smear.

	Total positive cases n=10	
Microscopy thick/thin smear	RDT	Centrifuge Buffy coat smear
6	10	10

Table 5: The comparative characteristics of the sensitivity, specificity & PPV, NPV of the following tests.

	Microscopy PBS	RDTs	CBS
Sensitivity	60%	80%	80%
Specificity	98.58%	98.58%	98.58%
PPV	70%	80%	80%
NPV	98.58%	98.58%	98.58%
Accuracy	96.02%	96.02%	96.02%

DISCUSSION

Plasmodium species could result in severe public health concerns due to inappropriate treatments, leading to recrudescence and even drug resistance (Kang *et al.*, 2017). In the present study, we found 7 (70%) males and 3 (30%) females in Positive cases. This result is nearly similar with the study from India by Prajapati *et al.*, 2018; they found 231 (69.17%) males and 103 (30.83%) females in the clinically suspected cases (Prajapati *et al.*, 2018). Since male are more exposed in outdoor work. In our study, we found 70% was positive for Malaria by microscopy PBS, 100% was positive by Centrifuge buffy coat smear and 100% RDTs. In a study from India in 2016 showed similar results as our study in 1982 clinically suspected cases 2.92% was positive by microscopic examination of peripheral blood film, 3.33% was positive by centrifuged buffy coat smear and 4.09% was positive by rapid card test (Singh and Vivek 2016). Another research from India showed similar results as our study 17.1% was positive by microscopic examination of peripheral blood film, 21.9% was positive by centrifuged buffy coat smear and 23.2% was positive by rapid card test (Mohanty *et al.*, 2015). Other study in 2013 from Saudi Arabia showed similar results as our study 19.9% was positive by microscopic examination of peripheral blood film, 24.13% was positive by centrifuged buffy coat smear and 27.09% was positive by rapid card test (Ebrahim *et al.*, 2013). In a study in 2010 from India showed similar results as our study 82.8% was positive by microscopic examination of peripheral blood film, 92.18% was positive by centrifuged buffy coat smear and 93.7% was positive by rapid card test (Plowe, 2003).

The sensitivity specificity PPV, NPV & accuracy of all the three different tests were compared. We found that RDT and CBS showed a sensitivity of (80%), specificity (98.58%) PPV (80%), NPV (98.58%) and accuracy (97.35%), the sensitivity of PBS is 60%, specificity 98.58%, PPV (70%), NPV (98.58%) and accuracy (96.02%) respectively. In the present study the sensitivity and specificity of PBS smear was less, it could be due to poor training of technical person. In a study in 2003 showed the similar results as our study with a sensitivity of centrifuged buffy coat 93.3%, specificity 95% & sensitivity of rapid card was 98.24% and specificity was 93.65% (Plowe, 2003).

CONCLUSIONS

In our study the sensitivity, specificity of PBS is lesser than RDT and CBS, hence owing to the high number of false negatives in microscopy, it is necessary to reinforce training in microscopy, the “Gold Standard” in endemic areas, especially for confirmation of clinical diagnosis.

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