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Comparative Evaluation of Centrifuged Buffy Coat Smear, Peripheral Blood Smear, and Rapid Antigen Detection Test in Malaria Diagnosis

Ritesh Kumar[1], Manisha Kumari[2], Dr. Harmesh Manocha[3*], Sri Ram Chaudhari[4]
[1,2,4]Postgraduate student, Department of Microbiology, Sharda University, Greater Noida, Uttar Pradesh, India
[3]Associate Professor and HOD, Department of Microbiology, GIMS, Kasna, Greater Noida, Uttar Pradesh, India

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***Corresponding author**
Dr. HarmeshManocha

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Abstract: The early diagnosis of malaria not only mitigates the sufferings but also reduces the transmission of the parasite in the community. Therefore precise laboratory diagnosis and species identification are very essential. The present study was conducted to compare the efficacy of centrifuged buffy coat smear, peripheral blood smear, and rapid antigen detection. A total of 399 samples were collected from all cases clinically suspected of malaria. Detection of malaria parasite was done by the following techniques: Staining of Peripheral blood smear with Leishman stain, Rapid antigen detection by chromatographic Immunoassay and centrifuged Buffy Coat Smear (CBCS). The total number of malaria positive cases was found to be 133 (33.33%).It was observed that CBCS had high sensitivity (85.38%) in detecting the malaria parasites as compared with PBS (83.84%), while PBS had high specificity (99.62%) in detecting the malaria parasites as compared with CBCS (98.88%). In comparison to the gold standard Rapid antigen test, it was observed that while both PBS and CBCS had excellent specificity, PBS thick smear had slightly higher sensitivity (83.9%) in detecting the malaria parasites as compared with PBS thin smear (81.5%), while CBCS thick smear had slightly higher sensitivity (85.61) in detecting the malaria parasites as compared with CBCS thin smear (85.60).
Keywords: Diagnosis, blood smear, rapid antigen, malaria, comparative.

INTRODUCTION

Infected mosquitoes carrying protozoal parasites of Plasmodium species causes this serious disease that is transmit from one person to another through the bites of infected mosquitoes, and if untreated can be life-threatening. It was in the year 1898, malaria was found to be transmitted among human by female Anophelesmosquitoes which typically bite between dusk and dawn[1,2]. Fever, chills, sweating, headache, vomiting, diarrhea, abdominal pain, and distension, cough, splenomegaly and hepatomegaly are some symptoms. There are 4 species of human malaria parasite Plasmodium *viva*, *P. falciparum*, *P. ovale*, and *P. malariae*. In India 60 to 65% of the infections are due to *P. vivax* and 35 to 40% due to *P. falciparum*[3].The onset of the symptoms of malaria is not specific and generally is accompanied by fever, body ache, malaise, fatigue, and headache. Therefore, it's quite troublesome to identify malaria, however, the treatment and prevention is efficient and needs to me opted immediately to prevent fatal situations [4]. Particulary, in themalaria-prone areas, the early clinical features can be easily misdiagnosed Early interventions not only ensures low sufferings but also reduces the transmission of the disease [5]. So, exact laboratory investigations to identify the species is prominent. Various laboratory diagnosis tools and techniques are available such as the conventional thin and thick peripheral blood smears (PBS), concentration techniques such as buffy coat smears and fluorescent (QBC) technique, serologic tests such as the detection of parasite-specific proteins (Dipstick)[6] and polymerase chain reaction(PCR) These tools have their own characteristics and utilities in terms of efficacy, sensitivity, specificity etc Advent of any new approach towards diagnosis as well as making the existing techniques more beneficial mineenhancing it would suit the demand of time. Usually, malaria is diagnosed in the laboratory using different techniques, eg. Conventional microscopic diagnosis by staining thin and thick peripheral blood smear, other concentration techniques, eg. Quantitative Buffy Coat (QBC) method, rapid diagnostic test and molecular diagnostic methods such as Polymerase chain reaction(PCR)[7].

MATERIALS AND METHODS

Study design: A Cross-Sectional study

Source of data

The proposed study was carried out in the Department of Microbiology, School of Medical Sciences and Research, Sharda University, Greater Noida.

Sample size: 399

Sample technique: Simple Random sampling

Inclusion criteria

Patient clinically suspected of malaria and patient who had given the consent.

Exclusion criteria

Patients who had not given consent for the study.

Duration of study

01/01/2015 **to** 01/01/2016

STUDY ANALYSIS

Sensitivity, specificity, positive predictive value, negative predictive value were calculated for each method by comparing the proportion of positive and negative results for each method with the gold standard,Leishman stained thick blood smear examination. Sensitivity, specificity, positive predictive value, negative predictive value were calculated for each method by comparing the proportion of positive and negative results for each method with the gold standard,Leishman stained thick blood smear examination. Sensitivity, specificity, positive predictive value, negative predictive value were calculated for each method by comparing the proportion of positive and negative results for each method with the gold standard,Leishman stained thick blood smear examination. Sensitivity, specificity, positive predictive value, negative predictive value were calculated for each method by comparing the proportion of positive and negative results for each method with the gold standard,Leishman stained thick blood smear examination.

Sensitivity, specificity, positive predictive value, negative predictive value were calculated for each method by comparing the proportion of positive and negative results for each method with the gold standard rapid antigen detection test.

SENSITIVITY

TP/TP+FN. This is a number of True Positives.

SPECIFICITY

TN/TN+FP. This is a number of True Negatives.

POSITIVE PREDICTIVE VALUE (PPV)

TP/ TP + FP.

NEGATIVE PREDICTIVE VALUE (NPV)

TN/TN+FN

The values obtained are multiplied by 100 and reported as a percentage.

Where, TP = True positive, TN = True negative,
FP = False positive, FN = False negative

RESULTS

Table-1: Demographic profile of malaria-positive patients (n=133) under study

	OPD (number of patients)						IPD (number of patients)					
Age (in years)	Male			Female			Male			Female		
	P.V	P.F	Mixed	P.V	PF	Mixed	P.V	P.F	Mixed	P.V	P.F	Mixed
0-14	12	0	1	5	0	0	20	0	2	8	0	0
14-60	33	0	1	8	0	0	27	0	0	12	0	1
>60	1	0	0	1	0	0	1	0	0	0	0	0
Total	46	0	2	14	0	0	48	0	2	20	0	1

OPD: Outpatient department, IPD: Inpatient department, P.V: Plasmodium vivax, P.F: Plasmodium falciparum

Table-2: Results of samples in five different methods [PBS (thick & thin), CBCS (thick & thin), and Rapid antigen] for detection of malaria

S.No [Total samples]	PBS		CBCS		Rapid antigen	
Thick	Thin	Thick	Thin			
1.	Negative	Negative	Negative	Negative	Negative	266
2.	Negative	Negative	Negative	Negative	Positive	19
3.	Negative	Negative	Positive	Positive	Positive	1
4.	Positive	Negative	Positive	Positive	Positive	3
5.	Positive	Positive	Positive	Positive	Positive	106
6.	Negative	Negative	Positive	Positive	Negative	2
7.	Positive	Positive	Positive	Negative	Negative	1
8.	Negative	Negative	Negative	Positive	Positive	1
Total positive samples	*110*	*106*	*113*	*113*	*130*	[399]

*Total number of samples positive for malaria = 133. PBS: Peripheral blood smear, CBCS: Centrifuged buffy coat smear

Table-3: Species distribution of malaria parasites in different methods (n=133)

Species (n=133)	PBS (n=133)		CBCS (n=133)		Rapid Antigen	
	Positive	Negative	Positive	Negative	Positive	Negative
P.vivax	101		108		126	
P.falciparum	0 (0.00)		0 (0.00)		0 (0.00)	
Mixed infection	5		5		4	
Total	106	27	113	20	130	3

Figures in parentheses indicate percentages. PBS: Peripheral blood smear, CBCS: Centrifuged buffy coat smear

Table 4: Sensitivity, specificity, and validity of PBS (Thick and Thin) and CBCS (Thick and Thin) in comparison to Rapid antigen test

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PBS (Thick)	83.84	99.62	99.09	92.73
PBS(Thin)	81.53	99.62	99.06	91.78
CBCS(Thick)	84.61	98.88	97.34	93.00
CBCS(Thin)	85.38	99.25	98.23	93.35

PBS: Peripheral blood smear, CBCS: Centrifuged buffy coat smear, PPV: Positive predictive value, NPV: Negative predictive value

DISCUSSION

The present study demonstrated the performance of a modified technique for diagnosis of malaria by incorporating a centrifugation-enhanced step into the conventional method of smear preparation and examination for malaria. It helps to concentrate the parasites, which are then easily visualized by microscopy. The results of the study show that as compared with thin PBS examination, the thick PBS examination detected 4 more cases as malaria-positive, while compared with thin CBCS examination, thick CBCS detected no any more cases as malaria-positive which also correlated with the rapid antigen test to a great extent. The thin PBS failed to detect true malaria infection in 24 (6.01%) samples, thick PBS, in contrast, failed to detect true malaria infection in 21 (5.26%) samples. The thin CBCS failed to detect true malaria infection in 19 (4.76%) patients, thick CBCS, in contrast, failed to detect true malaria infection in 20 (5.01). Now comparing PBS with CBCS, the CBCS detected 4 more cases as malaria-positive. The PBS failed to detect true malaria infection in 21 (5.26) samples, which is not at all desirable in a malaria-endemic country like India. The CBCS, in contrast, failed to detect true malaria infection in 19 (4.76%) patients.

Similar results were obtained in the study by Akhtar *et al.* [8] in which out of 120 patients, the CBCS detected 6 more cases (49%) as malaria positive as compared with the peripheral smear (44%). Similarly, in another study where the authors used centrifugation-enhanced heparinized capillary tubes for smear preparation and examination found that, out of 100 patients, the modified centrifuged buffy coat detected 7 more samples as malaria-positive as compared with the conventional smear technique. The addition of centrifugation to the conventional smear technique improved its sensitivity from 86.79% to nearly 100% [9]. In yet another study from north

India[10], out of 50 patients clinically diagnosed as cases of cerebral malaria, only 28 patients (56%) were positive by Leishman stained blood smear examination for various stages of *P. falciparum*, whereas QBC and ParaSight-F (antigen) test were positive in 47 (94%) and 46 (92%) patients, respectively. In the present study, we have used the antigen test as the gold standard. If, in contrast, we had used PBS as the reference standard, which is the conventional method, it would have rendered 21 samples detected as malaria-positive by the antigen test to be labeled as false-positive. This can act as a deterrent to malaria testing in field conditions where antigen detection systems are used as diagnostic tests for rapid diagnosis of malaria. However, the use of CBCS leads to the demonstration of the parasites in 1 (4.76%) of these 21 samples thereby providing an excellent correlation between the antigen test and direct demonstration of the parasites. Still, 19 cases were detected by antigen testing alone, which were microscopy negative. It is, however, probable that most of these apparently false-negative cases by CBCS were true-positives, which were not detected by microscopy, particularly in case of *P. falciparum* malaria due to sequestration limiting the number of circulating parasites at the time of blood collection. This is evident from the fact that CBCS as compared with the PBS enabled detection of 4 more cases of plasmodia infection.

CONCLUSIONS

Though the previous study concluded that CBCS is an easy, rapid and accurate technique and could be adopted for the reliable diagnosis of malaria in resource-limited settings where RDT and QBC may prove to be costlier options.

However, we find that CBCS though have slightly better sensitivity and specificity compared to thick PBS smear. The difference is not significant. So it should not be adopted as a method of diagnosis in a routine setting as it has little advantage over thick PBS smear as the cost of tedious technique which may not be acceptable in most of the laboratories.

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