

Original Research Article

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Usefulness of Modified Centrifuged Blood Smear in Diagnosis of Malaria

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Malaria is a heavy burden on tropical communities, a threat to non-endemic countries, and a danger to travellers. Keeping in mind the seriousness of the condition and the current availability of various diagnostic facilities; this study is proposed to compare the peripheral blood smear examinations (thick and thin), Quantitative Buffy Coat (QBC) examination, Rapid Antigen Detection Test (RDT), and Modified Centrifuged Blood Smear (MCBS) in clinically suspected cases of malaria. The study included 1982 blood samples of patients, clinically suspected to be of Malaria. All the samples were subjected to Thin Blood Smear, Thick Blood Smear, QBC, RDT and MCBS. Results were statistically compared considering MCBS as the standard method. Out of total 1982 samples, maximum 81(4.09%) were found positive by RDT, followed by MCBS 66(3.33%), QBC 65(3.28%), thick blood smear 63(3.18%) and thin blood smear 58(2.92%). Addition of centrifugation step in MCBS increases the sensitivity of the smear technique and at the same time preserves the morphology of the parasite, giving it more specificity. MCBS is an easy, cheap and accurate technique and could be adopted for more reliable diagnosis of malaria in resource-limited settings where expensive techniques like RDT and QBC are not feasible.

Introduction

Malaria is the most important protozoal parasitic disease of humans affecting more than one billion people worldwide causing 1 to 3 million deaths each year. Malaria remains today a heavy burden on tropical communities, a threat to non-endemic countries, and a danger to travelers (Fauci *et al*, 2008). In India about 27% population lives in malaria high transmission (≥ 1 case/1000 population) areas about 58% in low transmission (0-1 case/1000 population) areas (Park K, 2011).

Lack of proper infrastructure, inability to control the disease in endemic areas, and movement of the population are some important factors responsible for failure to curb malaria (NVBDCP, 2004). The non-specific nature of the clinical presentation of malaria may lead to over-treatment of malaria in malaria endemic areas; thereby resulting in misdiagnosis and non-treatment of other diseases. 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 is very essential (Estacio *et al.*, 1993). In the present study an attempt is made to compare the peripheral blood smear examinations (thick and thin), QBC examination, Rapid Diagnostic Antigen Test and Modified Centrifuged Blood Smear (MCBS) in clinically suspected cases of malaria.

Materials and Methods

The study was carried out from February 2014 to January 2015. A total of 1982 non repeat blood samples of clinically suspected malaria cases received in central clinical laboratory of tertiary care hospital in Meerut were studied.

Blood samples received were examined by Leishman stained thick and thin blood smears, QBC, pLDH test using Advantage MAL CARD and MCBS.

Inclusion Criteria

Patients of all age groups and both sexes, presenting with pyrexia as main symptom and attending various outpatient departments or admitted in various wards at Subharti Medical College & Hospital, Meerut (U.P).

Exclusion Criteria

Patients who were currently taking antimalarial therapy or who had been treated with antimalarial drugs within the past 2 weeks were excluded from the study.

Informed consent was obtained from all patients, included in this study.

The study has been approved by the research and ethical committee of the institute.

Test procedure of MCBS: Blood samples were collected into heparinized capillary

(Hematocrit) tubes and one end of the tube was immediately sealed using wax. The samples collected in the capillary tubes were centrifuged at 6000 rpm for 5 min. The capillary tube was cut by tube cutter at the required junction (buffy coat layer) (Bhandari *et al.*, 2008).

The following method was attempted: (Bhandari *et al.*, 2008)

The tube was cut just above the buffy coat layer, saving a small column of plasma. The retained plasma column, the buffy coat and an equal quantity of sub buffy coat RBC column were pushed on to the slide; they were gently and uniformly mixed, and smears were drawn. These smears had thickness equivalent to peripheral blood smears, providing an advantage of including the concentration of parasitized RBCs in a thin smear.

This technique combines the advantages of Peripheral Blood Smear and QBC. The parasites are observed directly without any aid and hence this technique is highly specific as far as diagnosis goes. Tube centrifugation adds to the sensitivity of Peripheral Blood Smear and makes it similar to QBC.

Test procedure for thin blood smear, thick blood smear, QBC were as per standard instructions. (Chakraborty, 2004; Estacio *et al.*, 1993).

Test procedure for Rapid antigen detection was as per instruction manual (J.MITRA).

Calculations: Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of Thin Blood Smear, Thick Blood Smear, QBC, and RDT were calculated by comparing their result with MCBS examination as the

standard reference in the present study, using 2x2 Table (Park K, 2011).

Results and Discussion

Out of total 1982 cases, 81 (4%) were positive by one or more methods i.e Thin Blood Smear, Thick Blood Smear, QBC, RDT and MCBS, while 1901(96%) cases were negative by all the five methods.

The age of the patients in this study ranged from infants to 89 years old with maximum (43.2%) between the age group of 20-29 years, with male to female ratio of 1.7: 1. There was an increase in the incidence of malaria positive cases during June to September period (3.28%). Out of total 1982 samples, maximum 81(4.09%) were found positive by RDT, followed by MCBS 66(3.33%), QBC 65(3.28%), thick blood smear 63(3.18%) and thin blood smear 58(2.92%). (Table 1)

Out of 66 cases positive by MCBS, 60(90.91%) were positive for *Plasmodium vivax*, 5 (7.58%) were positive for *Plasmodium falciparum* and 1 (1.52%) case was positive for mixed infection, both by *Plasmodium vivax* and *Plasmodium falciparum*.

Out of 81 cases positive by Rapid Antigen

Detection Method, 75(92.60%) were positive for non-*P.falciparum* species and 6 (7.41%) were positive for either *P.falciparum* or mixed infection.

Out of 58 samples positive by thin blood smear, 52 were identified as *P.vivax*, 5 were identified as *P.falciparum* and 1 sample was identified as mixed (*P.vivax* + *P.falciparum*) infection. (Table 2)

Out of 63 samples positive by thick blood smear, 38 were identified as *P.vivax*, 4 were identified as *P.falciparum* and in 21 samples species could not be identified. (Table 2)

Out of 66 samples positive by MCBS, 60 were identified as *P.vivax*, 5 were identified as *P.falciparum* and 1 sample was identified as mixed (*P.vivax* + *P.falciparum*) infection. (Table 2)

Out of 65 samples positive by QBC, 34 were identified as *P.vivax*, 4 were identified as *P.falciparum* and in 27samples species could not be identified. (Table 2)

Sensitivity, Specificity, PPV and NPV of Thin Blood Smear, Thick Blood Smear, QBC and RDT was compared taking MCBS as the standard method. (Table 3)

Table.1 Number of Cases, Positive by Various Diagnostic Methods. (n=1982)

Diagnostic Methods	Positive (n=1982)	% positive
RDT	81	4.09
MCBS	66	3.33
QBC	65	3.28
Thick blood smear	63	3.18
Thin blood smear	58	2.92

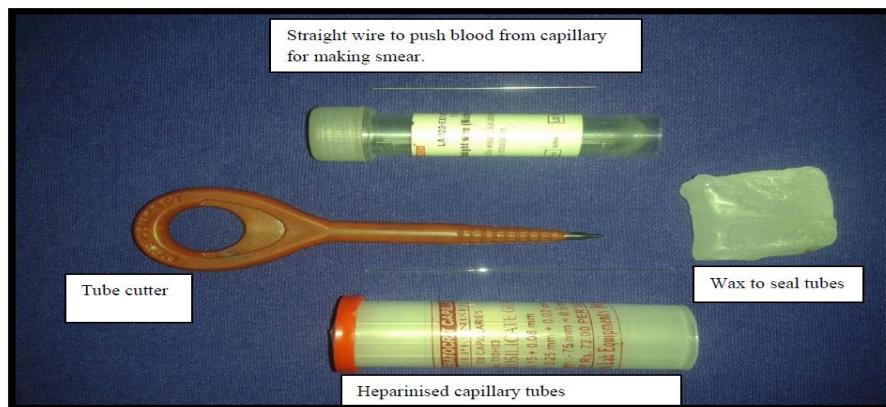
Table.2 Identification of Species by Various Diagnostic Methods

	Thin blood smear (n=58)	Thick blood smear (n=63)	MCBS (n=66)	QBC (n=65)
<i>P.vivax</i>	52	38	60	34
<i>P.falciparum</i>	05	04	05	04
Mixed (P.f + P.v)	01	-	01	-
Species unidentifiable	00	21	00	27
Total positive	58	63	66	65

Table.3 Sensitivity, Specificity, PPV and NPV of Thin Blood Smear, Thick Blood Smear, QBC, RDT Compared to MCBS as the Standard Method

	Sensitivity	Specificity	PPV	NPV
Thin blood smear	87.88%	100%	100%	99.58%
Thick blood smear	93.94%	99.95%	98.41%	99.79%
QBC	92.42%	99.79%	93.85%	99.74%
RDT	100%	99.22%	81.48%	100%

Figure.1 Equipments Used for Preparation of Modified Centrifuged Blood Smear



The absolute necessity for rational therapy in the face of rampant drug resistance places importance on the accuracy of malaria diagnosis (Wongsrichanalai *et al.*, 2007). Rapid detection and effective treatment is a pre-requisite for reducing the morbidity and mortality due to malaria. Newer techniques like QBC, Antigen detection assays are

rapid, simple and easy to interpret (Parija *et al.*, 2009). The blood smear examination is time tested and optimum method for diagnosis of malaria, but there are some operational difficulties, which results in delay of radical treatment in many cases. On this background the MCBS is relatively inexpensive and gives quite specific results.

Incidence of malaria cases varies from year to year and the reason for low positivity (4.09 %) in current study may be due to low incidence of malaria cases in Meerut this year.

In this study MCBS, gave positive result in 66% cases, which was 8 cases more than thin blood smear. This is distinctly advantageous as expenses for the two methods are comparable. Similar results were obtained in the study by Akhtar *et al.*, in which out of 120 patients, the MCBS detected 6 more cases as malaria positive as compared with the peripheral smear (Akhtar *et al.*, 2010).

Similarly, in another study where the authors used centrifugation-enhanced heparinised 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% (Bhandari *et al.*, 2008). Specificity and PPV of MCBS is better than thick film, QBC and RDT and is comparable to thin film. Thus MCBS being inexpensive; is distinctly advantageous compared to QBC and RDT. Sensitivity and NPV of MCBS is equal to RDT and is even better than thin film, thick film and QBC. Thus MCBS does not compromise on sensitivity compared to expensive techniques like QBC and RDT. (Table 3)

In conclusion, new simple method of MCBS followed in this study is as sensitive as RDT and eliminates the false positive results of RDT. The modified MCBS is cheap, cost effective, easy to perform and provides a permanent record of the smear. MCBS is also a rapid and accurate technique and

could be adopted for reliable diagnosis of malaria in resource-limited settings where RDT and QBC may prove to be costlier options. Some of the equipment like compound microscope, slides and stains are already available in rural health centers. Most of other material required like capillary tubes, centrifuge, tube cutter and steel wire, is also inexpensive and can be easily made available. Hence, it can also be used in malaria diagnosis at the rural level in our country.

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