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Feasible choices in diagnostic methods of malaria

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Abstract

Background & objectives: The present study was undertaken to find out a new easy method in the diagnosis of malaria by centrifuged buffy coat smear, which was found to be a feasible and reasonable procedure.

Methods: Blood samples collected from 120 patients suspected of malaria were subjected to all three diagnostic modalities—peripheral blood smear (PS), centrifuged buffy coat smear (CBCS) and antigen detection test using pLDH and aldolase (AG).

Results: The results of various methods were compared. It was seen that addition of centrifugation (i.e. CBCS) to conventional method of PS (i.e. thick and thin smears) improved its sensitivity from 85 to 93.3%. Antigen detection and CBCS were found superior to PS in sensitivity. CBCS gives combined sensitivity and specificity of both antigen and PS.

Conclusion: CBCS is as sensitive as antigen test and as specific as PS in species identification. It is a reasonable and feasible procedure too.

Key words Buffy coat smear; diagnostic methods; malaria; peripheral blood smear

Introduction

Malaria is the most common serious parasitic disease in human beings, killing one person every 12 sec. The disease now occurs in >90 countries worldwide, with >500 million cases of malaria and 3 million deaths per year in developing countries. The majority of these deaths are seen in pediatric age group and in pregnant women[1]. The definitive diagnosis of human malaria should be based on clinical criteria supported by laboratory information of parasitaemia. For decades, light microscopy of blood smears has been the gold standard in the diagnosis of malaria[2]. The diagnostic modalities which are available for malaria range from conventional thick and thin smears, quantitative buffy coat smears [QBC], to rapid and more reliable diagnostic modalities like antigen detection tests for detecting parasitic antigen like histidine-rich protein-2 (HRP-2), *Plasmodium* lactate dehydrogenase (pLDH) and pan specific aldolase. These techniques vary in sensitivity and specificity[3]. Newer diagnostic techniques have been developed based on antigen detection on the basis of detection of parasite specific nucleic acid sequences in the sample either by polymerase chain reaction (PCR) or by using specific complimentary biotinylated probes to detect parasite specific sequence of DNA in blood samples by non-isotopic identification methods[1].

All the techniques have their own advantages and disadvantages with respect to sensitivity, specificity, time consumption, cost-effectiveness and ease of procedure. It would be a great help if a new technique that utilizes most of the advantages of the above techniques is developed and standardized[4]. Though microscopy is considered as the "gold standard" for diagnosis of malaria, it requires up to 30 min for results. It is labour intensive and requires considerable expertise for its interpretation, particularly at low lev-

els of parasitaemia[1]. Keeping in mind the seriousness of the condition and the current availability of diagnostic facilities across India, the present study was undertaken to find the most feasible and reasonable technique from the commonly employed techniques in diagnosis of malaria.

Material & Methods

The study was conducted in central hospital laboratory of NKP Salve Institute of Medical Sciences after obtaining clearance from the Institutional Ethics Committee. Blood samples were collected from 120 patients attending the OPD and IPD with clinical suspicion of malaria, in a one year period. Approximately 2 ml of venous blood was collected from each patient.

Sample processing: Firstly, thick and thin smears were prepared as per the standard method described and the smears were stained with Leishman stain. The average time spent on screening each slide varied depending on parasite density. Thick smears were reported negative after examination of 200–300 oil immersion fields with no parasite. A thin smear was given negative when no parasites were observed in 200 oil immersion fields.

Secondly, centrifuged buffy coat smears were prepared by using 2 ml blood collected in a wide bore 4 ml tube with EDTA which was centrifuged (2000–3000 rpm for 15 min). The supernatant plasma was separated and layer of buffy coat and equal thickness of RBC layer just below was picked up to prepare smears which were stained by Leishman stain. We did not use the traditional Wintrobe tube to obtain a buffy coat since filling of the tube was found to be cumbersome.

Thirdly, antigen detection using pLDH and aldolase commercially available antigen detection kit detecting *Plasmodium* LDH and aldolase were used. The test was conducted using anticoagulated venous blood. The sample was added to test strip using a calibrated dropper provided with the kit, and the strip was placed in a micro well containing buffer. The result was read after 15 min as per manufacturer's instructions. It was interpreted as positive for *P. falciparum* if T1 and control (C) bands were seen. If only T2 and C bands were seen it was interpreted as positive for *P. vivax*. If T1, T2 and C bands were seen it was indicative of mixed infection. The data were analyzed statistically for specificity, sensitivity and predictive values.

Results

The three diagnostic modalities gave varied results as shown in Table 1. The incidence of malaria was found to be 53.33% (64/120). Table 2 shows that the PS was positive in 53 cases out of which 15 were positive for *P. falciparum*, 36 for *P. vivax* and 2 samples were positive for mixed infection. The CBCS showed 59 positive for malaria of which 16 cases were positive for *P. falciparum*, 41 for *P. vivax* and 2 for mixed infection. The antigen was positive in 60

Table 1. Result of samples isolated and in combination of various methods (PS, CBCS and antigen)

S.No.	PS	CBCS	Antigen	Cases	Interpretation
1.	Negative	Negative	Negative	56	PS+CBCS+AG negative
2.	Negative	Positive	Negative	2	Only CBCS positive
3.	Negative	Negative	Positive	3	Only AG positive
4.	Negative	Positive	Positive	6	CBCS+AG positive
5.	Positive	Negative	Negative	1	Only PS positive
6.	Positive	Positive	Negative	1	PS+CBCS positive
7.	Positive	Negative	Positive	1	PS+AG positive
8.	Positive	Positive	Positive	50	PS+CBCS+AG positive
Total	53 (44%)	59 (49%)	60 (50%)	120	64 (53.3%)

Table 2. Distribution of species in PS, CBCS and antigen tests

Species	PS (n = 64)		CBCS (n = 64)		Antigen (n = 64)	
	Positive	Negative	Positive	Negative	Positive	Negative
Pv	36 (56.2)	8 (12.5)	41 (64.06)	3 (4.68)	40 (62.5)	3 (4.68)
Pf	15 (23.4)	3 (4.68)	16 (25)	2 (3.12)	18 (28.12)	1 (1.56)
Mix	2 (3.12)	0 (0)	2 (3.12)	0 (0)	2 (3.12)	0 (0)
Total	53 (82.8)	11 (17.18)	59 (92.18)	5 (7.81)	60 (93.7)	4 (6.25)

Figures in parentheses indicate percentages.

cases with 18 being positive for *P. falciparum*, 40 for *P. vivax* and 2 for mixed infection.

Table 3 shows the specificity and sensitivity of PS, CBCS and antigen. We used the antigen test as gold standard to compare our results and calculate the sensitivity and specificity of methods used by us. CBCS had a sensitivity and specificity of 93.33 and 95% respectively, while PS showed 85 and 96% respectively for detection of malaria. AG had a sensitivity of 98.24% and a specificity of 93.65%. Hence, CBCS had the specificity of PS especially in species identification and a close sensitivity to AG. Table 3 shows the positive and negative predictive values of the various methods.

Discussion

In the present study we compared three methods for the detection of malaria, to find an easy but sensitive and cost-effective method. We found the CBCS, using wide bore tubes instead of Wintrobe tubes, to be as sensitive as antigen and as specific as the PS technique for the diagnosis of malaria.

By the process of CBCS, compared to conventional smear (PS) technique, more cases were detected. It is cheap, easy to perform and cost-effective. The simple equipment required for this technique is available even in peripheral laboratories. Hence, it can also be used in malaria diagnosis at the rural level in our country. The CBCS compared to the antigen test showed a sensitivity of 93.33% and specificity of 95%, with a positive predictive value of 94.91% and negative predictive value of 93.4% (Table 3).

The PS and CBCS negative patients were detected by antigen and were the ones who would be greatly benefited by undergoing the antigen test at the same time. However, one negative PS case was missed by the antigen test, but picked up on CBCS, which can be explained by insufficient enzyme production, which occurs during early malarial infection, or if the patient's blood sample contained parasites at concentration below the optimal test detection level (50–100 parasite/µl of blood). Thus, the antigen test is of importance only in PS negative cases and could be preferably used as a final diagnostic test and not as a screening test or first line of investigation considering its high cost. Though other studies have compared PS with QBC and AG [3] or with QBC and other modifications of the buffy coat smear and other methods[1,4,5], to the best of our knowledge no other studies have compared the simple CBCS as done by us

Table 3. Comparison of sensitivity, specificity and validity

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PS	85	96	96.2	86.6
CBCS	93.33	95	94.1	93.4

PPV—Positive predictive values; NPV—Negative predictive values.

Table 4. Comparison of modalities for reasonable choices in malaria diagnosis

Variable	PS	CBCS	Antigen
Method	Cumbersome	Cumbersome	Easy
Time	Long (20–30 min)	Long (30–35 min)	Fast (10–15 min)
Sensitivity	85%	93.3%	98.24%
Specificity	96%	95%	93.65%
Cost	INR 5/-	INR 7/-	INR 80/-
Availability	Everywhere	Everywhere	Limited

with PS and AG. The use of QBC in the rural parts of our country is difficult[6], and we feel that our method of CBCS could easily be done even in the rural areas, although PS is considered to be the gold standard[7]. More such studies would be required to establish this as a good screening test for malaria.

Conclusion

Our study proved that of the trial of the various diagnostic modalities in malaria, CBCS and antigen were found superior to PS. Our new simple method of CBCS is as sensitive as antigen and as specific as PS and eliminates the false positive results of antigen. It is also easy to perform and cost-effective. A comparison of the three methods of diagnosis of malaria used by us is given in Table 4. Buffy coat smears can be easily used in the diagnosis of malaria. However, more studies are required before we can establish CBCS as a new gold standard in the diagnosis of malaria.

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