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# STUDY OF USEFULNESS OF CENTRIFUGED BUFFY COAT SMEAR IN COMPARISION WITH PERIPHERAL BLOOD SMEAR IN THE DIAGNOSIS OF MALARIA

**Dr. Hamsini C.*[1] and Dr. P. Karkuzhali[2]**

[1]Postgraduate, Department of Pathology, SBMCH, Bharath University, Chennai.
[2]Professor and HOD, Department of Pathology, SBMCH, Bharath University, Chennai.

***Corresponding Author: Dr. Hamsini C.**

Postgraduate, Department of Pathology, SBMCH, Bharath University, Chennai.

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## ABSTRACT

**Introduction and Background:** Malaria continues to be a global public health challenge with more than 200 million cases and over 6,60,000 deaths annually, especially in the tropical and sub-tropical countries. The laboratory diagnosis of malaria is made by different techniques 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) and PCR. **Aim:** The present study was undertaken to assess the usefulness of a centrifuged buffy coat smear (CBCS) technique for diagnosis of malaria and to compare it with conventional PBS examination. **Materials and Methods:** Blood samples were collected from 100 malaria suspected patients who came to the department of pathology, SBMCH, Chennai, were subjected to these tests, that is PBS and CBCS. **Results:** The highest number of cases were detected by the CBCS method (25%) followed by the PBS (18%). It was observed that while both PBS and CBCS had excellent specificity, PBS had low sensitivity (72.8%) in detecting the malaria parasites as compared with CBC (90.9%). **Conclusion:** It was concluded that CBCS is an easy, rapid and accurate technique and could be adopted for reliable diagnosis of malaria, and especially useful in centres, where facility of QBC not available.

## INTRODUCTION AND BACKGROUND

Malaria remains one of the world's most important parasitic infections. The current social, economic, and medical impact of malaria in tropical underdeveloped settings is immense. The impact of malaria morbidity and mortality continues to increase across malaria risk areas. Malaria continues to be a global public health challenge with more than 200 million cases and over 6,60,000 deaths annually, especially in the tropical and sub-tropical countries.[1]

Even in developed countries where the disease is not endemic, increasing immigration and worldwide travel to endemic regions have led to an increased incidence of imported malaria. In India, malaria is endemic throughout the country and is a major public health problem accounting for 1-2 million cases and 1100 deaths annually.[1] However, according to recently published studies, the burden of malaria appears to be much higher than the previously reported figures (i.e. a 9- to 50-fold underestimation of malaria-related cases and 13-fold under-estimation of malaria-related mortality, respectively.[2,3]

The earliest symptoms of malaria are very nonspecific and variable such as fever, headache, body ache, malaise, fatigue and abdominal discomfort. Hence, there is difficulty in diagnosing malaria clinically but the treatment has to be started immediately in order to avoid complications.[14] The nonspecific nature of the clinical presentation of malaria may lead to over-treatment of malaria in malaria endemic areas and missing the diagnosis of malaria in low-transmission areas.

In malaria endemic areas, this may lead to 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.[15] Therefore precise laboratory diagnosis and species identification is very essential.

The World Health Organization[11] recommended prompt parasitological confirmation by microscopy or alternatively rapid diagnostic tests (RDTs) in all patients suspected of malaria before treatment is started and treatment on the basis of clinical suspicion alone, should only be considered when a parasitological diagnosis is not accessible. Although microscopy by thin and thick blood films is the gold standard of malaria diagnosis,[12]

the risk of false negative microscopy is higher if the patient has received recent doses of antimalarial drugs. The choice between RDTs and microscopy depends on local circumstances, *e.g.* the skills available.[13] However, a major drawback of RDTs is more expensive and the sensitivities and specificities are variable.

The laboratory diagnosis of malaria is made by different techniques 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)[16] and PCR. These techniques have their own advantages and disadvantages with respect to sensitivity, specificity, time consumption, cost effectiveness, ease of procedure etc. It would be of great help if a new technique that utilizes most of the advantages, while eliminating most of the disadvantages of the above techniques, is developed and standardized.

The thin smear technique has no false positivity but it is not very sensitive. Hence, false negativity is frequent and requires two or more repetitions that may not be well appreciated by patients. The thick blood smear has a better rate of parasite detection; however, species identification becomes difficult sometimes.

Dehemoglobinization is required if Leishman's stain is to be used. Jaswant Singh and Bhattacharya (JSB) and other field stains are not easily available to all laboratories. On the other hand, the fluorescent (QBC) technique, even though provides fast diagnosis with high sensitivity has disadvantages such as over-diagnosis (glowing particles other than actual malarial parasite are identified as positive), thereby leading to a risk of false positivity. Species identification is also difficult and leads to frequent errors, thereby tempting the technician to present a report of mixed infections (both *Plasmodium vivax* and *Plasmodium falciparum*). In fact, the percentage of mixed positivity reported in this area is many times more than the national average. The cost factor is also considerably high, which is not affordable to many poor patients.

The centrifuged buffy coat smear involves the collection of 2 ml of venous blood into anticoagulant bottles, filling the Wintrobe's tube and centrifugation for 20-30 min, finally obtaining a buffy coat layer onto a slide is a very cumbersome procedure. Although capillary tubes were used in the past, it was unacceptable due to problematic procedure and lack of standardization.[17]

The National Vector Borne Disease Control Programme (NVBDCP) of India reports about 2 million malaria parasite positive cases annually, of which about 50% are Plasmodium falciparum.[9]

An optimal diagnosis of malaria requires the detection and identification of each Plasmodium species present in the patient's blood and especially in cases of *P. falciparum* infection, an evaluation of the level of parasitaemia.

A previous study from India[5] had developed, standardised and reported on the feasibility of a modified centrifuged buffy coat smear (CBCS) examination for diagnosis of malaria in which the authors had used a wide bore 4 ml tube instead of a Wintrobe's tube to obtain a buffy coat. This new technique combined most of the advantages of the existing techniques.

## AIM

The present study was undertaken to assess the usefulness of a centrifuged buffy coat smear (CBCS) technique for diagnosis of malaria and to compare it with conventional PBS examination.

## MATERIALS AND METHODS

Blood samples were collected from 100 malaria suspected patients who came to the department of pathology, SBMCH, Chennai. Blood samples (2-3 ml per patient) collected in EDTA vials from patients with a clinical suspicion of malaria were subjected to PBS and CBCS.

First, thick and thin smears were prepared as per the standard method, stained with Giemsa's stain for 40-45 min, and examined under oil immersion at ×100 magnification. Levels of parasitaemia (asexual stages/µl) were calculated, using the thick smears, by counting asexual parasites against a fixed number of leucocytes (usually 200) and assuming each patient had 8000 leucocytes/µl blood.[4] A thick smear was considered negative if no parasites were seen in 200 oil immersion fields. Thin smears were used to confirm the Plasmodium species present. Samples with pure gametocytaemia were included among the positive samples.

Second, CBCSs were prepared as described previously.[4] Briefly, this consisted of centrifuging the 2 ml EDTA blood in a wide bore 4 ml tube at 2000-3000 rpm for 15 min. The supernatant plasma was separated and layer of buffy coat and equal thickness of red blood cells (RBC) layer just below was picked up to prepare smears, which were stained by Giemsa stain. The CBCS were examined for 200 oil immersion fields before being reported negative. Levels of parasitaemia in CBCS were calculated if PBS were negative.

## RESULTS

During the study period, a total of 100 samples from an equal number of patients were received for testing for malaria parasites. The two diagnostic modalities gave varied results as shown in [Table 1]. The total number of malaria positive cases was found to be (27) [Table 1]. Of these, 13(48%) were positive for *P. vivax*, 12 (44%) were positive for *P. falciparum* and 2 (7.4%) for mixed infections due to both *P. vivax* and *P. falciparum.*

The highest number of cases were detected by the CBCS method (25%) followed by the PBS (18%). However, the CBCS failed to detect malaria infection in 2 patients, which were diagnosed by the PBS and in contrast, the CBCS detected malaria infection exclusively in 9 patients.

Table-3, shows the specificity, sensitivity and area under the curve (AUC) of PBS in comparison to CBCS. It was observed that while both PBS and CBCS had excellent specificity, PBS had low sensitivity (72.8%) in detecting the malaria parasites as compared with CBCS (90.9%). The usefulness of CBCS in detecting malaria parasites were further revealed by a statistically significant difference ($P < 0.001$ by Chi-square test) in the AUC of PBS and CBCS.

Table-2, shows the results of PBS and CBCS according to the parasitaemia level in the microscopy-positive cases. Both the tests detected malaria infection equally when the sample had a high parasite count of >1000 parasites/µl. However, at lower levels of parasitaemia (<200 parasites/µl), the PBS failed to detect malaria infection as compared with the CBCS in 32 samples. Similarly, at moderate levels of parasitaemia (200-1000 parasites/µl), the PBS failed to detect malaria infection as compared with the CBCS in 28 samples. Thus, the addition of centrifugation (i.e. CBCS) to the conventional method of PBS enabled detection of 72 more cases of plasmodia infection, which included 32 cases with low levels of parasitaemia, 28 cases with moderate levels and 12 cases with pure gametocytaemia.

**Table-1.**

| Sl.No. | PBS | CBCS | No.of samples |
|---|---|---|---|
| 1. | Negative | Negative | 73 |
| 2. | Negative | Positive | 9 |
| 3. | Positive | Negative | 2 |
| 4. | Positive | Positive | 16 |
| Total | (18%) | (25%) | 100 |

Total no. of samples positive for malaria=27.

**Table-2.**

| Parasite Density (Parasites/microlitre) | Number of specimens with indicated density as determined by |
|---|---|
| | PBS CBCS |
| <200 | 4 36 |
| 200-1000 | 20 48 |
| >1000 | 42 42 |
| Gametocytes only | 14 26 |

**Table 3: Sensitivity, specificity and validity of PBS in comparison to CBCS test.**

| TEST Sensitivity Specificity AUC |
|---|
| PBS 72.8% 99.2% 0.82 |
| CBCS 90.9% 99.5% 0.93 |

## DISCUSSION

The accurate diagnosis of malaria is important for the timely treatment of febrile patients with antimalarial drugs to reduce their mortality and morbidity and also to effectively manage non-febrile illness. PBS microscopy is very tedious and time consuming. Various sensitive methods have been employed for the simple, reliable, and rapid diagnosis of malaria. The most promising of these, was the CBCS which was compared with Giemsa stained PBS microscopy for diagnosis of P. vivax and P. falciparum infections.

Results were also made available in just eight to 15 minutes which is a fraction of the time required for thick film methods. The resources for training were also reduced as trainees with 3–5 days of training could produce results comparable to an experienced microscopist.

Some studies found that Rapid diagnostic tests like Advantage mal card showed sensitivity of 97% for P. vivax, thus making it a good diagnostic tool in areas where the predominant species is P. vivax, as in Mumbai, India. Thus Advantage mal card clearly has advantage over Parahit total and also on other malaria pLDH detection tests especially for nonfalciparum infections. Few RDTs have reported over 90% sensitivity for P. vivax.

It is, however, probable that most of the 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 or due to the parasitaemia being below the detection limit of approximately 50 parasites/µl by microscopy. This is evident from the fact that CBCS as compared with the PBS enabled detection of 72 more cases of plasmodia infection particularly at low and moderate levels of parasitaemia.

Previous other studies also found, that among the numerous methods available for malaria diagnosis, one of the quick and new methods using the principle of centrifugation is the QBC assay. QBC, which utilises high-speed centrifugation along with a larger volume of blood (55-65 µl), has a definite advantage in detecting Plasmodium infection in samples with low levels of parasitaemia (1 parasite/µl).[8] However, the drawbacks of QBC are that it is expensive, chances of leaking and breaking of blood-filled QBC tubes in the centrifuge and inability to keep a permanent record of the test.

The modified CBCS, in contrast, is cheap and provides a permanent record of the smear. The results of this study show, that as compared with traditional PBS examination, the CBCS detected 72 more cases as malaria-positive, especially at low- and moderate-levels of parasitaemia.

The PBS failed to detect true malaria infection in 72 samples (Table-2), which is not at all desirable in a malaria endemic country like India. The CBCS, in contrast, failed to detect malaria infection in only 2 patients (Table-1). Similar results were obtained in the study by Akhtar *et al.*,[5] in which out of 120 patients, the CBCS detected 6 more cases as malaria positive as compared with the peripheral smear.

Similarly, in another study[6] 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%.[6] In yet another study from north India,[7] 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.

## CONCLUSION

Malaria continues to be a global public health challenge. Microscopic examination of peripheral blood smear (PBS) is the standard method for malaria diagnosis, which is easily available and has low cost but its reliability is questionable at low level of parasitaemia. Our study and prior studies showed that the CBCS, in contrast, is cheap and provides a permanent record of the smear. It was concluded that CBCS is an easy, 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.

## REFERENCES

1. World Health Organization. World Malaria report 2012. Available from: http://www.who.int/malaria/worldmalariareport2012/. [Last accessed on 2013 Aug 05].
2. Hay SI, Gething PW, Snow RW. India′s invisible malaria burden. Lancet, 2010; 376: 1716-7.
3. Das A, Anvikar AR, Cator LJ, Dhiman RC, Eapen A, Mishra N, *et al*. Malaria in India: the center for the study of complex malaria in India. Acta Trop, 2012; 121: 267-73.
4. Moody A. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev, 2002; 15: 66-78.
5. Akhtar S, Maimoon S, Wilkinson A, Gowardhan V, Mahore S. Feasible choices in diagnostic methods of malaria. J Vector Borne Dis, 2010; 47: 151-4.
6. Bhandari PL, Raghuveer CV, Rajeev A, Bhandari PD. Comparative study of peripheral blood smear, quantitative buffy coat and modified centrifuged blood smear in malaria diagnosis. Indian J Pathol Microbiol, 2008; 51: 108-12.
7. Shujatullah F, Malik A, Khan HM, Malik A. Comparison of different diagnostic techniques in *Plasmodium falciparum* cerebral malaria. J Vector Borne Dis, 2006; 43: 186-90.
8. Salmani MP, Preeti BM, Peerapur BV. Comparative study of peripheral blood smear and quantitative buffy coat in malaria diagnosis. J Commun Dis, 2011; 43: 57-9.
9. V. P. Sharma, "Battling the malaria iceberg with chloroquine in India," Malaria Journal, 2007; 6: article 105.
10. H. E. Ronaldo, E. R. Emmanuel, C. Shirley, F. C. Remedios, and T. A. Angeles, "The Quantitative Buffy Coat Technique (QBC) in early diagnosis of Malaria: The Santo Tomas University Hospital Experience," Journal of the Philippine Society for Microbiology and Infectious Diseases, 1993; 22(2): 56–59.
11. WHO. Basic malaria microscopy, part 1: learner guide. 2nd ed. Geneva: WHO, 2010; 8.
12. WHO. Guidelines for the treatment of malaria. Geneva: WHO, 2010; 14.
13. WHO. Malaria microscopy quality assurance manul, version 1. Geneva: WHO, 2009; 9–24.
14. White NJ, Breman JG. Malaria and babesiosis diseases caused by RBC parasites. *In*: Harrison's principles of internal medicine. Kasper DL, *et al*, editors. 16th ed (Vol. 1); McGraw Hill: New Delhi, 2005; 1224-6.
15. Govt of Karnataka; Circular No NVBDCP: HE: 27:04-05; Ministry Of Health and Family Welfare, 2004; 1-4.
16. Todd WT, Lockwood DN, Nye FJ, Wilkins EG, Carey PB. Infection and immune failure. *In*: Davidson's principles and practice of medicine. Haslett C, *et al*, editors. 19th ed; Churchill Livingstone: London, 2002; 54.
17. Woo PT, Hauck L. The haematocrit centrifuge smear technique for the detection of mammalian *Plasmodium*. Trans Royal Soc Trop Med Hyg, 1987; 81: 727-8.