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Comparative Evaluation of Various Methods for Detection of Malaria with Special Reference to Modified Buffy Coat Preparation

Authors

Sonal Bhati, Gunja Dwivedi, Sunita Bhargava, Narendra Mogra

Department of Pathology, R. N. T. Medical College, Udaipur, Rajasthan

Corresponding Author

Dr Sonal Bhati

D/O Dilip Bhati ,4 Badi Holi Marg, Opp Durga Restaurant,Udaipur Rajasthan

Email: dr.rahul9181@gmail.com

ABSTRACT

The present study was aimed to do comparative evaluation of various methods for detection of Malaria with special reference to Modified buffy coat preparation and thus to establish a more sensitive method of detection. Blood samples collected from 200 suspected malaria patients were subjected to all the four tests. The results obtained from Modified buffy coat preparation were compared with the conventional PBF, QBC and antigen detection test. Sensitivity of PBF, antigen detection test and modified buffy coat preparation method was 84.90%, 86.79%, 94.33% respectively and specificity was 100%, 95.74% and 97.87%, respectively. It was seen that by addition of centrifugation to the PBF, sensitivity improved from 84.90% to 94.33 %.

Key words: PBF- Peripheral blood film, QBC- Quantitative buffy coat

INTRODUCTION

Malaria is the most common serious parasitic disease in human beings. In India, 1.06 million cases have been reported in 2012 of which 0.53 million cases are caused by plasmodium falciparum⁽¹⁾.

The classic presentation of malaria consists of paroxysms of fever alternating with periods of fatigue. Symptoms associated with febrile

paroxysms include high grade fever, chills, rigor, sweat, headache, myalgia, back pain, abdominal pain, nausea, vomiting, diarrhea, pallor and jaundice.

Malaria caused by *P. vivax*, *P. Malariae* and *P. ovale* results in parasitemia of less than 2% cases whereas parasitemia in *P. falciparum* can reach up to 60% or more⁽²⁾. The early diagnosis of malaria not only mitigates the sufferings but also reduces

the transmission of the parasite in community. Therefore, precise laboratory diagnosis and species identification is highly significant.

The laboratory diagnosis of malaria can be made by different techniques such as conventional thick and thin peripheral blood smear examination, QBC method (fluorescent), Antigen detection method, Antibody detection test, PCR, buffy coat preparation and by other newer techniques.

Thin peripheral blood smear examination technique has little false positivity but it is not very sensitive; false negativity is frequent and requires two or more repetitions of the test that may not be well appreciated by the patients. Thick blood smear examination has better rate of parasite detection but species identification becomes sometimes difficult. QBC fluorescent technique provides faster diagnosis with high sensitivity but high false positivity in inexperienced hands; difficult species identification and high cost are the limiting factors with the technique⁽³⁾.

Antigen detection method of malaria parasite is easy to perform and rapid with high specificity and sensitivity⁽⁴⁾. Modified buffy coat preparation is based on concentration of blood thereby parasite detection becomes easy. It is more sensitive and specifically eliminates false positives of QBC method. The procedure is easy and cost effective and can be carried out at remote places⁽³⁾.

Hence the purpose of the present study is to do comparative evaluation of various methods for detection of Malaria with special reference to

Modified buffy coat preparation and thus to establish a more sensitive method of detection.

MATERIAL AND METHODS

Blood samples were collected from 200 patients presenting with clinical features suggestive of malaria in the outpatient / indoor departments of our institution, into an EDTA vial and heparinised QBC capillary tube.

One end of heparinised QBC capillary tube was immediately sealed by bees wax, centrifuged at 6000 rpm for 5 minutes and then was cut just above the buffy coat layer by Adam's plier. The sediment column of blood was pushed by a steel wire onto glass slides, smears prepared and stained by routine Leishman's stain.

Simultaneously, thick and thin smears were freshly prepared and stained with Leishman's stain. Blood samples were also collected into the QBC fluorescent tubes. The "Malaria" card, for antigen detection test is an immunoassay based on the "sandwich" principle. Anti-coagulated blood was gently mixed. Sample loop was dipped into the sample and blood was blotted onto the sample pad in the sample well 'A' of malaria card then 5 drops of assay buffer was added into the well 'B' and results were read after 20 minutes. Appearance of three pink coloured line each in P.falciparum region (F), pan region (P) and control region (c) indicates that the sample is reactive for P. falciparum and / or P. vivax/ P. malariae /P. ovale.

Appearance of two pink coloured line one in (P) and (c) regions indicates that the sample is reactive for P. vivax/ P. malariae /P. ovale only.

Appearance of one pink coloured line at control regions (c) indicates that the sample is nonreactive for *P. falciparum* and other Plasmodium species.

Test is invalid, if no line appears after the completion of test .

RESULTS

In the present study patients positive for malaria parasites ranged between 3 years to 70 years with

maximum cases between the age group of 21-40 years with the male to female ratio of 1.9:1 .

Total 200 samples were examined out of which 110 cases (55%) were positive for malaria parasites by various methods. Out of 110 positive cases, 64 cases (58.18%) were positive for *Pl. vivax*, 42 cases (38.18%) for *Pl. falciparum* and 4 (3.63%) cases for mixed *Pl. vivax* and *Pl. falciparum*.

Distribution of cases by thick and thin film, QBC test, Antigen detection test and modified buffy coat preparation method

S.No.	No. of cases	Thick and thin film (PS)	QBC (QBC)	Antigen detection test (AG)	Modified buffy Coat Smear Preparation Method (MBCP)	Interpretation
1.	90	+	+	+	+	PS+QBC+AG+MBCP all positive
2.	10	-	+	-	+	QBC+MBCP positive
3.	4	-	+	-	-	Only QBC positive
4.	2	-	+	+	-	AG+QBC positive
5.	2	-	-	+	-	Only AG positive
6.	2	-	-	+	+	AG+MBCP positive
7.	90	-	-	-	-	PS+QBC+AG+MBCP all negative
Total	200 (n)	90 (45%)	106 (53%)	96 (48%)	102 (51%)	110 (55%)

Out of 200 cases examined, 90 cases were diagnosed by all methods. Out of the remaining 20 cases, 10 cases were detected by both QBC and MBCP, 4 cases were diagnosed only by QBC, 2 cases were diagnosed by both QBC and antigen detection test. Further 2 cases were detected by

both antigen detection test and modified buffy coat preparation and 2 cases were diagnosed by only antigen detection test. Positivity of thick and thin smear, QBC test, antigen detection test and modified buffy coat smear preparation method was 45%, 53%, 48% and 51% respectively.

Out of 90 thick and thin smear positive cases *P. vivax* cases were 50 (55.55%), *P. falciparum* were 36 (40%) and mixed infections were present in 4 (4.44%) Malaria parasites were positive by QBC technique in 106 cases. Amongst which 60 cases (56.60%) were of *P. vivax*, 42 cases (39.62%) were of *P. falciparum* and 4 cases (3.77%) were of mixed infection type. Modified buffy coat preparation was positive in 102 cases. Out of 102, 57 cases (55.88%) were of *P. vivax*. *P. falciparum* was detected in 41 cases (40.19%) and mixed infections were detected in 4 cases (3.92%). Antigen detection test was positive in 96 cases. In

55 cases (57.29%) malaria infection could be *P. vivax/ P. ovale/P. malariae*. Further in PBF examination species were identified as *P. vivax*. But 2 cases in which only antigen detection test positive for *P. vivax/ P. ovale/ P. malariae* were considered *P. vivax* positive cases because *P. ovale* and *P. malariae* are very rare in this area. In 41 cases (42.71%) Antigen detection cards were positive for either *P. falciparum* or *P. falciparum* and *P. vivax/ P. ovale/ P. malariae*. Species of malaria parasites were later confirmed by other tests.

Comparison of sensitivity and specificity of the tests taking QBC as base parameter

S. No.	Name of test	Total positive	Total negative	TP	TN	FP	FN	SN	SP
1.	Thick and thin film examination	90	110	90	94	0	16	84.90%	100%
2.	Antigen detection	96	104	92	90	4	14	86.79%	95.74%
3.	Modified buffy coat smear preparation method	102	98	100	92	2	6	94.33%	97.87%

DISCUSSION

Rapid detection and effective treatment is a pre-requisite for reducing the morbidity and mortality due to malaria. Leishman's or Giemsa stained thick smears are considered to be the 'Gold standard' in diagnosis. However, the interpretation of thick smears is laborious thus we compared different methods available for rapid diagnosis.

In the present study sensitivity and specificity of peripheral blood film was 84.90% and 100%, respectively. This observation is in accordance

with the study done by Bhandari et al. 2008(3) and Akhtar et al.2010 (5).

Antigen detection test had sensitivity (86.79%) and specificity (95.74%) which is in consonance with the study done by Singh et al. 2010(6).

Modified buffy coat preparation method had sensitivity (94.33%) and specificity (97.87%) similar to findings of Akhtar et al. 2010(5).

In present study we observed that modified buffy coat preparation method has the advantages of both the peripheral blood film and QBC test. The

cost per test was just 2-3 Rs. more than peripheral blood film examination. The test is easy to perform and can detect more malaria positive cases with ease and even at remote places where proper adequate facilities are not available.

CONCLUSION

Since malaria is endemic in certain regions of India, we need to employ more sensitive tests, which are also rapid to detect low levels of parasitemia in population. Modified buffy coat preparation method was found to be simple and more sensitive, more specific and cost effective method for screening of malaria parasites as compared to other methods. It can be used as an alternative to QBC method for screening and typing of malaria parasites, where QBC facilities are not available such as at primary and secondary referral hospitals. Hence, we recommend modified buffy coat method as a more suitable alternative to QBC method for detection of malaria parasites.

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