

Original Research Article

Quantitative Buffy Coat: A Useful Tool for Diagnosis of Malaria

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ABSTRACT

Background: Malaria is one of the highest killer diseases affecting most tropical countries. It affects over 500 million people worldwide and over one million children die annually from malaria. The aim of this study was to evaluate the sensitivity and specificity of Quantitative Buffy Coat (QBC) test for diagnosis of malaria. **Materials and Methods:** This prospective study was carried out at Department of Microbiology, MGM Medical College and Hospital, Kamothe, Navi Mumbai, India. A total of 100 samples were examined for malaria parasites by both thick and thin smear and quantitative Buffy coat test. **Results:** We found that detection of malarial parasites by Quantitative Buffy Coat test was 100% positive for 50 positive by microscopy and 5 positive in 50 negative by microscopy. **Conclusion:** We concluded that finding of sensitivity and specificity of QBC was better than microscopic examination in confirmation of malaria. This will help early detection, proper diagnosis and treatment of malaria.

INTRODUCTION

Malaria is one of the highest killer diseases affecting most tropical countries. It affects over 500 million people worldwide and over one million children die annually from malaria. [1-2] According to the UNICEF, in every minute malaria kills a child in the world [3]. Of all the human malaria parasites, *Plasmodium falciparum* (*P. falciparum*) is most pathogenic and frequently fatal if untreated in time [3]. In India, according to Nandwani [4] a total of 1.82 million cases of malaria and 0.89 million cases of *P. falciparum* cases were reported in the year 2002. According to National Vector Borne Disease Control Programme [5] there were 10,66,981 malaria positive cases and 5,33,535 *P. falciparum* in the year 2012.

The increasing incidence of falciparum malaria, the need to identify and treat the additional infective carriers (reservoirs) and to reduce the chances of transmission has given an impetus for development

of simple and rapid methods for the diagnosis of falciparum malaria. Conventional Leishman's, Giemsa or Romanowsky's stained peripheral blood examination by light microscopy is the standard method for malaria diagnosis in malaria endemic countries. Conventional light microscopy has the advantages that it is relatively inexpensive, provides permanent record and can be shared with other disease control programmes. However, it suffers from disadvantages such as it is labour intensive and time consuming [6].

The diagnosis of malaria is conventionally established by microscopic examination of stained thick and thin blood smears. Considerable time and experience are required for adequate preparation and interpretation of the blood smears. A less labour-intensive alternative to this conventional technique is therefore long overdue. In 1983, a method using a capillary tube precoated with

acridine orange and containing a float was developed for the rapid quantification of leucocytes in peripheral blood. This method was termed "QBC" for "Quantitative Buffy Coat Analysis" [7]. The acridine orange stains all nucleic acid containing cells and the associated fluorescence is observable under blue-violet light through a microscope. This technique was then adopted for detection of malaria parasites as the acridine orange staining permits differential colouration of green (nuclei) and red (cytoplasm) in stained parasites. Spielman et al. [8] found that the QBC method is at least eight times more sensitive than Giemsa stained thick blood smears. Rickman et al [9] found that the QBC method is easier and faster to perform than the thick smear method yielding a specificity of 98.4%. In a field study, Mak et al [10] found 55.93% sensitivity and 94.92% specificity when the QBC tube method was compared with the blood smears.

The aim of our study is to determine the sensitivity and the specificity of the QBC tube method in the detection of malaria parasite in a tertiary care hospital.

MATERIALS AND METHODS

This prospective study was carried out at Department of Microbiology, MGM Medical College and Hospital, Kamothe, Navi Mumbai, India. A total of 100 samples were examined for malaria parasites by both thick and thin smear and quantitative Buffy coat test.

Ethical clearance was obtained from the Institutional Ethical committee of MGM Institute of Health Sciences (Deemed University), Navi Mumbai before starting the project.

Samples were collected by either capillary finger-prick or phlebotomy. The blood samples were collected in Vacutainer tube containing ethylenediamine tetraacetate (EDTA). Each sample was processed by conventional microscopic examination and QBC tube test.

Acridine orange (AO) binds deoxyribonucleic acids and ribonucleic acids. The malaria parasite binds Acridine orange in the nucleus and the cytoplasm and emits green and red fluorescence when excited at 480 nm allowing the detection and examination of parasite morphology by fluorescent microscopy.

The outlines of stained parasites are well preserved and the general morphology is similar to that in specimens stained by the Giemsa stain. [11] In the present study, fluorescent microscopy was through the use of a standard light microscope provided with a battery-powered light and a special blue-violet light. Red blood cells are not stained by the dye, hence remain inconspicuous under fluorescent light while the brightly fluorescent parasites are easily seen. The nuclei of the parasites emit yellowish green fluorescence whereas the cytoplasm exhibits bright red fluorescence.

The QBC glass capillary tube (Diagnour RFCL Ltd., India) is 75 mm in length and 1.677 mm in diameter [7]. The tubes are internally coated with EDTA and heparin at the fill end and with acridine orange stain and potassium oxalate at the other end. Samples of blood, 55-65 μ l, were transferred to the QBC tube by capillary action. Tube rolled between fingers at least 10 times or for at least 5 seconds to mix blood with coating of Acridine Orange.

Expansion of the centrifugally separated cell layers is achieved with a 20 mm plastic float. When the plastic float is inserted into the tube, there will be a 40 μ l wide space between the float exterior and the float interior. The plastic float having a specific gravity (1.055) that is midway between that of plasma (1.028) and red blood cells (1.090) is positioned. Tube sealed using tube plug and then tube suspender inserted into open end of QBC tube using clean forceps, provided with the kit. QBC tube centrifuged immediately. QBC tube Placed on rotor of micro centrifuge and centrifuged at 12,000 Revolutions per minute (RPM) for 5 minutes.

The area surrounding the float just beneath the buffy coat was examined under oil immersion. Individual cells within this layer were easily seen by microscopy; the malaria parasites staining green (DNA) and orange (RNA) under blue-violet light. The entire circumference of the tube was examined systematically while moving away from the buffy coat through the erythrocyte layer.

Each tube was examined until parasites were detected or for a maximum of 5 minutes. QBC tubes were examined within an hour of collection and centrifugation respectively. The QBC tube was examined under x 10 ocular lens and 60 x objective lens.

Thick and thin smears were prepared from patient blood samples and stained with Giemsa stain and examined under. Each blood smear was examined microscopically without the knowledge of the QBC test result. The parasite species was determined by examination of the thin blood film smears. In the event that no parasites were seen, the corresponding thick film was reexamined for a further 200 microscopic fields under x 1000 magnification.

RESULTS

A total of 100 samples were examined for malaria parasites by both thick and thin smear and quantitative Buffy coat test and the results were compared with peripheral blood smear examination. Blood smear results indicated that 55 (55%) cases were found to be positive for malarial parasites and the rest 45 (45%) were negative. QBC test showed that 60 (60%) cases were positive for malarial parasites and 40 (40%) were negative.

Among the positive patients *Plasmodium vivax* was detected in 40 (72.73%) cases and *Plasmodium falciparum* in 15 (27.27%) cases by blood smear. The Quantitative Buffy Coat test showed *Plasmodium vivax* 43 (71.66%), *Plasmodium falciparum* 17 (28.34%).

DISCUSSION

Malaria is a well-known disease and it continues to be a major public health problem at the start of new millennium. Reliable diagnosis of malaria requires laboratory confirmation of the presence of malaria parasites in the blood of a febrile patient [12]. Although microscopic examination of blood smear continues to be the gold standard, it has a drawback that it is time consuming and requires an expert microscopist and less sensitive in cases of low parasitemia [13].

Various sensitive methods have been employed for the simple, reliable, and rapid diagnosis of malaria, the most promising of these is the rapid diagnostic test and quantitative buffy coat [14]. We employed these tests and compared with Giemsa stained peripheral blood smear for the diagnosis of *P. vivax* and *P. falciparum* infections.

Parzy et al found QBC to be more sensitive than blood smear examination and advocated its use for urgent diagnosis [15]. Study by (Ye Htut et al. 2002) had a sensitivity of 82.8% and 100% for

P.falciparum and *P.vivax* and specificity of 97.1% and 98.6% [16].

One of the major advantages of the QBC technique is rapidity and reliability in diagnosis of malaria even under field conditions. In addition, it requires less training and experience than blood smears. Its chief drawback is its high cost and in the identification of *Plasmodium* species. Ring stages of *P. falciparum* and *P. vivax* are difficult to distinguish by the QBC. This problem is particularly important in endemic areas where *P. falciparum* coexists with *P. vivax* [17].

However, the rapid diagnostic test was found to be user friendly and interpretation was more objective as compared to smear and QBC [18]. Although no single test can replace the conventional method of peripheral blood smear examination, these newer diagnostic tests can be used as supplement to microscopic examination of peripheral blood smear where the diagnosis cannot be made on microscopy and an experienced microscopists are not available. The high cost of the test may prevent routine use in many laboratories. However it is a valuable adjuvant at the time of emergency for rapid diagnosis, although microscopy remains the main stay for the diagnosis of malaria.

There are reports or studies on diagnosis of malaria by different methods from India and other countries. There are some differences depending on geographic location, disease burden, endemicity and methods followed.

Our results of QBC test (60%) were higher than Aparna Y et al. [20] (20.44%). Other workers do not have data for QBC test. QBC test is more sensitive than light microscopy as the blood sample is centrifuged at 12,000 rpm and RBCs are concentrated.

As regards, species wise detection of malarial parasites by different methods, in our study, microscopic findings were *Plasmodium vivax* 40 (72.73%), *Plasmodium falciparum* 15 (27.27%). In Quantitative Buffy Coat test findings, positive results were *Plasmodium vivax* 61.11%, 43 (71.66% and *Plasmodium falciparum* 17 (28.34%)

Microscopy is subjective and there is possibility of over reporting of mixed malarial parasitic infection.'

The sensitivity of QBC has been reported to be as high as 90%, by Gurung et al. [22], 96.22%, by Bhandari et al. [23], and 99.7%, by Benito et al. [24].

Sreekanth B et al. reported that total of 74 (74%) cases were positive by blood smears, while 80 (80%) were positive by QBC. Blood smears indicated that 74% (55 Of 74) of the patients were positive for *P. vivax* and 25% (19 of 74) were infected with *P. falciparum*. QBC showed that 75 % (60 Of 80) were positive for *P.vivax* and 25% (20 of 80) were infected with *P.falciparum*. QBC had a sensitivity and specificity of 74.3% and 80.7% for *P.vivax* and 100% and 98.7% for *P.falciparum*. [25]

Binesh Lal Y et al. reported on 572 samples, 92 (16.08 %) samples were found to be positive for malarial parasite. Of the positive samples, 90 (97.82%) samples were positive by smear, 78 (84.78%) samples were positive by QBC, 69 (75%) samples were positive by antigen detection test. [26]

CONCLUSION

QBC test is more sensitive than microscopy, but requires specialized equipment and training. Light microscopy is less sensitive and subjective. Hence, when microscopic examination is negative, it is necessary to perform QBC test, which has high sensitivity and specificity. This will help early diagnosis of malaria. The QBC test is not a substitute for the blood smear in the overall detection and control of malaria. However the speed and ease of use of this technique make it an important new tool for the diagnosis of malaria.

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Table 1: Showing comparison of different methods for diagnosis of malarial parasites.

Blood smear	QBC	
	Positive	Negative
Positive 55	55	0
Negative 45	5	40
Total 100	60	40

Table 2: Showing speciation of malarial parasites by different methods.

Malarial parasites	Blood smear	QBC
<i>Plasmodium vivax</i>	40 (72.73%)	43 (71.66%)
<i>Plasmodium falciparum</i>	15 (27.27%)	17 (28.34%)
Total	55 (100%)	60 (100%)

Table 3: Showing comparison of work of different workers.

Workers	Place	No. of samples	Leishman's	QBC test
DK Mendiratta et al. [6]	Sevagram Maharashtra	443	18.28%	
Azikiwe CCA et al. [19]	Nigeria	200	59%	
Aparna Y. et al.[20]	Karnataka India	137	13.87%	20.44%
Ali Akbar et al. [21]	Kahnuj, Iran	124	77%	
Our study	Navi Mumbai India	100	55%	60%

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