A Comparative study of peripheral blood smear, QBC and Antigen Detection test in Diagnosis of Malaria, in a tertiary care hospital

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ABSTRACT

Background: Rapid diagnosis of malaria is pre-requisite for effective treatment and reducing mortality and morbidity of malaria.

Aim: To compare the efficacy of various methods i.e., thick and thin peripheral blood smear, Quantitative Buffy Coat (QBC), plasmodium lactate dehydrogenase for diagnosis of malaria.

Methods: A prospective study comprising of 400 samples were collected from patients presenting with classical symptoms of malaria. Thick and thin smears were prepared and stained with Leishman's stain. QBC and antigen detection tests were done using commercially available kits.

Results: Considering thick smear as gold standard thin smear had a sensitivity and specificity of 66.12% and 100% respectively. QBC and Malaria antigen tests were positive in 84 (21%) and 76 (19%) cases, with a sensitivity of 96.7% and 93.0% respectively.

Conclusion: Leishman's thick smear although cost effective, but difficult to interpret. QBC method has its advantages in terms of speed, sensitivity and ease of diagnosis; so if facilities are available, QBC should be used for routine diagnosis. However, Leishman stained thin blood smear still appear superior for species identification. In places where facilities are not available, rapid, simple and easy to interpret antigen detection test can be used.

Keywords: malaria, peripheral blood smear (PBS), quantitative buffy coat (QBC), antigen detection

INTRODUCTION

Malaria poses diagnostic challenge to the medical community worldwide. Its occurrence is noted in more than 90 countries. It is estimated that there are more than 50 million cases and 1.1 -2.2 million deaths occur due to malaria every year.[1] It continues to be one of the major health burden in developing countries. The earliest symptoms of malaria are very non-specific and variable which poses difficulty in clinical diagnosis. Plasmodium falciparum malaria is a medical emergency that requires accurate diagnosis and appropriate treatment.[1-4] Therefore precise laboratory diagnosis and species identification is essential.

The diagnostic modalities which are available for malaria range from conventional thick and thin smear to rapid modalities like fluorescent staining (QBC) and antigen detection tests detecting parasitic antigens like histidine-rich protein-2 (HRP 2), plasmodium lactate dehydrogenase (pLDH), pan-specific aldolase and molecular methods like PCR.

The commonly employed method comprises microscopic examination of Romanowsky stained blood films.[2] Thin PBS carry a disadvantage of not detecting low parasitemia.[5] In recent years, quick and new techniques for malaria diagnosis have been developed. QBC (quantitative buffy coat) technique is one of them in which malarial parasite is detected even when there are only 1-2 parasites/µl of blood; it stains nucleic acid with fluorescent dyes.[6] Other newer technique is Rapid Diagnostic Tests (RDT's) for detection of malaria antigen and enzymes. The antigen detected is histidine rich protien-2 (HRP-2) and enzymes detected are plasmodium lactate dehydrogenase (pLDH) and pan-specific aldolase. All these techniques vary in their sensitivity, specificity, positive and negative predictive values. This study was aimed to compare the efficacy of various methods for diagnosis of malaria.

MATERIALS AND METHODS

This prospective study was conducted during

Aug'2009 –July'2010.The study group comprised of 400 patients presenting with fever, chills and rigor and other suggestive symptoms of malaria attending various outpatient and inpatient departments of M.K.C.G. Medical College and Hospital.

Samples collection, smear preparation and reporting

Consent was obtained from the patients prior to the collection of samples. Approximately 5 ml of venous blood was collected from each patient during the peak of fever and transported to the laboratory. Thick and thin blood smears were prepared as per the standard method. The smears were stained with Leishman's stain. Approximately 80- 100 oil immersion fields were examined over 8- 10 minutes for reporting.

QBC: [Lilac Medicare (p) LTD]

In the QBC technique, approximately 55-65 µl of blood was taken into a capillary tube coated with acridine orange, Potassium oxalate and fitted with a cap. A plastic float was inserted inside the tube and spun in the QBC microhaematocrrit centrifuge at 12,000 rpm for 5 minutes. The principle of QBC technique is based on the fact that on centrifugation at a high speed, the whole separates into plasma, buffy coat and packed red cell layer. The float gets buoyed by the packed blood cells and is automatically positioned within the buffy coat layer. Blood cells in the buffy coat layer separate according to their densities, forming visibly discrete bands. Due to acridine orange, the malaria parasite stains green (nucleus) and orange (cytoplasm). The tube is examined in the region between the red blood cells and granulocytes and within the granulocytes and mononuclear cell layer, where the parasites are more abundant.

Antigen detection using pLDH:

Commercially available antigen detection kit detecting plasmodium LDH [MAL CARD, (J. Mitra & Co. Pvt. Ltd)] was used. The test was done using anticoagulated blood. The interpretation of the test result was done as; when one control band and two test bands appeared the test was considered to be positive for P. falciparum, P.vivax/malariae/ovale; When one control band and one test bands appeared the test was considered to be positive for P. falciparum and when only control band appeared without test band, the test was considered negative.

RESULTS

QBC was found to be the most sensitive method and thin smear was the most specific method.

Table.1. Comparison of Sensitivity, Specificity, Positive Predictive value and Negative predictive values of thin smear, QBC, Antigen detection methods

Test	Sensitivity (%)	Specificity (%)	Positive predictive value (PPV) (%)	Negative Predict Predictive value (NPV) (%)
Thin smear	66.12	100	100	94.12
QBC	96.7	92.89	71.42	99.3
Antigen detection	93.0	94.67	76.13	98.7

In our study, detection of *falciparum spp*. of plasmodium was highest by QBC method as compared to other tests. Detection of *vivax spp*. was highest by PBS. Species diagnosis was 100% by all the tests except QBC where 5.95% remained undiagnosed.Table.2.

Table 2. Comparison of different test methods in species detection of Plasmodium

Plasmodium spp.	Thin smear	Thick smear	QBC	Antigen test
Falciparum	26 (63%)	40 (65.12%)	60 (72.24%)	51 (67.1%)
Vivax	12 (28.8%)	(28.57%) 13 (15.4.0%)	17 18	(23.7%)
Mixed infection	3 (8.43%)	5 (8.0%)	6 (7.1%)	7 (9.2%)
Species undiagnosed	0	0	5 (5.95%)	0
Total	41	62	84	76

We observed that smear examination required on an average 10 to 12 minutes in contrast to 2 to 3 minutes to report a QBC tube.

DISCUSSION

Rapid detection and effective treatment is a pre-requisite for reducing the morbidity and mortality in malaria cases. Leishman or Giemsa stained

blood smears are considered to be the 'Gold standard' in diagnosis. However, the interpretation of thick smear is laborious and results depend on the quality of microscope, staining technique with which blood film is prepared and also the concentration and motivation of microscopist.[8,9] This is time consuming and therefore delays diagnosis.[10] However the advantages of Leishman stained smear is that a permanent record of the smear can be kept, it's low cost and species identification is done without much difficulty in most of the cases. Newer techniques like QBC and Antigen detection assays are rapid, simple and easy to interpret.

In this study the result positivity of QBC technique is found to be higher 84/400 (21%) and thin smear is lower i.e. 41/400 (10.25%). The sensitivity of Leisman-stained thin smear was found to be lowest (66.12%); however, this method had a high specificity and positive predictive value (100%). QBC, had a sensitivity of 96.7%, specificity of 98.2%, PPV and NPV were found to be 71.42%, and 99.3%, respectively. Our results demonstrated a higher sensitivity and greater rapidity of QBC technique as compared to Leishman stained thin blood films in comparing the results of other studies.[2,11,13] Our results were comparable with results of other studies.[2,5,11,12]

The speed of QBC method (15 min) in detecting malarial parasites is a definite advantage in laboratories which screen large number of samples. In addition, low levels of parasitemia(2 parasites/µl) can easily be detected as more blood is being used per sample (55-65µl). There is no loss of parasites during the procedure. The parasitised eryrhrocytes are concentrated in the small area of buffy coat, which helps in rapid scanning of the parasite.[7] Other advantages of QBC are its ease of interpretation and it being technically easy to perform. In our study, by QBC method, species is identified in 76 (86%) cases. It is more sensitive for detection of falciparum spp. of plasmodium which was highest [72.24%] as compared to other tests. Species diagnosis was 100% by all the tests except QBC where 5.95% remained undiagnosed. Studies also reported the ability of QBC method in species identification ranges from 75-93%.[14] The difficulty encountered is due to the morphology of the infected erythrocyte remains occult in QBC technique.[4] There are six cases found to be positive by QBC, which are subsequently found to be negative for thick and thin smear and Malacard test. This can be explained by the fact that certain artifacts in blood might resemble the ring forms of P. falciparum.

Malacard test for detection of malaria antigen had a sensitivity, specificity and PPV of 93.0%, 94.67% and 76.13% respectively. This test was based on detection of pLDH with the help of monoclonal antibodies. The values obtained for this kit-based procedure were much lower than those observed for other tests. This low sensitivity could be attributed to low parasitemia levels as observed by Iqbal et al.[15] The specificity was comparable to other observers using the tests based on similar principle.[15,16] However, the test was found to be more user friendly and interpretation was more objective as compared to smear and QBC.

CONCLUSION

QBC provides a reliable, quick, and easily mastered method for diagnosis of malaria; it is of much use in laboratories which screen large number of samples and in endemic areas where parasite level is low. In situations where adequate laboratory back up is not available, antigen detection test can be employed despite having low sensitivity. However, Leishman stained thin blood film still appears superior for species identification.

AUTHOR NOTE

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