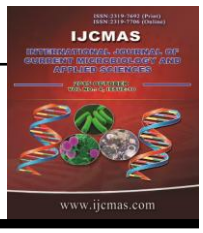
Int.J.Curr.Microbiol.App.Sci (2015) 4(10): 960-963

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 10 (2015) pp. 960-963**
http://www.ijcmas.com



Original Research Article

Seroprevalence and Comparative Study of Malaria in a Tertiary Care Centre in Vijapur: a Northern District of Karnataka, India

Manjunath P. Salmani[1]* and Preeti B. Mindolli[2]

[1]Department of Microbiology, Shri B.M. Patil Medical College, H&RC, Solapur Road, Vijaypur-586103, Karnataka, India
[2]Department of Microbiology, Al Ameen Medical College, H&RC, Athani Road, Vijaypur-586108, Karnataka, India
**Corresponding author*

A B S T R A C T

Malaria occurs throughout the tropics causing over 100 million cases and over 1.2 million deaths every year. The earliest symptoms of Malaria are very non-specific and variable. Therefore precise laboratory diagnosis and species identification is essential. Aim of the study is to estimate the prevalence of malaria and to compare Peripheral Blood Smear, QBC and Antigen detection methods in Malaria Diagnosis. The study was conducted from July 2014 to June 2015 in the Department of Microbiology, Shri BM Patil Medical College, H&RC (SBMPMC, H&RC), Vijaypur, Karnataka. The study group comprised of 357 patients presenting with pyrexia with chills and rigor attending the outpatient and inpatient departments of SBMPMC, H&RC, Vijaypur, Karnataka. Malarial parasite was detected in 50 (14.0%) cases by Leishman stained thick smear and in 32 (9.0%) cases by Leishman stained thin peripheral blood smear. These cases were also positive by the QBC method. An additional 12 cases were diagnosed as malaria by QBC technique. In total, QBC technique detected 62 (17.4%) cases of malaria. Antigen detection test (using pLDH) detected 46 cases (12.9%). Estimating the seroprevalence of Malaria helps in knowing the disease burden in a geographical area. QBC method provides a reliable and quick method for diagnosis of malaria. QBC method is useful in laboratories which screen large number of samples and endemic areas where parasite level is low. In situations where adequate laboratory back up is not available, antigen detection test can be employed. However, Leishman stained thin blood film still appear superior for species identification.

Keywords

Sero-prevalence, Malaria Leishman stain

Introduction

Malaria occurs throughout the tropics causing over 100 million cases and over 1.2 million deaths every year.

The earliest symptoms of Malaria are very non-specific and variable. Therefore precise laboratory diagnosis and species

identification is essential. The commonly employed method for diagnosis of malaria involves the microscopic examination of Romanowsky stained blood films. In recent years, numerous quick and new techniques for malaria diagnosis have been developed, one such being the QBC (quantitative buffy coat) technique.

The 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. In this study we estimated prevalence of Malaria and compared Peripheral Blood Smear, QBC and Antigen detection methods in Malaria Diagnosis

Materials and Methods

The study was conducted from July 2014 to June 2015 in the Department of Microbiology, Shri BM Patil Medical College, H&RC (SBMPMC, H&RC), Vijaypur, Karnataka.

The study group comprised of 357 patients presenting with pyrexia with chills and rigor attending the outpatient and inpatient departments of SBMPMC, H&RC, Vijaypur, Karnataka. Oral consent was taken from the patient prior to the collection of specimens. The specimen collected was 5ml of blood in an EDTA bulb. The age group of these patients varied from 3-78 years.

Thick and thin blood smears

Thick and thin blood smears were prepared as per the standard method. The smears were stained with Leishman's stain. Approximately 80-100 fields were examined over 8-10 minutes by an experienced microscopist.

QBC technique

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 then spun in the QBC microhaematocrit centrifuge at 12000 rpm for 5 minutes. The tube was then mounted on a small plastic holder and examined through an ordinary light microscope with customized fluorescence (paralens attachment). Approximately 10-20 fields were examined over 1-2 minutes. The principle of QBC technique is based on the fact that on centrifugation at a high speed, the whole blood 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; platelets remaining at the top, lymphocytes and monocytes within the middle layer and granulocytes at the bottom. Due to acridine orange, the malarial 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 parasites are most abundant.

Antigen detection using pLDH

Commercially available antigen detection kit detecting plasmodium LDH (Optimal) was used. The test was done using anti-coagulated blood. The test was done according to manufacturer's instructions.

Results and Discussion

357 samples were evaluated by thick and thin Leishman stained peripheral blood smear, QBC technique and antigen detection

test. Malarial parasite was detected in 50 (14.0%) cases by Leishman stained thick smear and in 32 (9.0%) cases by Leishman stained thin peripheral blood smear. These cases were also positive by the QBC method. An additional 12 cases were diagnosed as malaria by QBC technique. In total, QBC technique detected 62 (17.4%) cases of malaria. Antigen detection test (using pLDH) detected 46 (12.9%) cases (Table 1 and Table 2).

In the present study the seroprevalence of malaria varied from 9% to 17.4% by different methods which co-relates well with the studies conducted by other researchers. Our results demonstrated a higher sensitivity and greater rapidity of QBC technique as compared to Leishman stained thin blood films, confirming the results of other studies.

Table.1 Seroprevalence of Malaria by different methods

Name of the test	Seroprevalence
Thick Peripheral Blood Smear	50 (14.0%)
Thick Peripheral Blood Smear	32 (9.0%)
Quantitative Buffy Coat	62 (17.4%)
Antigen detection test (pLDH)	46 (12.9%).

Table.2 Comparison of Leishman stained thin and thick film with QBC and antigen detection test

Result	Thick blood film	Thin blood film	QBC	pLDH
Positive	50	32	62	46
Negative	307	325	295	311
Total	357	357	257	357

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 parasitaemia (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. Another advantage of QBC is its ease of interpretation and it being technically easy to perform. Concern over the ability of the QBC method in identification of species has been noted, with success claims ranging from 75% to 93%. In our study species identification was possible only in 63.9% cases. This difficulty is encountered because the morphology of the infected erythrocyte remains occult in QBC technique. Other drawbacks of the QBC are that it is expensive, and there are chances of leaking and breaking of blood filled QBC tubes in the centrifuge.

Leishman stained blood smear examination is labour intensive and time consuming (60 minutes). Another drawback of this method is that only a small volume of blood (10µl in thick smear and 1µl in case of thin smear) is examined and during staining process 40-60% of parasites may be lost. Because of this, cases of low parasitaemia go

undetected. Leishman stained thick blood film detects malarial parasite when there are 5-20 parasites/µl and thin blood film detects malarial parasite only when there are 50 parasites/µl of blood. The advantages are that a permanent record of the smear can be kept and its low cost. Another advantage is that species identification is done without much difficulty in most of the cases.

The sensitivity of antigen detection test is lower compared to thick film and QBC technique. However, the test was found to be more users friendly and interpretation was more objective as compared to peripheral blood smear and QBC.

In conclusion, estimating the seroprevalence of Malaria helps in knowing the disease burden in a geographical area. QBC method provides a reliable and quick method for diagnosis of malaria. QBC method is useful in laboratories which screen large number of samples and endemic areas where parasite level is low. In situations where adequate laboratory back up is not available, antigen detection test can be employed. However, Leishman stained thin blood film still appear superior for species identification.

Reference

Bruce-Chwatt LJ. 1984. DNA probes for malaria diagnosis. *Lancet* . 795.

Bhandari PL, Raghuveer CV, Rajeev A, Bhandari PD. 2008. Comparative study of peripheral blood smear, quantitative buffy coat and modified centrifuged blood smear in malaria diagnosis. *Indian J Pathol Microbiol.* 51: 108-12.

Chatterjee KD, Examination of blood for parasites. In: *Parasitology*. 12th ed; Calcutta: 1980. p 212-17.

Gay F, Traore B, Zanoni J, Danis M, Fribourg-Blanc A. 1996. Direct acridine orange fluorescence examination of blood slides compared to current technique for malaria diagnosis. *Transactions of Royal Society of Tropical Medicine and Hygiene.* 90: 516-18.

Kevube RA, Wardlaw SC, Patton CL. 1989. Detection of haemoparasites using quantitative buffy coat analysis tubes. *Parasitology Today.* 5: 34.

Krishna BV, Deshpande AR. 2003. Comparison between conventional and QBC methods for diagnosis of malaria. *Indian J Pathol Microbiol.* 46: 517-20.

Long GW, Jones TR, Rickman LS, Fries L, Egan J, Wellde B et al. 1994. Acridine orange diagnosis of *plasmodium falciparum*; evaluation after experimental infection. *The American Society of Tropical Medicine and Hygiene.* 51(5): 613-16.

Manjunath P. Salmani, Preeti B. Mindolli, Basavaraj V. Peerapur. 2011. Comparative Study of Peripheral Blood Smear, QBC and Antigen Detection in Malaria Diagnosis. J Clin and Diagn Res. 5(5): 967-969

Mendiratta DK, Bhutada K, Narang R, Narang P. 2006. Evaluation of different methods for diagnosis of P. falciparum malaria. *Indian J Med Microbiol.* 24: 49-51.

Pinto MJW, Rodrigues SR, Desouza R, Verenkar MP. 2001. Usefulness of quantitative buffy coat blood parasite detection system in diagnosis of malaria. *Indian J Med Microbiol.* 19: 219-21.