Original Article

Microbiology Section

Comparative Study of Peripheral Blood Smear, QBC and Antigen Detection in Malaria Diagnosis

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ABSTRACT

Rapid diagnosis of malaria is pre-requisite for effective treatment and reducing mortality and morbidity of malaria. In this study, Quantitative Buffy Coat (QBC) was compared with thick and thin peripheral blood smears and malaria antigen test. A total of 387 samples were collected from patients presenting with fever and chills. Malaria was diagnosed in 60, 72 and 56 patients by Leishman staining technique, QBC method and malaria antigen test respectively. The QBC method allowed an additional 12 cases. Thus the prevalence rate of malaria during the study was 18.6%. In 315 patients who were negative by the QBC, malaria antigen test and the Leishman stained smears were also negative for malarial parasite. Although QBC method was superior to the smear for malarial parasite detection, species identification was not possible in 32 cases by this technique.

The QBC method has its advantages in terms of speed, sensitivity and ease, especially in an endemic area as ours. The QBC method helps in the diagnosis of jaundice, aplastic anaemia and kala-azar. The QBC system can also be used in the diagnosis of other parasitic diseases such as filariasis. However, Leishman stained thin blood smear still appear superior for species identification.

Key Words: Malaria; Peripheral Blood Smear; Quantitative Buffy Coat; pLDH

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. Hence there is difficulty to clinically diagnose malaria but the treatment has to be started immediately in order to avoid complications. Therefore precise laboratory diagnosis and species identification is essential [1].

The commonly employed method for diagnosis of malaria involves the microscopic examination of Romanowsky stained blood films [2]. An expert microscopist can detect 20-40 parasites/µl on standard blood films. Although a thick peripheral blood smear (PBS) allows identification of the plasmodial parasite and stages, the technique is laborious, time consuming and requires a well trained microscopist for accurate identification [3].

In recent years, numerous quick and new techniques for malaria diagnosis have been developed, one such being the QBC (quantitative buffy coat) technique. Malarial parasite, unlike their red blood cell host, contains nucleic acid that stains with fluorescent dyes, such as acridine orange. As the parasites within erythrocytes mature, they reduce the buoyant density of infected erythrocyte. These two properties are exploited in QBC technique for malaria diagnosis [4].

Thin PBS detects malarial parasites only when 40-60 parasites/µl of blood are present [3]. However, QBC technique detects malarial parasite even when there are only 1-2 parasites/µl of blood [5].

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.

All these techniques vary in their sensitivity, specificity, positive and negative predictive values. Objective of the study was to compare Leishman stained thick and thin PBS with that of QBC and malaria antigen test using pLDH in the diagnosis of malaria.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Microbiology, Shri BM Patil Medical College Hospital and Research Centre, Bijapur, Karnataka. The study was conducted from August 2009 to July 2010.

The study group comprised of 387 patients presenting with pyrexia with chills and rigor attending the various outpatient and inpatient departments of SBMP Medical College Hospital and Research Centre, Bijapur.

Sample collection: Oral consent was taken from the patient prior to the collection of specimens. The specimen collected was 5ml of blood in a 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 [6].

QBC: 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 anticoagulated blood. The test was done according to manufacturers instructions. Interpretation of the test result was done as below:

1. When one control band and two test bands appeared the test was considered to be positive for P. falciparum.
2. When one control band and one test band appeared the test was considered positive for P. vivax
3. When only control band appeared at the top of the test strip without test band the test was considered negative.

RESULTS

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

Malarial parasite was detected in 60 (15.5%) cases by Leishman stained thick smear and in 41 (10.6%) cases by leishman stained thin peripheral blood smear [Table/Fig-1]. 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 72 (18.6%) cases of malaria. Thus the prevalence rate of malaria was 18.6%. All patients who were malaria parasite negative by QBC method were also smear negative.

Result	Thick blood film	Thin blood film	QBC	pLDH
Positive	60	41	72	56
Negative	327	346	315	331
Total	387	387	387	387

[Table/Fig-1]: Comparison of Leishman stained thick and thin blood film with QBC method

The species of malarial parasite encountered by PBS examination were Plasmodium falciparum (19; 43.9%) and Plasmodium vivax (22; 48.8%).

Mixed infections (P. vivax and P. falciparum) accounted for 3 cases (7.3%). By the QBC method species identification was possible only in 46 (63.9%) cases. The species of malarial parasite encountered by QBC method were Plasmodium falciparum (21; 29.2%) and Plasmodium vivax (18; 25.0%). Mixed infections (P. vivax and P. falciparum) accounted for 7 cases (9.7%). In Optimal test results, P. falciparum accounted for 26 (36.1%) cases and P. vivax accounted for 30 (41.7%) cases. 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 of falciparum malaria is a prerequisite in reducing the morbidity and mortality due to the disease. Leishman stained blood smear examination which is a cornerstone in the laboratory diagnosis of malaria has undergone little improvement since its inception. This is labour intensive and time consuming and therefore delays diagnosis [8].

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 [1, 7].

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 [5]. Another advantage of QBC is its ease of interpretation and it being technically easy to perform. A technician can be taught to carry out the QBC test and detect malarial parasite accurately, in less than a day, in contrast to smear examination and interpretation which takes weeks [3]. Concern over the ability of the QBC method in identification of species has been noted, with success claims ranging from 75% to 93% [9]. 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 [3]. 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. One more disadvantage of QBC technique is that a permanent record of test cannot be kept [5].

Leishman stained blood smear examination is labour intensive and time consuming (60 minutes) [7]. 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 [5]. 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 user friendly and interpretation was more objective as compared to smear and QBC.

In conclusion, QBC method provides a reliable, quick, easily mastered method for diagnosis of malaria. QBC method is useful in laboratories which screen large number of samples and in endemic areas where parasite level is low. The QBC system can also be used in the diagnosis of other parasitic diseases from blood like filariasis and kala-azar. 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 appear superior for species identification.

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