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Comparative Study of Peripheral Blood Smear and Quantitative Buffy Coat in Malaria Diagnosis

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Abstract

A rapid test for diagnosis of malaria based on acridine orange staining of centrifuged blood samples in a microhaematocrit tube (QBC) was compared with Leishman stained thin peripheral blood smear in 287 samples. Malaria was diagnosed in 44 patients by Leishman staining technique and in 65 patients by QBC method. The QBC method allowed detection of an additional 21 cases. Thus the prevalence rate of malaria during the study was 22.65%. In 222 Patients who were negative by the QBC technique, 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 difficult by this technique. The QBC method provides a reliable, quick, easily mastered, accurate method for diagnosis of malaria. The QBC system can also be used in the diagnosis of other parasitic diseases from blood (Filariasis). However, Leishman stained thin blood film still appear superior for species identification.

Keywords : Malaria; Peripheral Blood Smear; Quantitative Buffy Coat

INTRODUCTION

Malaria occurs throughout the tropics causing over 100 million cases and over 1 million deaths every year. The earliest symptoms of Malaria are very nonspecific 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]

BACKGROUND

The commonly employed method for diagnosis of malaria involves the microscopic examination of Romanowsky stained blood films. Although a thick peripheral blood

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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.[2]

Thin PBS detects malarial parasites only when 40-60 parasites/µl of blood are present. In the recent years, numerous quick and new techniques for malaria diagnosis have been developed, one such being the Quantitative Buffy Coat (QBC).[2] This QBC technique detects even when there are only 1-2 parasites/µl of blood.[3]

Hence, this study was carried out to comparison of Leishman stained thin PBS and QBC technique in the diagnosis of malaria.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, Shri BM Patil Medical College, Bijapur, Karnataka. The study was conducted from June 2008 to May 2009.

The study group comprised of 287 patients presenting with pyrexia attending the various outpatient and inpatient departments of SBMP Medical College, Bijapur. The specimen collected was 1ml of EDTA blood. The age group of these patients varied from 3-78 years.

Blood samples were subjected to thin smear examination after staining with Leishman stain. Approximately 100 fields were examined over 8-10 minutes.[4] In the QBC technique, approximately 55-65 µl of blood was taken into a capillary tube coated with acridine orange 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.

RESULTS

287 samples were evaluated by Leishman stained thin peripheral blood smear and QBC technique.

Malarial parasite was detected in 44 cases by Leishman stained thin peripheral blood film. These cases were also positive by the QBC method. An additional 21 cases were diagnosed as malaria by QBC technique. Thus the prevalence rate of malaria was 22.65 %. All patients who were malarial parasite negative by QBC method were also smear negative.

The species of malarial parasite encountered by thin PBS examination were *Plasmodium falciparum* (16; 36.36%) and *Plasmodium vivax* (22; 50%). Mixed infections (*P. vivax* and *P. falciparum*) accounted for 4.62 % (n=3) cases. By the QBC method only *P. falciparum* could be identified (8; 12.30 %). Species identification was not possible in 87.70 % cases.

Table 1 : Comparison of Leishman stained thin blood film and QBC method for malaria diagnosis

Leishman stained blood film	QBC positive	QBC negative	Total
Positive	44	00	44
Negative	21	222	243
Total	65	222	287

P value < 0.05

Table 2 : Results of samples in all combinations

PBS	QBC	No.
Negative	Negative	222
Negative	Positive	24
Positive	Positive	41
Positive	Negative	00

DISCUSSION

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.[1,5]

The speed of QBC method (10 min) in detecting malarial parasites is a definite advantage in laboratories which screen large number of samples. In addition, low levels of parasitaemia (1 parasite/µl) can easily be detected as more blood is being used per sample (55-65µl). Another advantage of QBC is its ease of interpretation and it being technically easy to perform. 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. Other disadvantages of QBC are that a permanent record of test cannot be kept and difficulty in identifying the species of malarial parasite. In some cases, only *P. falciparum* could identified because of the typical morphology of their gametocytes.This difficulty is encountered because the morphology of the infected erythrocyte remains occult in QBC technique.[2]

Leishman stained blood smear examination is labour intensive and time consuming (30 min).[6] Another drawback of this method is that only a small volume (4-5µl) of blood is examined and during staining process 40 % of parasites may be lost. Because of this, cases of low parasitaemia go undetected. Leishman stained 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.

CONCLUSION

QBC method provides a reliable, quick, easily mastered, accurate method for diagnosis of malaria. The QBC system can also be used in the diagnosis of other parasitic diseases (Filariasis) from blood. However, Leishman stained thin blood film still appear superior for species identification.

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