Original Article

Microbiology Section

Correlation of Quantitative Buffy Coat, Blood smear and Antigen Detection in Diagnosing Malarial Infection

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ABSTRACT

Objective: To correlate the diagnostic methods such as Quantitative Buffy coat (QBC), Blood smear and Antigen detection in diagnosing malarial infection.

Design: A hospital based prospective study

Place and Duration of Study: Department of Microbiology, Saveetha medical college, Thandalam, Kanchepuram district. From April 2010 to September 2010.

Materials and Methods: A total of 572 blood samples from clinically suspected malaria patients were included in this study. All the samples were subjected to three different techniques such as staining technique (thick and thin smear), antigen detection test and Quantitative Buffy Coat technique (QBC).

Results: Among the 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.

Conclusion: In conclusion we suggest using two diagnostic tools, antigen detection and smear in conjunction for the early diagnosis of malarial infection or use antigen detection as a primary test as well as a screening tool for obtaining a fast positive or negative result and confirming it with gold standard test.

Key Words: Plasmodium species, Quantitative Buffy Coat technique, Blood smears, Antigen detection test

KEY MESSAGE

- Blood smears and QBC were found superior to antigen detection assay.
- Malarial antigen detection test was less time consuming.

INTRODUCTION

Malaria is one of the major parasitic diseases affecting 300-500 million people annually worldwide and accounts for over 1 million deaths [1]. It is caused by parasites of the species *Plasmodium* that are spread from person to person through the bites of infected mosquitoes. In the year 1898, malaria was shown to be transmitted among humans by female Anopheles mosquitoes [2]. Approximately, 40% of the world's population, mostly those living in the world's poorest countries, are at risk of malaria. Every year, more than 500 million people become severely ill with malaria. Most cases and deaths are in sub-Saharan Africa. Malaria afflicts 90 countries and territories in the tropical and subtropical regions and almost one half of them are in Africa, south of Sahara. About 36% of the world population (i.e., 2020 million) is exposed to the risk of contracting malaria [3]. Pregnant women and children have an increased susceptibility to malaria with mortality predominant in children [1].

Natural transmission of malaria depends on the presence of, and relationship between the three basic epidemiological factors: the agent, the host and the environment. While the malaria parasite is the true agent of infection, the female anopheles mosquito is the agent of transmission. There are four species of human malaria parasites *Plasmodium vivax, P. falciparum, P. malariae* and *P. ovale.* In India 60 to 65 % of the infections are due to *P. vivax* and 35 to 40% due to *P. falciparum*. Only few cases of *P. malariae* have been reported from Orissa and Karnataka [4].

A presumptive diagnosis of malaria is based upon the presence of fever with chills and rigors alone. The clinical suspicion of malaria would be confirmed by a laboratory test which is simple to perform, rapid, sensitive, specific, and less expensive. At the present time, no such test exists. The most common test for malaria diagnosis remains the microscopic examination of, Leishman or Fields stained blood smears. Microscopy has historically been the mainstay of the diagnosis of malaria. Clinical diagnosis of malaria currently depends on the visualization of parasites by light microscopy of Giemsa-stained thick and thin blood smears. This procedure is cheap and simple, but it is a labour intensive procedure and requires well-trained personnel [5]. Microscopy is also time-consuming and has limited sensitivity when parasitemia is low. Malaria microscopy, currently considered the "gold standard" [6].

During the last decade, several new diagnostic methods for malaria have been developed, including antigen detection, fluorescence-based assays (e.g., quantitative buffy coat) and PCR. Each of these tests has strengths and weaknesses in terms of test parameters, cost, and technical complexity [7].

With the spread of parasite resistance to antimalarial drugs and the increasing difficulty in controlling malaria, it is a potential medical

emergency it is important to diagnose malaria accurately and to treat it correctly. Delays in diagnosis and treatment are leading causes of death in many countries. In the laboratory, malaria is diagnosed using different techniques, e.g. conventional microscopic diagnosis by staining thin and thick peripheral blood smears, other concentration techniques, e.g. Quantitative Buffy Coat (QBC) method, rapid diagnostic tests and molecular diagnostic methods, such as polymerase chain reaction (PCR). Rapid Diagnostic Tests have been used experimentally since the early 1990s [8].

In this study we correlate different diagnostic methods for the early and accurate detection of *Plasmodium species* in a tertiary care hospital.

MATERIALS AND METHODS

A total of 572 blood samples were collected from clinically malaria suspected patients at Saveetha Medical College for a period of six months (April-Sept). Five ml of blood was obtained by venepuncture from the cubital vein with aseptic precautions and anti-coagulated with EDTA. Collected samples were received in the clinical parasitology laboratory and detection of malarial parasite was done by the following techniques.

STAINING [6]

Thick and thin blood smears were prepared on two slides and stained by Leishman's stain. All stained slides were screened for malarial parasites using microscope (100X).

ANTIGEN DETECTION TEST [9]

Blood samples were subjected to antigen detection using SD kit as per kit instructions. All kit components were brought to room temperature. The anti-coagulated blood sample was mixed by gentle swirling. 5 µl of whole blood was added into the sample well and 2 drops (60 µl) of assay buffer into the buffer well. The test result was read after 20 minutes.

QUANTITATIVE BUFFY COAT TECHNIQUE (QBC) [6]

Specially designed microhematocrit tubes coated with acridine orange were used. Approximately, 55-60 µl of blood was loaded into the tubes and stopper and float were applied at either ends. The tubes were centrifuged at 12000 RPM. The interpretation was done using a standard microscope fitted with Para Lens ultraviolet microscope adaptor and a × 60 objective connected to fibre optic ultraviolet light module.

RESULTS

Of the 572 samples, 92 (16.08 %) samples were found to be positive for malarial parasite. Among this 92 positive samples, 90 (97.82%) were positive by smear and the positivity of QBC and antigen detection test were 78 (84.78%) and 69 (75%) respectively.

Out of 92 positive samples, 90 were identified as *Plasmodium vivax* using staining technique and out of this 78 were positive by QBC. Among the 69 samples which were positive for antigen detection, 67 showed positive for other *Plasmodium sp* and only two cases of *Plasmodium falciparum* (2.17%) which was detected by antigen detection alone. Comparison of peripheral blood smear examination with QBC & HRP II Antigen for malarial parasite detection is shown in [Table/Fig-1].

Sensitivity, specificity Positive predictive value (PPV) and Negative predictive value (NPV) of QBC and Antigen detection methods is calculated using 2 × 2 table with Leishman's stain smear as the gold standard is shown in [Table/Fig-2].

Smear	QBC		Antigen Detection test	
	Positive	Negative	Positive	Negative
Positive - 90	63	27	67	23
Negative - 482	15	467	2	480
Total -572	78	494	69	503

[Table/Fig-1]: Comparison of peripheral blood smears examination with QBC & HRP II Antigen for malarial parasite detection.

Test	QBC	Antigen Detection test
Sensitivity (%)	80.76	97.10
Specificity (%)	94.53	95.42
PPV (%)	70.00	74.44
NPV (%)	96.88	99.58

[Table/Fig-2]: Sensitivity, specificity Positive predictive value (PPV) and negative predictive value (NPV) of QBC and Antigen detection test.

DISCUSSION

Accurate diagnosis is essential both to target antimalarial drugs and to enable effective management of the frequently fatal nonmalarial febrile illnesses that share signs and symptoms with malaria [10]. Early detection and effective management of malaria is very much needed in reducing the morbidity and mortality due to the malarial disease. The development of easy, rapid, and accurate tests for the detection of plasmodial infection is highly desirable [11].

Ninety eight percent of the positive samples were found to be *Plasmodium vivax* by Leishman's staining. The proportion of *P. vivax* and *P. falciparum* varies in different parts of India. Epidemiological survey by Kumar *et al* on true malaria burden in India stated that the southern Tamil Nadu state have less than 10% of infection by *P. falciparum* and the rest are *P. vivax* infections [12]. In the present study, total incidence of malaria parasite was found to be 16.08 % (92/572) almost similar observation (19.95%) was made in South Indian study by Parija *et al* [13].

In our study, the sensitivity was 97.77% and specificity was 100% for smear. Similar study done by Bhandari *et al* [14] has quoted the sensitivity percentages as 85% and 86.79%. The specificity was mentioned 100% each respectively. Sensitivity of smear in our study was slightly higher compared with the above studies.

Quantitative Buffy Coat technique had a sensitivity of 80.76% and specificity of 94.53%. The sensitivity of QBC has been reported to be as high as 90% by Bimala Gurung *et al* [13], 96.22% by Bhandari *et al* [14] and 99.7% by Benito *et al* [15]. But relatively low sensitivity of QBC was observed in our study. The reasons for low sensitivity of QBC could be (1) If the parasitic level is <100 parasites/µl (0.002% parasitemia) the sensitivity range from 41 to 93% [2]. The specificity of QBC has been found to be lower (52%), particularly for the centrifugal method, where the denser late-stage parasites may be hidden in the separated mononuclear cell layer [16]. Moreover, false negative results may be due to difficulty in identifying parasites by the first-time users [13]. Despite this, the specificity of the test as yielded (94.53%) by this study which shows it is good diagnostic test.

Antigen detection test showed a sensitivity of 97.10% compared with thick smear and specificity was found to be 95.42%. Sensitivity of antigen detection test was much higher when compared to

QBC which shows antigen detection test is a better screening test. On comparing it with smear, both had almost similar sensitivity but one limitation of the antigen detection kits is that they cannot diagnose relapse in patients with *P. vivax* infection, which still remains an important advantage of microscopy [17].

Interestingly, we were able to detect two *Plasmodium falciparum* infection in antigen detection alone which was not detected by smear and QBC. In these two cases probably the parasite sequestered and this prevented its detection in smear and QBC examination. Similar observation was also made by Mendiratta *et al* [18]. This result is partly explained by the fact that the body slowly eliminates HRP-2 after parasite clearance. Also the HRP-2 has been shown to persist and is detectable after the clinical symptoms of malaria have disappeared and the parasites have apparently been cleared from the host [19].

Humar *et al* [20], detected circulating HRP-2 antigen in 68% of treated patients on day 7, and in 27% it was still present on day 28. The reason for the persistence of the HRP-2 antigen is not well understood and may reflect the presence of latent, viable parasites. In addition, the action of antimalarial therapy may influence the persistence of HRP-2.

In spite of all above facts, the two cases detected by antigen test were treated with suitable antimalarial drugs because patient was symptomatic.

The gold standard for laboratory diagnosis is still microscopic examination of thick and thin blood films. However, in the hospital environment as well as in the field, this is time-consuming and does not allow for a quick preliminary diagnosis. The Rapid Diagnostic Tests (RDT), therefore, has enabled a prompt positive or negative result to be available to the clinician within minutes. The only drawback to these tests is the inability to clearly differentiate between all the human *Plasmodium* species.

In conclusion, taking all possible pros and cons of the diagnostic methods and patients care, we suggest using two diagnostic tools, antigen detection and smear in conjunction for the early diagnosis of malarial infection or use antigen detection as a primary test as well as a screening tool for obtaining a fast positive or negative result and confirming it with gold standard test. The antigen detection test had high sensitivity and specificity. In addition it is less time consuming and easy to perform.

In a country where malaria is endemic, opting for two different test for a diagnosis will not be a concern when it comes in managing the patient where early diagnosis and timely therapy is required, thus averting the florid manifestations of malaria and reducing morbidity and mortality.

REFERENCES

[1] Ashley E., McGready R, Proux S and Nosten F. Malaria. *Travel Med Infect Dis* 2006; 4 (3-4): 159-73

[2] Pim Martens and Lisbeth Hall., Malaria on the Move: Human Population Movement and Malaria Transmission- *Emerg Infect Dis* 2000; 6(2):103-9.

[3] Karunamoorthia K, Bekelea M. Prevalence of malaria from peripheral blood smears examination: A 1-year retrospective study from the Serbo Health Center, Kersa Woreda, Ethiopia. *J Infect Public Health* 2009; 2(4): 171-76.

[4] Shiv Lal, G.S. Sonal, P.K. Phukan., Status of Malaria in India. *J Indian Acad Clin Med* 2000;5(1):19-23

[5] Zakeri S, Najafabadi S T, Zare A,Djadid N.Detection of malaria parasites by nested PCR in south-eastern, Iran: Evidence of highly mixed infections in Chahbahar district. *Malar J* 2002, 1:1-6.

[6] Warhurst C, Williams JE. Laboratory diagnosis of malaria. *J Clin Pathol* 1996;49:533-8

[7] Shenoi UD, Laboratory diagnosis of malaria. *Indian J Pathol Microbiol* 1996; 39:443-5

[8] Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria Diagnosis: A Brief Review. *Korean J Parasitol* 2009; 47(2): 93-102.

[9] Singh N, Shukla M, Shukla M K, Mehra R J, Sharma S, Bharti P K *et al.* Field and laboratory comparative evaluation of rapid malaria diagnostic tests versus traditional and molecular techniques in India. *Malar J* 2010; 9(191): 1-13.

[10] Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, et al. Global, regional, and national causes of child mortality in 2008: A systematic analysis. *Lancet* 2010; 375:1969–87.

[11] Gurung B, Bairy I, Jagadishchandra, Manohar C. Evaluation of Falcivax against Quantitative Buffy Coat (QBC) for the Diagnosis of Malaria. *Int J Collab Res Intern Med Public Health* 2010; 2(5); 132-40.

[12] Kumar A, Valecha N, Jain T, Dash A P. Burden of Malaria in India: Retrospective and Prospective View. *Am J Trop Med Hyg* 2007; 77(S6):69–78.

[13] Parija S C, Dhodapkar R, Elangovan S, Chaya D R. Comparative Study Of Blood Smear, QBC and Antigen Detection For Diagnosis Of Malaria. *Indian J Pathol Microbiol* 2009; 52(2): 200-2

[14] Bhandari PL, Raghuveer C V, Rajeev A, Bhandari P D. Comparative study of peripheral blood smear, Quantitative buffy coat and Modified centrifuged blood smear in malaria diagnosis. *Indian J Pathol Microbiol* 2008; 51(1): 108-12

[15] Benito A, Roche J, Molina RA, Amela C, Altar J. Application and evaluation of QBC malaria diagnosis in a holoendemic area. *Appl Parasitol* 1994; 35: 266-72.

[16] Moody A. Rapid Diagnostic Tests for Malaria Parasites. Clin Microbiol Rev 2002; 15(1): 66–78.

[17] Belizario VY, Pasay CJ, Bersabe MJ, De Leon WU, Guerrero DM , Bugaoisan VM., Field Evaluation Of Malaria Rapid Diagnostic Tests For The Diagnosis Of P. Falciparum And Non-P. Falciparum Infections- *Southeast Asian J Trop Med Public Health* 2005; 36(3): 552-61.

[18] Mendiratta.K, Bhutada K, Narang R, Narang P. Evaluation of different methods for diagnosis of P. falcipaurm malaria. *Ind J Med Micrbiol*: 2006; 24(1); 49-51.

[19] Laferi, H, Kandel K, Pichler H. False positive dipstick test for malaria. *N Engl J Med.* 1997; 337:1635–6.

[20] Humar, A, Ohrt C, Harrington M. A, Pillai D, Kain K. C. ParaSight® F test compared with the polymerase chain reaction and microscopy for the diagnosis of Plasmodium falciparum malaria in travelers. *Am J Trop Med Hyg* 1997; 56: 44–8.

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