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Research Article

Comparative Study of Various Techniques in Diagnosis of Malaria

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Abstract: The present study was undertaken to evaluate the effectiveness of Enzyme linked Immuno Sorbant Assay (ELISA), Quantitative buffy coat(QBC) , SD Bioline Malaria Antigen P.f/Pan (Histidine Rich protein II (HRP II) & Lactate Dehydrogenase (pLDH)) as compared to peripheral smear examination in the diagnosis of malaria. 80 samples were collected from clinically suspected cases of malaria during March 2012 to February 2013 at Era's Lucknow Medical College& Hospital, Lucknow. Microscopic examinations of Leishman stained smears, ELISA, QBC, SD Bioline Malaria Antigen P.f/Pan test were done. 49(61.25%) samples were positive by peripheral smear, 74 (92.5%) were positive by ELISA, 77(96.25%) by QBC and 71 (88.75%) by SD Bioline Malaria Antigen P.f/Pan tests. Both ELISA & QBC are advantageous where work load is high, but are costly. Peripheral blood smears remained the gold standard for malaria species diagnosis in routine diagnostic laboratories but in this study the efficacy of newer malaria rapid diagnostic tests (RDT) surpassed the diagnostic efficacy of clinical microscopy and hence these RDT's will have a greater role in clinical practice.
Keywords: Malaria, Microscopy, QBC, ELISA, Rapid Diagnostic Tests

INTRODUCTION

Prevalence of malaria is around 300 million people worldwide with a global death rate over 1.5 million/year [1, 2]. Malaria is a serious disease caused by the protozoal parasite Plasmodium species, and if left untreated, can be fatal. Four of the known species of Plasmodium commonly infect humans: *P. falciparum, P. vivax, P. ovale, and P. malariae*. The symptoms of malaria include high fever, chills, rigors, and flu-like illness. Because of these common symptoms the disease is often misdiagnosed. With the increasing incidence of malaria, the need to identify and treat the additional carriers (reservoirs) and to reduce the chances of transmission has given an impetus for development of simple and rapid methods for the diagnosis of malaria. Leishman and Giemsa stained peripheral blood smear examination remain the gold standard for the diagnosis of malaria in endemic countries. But there are lots of disadvantages of these methods [3].

QBC, ELISA, Acridine Orange (AO) stain, detection of soluble histidine rich protein II antigen (HRP2 Ag), pLDH and aldolase enzymes in whole blood have been evaluated to diagnose malaria. Malaria presents a diagnostic challenge to laboratories in most countries. The urgency and importance of obtaining results quickly from the examination of blood samples from patients with suspected acute malaria render some of the more sensitive methods like PCR, DNA probe assay for malaria diagnosis impractical for routine laboratory use, although they may be considered reference procedures [3-5]. Thus, as the present study an attempt is made to evaluate and compare maximum number of rapid, feasible and economical tests for diagnosis of malaria with the Leishman stained blood smears which is considered as Gold standard.

Due to current scenario, the present study was carried out in a tertiary care hospital in Lucknow to evaluate the effectiveness of Enzyme linked Immuno Sorbant Assay (ELISA), Quantitative buffy coat(QBC) , SD Bioline Malaria Antigen P.f/Pan (Histidine Rich protein II (HRP II) & Lactate Dehydrogenase(pLDH)) against peripheral smear examination in the diagnosis of malaria.

This study attempts to review the current methodology and approach to the diagnosis of malaria in a practical and helpful way for the laboratory and for the physician caring for the society.

MATERIALS AND METHOD

The study was conducted on 80 patients admitted in the paediatric and medicine wards of Era's Lucknow Medical College& Hospital, Lucknow, India who were clinically suspected as cases of malaria from March 2012 to February 2013. A detailed clinical history

regarding the duration of fever, its nature and associated symptoms was taken from each patient. All these patients were of acute febrile illness and had no obvious focus of bacterial, viral or fungal infection.

Sample collection

Oral consent was taken from the patients prior to the collection of specimens. 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 films were made on clean glass slides by pricking the finger. Leishman staining was performed [4, 5].

The SD Malaria antigen test contains a membrane strip, which is precoated with two polyclonal antibodies as two separate lines across a test strip. One polyclonal antibody (test line Pf) is specific to lactate dehydrogenase of *P. falciparum* and the other polyclonal antibodies (test line P.v/ pan) are Pan specific to the lactate dehydrogenase of Plasmodium species. Test procedure was performed as per kit literature.

QBC was performed by pricking a finger and filling QBC tube with 55 ml blood stopper and float was placed at either end of the tube and then centrifuged at 12,000 rpm for 5 mins. The QBC tube after centrifugation was placed in para viewer and examined using a standard microscope equipped with the Paralens ultraviolet microscope adaptor and a 60 x objective connected to fibre optics ultraviolet light module. Parasites were observed in buffy coat. Cytoplasm of parasite appears red and nuclear chromatin appeared green.

The ELISA kit (Malaria Ag CELISA; Cellabs) evaluated in this study is a commercial ELISA test kit designed as a confirmatory test for *P. falciparum* malaria and is similarly used in recently developed drug sensitivity assays [6]. It is based on the detection of HRP2, a highly sensitive marker of falciparum malaria, in blood samples. The EDTA blood samples were frozen-thawed twice to obtain full hemolysis before being tested in the ELISA [7]. One hundred microliters of the samples was transferred into the ELISA in duplicate. Positive and negative controls, as well as serial dilutions of positive controls, were tested on every plate. Forty samples were tested on each 96-well plate. The plates pre-coated with monoclonal antibodies against PfHRP2 (capture antibody of IgM class; code: CPF4) were incubated at room temperature for 1 hour. Subsequently, the plates were washed five times with the provided washing solution, and 100 uL of the diluted Ab-conjugate (indicator antibody of IgG1 isotype; code: CPF6) was added to each well. After further incubation for 1 hour, the plates were once again washed five times, and 100 uL of the diluted TMB chromogen (1:20) was added to each well. The plates were incubated for another 15 minutes in the dark, and 50 uL of the stopping solution was added. Spectrophotometric analysis was performed with an ELISA plate reader (SpectraMAX340 Microplate Spectrophotometer; Molecular Devices, Sunnyvale, CA) at an absorbance maximum of 450 nm. The complete ELISA takes 3 hours to perform.

RESULTS

In our study, out of total 80 cases 44 were males and 36 were females. Maximum numbers of cases were in age group 1-10 followed by age group 11-20.

Table 1: Age wise distribution of cases

Age Groups (years)	Positive cases	Males	Females
0-10	28	16	12
11-20	18	10	8
20-30	6	2	4
30-40	18	12	6
40-50	6	4	2
50-60	2	0	2
>60	2	0	2
Total	80	44	36

Table 2: Month wise distribution of cases

Months	Cases	Percentage
March 2012	0	0
April 2012	0	0
May 2012	0	0
June 2012	0	0
July 2012	10	12.5
August 2012	16	20
September 2012	38	47.5
October 2012	14	17.5
November 2012	2	2.5
December 2012	0	0
January 2013	0	0
February 2013	0	0
Total	**80**	**100**

38 cases were in September followed by August, October, July and November viz 16, 14, 10 and 2 respectively.

Table 3: Comparison of Methods

Methods	Positive	%	Technical experience	Equipment required	Finance (Rs)	Time (mins)
PBS	49	61.25	Lots of Practice	Microscope, Electricity	15	35-40
Malaria kit	71	88.75	No Practice	No equipment Required	70-80	10
QBC	77	96.25	Lots of practice	QBC kit, Fluorescent objective, Centrifuge, Electricity	250-300	10
ELISA	74	92.5	Lots of practice	Microtitreplate, washer, Elisa reader	150-200	180

QBC showed highest positivity i.e. 77(96.25%) followed 74(92.5%) by ELISA and 71 (88.75%) by Kit. The conventional method of Blood film showed positivity of 49(61.25%) cases.

Table 3 further differentiates the four techniques on the basis of technical assistance, equipment required, cost and time.

DISCUSSION

Clinical diagnosis and microscopy are most commonly used methods for treatment of malaria. Although microscopic examination of blood smear continues to be the gold standard, it has a drawback that it is time consuming and requires an expert microscopist and results are poor in cases of low parasitaemia. The ability to diagnose malaria by blood film examination alone is about 75% for *P. falciparum* as shown by several studies [8]. Rapid detection and effective treatment of malaria is a prerequisite in reducing the morbidity and mortality due to the disease. In the present study, we included 80 patients attending hospital over a period of one year for different complaints suggestive of malaria. We evaluated and compared four different techniques for diagnosis of malaria.

QBC showed highest positivity i.e. 77(96.25%) followed 74(92.5%) by ELISA and 71 (88.75%) by SD Bioline Kit. The conventional method of Blood film showed positivity of 49(61.25%) cases.

Antigen Kit SD bioline (88.75%) detected more positive no. of than by Leishman stain (61.25%). Our results were in agreement with other studies [5, 8, 9]. In our study there were no false positive results by any of the methods suggesting 100% specificity.

The QBC method is more sensitive, rapid, and practical than thick blood film for the diagnosis of malaria [10]. The main concerns of this test are cost and the need for special equipment (centrifuge, fluorescence microscope, and capillary tubes). These features are clearly undesirable for fieldwork conditions [11, 12]. A study conducted in 2001, reported a sensitivity of 91.7% and a specificity of 88.9%, with the QBC assay when compared with microscopy [10]. In general, the QBC results are correlated by previous studies being in the same range [13, 14]. In our study it was 100% sensitive and specific probably because of the high parasite load, and there were no false identification. The discrimination of Plasmodium species by the QBC assay poses some difficulties because the discrimination of *P. vivax* from, *P. falciparum* is very difficult in some of the patients; hence identification of the species by QBC was not attempted in our study.

Few situations are conceivable where ELISA would be used in everyday clinical diagnosis of malaria. However, in research settings and similarly for blood bank screening, where large numbers of samples have to be screened, ELISA can provide a fast, relatively inexpensive, and reliable way for diagnosis [15]. In these settings, ELISA may serve as a suitable adjunct to microscopy. The fact that HRP2-based ELISA test kits and monoclonal antibodies are available on the market greatly improves the applicability of these assays for the diagnosis of *P. falciparum.*

CONCLUSION

Since malaria is endemic in certain regions of India, we need to employ more sensitive tests, which are also rapid to detect low levels of parasitemia in population. Therefore, we recommend QBC and ELISA to be used in setups where appropriate facilities are available and are advantageous where work load is high, but are costly; however, in situations where adequate laboratory back up is not available, simpler and easy to use techniques like antigen detection can be employed despite having lower sensitivity. Peripheral blood smears remained the gold standard for malaria species diagnosis in routine diagnostic laboratories but in this study the efficacy of newer malaria rapid diagnostic tests (RDT) surpassed the diagnostic efficacy of clinical microscopy and hence these RDT's will have a greater role in clinical practice.

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