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COMPARATIVE STUDY OF THREE DIFFERENT METHODS FOR THE RAPID DIAGNOSIS OF PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX MALARIA

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ABSTRACT

Objectives: The present study was undertaken to evaluate the effectiveness of Rapid screening methods like Quantitative Buffy Coat (QBC) and Antigen detection assay- pLDH (plasmodium lactate dehydrogenase) and HRP2 (Histidine rich protein 2) as compared to Peripheral smear examination in the rapid diagnosis of malaria.

Methodology: A total of 137 samples were collected from clinically suspected cases of malaria during May2010 to July 2010 and malaria microscopy with Leishman stained smears, QBC and antigen detection test (pLDH & HRP2) was done. Sensitivity and specificity was calculated. Z test was applied to find out the difference between any two tests.

Results: Of the total number of samples, 28 were positive; out of which 19 (13.87%) samples were positive by peripheral smear, 28 (20.44%) were positive by QBC and 21 (15.33%) by antigen detection tests. 19 were positive by both QBC and Peripheral smear and 109 were negative by both. QBC detected additional 09 positive cases which were negative on peripheral smear. 19 were positive by both Antigen test and peripheral smear, but antigen test detected 02 additional positive cases than peripheral smear. The QBC test was 100% sensitive, 92.37% specific with Positive Predictive Value of 67.86% and Negative Predictive Value of 100%.The antigen test was 84.21% sensitive, 95.76% specific with Positive Predictive Value of 76.19% and Negative Predictive Value of 97.47%. Out of 25 P. vivax positive cases, 16 were positive by peripheral smear and 16 were positive by Antigen test. Out of 05 positive cases of P.falciparum by Antigen test only 03 were positive by peripheral smear and 03 were positive by QBC. Among the peripheral smear negative cases, QBC could detect additional 09 cases out of 11 cases i.e. 81.81%.

Conclusion: We did not find any significant difference between Peripheral smear and Quantitative buffy coat (QBC) and Peripheral smear and Antigen detection assay. Quantitative buffy coat is advantageous where work load is high, but is costly and gives false positive report. Antigen detection test is useful when microscopy is not available and immediate clinical diagnosis is required especially for P. falciparum cases, but gives false positive results even after treatment. Both the methods cannot replace microscopic method for identification of species and for determination of parasitaemia.

Key Words: Quantitative buffy coat (QBC), Peripheral smear, pLDH (plasmodium lactate dehydrogenase), HRP 2 (Histidine rich protein 2), malarial parasite.

INTRODUCTION

Malaria presents a diagnostic challenge to the medical community worldwide [(1)]. Resurgence has occurred in many parts of the globe due to insecticide and drug resistance, social instability and non-availability of anti-malarial vaccine [(2)]. Non-specific nature of the symptoms and signs of malaria results in mis-treatment; both over-treatment with anti-malarial agents and under-treatment of patients with non-malarial illnesses.[(3)] According to the World Malaria Report released in 2006 by the World Health Organisation, there were 247 million malaria cases, 3.3 billion people at risk and 8, 81,000 deaths from 109 countries. In 2008, India had an estimated 1.52 million malaria cases accounting for 60% of cases in the WHO South East Asian Region. [(4)] Due to the serious nature of P. falciparum infections, prompt and accurate diagnosis is essential for effective malaria management [(1)]. The commonly employed method for the diagnosis of malaria include microscopic examination of Romanowsky stained blood films (5) which is labour-intensive, time consuming and requires experienced microscopist for accurate identification and its sensitivity decreases in parallel with the density of malarial parasites in blood.([6,)] Newer techniques such as hybridisation with DNA probes are too sophisticated for routine use in the field. [(7)]

In recent years, numerous quick and new techniques like Fluorescent staining (QBC) and Antigen detection tests detecting parasite antigens like Histidine rich protein -2 (HRP-2), Plasmodium lactate dehydrogenase (pLDH) and PAN specific aldolase have been developed.**[(1,5)]**

WHO has recently reiterated "the urgent need for simple and cost effective diagnostic tests for malaria to overcome the deficiencies of (both) light microscopy and clinical diagnosis." [(3)] So the present study was undertaken to evaluate the effectiveness of Rapid screening methods like QBC and Antigen detection assay (pLDH and HRP2) as compared to Peripheral smear examination in the rapid diagnosis of malaria.

METHODOLOGY

This study was conducted in the Department of Microbiology of Shri. B. M. Patil Medical College, Hospital and Research Centre, Bijapur, Karnataka, India from May 2010 to July 2010.

Study design: It was a cross-sectional study.

Study Type: It was an observational and analytical type of study.

Statistical test: Z test.

Inclusion criteria: A total of 137 samples were collected from clinically suspected cases of malaria of all the age groups in both the sexes attending our hospital and for whom malaria microscopy or QBC or antigen detection test had been requested.

Exclusion criteria: Patients already on anti-malarial drugs were excluded from the study.

Ethical clearance was obtained from the Institutional Ethical committee before starting the project.

Informed and written consent was obtained from all the patients.

The detailed history, clinical signs and symptoms were recorded in the proforma. 2ml venous blood was collected under aseptic precautions. Standard thick and thin smears were prepared and the remaining sample was collected in a sterile EDTA bottle. The smears were stained with Leishman's stain and observed under oil immersion objective by a trained microscopist who was blinded with the results of QBC and Antigen detection test. The blood collected in EDTA was subjected to Quantitative buffy coat method and antigen detection test.

QBC was done using QBC malaria test kits provided by BD (Becton Dickinson) Diagnostics. The QBC malaria tube was filled from the end nearest to two blue lines from a collection tube of well mixed venous blood, to a level between the two blue lines. The tube was held horizontal and rolled between the fingers to mix the blood with

anticoagulant coating and staining agent. Tube was tilted slightly so that blood flows away from the orange coated end and closed by pressing a plastic closure. With a clean forceps, a float was inserted into the unsealed end of the tube. Then the tube was labelled and placed into slots of centrifuge rotor. After proper balancing, the tube was centrifuged at rate of 12000 rpm for 5 minutes. Centrifugal tube was inserted into the groove of Para viewer. Para viewer with QBC tube was placed on the stage of a white light microscope fitted with a paralens adaptor. About 2-3 drops of fluorescence optical immersion oil was added over buffy coat area of the tube. Using 60X objective and a minimum working distance of 0.34mm, the buffy coat of the tube was brought into focus and the entire circumference of the tube was examined. The total examination time to exclude negative was approximately 2 minutes. The presence of malaria parasite was indicated by the distinct bi-coloured signet forms of trophozites strikingly apparent in cells near the granulocyte layer. Gametocyte of P. falciparum appears as yellow sickle-shaped bodies. Schizonts of P.vivax can be recognised by the presence of malaria pigment which appears dark brown in colour. [8]

Malaria pLDH/HRP2 was detected according to manufacturer's instruction using SD BIOLINE Malaria Antigen P.f/Pan rapid kit test" manufactured by SD Bio Diagnostics Pvt. Ltd.

RESULTS

Of the 137 patients studied the maximum number of patients i.e. 51% of the patients belonged to the age group 16-30 years. The male and female ratio was 1.4:1.

Of the total number of samples, 19 (13.87%) samples were positive by peripheral smear, 28 (20.44%) were positive by QBC and 21 (15.33%) by antigen detection tests. (Table 1)

19 were positive by both QBC and Peripheral smear and 109 were negative by both. QBC detected additional 09 positive cases which were negative on peripheral smear. (Table 2)

19 were positive by both Antigen test and peripheral smear, but antigen test detected 02 additional positive cases than peripheral smear. (Table 3)

The QBC test was 100% sensitive, 92.37% specific with Positive Predictive Value of 67.86% and Negative Predictive Value of 100%. (Table 4)

The antigen test was 84.21% sensitive, 95.76% specific with Positive Predictive Value of 76.19% and Negative Predictive Value of 97.47 %. (Table 4)

Out of 25 P.vivax positive cases, 25 were positive by QBC, 16 by peripheral smear and 16 by antigen test. Out of 05 positive cases of P. falciparum, 05 were positive by antigen test and only 03 cases were positive by peripheral smear and 03 were positive by QBC. (Table 5)

Among the peripheral smear negative cases, QBC could detect additional 09 cases out of 11 cases i.e. 81.81%. (Table 5)

DISCUSSION

Majority of the patients in our study were adults. The mean age was 35.02 years and SD of 17.97 years. The results are in consistent with Sangeeta Gupta et al. A cautionary note is however warranted in generalising from these data because the available national data provides very little information on age-specific prevalence for India.[9]

In the present study QBC detected more number of positive cases i.e. 28 (20.44%) than peripheral smear 19 (13.87%) which is consistent with H.Singh et al [10], MJW Pinto et al[5] and BVS Krishna et al.[11](Table 1).

We also found that Antigen test detected more positive cases – 21(15.33%) than Peripheral smear. This is in consistent with findings of C. Rajendran et al.[12]

The total incidence of malaria in our study was 13.86% (19/137). (Table 1)

In the present study only 19 (13.87%) cases were positive by peripheral smear. This is in agreement with Pinto MJW et al. [5] This could be due to the fact that in 100 fields of a thick blood films, approximately 0.25ul of blood is examined and during staining 60-80% of parasites may be lost. Hence the detection limit of thick blood films is about 5-20 parasites /µl. [11] (Table 1)

Compared to Peripheral smear, QBC was found to be 100% sensitive as it could detect additional 09 cases which were negative on peripheral smear. All the blood samples which were negative by QBC were also negative by peripheral smear. This is in agreement with Bhandari et al [13] who had 100% sensitivity with QBC. QBC is of great importance in peripheral smear negative cases and should be preferably used as a final diagnostic test and not as a screening test or first line investigation considering its high cost and tendency to report false positives.[13](Table 2)

Antigen test was superior to Peripheral smear study in our study as it could detect 9.05% more cases than peripheral smear. However, it does give the remainder 9.523% false positive result. We observed low sensitivity (84.21%) with antigen test. This could be due to low parasitaemia levels as observed by Iqbal et al [6] who observed 75% sensitivity at parasitaemia of 100 parasites/µl. (Table 3)

Using Peripheral smear study as the 'gold standard', the QBC with respect to peripheral smear was found to be 100% sensitive which is in agreement with Bhandari et al [13] who had 100% sensitivity with QBC and specificity of 93.61%. This could be because in the QBC method approximately 65-75ul of blood is used. Due to the high concentration of parasitized erythrocytes in a small region, there is more probability of detecting the parasites within a short time. There is no loss of parasites during the procedure and hence the detection limit is 2 parasites/µl of blood or lower. [13] But the specificity of the QBC test was low in our study as shown in other studies. [13, 14, 15] The Positive Predictive Value of QBC did not reveal the absolute certainty of diagnosis. However, the claim of 100% sensitivity was proven in this study. This may be due to the fact that Howell-jolly bodies, artefacts such as cell debris and bacterial contamination may give false positive results. [13]

The 'z' value was 1.45 (6.57/4.53) i.e. numerator < 2 x denominator. Therefore, there is no significant difference at p=0.05 i.e. p>0.05 (Table 4)

Antigen detection test was superior to peripheral smear in our study as it could detect 9.05% more cases than Peripheral smear. This could be due to persistence of HRP2 following clearance of P.falciparum.

The antigen test has got high specificity of 95.76%, however it does give the remainder 9.523% false positive results, but the sensitivity was low (84.21%). This might be due to low parasitaemia and the sensitivity of the test increases with increase in parasite density as also observed by Iqbal et al [6] and C. Rajendran et al. [12]

The 'z' value was 0.34 (1.46/4.26) i.e. numerator < 2 x denominator. Therefore, there is no significant difference at p=0.05 i.e. p>0.05 (Table 4)

The QBC method was 100% sensitive than antigen test. Also QBC positive cases with low parasitaemia (grade 1 & grade 2) gave negative result with antigen test.

The 'z' value was 1.12 (5.11/4.61) i.e. numerator < 2 x denominator. Therefore, there is no significant difference at p=0.05 i.e. p>0.05(Table 4)

Species identification especially gametocytes of P. falciparum was not possible in 03 cases with QBC which was confirmed by peripheral smear examination. Concern over the ability of QBC method to enable species identification has been expressed. [5, 14] This could be attributed to the morphology of the erythrocytes being not apparent in QBC [13] also the gametocytes have a

buoyant density similar to that of leucocytes and are found within the buffy coat, where it is difficult to distinguish parasites from leucocytes.[14](Table 5)

CONCLUSION

In the present study of two months duration, we compared Peripheral smear a known 'Gold Standard' with Quantitative buffy coat (QBC) and Antigen detection assay.

We did not find any significant difference between Peripheral smear and Quantitative buffy coat & Peripheral smear and Antigen detection assay.

Quantitative buffy coat is advantageous where work load is high, but it is costly and gives false positive report.

Antigen detection test is useful device when microscopy is not available and immediate clinical diagnosis is required especially for P. falciparum cases which may develop cerebral complications. But it gives false positive results even after treatment.

Both the methods cannot replace microscopic method for identification of species and for determination of parasitaemia. Therefore, further studies should be done with large number of samples for the evaluation of Quantitative buffy coat and Antigen detection test.

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Table: 1 Showing the result of Peripheral smear, QBC test and Antigen detection assay

Results	**Peripheral smear**	**QBC**	**Antigen assay**
Positive	19 (13.87%)	28 (20.44%)	21 (15.33%)
Negative	118 (86.13%)	109 (79.56%)	116 (84.67%)
Total	137	137	137

Table: 2 Validity of QBC test versus Peripheral smear study

Test	**Peripheral smear positive**	**Peripheral smear negative**
QBC positive	19	**09**
QBC negative	00	109

Table: 3 Validity of antigen assay versus peripheral smear

Test	**Peripheral smear positive**	**Peripheral smear negative**
Antigen test positive	19	**02**
Antigen test negative	00	116

Table: 4 Comparison of validity of Antigen test versus QBC test

Test	**Sensitivity**	**Specificity**	**Positive Predictive value**	**Positive Predictive value**
QBC	**100**	92.37	67.86	**100**
Antigen test	84.21	**95.76**	76.19	97.47

Table: 5 Comparison of Peripheral smear, QBC and Antigen detection assay

	Peripheral smear			**QBC**			**Antigen assay**		
	Pf	Pv	Mixed	Pf	Pv	Mixed	Pf	Pv	Mixed
Positive	03	16	00	03	**25**	00	**05**	16	00
Negative	02	09	00	02	00	00	00	09	00