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The diagnostic and prognostic value of conventional and rapid diagnostic tools in malaria

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Abstract: *Background:* The burden of malaria is raising all over the world and India is no exception. Despite well established treatment regimens and diagnostic tools, Malaria is thought to kill between1.1 to 2.7 million people worldwide each year. Rapid diagnosis and early treatment are one of the key factors in controlling the disease burden of malaria. *Objective:* The study was conducted to investigate the diagnostic and prognostic utility of rapid test (QBC, PLDH, HRP2) with conventional thick and thin films *Methods:* The present study included clinically suspected cases of malaria referred to Microbiology laboratory at Kempegowda Institute of Medical Sciences, Bangalore during the period from April 2004 to April 2005. Blood samples were collected and were subjected to conventional peripheral smear tests as well as rapid tests by using quantitative buffy coat and Paramax-3 kits. *Results:* Peripheral smear was 60.78% sensitive to Paramax -3 kit with 59.21% sensitivity for *P falciparum* and 65.38% for *P vivax*. Peripheral smear showed more number of false negatives as compared to Paramax-3 kit. QBC is 67.64% (69/102) sensitive to Paramax -3 kit with 60.81% sensitivity for *P falciparum* and 92.31% for *P vivax*. Although specificity and sensitivity of QBC higher than peripheral smear, it was not on par with Paramax-3 kit. *Conclusion:* The QBC method is highly sensitive and specific and provides a reliable, rapid and accurate method for diagnosis of malaria. However, Paramax-3 test was the most sensitive for *P falciparum* and *P vivax*.

Keywords: Malaria, *P vivax*, *P falciparum.*

Introduction

More than 3 million people worldwide live in malarious regions. Every year 500 million people are infected with malaria and 2 million of these die from disease [1]. In India the most important among the entire vector born diseases is malaria. In 1995, Malaria Action Programme has been launched with emphasis on early diagnosis and prompt treatment, selective and sustainable vector control, out of all these, prompt treatment is of first priority. In the malaria eradication programme, case detection through laboratory services is a key element of malaria surveillance [2].

Laboratory confirmation of malaria infections requires the availability of a rapid, sensitive and specific test at an affordable cost. Conventional methods of laboratory diagnosis for malaria using microscopic examination of stained thick and thin blood films. However, examination of thick blood films requires technical expertise and availability of good quality microscope. It is also time consuming and of limited sensitivity in the detection of low parasitemia [3]. Alternative method for malaria diagnosis appropriate for the out patient setting have been introduced to overcome limitations of conventional microscopy. Concentration of malaria parasite infected red blood cell by centrifugation coupled with staining with acridine orange and fluorescence microcopy (quantitative buffy coat [QBC]) [3].

Most new technology for malaria diagnosis incorporates immuno chromatographic capture procedure with conjugated monoclonal antibodies providing the indicator of infection. Preferred target antigens are those, which are abundant in all asexual and sexual stages in the parasites. Currently, interest is focused on the detection of Histidine rich protein 2 (HRP2) from plasmodium falciparum and parasite specific lactate dehydrogenase (PLDH) from the parasite glycolytic pathway found in all species [4]. Plasmodium falciparum is most

pathogenic of malaria species and is frequently fatal if untreated. Hence, early and rapid diagnosis is required for effective management of patients. This study investigated the diagnostic and prognostic utility of rapid test (QBC, PLDH, HRP2) with conventional thick and thin films

Material and Methods

The present study included clinically suspected cases of malaria, which were referred to Microbiology laboratory at Kempegowda Institute of Medical Sciences, Bangalore during the period from April 2004 to April 2005. Detail clinical history including age, sex, presenting complaints were taken from the patients. General physical examination and systemic examination were done. Hemoglobin percentage values were taken from case records. 2 ml of anti-coagulated blood from these patients were subjected to various techniques used for diagnosis of malaria like peripheral smear examination (both thick and thin films), QBC and Paramax –3 kit.

Study design: A prospective clinical study consisting of 102 subjects is undertaken to screen the Peripheral smear of patients with clinical diagnosis of malaria and to compare the Rapid Diagnostic test QBC and Paramax-3 kits and to evaluate the diagnostic and prognostic utility of Rapid Diagnostic test with conventional thick and thin Smear.

Preparation of thin blood smear: A drop of blood not larger than a pins head is taken on a grease-free clean slide, at a distance of about half an inch from the right end and spread towards the right side of the slide with a spreader to form uniform tails. All the blood films were uniform and spread carefully such that the tail in all the smaples ended near about the center of the slide so that they contain a single layer of red blood cells. The film is allowed to dry and labeled. Leishman's stain is poured by means of a pipette over the dried film and is allowed to remain for 30 seconds. It is then diluted with twice its volume of distilled water (pH 7-7.2), and is allowed to remain on the slide for 10 to 15 minutes. The stained slide is then washed and examined with 1/12-inch oil-immersion lens [5].

Preparation of thick blood smear: A big drop of blood is taken on a slide and spread with a needle or with the corner of another slide to form an area of a half-inch square. The film is dried in a horizontal position and kept covered by a petri dish. The film is then subjected to de-haemoglobinization by placing the film in a vertical position in a glass cylinder of distilled water for 5 to 10 minutes. After dehaemoglobinisation, the film is stained with Leishman'sstain as mentioned above for staining thin blood smear [5].

QBC Technique: The QBC capillary is filled from the AO stained end with the blood sample up to the blue lines, the outer surface is wiped with tissue paper. The capillary is tilted so that the blood flows to the other end, the capillary is tilted for about 10-15 times. The capillary is held horizontally so that the column of blood moves away from the edge of the AO stained end. This end is closed with the finger and the other end is plugged with the plastic closure. The float is inserted inside the capillary using the forceps; the capillary is gently tapped so that the float moves down. The capillary is placed in the QBC centrifuge, which is set at the speed of 12,000 revolutions per minute and spun for 6-8 minutes. The spun capillary is removed and placed in the groove of the capillary holder [6-7].

Paramax 3 Kit: The standard operating procedure instructed by the manufacturer was used for diagnosis. HRP-2 antigen and PLDH were detected by the kit.

Statistical analysis: Chi square and Fisher Exact test has been used to find the significant proportion of *P falciparum* and *P vivax* in association symptoms, Sex and pulse. The Similar tests have been used to find the association of three type of diagnosis namely, Peripheral Smear, QBC and Paramax- 3 kit. The Diagnostic statistics have been used to find the diagnostic value of Peripheral smear and QBC. The data was analyzed by using SPSS 11.0 and Systat 8.0.

Results

Patient characteristics: In the present study the patients age group ranged from infants to elderly (table 1) and the incidence of malaria was more in male patients in this study group with M : F ratio of 2.1:1. The maximum

number of cases were seen in the age group of 20-30yrs (figure 1).

Table-1: Baseline demographic characters

Age in years	Sex		Total
	Male	Female	
0-10	9 (13.04)	5 (15.2)	14
10-20	8 (11.59)	6 (18.2)	14
20-30	24 (34.8)	10 (30.3)	34
30-40	7 (10.14)	4 (12.12)	11
40-50	12 (17.39)	5 (15.15)	17
50-60	2 (2.9)	2 (6.06)	4
60-70	3 (4.34)	1 (3.03)	4
70-80	4 (5.8)	-	4
Total	69 (100.00)	33 (100.00)	102

Fig-1: Prevalence of malaria in the patient population

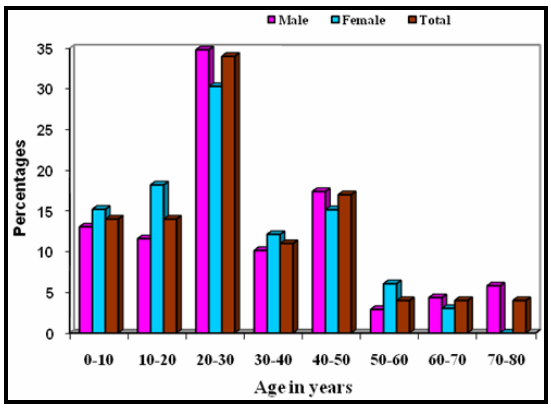


Table-2: Comparison of peripheral smear with Paramax –3 kit

Peripheral Smear	Paramax –3 kit			Total (n=102)
	P falciparum (n=74)	*P vivax* (n=26)	Both (n=2)	
Negative	31 (41.33)	9 (34.62)	-	40 (39.22)
P falciparum	43 (57.33)	-	2 (100.00)	45 (44.12)
P vivax	-	17 (65.38)	-	17 (16.67)

Prevalence: Fever was present in all the 102 cases studied. The other common presenting symptoms included headache, nausea and vomiting. Symptoms like convulsions, altered sensorium, bleeding episodes and jaundice were encountered. On systemic examination 90 (88.23%) cases presented with splenomegaly and 14 (13.72%) with hepatomegaly. Anemia was a common condition in both *P falciparum* and *P vivax.*

Comparision of peripheral smear with paramax-3: Peripheral smear was 60.78% sensitive to Paramax -3 kit with 59.21% sensitivity for *P falciparum* and 65.38% for *P vivax*. The Overall False negatives of peripheral smear in relation to paramax-3 kit were 40.0% with 41.33% for *P falciparum* and 34.62% for *P vivax* (Table 2). In the present 62 cases were positive by peripheral smear out of which 43 cases (57.3 %) were *P falciparum* and 17 cases (65.3%) were *P vivax* and in 2 cases mixed infection was seen. Paramax 3 kit detected all the cases in which 74 cases were *P.falciparum* and 26 cases were *P vivax* with 2 mixed infection (Figure 2).

Fig-2: Comparison of peripheral smear with Paramax –3 kit

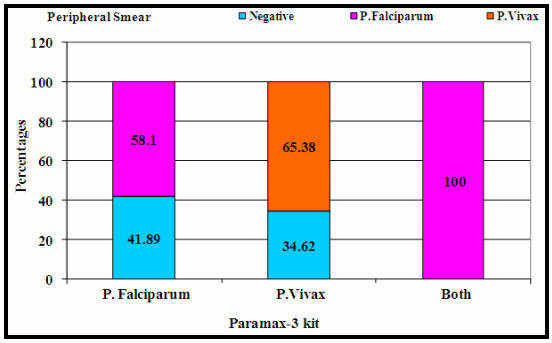
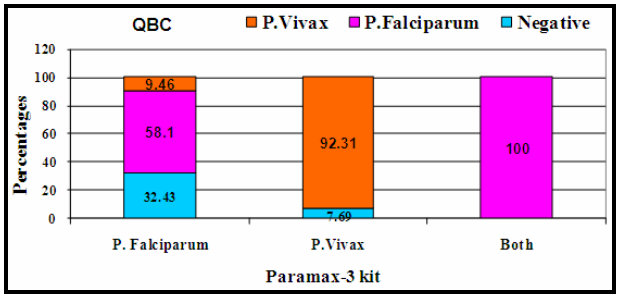


Table-3: Comparison of QBC with Paramax –3 kit				
QBC	**Paramax- 3 Kit**			**Total (n=102)**
	***P falciparum* (n=74)**	***P vivax* (n=26)**	**Both (n=2)**	
Negative	24 (32.43)	2 (7.69)	-	26 (25.49)
P falciparum	43 (58.10)	-	2 (100.00)	45 (44.12)
P. Vivax	7 (9.46)	24 (92.31)		31 (30.39)

Comparision of QBC with Paramax-3: QBC is 67.64% (69/102) sensitive to Paramax -3 kit with 60.81% sensitivity for *P falciparum* and 92.31% for *P vivax*. The overall false negatives of QBC in relation to paramax-3 kit is 31.0% with 32.43% for *P falciparum* and 7.69% for *P vivax*. Further, 7 (9.46%) cases were wrongly diagnosed by QBC as *P vivax* (Table 3). 69 cases were detected by QBC in which 43 were *P falciparum* and 24 were *P vivax*. 7 cases were wrongly diagnosed as *P vivax* but Paramax 3 kit diagnosed them as *P falciparum*. Paramax 3 kit showed 2 case of mixed infection while QBC diagnosed them as *F. falciparum* (figure 3).

Fig-3: Comparison of QBC with Paramax –3 kit



Prognostic improvement: In post treatment follow up 15 cases showed P falciparum persistence in peripheral smear and 11 cases of P vivax persistence. Paramax 3 kit showed persistence of HRP 2 in 25 cases and none of the cases showed persistence of PLDH (Table 4).

Table-4: Prognostic value of peripheral smear and Paramax-3 kit		
Prognostic value (*P falciparum*)	**Number of cases Persist**	**%**
Peripheral Smear	15/43	7534.88
Paramax-3 kit	25/74	33.78

Discussion

Although malaria prevalent in all age groups, it was commonly seen in the age group of 20-30 years with a mean age of 29.96. This was similar to other studies which reported prevalence of malaria in the age groups of 30 and 26 [8-9]. Males are more frequently exposed to the risk of acquiring malaria than females with a male preponderance with a ratio of 2.1:1 which is again similar to that reported by Mishra et al [10] and Singh et al.[11].

Quantitative buffy coat method is a highly sensitive and specific diagnostic technique. It has the advantage of rapid, easy interpretation and the cases can be diagnosed inspite of low parasitemia. It is more sensitive in detecting *P falciparum* gametocytes, *P vivax* schizont and less sensitive in detecting ring stages of *P vivax* and *P falciparum* and cases of mixed infection. The only draw back is its cost factor. In the present study 69 cases were diagnosed out of which 43 (58.1%) were *P falciparum* and 24 cases (92.3%) were *P vivax.*

The sensitivity and specificity of the QBC method in the present study were found to be 100 and 65% respectively. This was in accordance to other studies which reported a sensitivity in the similar range but a higher specificity [12-14]. In the present day paramax-3 test is the more accurate diagnostic technique as it is based on presence or absence of Ag of the parasite, HRP2 and PLDH.It requires a small amount of (5-6 µL) blood for the test to be performed, the results are obtained within 3-5 minutes and interpretation is easy depending on the presence or absence of a line on the test strip. Further its need increases in cases of cerebral

malaria, intravascular haemolysis where immediate and reliable diagnosis is important. The disadvantages include cost factor, persistence of HRP_2 Antigen even after effective treatment due to sensitivity and specificity of PLDH compared with other studies.

Our study found that the sensitivity and specificity of PLDH and HRP2 antigen were 100% with Paramax-3 kit. Similar results were found in other studies which used Paramax-3 kit.

Conclusion

Peripheral smear analysis is least expensive, species differentiation is clear and quantitation of parasitaemia is possible. Though the technique of staining is simple, it is time consuming, labour intensive and requires the service of a skilled technician. The QBC method is highly sensitive and specific. It provides a reliable, rapid and accurate method for diagnosis of malaria. The major drawbacks are the cost of the equipment and the identification of species, which is not always reliable. However Paramax-3 test was the most sensitive for *P falciparum* and *P vivax*.

Paramax-3 test meets many of the criteria for an ideal diagnostic test, it is simple, rapid, sensitive, specific, easy to perform, does not require any equipment. The test is very useful in diagnosis of mixed infections also. The major advantage of paramax-3 test is, it can used for post treatment out come.

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