COMPARATIVE EVALUATION OF VARIOUS LABORATORY METHODOLOGIES IN THE DIAGNOSIS OF MALARIA

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ABSTRACT: BACKGROUND: Malaria is the most important protozoan disease in India. The mortality and morbidity associated with this disease is considerably high whereas only limited diagnostic modalities are available in India. **AIMS:** To compare the conventional peripheral smear examination with Quantitative buffy coat (QBC) and rapid antigen detection kits. **MATERIALS & METHODS:** In the present study a total of 200 patients with clinically suspected malaria are screened with various techniques including conventional peripheral smear examinations with Giemsa, Leishman and JSB stain and newer modalities like Quantitative buffy coat testing and rapid antigen detection kits detecting PLDH. **RESULTS:** Among all these methodologies QBC test has shown highest sensitivity and antigen detection kit showed least sensitivity in the diagnosis of malaria.

KEYWORDS: Malaria, QBC, Rapid antigen detection.

INTRODUCTION: Malaria is an age old disease affecting man kind since millennia and continues to do so till today. There are about 300-500 million cases of malaria world wide and 1.1-2.7 million deaths occur annually.¹ In India, malaria is the most important disease among all the vector borne diseases. Prompt diagnosis of malaria is of most important in the patient management. If the diagnosis is missed or delayed especially with *P. falciparum* infection, potentially fatal complications like cerebral malaria, jaundice and severe anemia may develop. Though conventional diagnostic methods of peripheral smear examinations are widely used in India, rapid tests have been developed in the recent years for early detection of this dreadful infection. In the present study different modalities of malaria diagnosis which include the conventional peripheral smear examination with different staining techniques and the modern Quantitative buffy coat test (QBC), rapid antigen detection testing are evaluated in clinically suspected cases.

MATERIALS AND METHODS: The study was conducted in the Dept. of Microbiology, Kurnool Medical College for a period of 1 year from December 2013 to November 2014. A total of 200 blood samples were collected from clinically suspected cases and were processed further.

Selection criteria for Inclusion: The cases were selected as per the case definition of malaria according to WHO (2006). The criteria are as follows:

1. High index of suspicion.
2. Fever or history of fever within last 48 hours.
3. Fever with chills and rigors.
4. Fever with severe headache.
5. Absence of signs of other diseases.
6. Had antimalarials – inadequate or vomited out.

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Patients with at least four symptoms out of 6 were included in the study and the persons with joint pains, fever without chills, fever for more than 1month duration and fever with rash were excluded.

In these 200 samples 100 were collected from patients attending the malaria clinic in Govt. General Hospital and the remaining 100 were collected from the fever cases living in tribal villages surrounding Kurnool. All the samples were stained with conventional staining methods like Giemsa's staining, Leishman's staining and Jaswant singh & Bhattacharjee staining following the standard procedures.²

Quantitative buffycoat testing was done for all the samples according to the standard protocol and rapid antigen detection testing was done by using Malariagen rapid test kit (Aspen Laboratories).^{3,4}

Rapid Antigen Detection (Malarigen Rapid Test) :(Aspen Laboratories Pvt. Limited).

PRICIPLE OF THE TEST: The Malarigen Pf/Pv rapid test is a chromatographic immunoassay designed for detection of malarial parasite infection in human beings. Malarial antigens, LDH (Lactate Dehydrogenase) and aldolase are allowed to react with the Anti-LDH and Anti-Aldolase monoclonal antibody-coupled gold conjugate, followed by reaction with Anti-LDH and Anti-Aldolase monoclonal antibody immobilized at T1 T2 as test lines. When the blood sample is positive for malarial parasite, a pink/purple visible line appears at the test regions on the membrane. Appearance of the line (control) validates the procedure. The test can also discriminate between *P.falciparum* and *P.vivax*.

RESULTS: Out of 200 samples processed, majority were obtained in the age group of 11-30 years accounting to 120(60%) of the total samples followed by 31-50 years were 36 cases (18%). 13.5% were obtained below the age of 10 years i.e, 27 cases and Only 8.5% of the samples were obtained from the age group of >50 years i.e, 17 cases.

When different diagnostic modalities were compared, parasite detection rate was highest by QBC method.

Negatives (%)	Positives (%)	Method
164 (82)	36 (18)	Giemsa's staining
164 (82)	36 (18)	Leishman's staining
164 (82)	36 (18)	JSB staining
162 (81)	38 (19)	QBC method
165 (82.5)	35 (17.5)	Antigen detection
Method wise malaria positivity		

QBC method identified 38(19%) of the samples as positive whereas peripheral smear examination by different staining methodologies identified 36(18%) of the positive samples. The least sensitivity was seen with rapid antigen detection tests where only 35 samples (17.5%) were identified as positive.

Though peripheral smear examination is considered as gold standard in the developing countries, in view of the higher sensitivity of QBC and the clinical response of the patients for anti-malarial treatment, the total positivity was considered that of QBC which is positive in 38 cases.

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Out of 200, a total of 38(19%) blood samples were positive for malaria parasite. Out of these 38 positive samples 30(79%) were obtained from the tribal areas and only 8(21%) were from the urban region

Negatives (%)	Positives (%)	Area (n)
92 (92)	8 (8)	Urban(100)
70 (70)	30 (30)	Tribal(100)
162 (81)	38 (19)	Total(200)
Area wise malaria positivity		

25 out of the 38 positive samples were in the age group of 11-30years. Only 4 samples were positive for malaria above 50 years.

Positives (%)	Name of the species
20 (52.63)	P.falciparum
15 (39.47)	P.vivax
3 (7.8)	Mixed infections(both)
38 (100)	Total
Species wise distribution	

Plasmodium falciparum was the predominant species identified accounting to 20(52.6%) out of the 38 positive samples followed by P.vivax 15(39.4%). Mixed infections were observed in 3 samples (7.8%). These mixed infections include infection with both P.vivax and P.falciparum in the same patient.

Mixed infections	P.vivax	P.falciparum	Total Positives	Area
-	7 (87.5)	1 (12.5)	8	Urban
3 (10)	8 (26.7)	19 (63.3)	30	Tribal
3 (7.8)	15 (39.4)	20 (52.6)	38	Total
Area wise distribution of different species				

P.vivax was the most common species identified from the urban samples with 7 out of total 8 positives were identified as P.vivax and only 1 was identified as P.falciparum. In contrast, among tribal samples, 19 out of the 30 positives were P.falciparum and only 8 were P.vivax. All the three mixed infections were observed among tribal patients.

DISCUSSION: Accurate and early diagnosis of Malaria is the key stone for effective rationale therapy, which in turn decreases the mortality and morbidity, prevents emergence of drug resistance and avoid non-targeted effects. Different modalities are available for the early diagnosis of malaria and new methodologies are still up coming. India being an endemic country for malaria with a huge number of cases, low cost traditional methodologies like peripheral smear examinations are still widely practiced over the newer methodologies. Different staining techniques are used in India which

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include Giemsa, Leishman and JSB staining. QBC method and rapid kit based methodologies are used in higher centers and urban areas.

In the present study, the positivity of malaria in tribal population was 30% which is similar to neeru singh ET al⁵ study where an overall tribal incidence of 30% was observed. Das M. K and Joshi et al⁶ also observed similar findings in their study which was conducted in Andaman and nicobar islands. The positivity of malaria in urban population in the present study was 8%. This is in concurrence with the studies of Pinto et al (10.5%), Nandawani et al (15%) and Henavani et al (4.25%).^{7,8,9}

The higher incidence of malaria in the tribal population may be due to various factors like their occupation, lack of awareness, depending predominantly on forest produce or on forest labour, non-usage of personal protective methods like repellents, coils and bed nets.

In the present study the overall malaria positivity was 52.6% for *P.falciparum* which is similar to Gokhale et al study (56.75%). The incidence of *P.vivax* in our study was 39.5% which is in conformity with the studies of Gokhale et al (40.5%)¹⁰ and MJR pinto et al (42.7%).⁷

The incidence of 12.5% of *P.falciparum* in the urban community in our study is in accordance with the study of Das Chyani ET al¹¹ where the incidence was 21.4%. The *P.vivax* incidence of 87.5% was also in accordance with Das Chyani ET al.¹¹

In our study, the incidence of *P.falciparum* in tribal communities was 63.3%, The incidence of *P.vivax* is 26.7% which is consistent with onkarnath (20-30%) study. 10% of mixed infections in the present study is consistent with the results of Srinivasa murthy et al study (12.5%).¹²

In the present study all the three staining methods showed similar sensitivity and specificity. Whereas QBC detected an extra two cases (5.5%) which were negative by smear and antigen tests? A study conducted by Pinto et al showed an additional detection of 3.9% cases with QBC than smear examination. Similarly Srinivasa Murthy¹² revealed an additional 8% cases with QBC than smear examination.

The sensitivity of the rapid test kit in the present study was 97.2% which is consistent with the results of Chyani ET al¹¹ (96.7%). This lower sensitivity when compared to other methodologies can be explained by the fact that increased awareness of malaria has led to rampant misuse of anti-malarial drugs in inadequate doses empirically for any fever. Since the test detects PLDH which is produced only by living parasites, the blood samples judged negative by antigen detection may have been dead parasites and not yet cleared from the host.

To conclude, conventional method of blood smear examination requires technical expertise and the availability of good quality microscope. It is also time consuming and labour intensive except JSB staining which takes little time and gives consistent results with other staining methods which is seen in our study. Alternative methods of diagnosis appropriate for outpatient settings like QBC method have been used extensively since last decade. It is easier to use and more sensitive and faster in detection even with low level of parasitemia. The disadvantage being that it requires costly equipment and it may be difficult to differentiate species.

Rapid kit based tests are simple to perform, does not require technical expertise or instrumentation and are equally effective that of other methodologies. But the high cost of the kit may be a constraint for its regular and routine use. However it is a valuable adjunct at the time of emergency for rapid diagnosis, although microscopy remains the main stay for diagnosis in countries like India.

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Present study revealed that among the various diagnostic methods used in malaria, QBC method seems to be very sensitive method which can be recommended as a screening test for malaria. Kit based detection tests can be used as a rapid diagnostic methodology in emergency cases.

Various diagnostic methods used in our study.

Fig. 1: Giemsa stain-ring forms of *P.falciparum*

Fig. 2: Leishman stain-gametocytes of *p.falciparum*

Fig. 3: JSB Stain-schizonts with merozoites of *P.vivax*

Fig. 4: Rapid antigen detection kit

Fig: 5 QBC Test

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