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Research Paper

COMPARISON OF PERIPHERAL BLOOD FILM STAINED BY GIEMSA STAIN, ACRIDINE ORANGE STAINING AND RAPID DIAGNOSTIC TESTS FOR DETECTION OF *P. VIVAX* AND *P. FALCIPARUM* IN CLINICALLY SUSPECTED CASES OF MALARIA

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Malaria is one of the major public health challenges and syndromic approach is unreliable because of non-specific and overlapping symptoms with other febrile diseases. Due to emerging drug resistance, accurate diagnosis of malaria is essential to start rational therapy for malaria. Various diagnostic techniques available include: Peripheral blood film, acridine orange staining, rapid diagnostic tests. Materials and Methods: This three years study was conducted in department of microbiology, Maharishi Markandeshwar Institute of Medical Research & Sciences (MMIMSR), Mullana, Ambala. The blood samples collected from 218 clinically suspected cases of malaria were subjected to Giemsa staining, acridine orange staining and Rapid Diagnostic Test (RDT) as per standard procedures. Results: In the present study, out of total 218 cases tested, 19.7%, 18.8% and 17.% were positive by Giemsa stain, acridine orange staining procedure and RDT, respectively. On further examination of positive cases; *P. falciparum was* detected in 70%, 80% and 90% cases and *P. vivax* in 90%, 82.5%, and 75% cases by Giemsa staining, acridine orange and RDT, respectively. Conclusion: For *P. vivax* and for *P. falciparum*, Giemsa stain and RDT have better diagnostic accuracy, respectively.

Keywords: Acridine orange staining, Comparison, Giemsa staining, Malaria, Rapid Diagnostic Tests (RDTs)

INTRODUCTION

Charles Louis alphonse laveran, a French scientist, discovered the malaria parasite almost 125 years ago. Yet even today, malaria as a

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disease continues to be the world's foremost tropical disease and a major public health challenge (Sharma, 2007). Due to the rapid expansion of resistance to anti malarial drugs, the battle against malaria has become even more urgent (Mugisha, 2003).

Syndromic approach is unreliable because of the non specific and overlapping symptoms with other febrile diseases resulting in over diagnosis of malaria, over prescription of anti malarial drugs, under diagnosis and inappropriate treatment of non malarial febrile illnesses. Thus, a diagnosis of malaria infection based on clinical decision alone is unreliable and, if possible, should be supported and verified with a laboratory based confirmatory test (Uzochukwu, 2010).

In malaria patients prompt and accurate diagnosis and treatment with appropriate anti-malarial drugs is the most important strategy for effective case management (Tekola, 2010) . Failure to diagnose malaria correctly can lead to omission of a drug when a drug is required, administration of drug when no drug is required, or administration of an ineffective drug. Non-rational drug use, in turn, can promote drug resistance (McKenzie, 2003).

The diagnostic modalities which are available for malaria range from conventional thick and thin smear to rapid modalities like fluorescent staining and antigen detecting test detecting parasitic antigens like histidine rich protein-2 (HRP-2), plasmodium lactate dehydrogenase (pLDH) and pan specific aldolase (Wongsrichanalai, 2007). Keeping an eye on these state of the art techniques in the diagnosis of malaria, comparative study of the commonly employed diagnostic techniques in diagnosis of malaria, i.e., giemsa stained thick and thin smear, acridine orange staining and antigen detection using malaria pLDH /HRP2 combo test kit was planned.

This study attempts to compare the current methodologies- traditional microscopy using giemsa stain, acridine orange fluorescent staining procedure and a rapid detection test thereafter approach to the diagnosis of malaria in a practical and helpful way for the laboratory and for the physician caring for the patient .

MATERIALS AND METHODS

The present study was conducted in department of microbiology, MMIMSR, Mullana during a period of three years, i.e., from March 2009 to March 2012.

1. A total of 218 clinically suspected cases of malaria were enrolled in the study. 5 ml of whole blood was collected into the collection tube containing EDTA by venepuncture from patients presenting clinically with fever with chills and rigor and other suggestive symptoms of malaria (Mackie and MacCartney). Confirmation of diagnosis of malaria was done using three available techniques, the criteria for confirmation of positivity was positive by any of the three following methods.
2. Thick and thin blood smears (Mackie and MacCartney): For prepration of blood films, thoroughly cleaned 25 mm × 75 mm glass slides which were free of grease and scratches were used. Thick and thin blood smears were prepared as per the standard method. The smears were stained with giemsa stain. Thick smears were reported negative after examination of 200-300 oil immersion fields with no parasites observed; a thin smear

Table 1: Comparison of Various Methods for Detection of Different Species of Malaria Parasite (n=50)

S. No.	Method	Malarial Parasite Species									
		None		*P. vivax*		*P. falciparum*		*P. malariae*		*P. ovale*	
		n	%	n	%	n	%	n	%	N	%
1.	Giemsa (n=50)	7	14	36	72	7	14	0	0	0	0
2.	Acridine orange (n=50)	9	18	33	66	8	16	0	0	0	0
3.	RDT (n=50)	11	22	30	60	9	18	0	0	0	0
Note: c2=1.68 (df=4); p=0.794											

was given negative when no parasites were observed in 200 oil immersion fields.

3. Acridine orange staining technique: 75 µL of blood was mixed with 10 µL of acridine orange stain on a glass slide and covered with a coverslip and prepration was examined under fluorescent microscope (Lowe, 1996).
4. Detection of antigen: The malaria pLDH /HRP2 combo test kit contains a membrane strip, which is precoated with two antibodies as two separate lines across a test strip. One monoclonal antibody is panspecific to pLDH of the *Plasmodium vivax* and the other line consists of a monoclonal antibody specific to the HRP2 of the *Plasmodium falciparum* species.
5. Microlitres of whole blood was added into the sample well. Two drops of assay buffer were added into the buffer well. The result was read in 20 min (Moody, 2002).

STATISTICAL ANALYSIS

Data was statistically analyzed by using spss/pc + (statistical package for social sciences). Chi-square test (χ^2) was applied whenever possible and p-value was calculated.

RESULTS

A total of 50 cases were found to be positive using any of the three methods (Giemsa stain, Acridine orange fluorescent stain or RDT). Thus the prevalence of malaria among clinically suspected cases was 22.9%.

In the present study out of total 218 cases tested, 43 (19.7%), 41(18.8%) and 39 (17.%) were positive by Giemsa staining, acridine orange staining procedure and RDT respectively.

On examination of positive cases; Giemsa, acridine orange and RDTs could confirm the diagnosis of *P. vivax* in 36 (90%), 33 (82.5%), 30 (75%) cases and that of *P. falciparum* in 7 (70%), 8 (80%), 9 (90%) cases, respectively.

While sensitivity of Giemsa staining, acridine orange staining and RDT for detection of *P. vivax* is 90%, 82.5%, 75% and specificity is 100%, 100%, 100% and for *P. falciparum* sensitivity of Giemsa staining, acridine orange staining and RDT 70%, 80%, 90% and specificity of 100%,100%, 100%, respectively (Table 2).

DISCUSSION

The results of present study indicated that 50 (22.9%) were infected with malaria and the rest

Table 2: Diagnostic Efficacy of Different Methods for Malarial Parasite Species

Method	Diagnostic Efficacy				
	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
For *P. vivax*					
Giemsa stain	90.0	100.0	100.0	71.4	92.0
Acridine orange	82.5	100.0	100.0	58.8	86.0
RDT	75.0	100.0	100.0	50.0	80.0
For *P. falciparum*					
Giemsa stain	70.0	100.0	100.0	93.0	94.0
Acridine orange	80.0	100.0	100.0	95.2	96.0
RDT	90.0	100.0	100.0	97.6	98.0

168 (77.1%) were malaria negative. (Table 1) as seen in studies conducted by Iqbal *et al.* (2003), Htut *et al.* (2002), Iqbal *et al.* (2002), Endeshaw *et al.* (2010), Lema *et al.* (1999).

Gay *et al.* (1996) and Craig *et al.* (1997) found the Giemsa to be 72.9% , 93.1% sensitive and 100%, >95% specific for diagnosis of malaria, respectively. While, Gay *et al.* (1996) showed the sensitivity of fluorescence acridine orange technique (AO) to be 96.4%, and specificity of 95.1%. Stauffer *et al*. (2014) found the RDT Malaria P.f./P.v. test to be 100% sensitive and 99% specific for *P. falciparum*, and 86% sensitive and 99% specific for *P. vivax.*

CONCLUSION

Giemsa staining is the Gold standard as compared to existing techniques available for diagnosis of malaria parasite. For *P. vivax,* Giemsa stain is most sensitive and have better diagnostic accuracy whereas for *P. falciparum*, RDT is most sensitive and have better diagnostic accuracy. Half of the world's population is at risk of malaria, Rapid Diagnostic Tests (RDTs) is recommended for all patients with suspected malaria before treatment is started.

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