ISSN 1019-8555
The Journal of
Teachers Association
RMC, Rajshahi

Original Article

Acridine Orange Fluorescence Stain for the Diagnosis of Malarial Parasite

Md. A Siddique[1], Md. A Alam[2], Md. Tipu Sultan[3], Md. Q Uddin[4], S M A Hossain[5], S Naaz[6]

Summary

A total of six hundred suspected cases of malaria (150 from each centre of Rajshahi Medical College Hospital and three thanas under Rajshalhi district viz. Paba, Godagari and Charghat) were examined through both thick and thin film preparation and stained by both Giemsa and acridine orange fluorescence staining respectively. *All the slides in both stained preparation* were examined by three competent microbiologists in three different centres. Malarial parasite detection in cases of Rajshahi Medical College Hospital. Paba, Godagari and Charghat. In Giemsa staining parasites were positive in 14 (9.33%), 16(10.66%),26(17.33%) and 41(27.33%) cases respectively while it was found to be positive in 21(14.00%), 36(24.00%),49(32.66%) and 61(40.66%) cases in acridine orange fluorescence staining respectively. In comparison acridine orange fluorescence staining was found to be more sensitive over Giemsa staining especially in low parasitaemic cases.

TAJ 2003; 16(2): 58-61

Introduction

Malaria is caused by a sporozoan parasite of the genus plasmodium. It is transmitted from carrier to susceptible person by the bite of infected female anopheles mosquitoes. Relapse of malaria cases occur in *P. vivax, P. malariae and P. ovale* due to reactivation of phaenerozoite in the liver. Recurrence of malaria cases due to *P. falciparum* occur as a result of persistence blood infection.[1] Plasmodium can produce congenital and neonatal malaria among infected pregnant mothers.[2] t can cause post transfusion malaria also. Complications of malaria are cerebral malaria, severe anaemia, renal failure, black water fever and in pregnancy, abortion, still birth, and premature labour. Cerebral malaria is also common in pregnancy[3]. Nearly 2000 million people i.e. 41% of the world population remain exposed to malaria and among them, 300 to 500 million are clinical cases. Among the clinical cases, 1.5 to 2.7 million deaths occur each year affecting 90 countries or territories of the world[4]. In India, the annual incidence of malaria was 2 to 2.8 million in 1984. Now it is around 2 million every year[5]. In Bangladesh, annual malaria incidence was 0.21, 0.20, 0.46, 1.10 and 0.54 million in the year 1963, 1973, 1983, 1993 and 1997 respectively.[6] Now malaria is

[1] Assistant Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi
[2] Assistant Professor, Department of Microbiology, MAG Osmani Medical College, Sylhet.
[3] Assistant Professor, Department of Microbiology, Chittagong Medical College, Chittagong.
[4] Associate Professor, Department of Neuro Medicine, Rajshahi Medical College, Rajshahi
[5] Lecturer, Department of Microbiology, Rajshahi Medical College, Rajshahi
[6] Curator, Department of Pathology, Rajshahi Medical College, Rajshahi.

very common in hilly areas of Bangladesh and *P. falciparum* is the commonest species. Sporadic cases are fomd throughout the country. *P. falciparum* and *P. vivax* show frequent resistant to common antimalarial drugs and mosquitoes are also becoming increasing resistant to insecticides.

Several strategies are available for the control of malaria and one such approach is to eradicate the reservoir of infection. Rapid case detection followed by prompt and effective radical treatment constitutes an important measure in the control of malaria. It also reduces morbidity and mortality. Since 1903, demonstration of malaria parasite by giemsa staining microscopy (GSM) in the peripheral blood, either in thick or thin film was the conventional method for the diagnosis of malaria. The development of near techniques in the rapid diagnosis of malaria such as the technique of acridine orange fluorescence microscopy (AOFM) has opened a new door for malaria diagnosis. It has been found that AOFM is 8 times more sensitive than conventional GSM[8]. Therefore, a study was conducted with both thick and thin film, to assess the effectiveness and usefulness of AOFM over giemsa stain for the diagnosis malaria in our country.

Materials and Methods

Six hundred (150 for each centre) blood samples were collected from both in and out patient departments of Rajshahi Medical College Hospital (RMCH) and three thanas viz Paba, Godagari, Charghat under Rajshahi district over a period of one year. During febrile attack two thick and two thin films were prepared from finger prick samples of blood from the suspected malarial cases. All dried thick films were dehaemoglobinized by immersing in distilled water for 5 minutes. One dehaemoglobinized thick and one normal thin film (Naemoglobinised) were stained with giemsa stain. Three by traditional method competent microbiologists working independently in three different centre examined both the films. 100 positive films, to determme the parasite load parasite count was done per 100 WBCs.The other set of dehaemoglobinized thich and one normal thin film previously fixed by methanol were stained with acridine orange fluorescence stain by putting one drop of stain on the film and covered it with a cover slip. Excess stain was soaked with tissue paper. A drop of oil was put on the upper surface of cover slip and examined under fluorescence microscope by the same microbiologists separately. Parasite count was done accordingly in positive films. The positive reports from at lease two centres were taken as positive for both the stains.

Results

Positivity of malarial parasites in GSM and AOFM are presented in Table-1. The number of study population of each center was 150. Majority of positive cases were from Charghat followed by Godagari, Paba and least from RMCH. Parasite detection rate was more in AOFM than conventional GSM. Malarial parasite count in two staining methods and shown in Table-2. GMS detected only 2 (0.33%) cases when parasite count was low (1 to 10 parasites per 100 WBCs were AOFM detected 22 (3.66%) cases. On the other hand, in cases at more parasite count films, the detection rate of two Methods were comparable.

Table-1: Positivity of Giemsa stain microscopy and Acridine orange Fluorescence microscopy in 4 centers. (n=600)

Centers	Giemsa stain microscopy		Acridine orange Fluorescence Microscopy	
	Number	Percentage	Number	Percentage
RMCH (n=150)	14	9.33	21	14.00
Paba (n=150)	16	10.66	36	24.66
Godagari (n=150)	26	17.33	49	32.66
Charghat (n=150)	41	27.33	61	40.66
Total (n=600)	97	16.16	167	27.83

Table-2: Malaria parasite Count per 100 WBCs in Giemsa stain microcopy and Aridine orange Fluorescence microscopy (n=600)

Parasite count per 100 WBCs	Giemsa stain microscopy		Acridine orange Fluorescence microscopy	
	Number	Percentage	Number	Percentage
01-10	02	0.33	22	3.66
11-20	08	1.33	36	6.00
21-30	12	2.00	25	4.16
31-40	20	3.33	26	4.33
41-50	28	4.66	30	5.00
>51	27	4.50	28	4.66
Total	97	16.16	167	27.83

Discussion

Ross, in 1903, was the first man who demonstrated the malarial parasite by microscopic examination of Giemsa stained blood film and currently this is the standard technique for diagnosis of malaria. This technique is reliable and inexpensive. But this technique has a limitation that it can detect plasmodium only when the parasite count is at least 10 to 20 plasmodia/microliter of blood.[8] Sometimes inexperienced microscopists may misinterpret various artifacts commonly found in stained blood film, even they may fail to recognize some typical parasites with a high parasitaemia. Acridine orange (AO) Fluorescence stain has an advantage over Giemsa stain in rapidity, sensitivity and ease of use. But it needs a fluorescence microscope. In this study percentage of positive cases in GSM at RMCH, Paba, Godagari and Charghat thanas were 9.33%, 10.66%, 17.33% and 27.33% and in AOFM were 14.00%, 24.00%, 32.66% and 40.66% respectively. Maximum number of positive cases were from Charghat and Godagari thanas. In Paba centre parasite detection by AOFM was higher than GSM. this is due to low parasite count. Parasite detection by AOFM (27.83%) was better over GSM (16.16%) which is almost similar with the findings of Thomas *et.al.*[9] In our study, GSM detects only 2 (0.33%) cases when the parasite count was low (1-10 parasites per 100 WBCs) but AOFM detected 22 (3.66) such cases. In cases of high parasitaemic the detection by two methods was comparable. This is also similar with the report of A J Ollo *et al.*[10] Like Giemsa stain, Acridine orange fluorescence stain can also identify the species of plasmodium.

Conventional blood film examination (Giemsa stain) remains the standard diagnostic test for diagnosis of malaria but because of lower sensitivity, especially in sparsely parasitaemic cases, repeated tests and prolonged time are necessary to detect scanty parasitaemic cases. There is also the possibility of parasite loss during processing and masking of parasite by stain. Sometimes artifactual bodies contribute to background hazy during microscopy. AO staining is very simple, less time consuming, sensitive for the diagnosis of malaria in a clinical setting, especially in low parasitaemic cases.

Farther, the preparation of Acrinide orange stain is simple and the stain is stable for years when kept in dark at room temperature. AO stained slides can be showed several times with AO and with Giemsa after rinsing in methanol or distilled water. On the other hand Giemsa stained slides can not be restrained by AO.

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All correspondence to:
Md. A Siddique
Assistant Professor
Department of Microbiology
Rajshahi Medical College
Rajshahi.