ORIGINAL ARTICLE

Comparison of Acridine Orange with Giemsa Staining for Diagnosis of Malarial Parasite

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ABSTRACT

Aim: To compare Acridine orange stain with Giemsa stain for diagnosis of malarial parasites.
Study design: Cross sectional study
Methods: A total of 200 subjects having malarial symptoms were collected from the outpatients departments of various hospitals. The subjects taking anti malarial drugs were excluded from the study. 2ml of venous blood was drawn aseptically in sterile EDTA vial duly labeled. Slides of Thin and thick smears with Giemsa stain for MP and Thin and thick smear with Acridine Orange stain for MP were prepared. Data collected was entered and analyzed in SPSS version 15.0. The positive and negative cases by Giemsa staining and Acridine Orange staining were described by the frequencies and percentage. The comparison between two methods was performed by using Z-test for proportion. P value was calculated between the two means.
Results: Among these 200 subjects, malaria positive cases (Group 1), on both staining methods, were 170(85%) and malaria negative cases (Group 2) were 30(15%).
Conclusion: The Acridine Orange stain revealed reduced number of fields examined for the MP detection (P<0.01), the minimum time consumed for the first MP detection, more percentage of case detection at low parasitemia, easy to operate and the results were immediately available. The statistical difference in the detection of number of positive cases was non-significant between AO staining and Giemsa staining (P>0.05).
Keywords: Malaria, acridine orange

INTRODUCTION

The most common method of direct microscopy is examination of thick and thin blood films. The thick smear preparation may take 30-60 minutes and interpretation requires considerable experience. Thick films are stained unfixed by Giemsa and it provides sensitivity to the technique. Thin film should be fixed and stained by Giemsa which provides specificity for species identification and helps evaluating the intensity of parasitemia[1]. In thick blood film preparation, detection of parasites is often hampered by the presence of cellular debris[2]. Since the plasmodium Falciparum is trapped in microcirculation for more than half its asexual cycle of 48 hours, even in high parasitemia, only rare parasites may be detected on blood film examination during period of sequestration[3]. Moreover, the positive cases with low parasitemia may be missed due to lack of experienced personnel's. The main problem with microscopic diagnosis is that it is time consuming and must be performed by the skilled microscopists[4].

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METHODOLOGY

Patient with history of headache, shivering, high grade fever, vomiting, nausea were included in this study. A total of 200 cases were included in the present study. A detailed history was taken and physical examination carried out and recorded in proforma. 2ml of venous blood was drawn aseptically in sterile EDTA vial duly labeled. Thin smears with Giemsa stain for MP, Thick smear with Giemsa stain for MP, Thin smear with Acridine orange stain for MP and Thick smear with Acridine orange stain for MP were prepared.

RESULTS

Detail of results is given in tables 1,2 and 3

Table 1: Number of fields examined with Giemsa and Acridine Orange Staining

No. Of Fields Examined	Giemsa Staining (A)		Acridine Orange Staining (B)	
	Giemsa/thin	Giemsa/thick	AO thin	AO thick
Mean	50±15.3	30±12.2	20±5.0	10±6.9
Range	20-80	10-50	3-35	1-30

Statistical Analysis: A vs B = p<0.01 (Highly significant

Table 2: Time Consumed (min) for First MP Detection Using Giemsa and Acridine Orange Staining

Time consumed (Minutes)	Giemsa Staining (A)		Acridine Orange Staining (B)	
	Giemsa/thin	Giemsa/thick	AO thin	AO thick
Mean	23±9.1	16±7.6	7.1±3.7	1.9±2.1
Range	6-48	3-36	0.5-12	0.25-8
Total Subjects	164	164	170	170

Statistical Analysis: A vs B = $p<0.01$ (Highly significant)

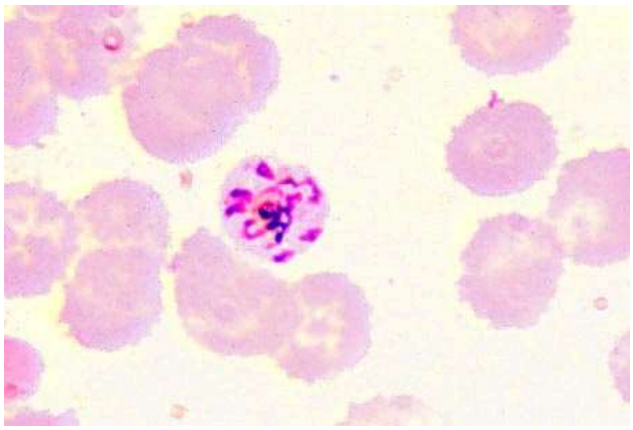

Fig 2: Giemsa stained thin blood film showing immature schizont, 2-12 nuclei. Infected red cell is smaller than uninfected red cell

DISCUSSION

Low parasitemia is major challenge in the diagnosis of malaria. The study conducted by Siddique et al [6] showed that Giemsa staining detection rate was 0.33 % when the parasite count was low (1-10 per 100 WBC) while the detection rate by AO staining was 3.66% at the same parasite level. In our study the detection rate by Giemsa staining at 1-10 parasites per 100 WBC was 28% and with AO staining it was 29.4% and we agree with them that the AO staining has high sensitivity as compared to Giemsa staining and we disagree with the study of Keiser J et al who say that the AO staining has low sensitivity rate at low parasitemia. Results of Htut et al[7] are similar that AO method has higher sensitivities than Giemsa staining at low parasitemia. However we agree with Keiser J et al[5] in which AO results are readily available within 3-10 min, whereas Giemsa staining may take 45 min or even longer.

Mean±SD of time consumed for the detection of first MP with Giemsa thin smear was 23±9.1 minutes ranging from 6-48 min. and Mean time consumed with Giemsa thick smear was 16±7.6 minutes ranging from 3-36 minutes. Mean±SD of time consumed for the detection of first MP with AO thin smear was 7.1±3.7 minutes with a range of 0.5-12 minutes and with the AO thick smear it was 1.9±2.1 minutes ranging from 0.25-8 minutes. Statistically when the time consumed for the detection of first MP with Giemsa staining was compared with the AO staining the difference was highly significant and in this respect our study results match the study results of Chiodinni et al[8] and Kawamoto et al[9] who have stated that the time for the detection of first parasite was only 1/3 by AO staining as compared to Giemsa method.

CONCLUSION

The Acridine Orange stain revealed reduced number of fields examined for the MP detection ($P<0.01$), the minimum time consumed for the first MP detection, more percentage of case detection at low parasitemia, easy to operate and the results were immediately available. The statistical difference in the detection of number of positive cases was non-significant between AO staining and Giemsa staining ($P>0.05$).

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