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Detection of Malaria through Light Microscope with Fluorescent Microscopy and Interference Filter

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ABSTRCT

The Aim of the Current study to compare Giemsa staining with acridine orange (A.O) staining by using light microscopy with IF and also with fluorescent microscopy for diagnosis of parasites in peripheral blood of patients suffering from clinically suspected cases of malaria at Saidu Teaching Hospital Swat Pakistan. in this study 350 patients with fever and shivering were included. General investigations like Hb, TLC and platelets were done by sysmex K-1000. Thin and thick blood films were made and stained according to protocol given i.e. by Giemsa and AO stains and slides were examined by different microscopes i.e. light microscope, light microscope with IFS and fluorescent microscope. Out of 350 subjects, 297 (85%) patients showed positive parasitemia and 53(15%) subjects were negative for malaria parasites. fib, TLC and platelets were reduced when comparing with MP negative cases. IFS microscope with acridine orange staining showed early detection of malaria parasites by counting fewer fields as compared to light microscopy with Giemsa stains. Time consumed for detection of parasites was also significantly reduced in IFS microscope by using AO stains.

Key Words: Giemsa Stain, Malarial parasite, Acridine Orange.

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Introduction

Malaria is defined as an acute or chronic disease caused by obligate intracellular protozoa of the genus plasmodium (1).The clinical course is characterized by paroxysms of high fever, chills, anemia and splenomegaly (2). Malaria has been a major medical problem in the subcontinent for thousands of years. In the plains of subcontinent, malaria has traditionally been seasonal and shown a marked tendency to be unstable (3). The overall slide positivity rate in Pakistanis 3.8% and predominant species is vivax being 60% and falciparum being 40%. In some studies, falciparum was more common (64.4% compared with vivax being 34.6°/x) (4). Different Previous study has been reported the detection of malarial parasites in A.O stained peripheral smears by fluorescent microscope examination (5). In one study, interference filter system was used in the standard light microscope (6). Another study shows that a new interference filter to be used with A.O stained smears in a standard light microscope for rapid diagnosis. Even a low parasitemia (<0.0002% with one to several parasites per thin film) could be detected. The time consumed was shortened as compared to Giemsa stained smears (7). It was also reported that time for identification of first parasite was only 1\3 by acridine orange staining as compared to Giemsa stained smears. The purpose of study is to compare acridine orange staining method with Giemsa staining by using light microscopy with IF and also with fluorescent microscopy for detection of parasites in peripheral blood of patients suffering from clinically suspected cases of malaria and to compare time taken for identification of first parasite.

Material and Methods

A total of 350 patients were included in the present study. All the patients were with high fever and shivering for the last 2-3 days without anti-malarial therapy. The subjects were selected from different wards of Saidu Sharif Teaching Hospital Swat Pakistan.

Specimen collection:

2ml of venous blood was withdrawn, thick & thin peripheral blood smears were prepared and blood was placed in EDTA vials. Laboratory Investigations Following investigations were carried out: Hb, TLC, Platelets, thin and thick smears for malarial parasites.

Fitting of Light microscope:

Light microscope was fitted with interference filter for excitation light of 480 nm wavelength and a barrier filter with a cut off wave length of 515nm was inserted into eyepiece. Light source was 100 watts halogen lamp.

Results

A total of 350 patients with fever & shivering were included in this study. Clinical features of the patients were recorded and are given in table 1. Table 2 shows the results of laboratory investigations. Haemoglobin levels of MP+ve cases were lower when comparing with MP negative cases. Mean TLC in group I (MP+ve cases) was 7.2 ± 2.47 and in group II (MP-ve

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cases) was 8.5±2.3. Comparison of group I and II shows highly significant difference (p<0.001). Mean platelet count in MP+ve cases was 142.4±73.9 and it was 267±85.1 in MP-ve cases. Difference between the two groups was found to be highly significant (p<0.001).Table 3 shows mean ± SD of number of fields examined and mean ± SD of time consumed for detection of first MP by using different stains and microscopes. When comparing two microscopes i.e. light microscope with Giemsa stain and light microscope with IFS by using AO stain, number of fields were significantly reduced by using IFS microscope with AO staining (p<0.001). When comparing light microscope with Giemsa stains and IFS microscope with AO stains, there were six Giemsa negative cases which became positive when examined by IFS microscope with AO stains (Table 4).

Table 1: Clinical features of patients

Symptoms/Signs	**No. of Patients**	**%age**
Rigors	320	90
Headache	212	59.5
Nausea	214	61.30
Vomiting	242	68
Body aches	234	65.5
Fever	350	100
Splenomegaly	62	17.71

Table 2 Comparison of TLC, HB and Platelets found in Group I and II

Parameters	**Group I (MP +ve case**	**Group II (MP –ve case**	**Statistical analysis**
Haemoglobin (gm/dl)	11.9±1.92 (7.2–16.0)	12.3±2.14 (8.4–16.0)	p>0.05 (NS)
TLC (109/L)	7.2±2.47 (2.9–13.9)	8.5±2.3 (2.9–13.9)	p<0.05 (HS)
Platelets count (109/L)	142.4±73.9 (16–364)	267.5±85.1 (103–470)	p<0.05 (HS)

Table 3: Number of fields examined & time consumed for parasite detection

Microscope	Light microscope (A)	Light microscope IFS (B)	Fluorescent microscope

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					(C)	
No. of fields examined	47.6 ± 15.3 (15 – 80)	28.8 ± 10.9 (10 – 50)	20.4 ± 8.8 (5– 40)	12.6 ± 6.02 (3 – 30)	17.5 ± 8.2 (4 – 35)	8.9 ± 6.9 (1 – 32
Time (Min) consumed for parasite detection	23.9 ± 9.1 (5 – 41)	16.5 ± 7.6 (2 – 35)	9.5 ± 3.7 (1– 15)	5.5 ± 3.4 (1 – 14)	7.1 ± 3.7 (0.5 – 15)	1.9 ± 2.1 (0.25 – 10)

Table 4: Comparison of light microscope with microscope

Types of stains with microscopes used	**MP+ve No. of cases**	**MP-ve No. of cases**
Light microscope with Giemsa stains	164 (82%)	36 (18%)
IFS microscope with AO stains	170 (85%)	30 (15%

Discussion

The reported slide positivity rate in Pakistan has been 3.8% and in our population of over 140 million people, more than five million people are exposed to the risk of infection and its complications. This study is perhaps the first of its kind regarding comparison of different microscopes with different stains. Different study show that fluorescent stained smears of AO were used and number of fields for detection of malarial parasite were calculated which were significantly reduced by IFS microscope with AO staining when comparing light microscope with Giemsa stains (8). The difference of IFS microscope with fluorescent microscope by using AO stains was also highly significant in early detection of parasitemia (9). Haemostatic system defects in malaria patients are well documented in literature. Thrombocytopenia was found in patients with malarial parasite positive cases which was highly significant statistically ($p<0.001$) (10). Anemia has been a consistent feature of malaria infection. In this study, Hb values are reduced in MP+ve cases as compared to MP-ve cases but difference is non-significant statistically. Anemia may be mainly due to mechanical destruction of parasitized red cells (11). Leukopenia is also observed in MP+ve cases and difference was highly significant statistically.

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Conclusion

The current Study Conclude that Hb values are reduced in MP+ve cases as compared to MP-ve cases but difference is non-significant statistically and IFS microscope with acridine orange staining showed early detection of malaria parasites by counting fewer fields as compared to light microscopy with Giemsa stains.

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