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PROMPT DETECTION OF MALARIA BY FLUORESCENT MICROSCOPY WITH INTERFERENCE FILTER AND LIGHT MICROSCOPE

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Abstract:
Aim: *The present study is planned to compare acridine orange (A.O) staining 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.*
Place and Duration: *In the Pathology department of Allama Iqbal Medical College, Lahore/ Jinnah Hospital Lahore for one-year duration from April 2019 to April 2020.*
Methods: *200 patients with fever and chills were included. General tests such as Hb, TLC, and platelets were performed by the sysmex K-1000. Blood thin and thick membranes were prepared and stained according to the protocol given, ie, by Giemsa and AO staining, and the slides were examined with various microscopes, ie light microscopy, IFS light microscope and fluorescence microscope.*
Results: *Of the 200 patients, 170 (85%) patients tested positive for parasitemia and 30 (15%) patients were negative for the malaria parasite. fib, TLC and platelet counts were all decreased compared to MP negative cases.*
Conclusion: *IFS microscopy with acridine staining showed early detection of malaria parasites by counting fewer fields compared to light microscopy with Giemsa staining. The time spent detecting parasites was also significantly reduced in the IFS microscope thanks to the use of AO dyes.*
Key words: *malaria parasite, Giemsa stain, acridine orange.*

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INTRODUCTION:

Malaria is as old as man himself. Malaria is defined as the acute or chronic disease caused by obligate intracellular protozoa of the genus Plasmodium. The clinical course is characterized by bouts of high fever, chills, anemia and enlargement of the spleen. Malaria has been a major medical problem in the subcontinent for thousands of years. In the plains of the subcontinent, including Punjab, malaria was traditionally seasonal and tended to be volatile. The overall slip positivity index in Pakistan is 3.8%, with vivax being the dominant species at 60% and falciparum being 40%. In some studies, falciparum was more frequent (64.4% compared to vivax 34.6 ° / x). Thrombocytopemia is a permanent symptom and is believed to be of immunological origin.

Previously, workers reported the detection of malaria parasites in A.O-stained peripheral smears using fluorescence microscopy. In one study, an interference filter system was used in a standard light microscope. Kawamoto (1991) described a new interference filter for use in A.O stained smears in a standard light microscope for rapid diagnosis. Even a low parasitemia (<0.0002% with one or more parasites per thin layer) could be detected. The time spent was shorter compared to Giemsa's stained smears. It was also reported that the identification time of the first parasite was only 1 µl after acridine staining compared to the Giemsa stained smears.

The aim of the study is to compare the method of acridine staining with Giemsa staining using light microscopy with IF and fluorescence microscopy to detect parasites in the peripheral blood of patients clinically suspected of malaria, and to compare the time needed to identify the first parasite.

THEMES AND METHODS:

This study was held in the Pathology department of Allama Iqbal Medical College, Lahore/ Jinnah Hospital Lahore for one-year duration from April 2019 to April 2020.

A total of 200 patients were enrolled in the study. All patients had high fever and chills for the last 2-3 days without antimalarial treatment. The subjects were selected from the departments of various hospitals, outpatient departments of various hospitals and private clinics.

Sampling: 2 ml of venous blood were collected; thick and thin smears of peripheral blood were prepared and blood was placed in EDTA vials. Laboratory Tests were carried out on: Hb, TLC, Platelets, thin and thick smears of malaria parasites.

Light microscope fit: The light microscope was equipped with an interference filter for excitation light with a wavelength of 480 nm, and a barrier filter with a wavelength cut-off of 515 nm was inserted into the eyepiece. The light source was a halogen lamp with a power of 100 watts.

RESULTS:

A total of 200 patients with fever and chills were enrolled in the study. The clinical characteristics of the patients are recorded and presented in Table 1. Table 2 presents the results of the laboratory tests. The hemoglobin level in MP + ve cases was lower compared to MP-negative cases. Mean TLC in group I (MP + ve cases) was 7.2 ± 2.47, and in group II (MP-ve cases) 8.5 ± 2.3. The comparison of groups I and II shows a highly significant difference ($p < 0.001$). The mean platelet count in MP + ve cases was 142.4 ± 73.9, and in MP-ve cases 267 ± 85.1. It was found that the difference between the two groups was highly significant ($p < 0.001$). Table 3 shows the mean SD of the number of fields tested and the mean SD of the time spent detecting the first MP using the various dyes and microscopes.

When comparing the two microscopes, ie the Giemsa light microscope and the IFS light microscope using AO staining, the number of fields was significantly reduced using an IFS microscope with AO staining ($p < 0.001$).

Comparing the light microscope with the Giemsa stains and the IFS microscope with the AO stains, six cases of Giemsa negative were found, which turned positive when examined under the IFS microscope with AO stains (Table 4).

Table 1: Clinical features of patients

Symptoms/Signs	No. of Patients	%age
Rigors	180	90
Headache	119	59.5
Nausea	120	60
Vomiting	138	69
Body aches	131	65.5
Fever	200	100
Splenomegaly	32	16

Table 2: Comparison of Hb, TLC and platelets count in group i & ii

Parameters	Group I (MP +ve case	Group II (MP –ve case	Statistical analysis
Hemoglobin (gm/dl)	11.9±1.92 (7.2–16.0)	12.3±2.14 (8.4–16.0)	p>0.05 (NS)
TLC (10^9/L)	7.2±2.47 (2.9–13.9)	8.5±2.3 (2.9–13.9)	p<0.05 (HS)
Platelets count (10^9/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 (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 was 3.8%, and in our population of over 140 million people, over five million people are at risk of infection and its complications. This study is possibly the first of its kind to compare different microscopes to different stains.

Fluorescent stained AO smears were used in this study and the number of parasite malaria detection fields was calculated, which was significantly reduced by an IFS microscope with AO staining compared to a Giemsa stained light microscope. The difference between the IFS microscope and the fluorescence microscope using AO dyes was also very important in the early detection of parasitemia.

The disadvantages of the hemostatic system in malaria patients are well documented in the literature. Thrombocytopenia was found in patients with positive results of parasite malaria infection, which was highly statistically significant (p <0.001) 14. Thrombo-cytopenia may result from the destruction of platelets by the immune system.

Anemia is a constant feature of malaria infection. In this study, Hb values are reduced in MP + ve cases compared to MP-ve cases, but the difference is not statistically significant. Anemia can be mainly caused by mechanical destruction of the parasitic red blood

cells16. Leukopenia is also observed in MP + ve cases, and the difference was highly statistically significant.

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