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A COMPARATIVE STUDY OF DARK GROUND MICROSCOPY & IgM ELISA FOR THE DETECTION OF LEPTOSPIROSIS IN ACUTE FEBRILE ILLNESS CASES AT A TERTIARY CARE HOSPITAL IN KANPUR

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ABSTRACT

Introduction: Most developing countries are found in tropical regions, where the incidence of leptospirosis is higher than that of temperate regions. Leptospirosis is thought to be the most common zoonosis. In a setting with limited resources like India, underreporting is typically caused by a lack of knowledge, unusual presentations, and a lack of diagnostic facilities. For proper care and control, it is critical to understand the incidence of these illnesses in the area.

Aim: A comparative study of dark ground microscopy &Ig M ELISA for the detection of leptospirosis in acute febrile illness cases at a tertiary care hospital in Kanpur.

Methods: The present study was conducted in Department of Microbiology and Medicine Rama Medical college hospital and research centre Kanpur. Type of study is cross sectional observational study conducted between April 2023 to March 2024. Blood sample was collected in a plain vial by aseptic precautions and then centrifuge the sample for plasma. The slide was focused under low and high power magnification in Dark ground microscopy (DFM) and perform IgM ELISA test for the detection of Leptospirosis.

Results: Out of 50 blood samples 22 were male and 28 females. In the age group between 31-40 years observed in leptospirosis. Fever (100%), Myalgia (78.57%), Nausea (71.42%) Vomiting, were common clinical features observed in leptospirosis cases. 14 were positive by IgM ELISA and 6 were positive by Dark ground microscopy. The sensitivity and specificity of IgM ELISA and Dark ground microscopy (DFM) were 100%; 42.85% and 81.81%; 100% respectively. The NPV and PPV of IgM ELISA and Dark ground microscopy (DFM) is 100%, 81% and 42% ,100% respectively.

Conclusion: In our study IgM ELISA is more effective method when compared to Dark Ground Microscopy with higher sensitivity and specificity than the DGM. Hence, IgM ELISA is rapid and relevant method for detection of Leptospirosis.

Keywords: *Leptospirosis* , Dark-ground Microscopy, Ig M ELISA, Acute febrile illness, High Power Field .

INTRODUCTION

A zoonosis called leptospirosis is brought on by harmful strains of the Leptospira bacteria. In tropical and subtropical regions, it is becoming more widely recognised as a cause of acute febrile illnesses in the world.(1,2) This disease can present as a moderate, self-limited pyretic illness or as a serious, potentially fatal illness with jaundice, renal failure, thrombocytopenia, and bleeding. When leptospirosis first appears, it might be mistaken for other frequent acute febrile illness causes that have similar clinical presentations, such as dengue, malaria, scrub typhus, typhoid, and others.(3) In order to treat a patient with proper medications for acute leptospirosis, early identification is critical. (4)

Nevertheless, the nonspecific presentation of this illness makes an early diagnosis difficult. Numerous commercially available diagnostic test kits are designed to identify particular antibodies against the pathogenic *Leptospira*. Since these tests are not very accurate, treating a patient with the right antibiotics based on their results is never certain.(5)

We have conducted the cross sectional study to assess the role of Dark Filed Microscopy (DFM) and Ig M ELISA for the early diagnosis of *Leptospira*.

MATERIAL AND METHODS

An observational cross- sectional study was carried out between April 2023 to March 2024 at Department of Microbiology and Medicine Rama Medical College Hospital and Research Centre in Kanpur, Uttar Pradesh India. A total of 50 blood samples from acute febrile illness cases (showing sign of fever, abdominal pain, decreased urine output, Atalagia Neurological symptoms, Headache) were showed. Patients diagnosed with other cause of fever and patients without case definition of leptospira were excluded. After obtaining informed consent from the patients 5 ml blood sample is collected aseptically in a 2 separate vials one vial contain 500 µl of 1% sodium oxalate solution as anticoagulant and the other one was a plain vial. The former sample with anticoagulant was used for dark field microscopy and the other one was used for ELISA. Data were collected as age, sex, occupation and exposure history of the patient. The ethical committee clearance certificate was taken before starting of study by Institutional Medical Ethical Committee (IMEC).

Dark Field Examination

To separate the undesirable cellular components, the blood samples were centrifuged for five minutes at about 3000 rpm using a 1% sodium oxalate solution. A slide with a thickness of 1 mm was coated with 10 µl of supernatant plasma. To create a thin layer devoid of air bubbles, a cover slip was placed over the drop and compressed. We employed a binocular dark field microscope equipped with a high power objective. The *Leptospires* were visualized by carefully scanning the slide. Depending on the concentration, the number of *Leptospires* observed in 40X HPF (high power field) was determined by simply counting as *Leptospira* positive.

Enzyme Linked Immunosorbent Assay (ELISA)

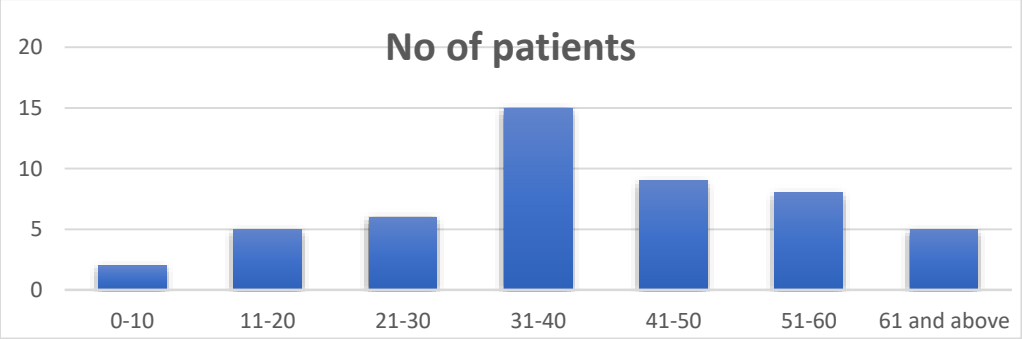
Leptospira Ig M antibody was detected in the serum using Vircell Microbiologist *Leptospira* ELISA IgM for in vitro diagnostic use.. SELISA procedure was followed as per the instructions provide in the kits. Optical density was recorded in an ELISA reader by using 405 nm filters.

Statistical analysis:

Data recorded on the case report from and structured proforma were subsequently entered into a spreadsheet. Data management and analysis were performed using Microsoft Excel.

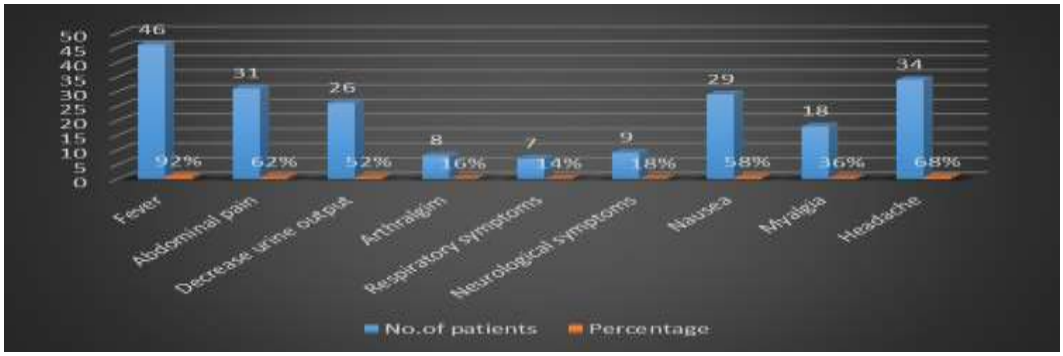
RESULTS

A total 50 blood sample were collected from patients in which *Leptospira* suspected cases which were studied between the age group 0-60 & above years with mean age 31-40 years (30%) as shown in Graph 1.



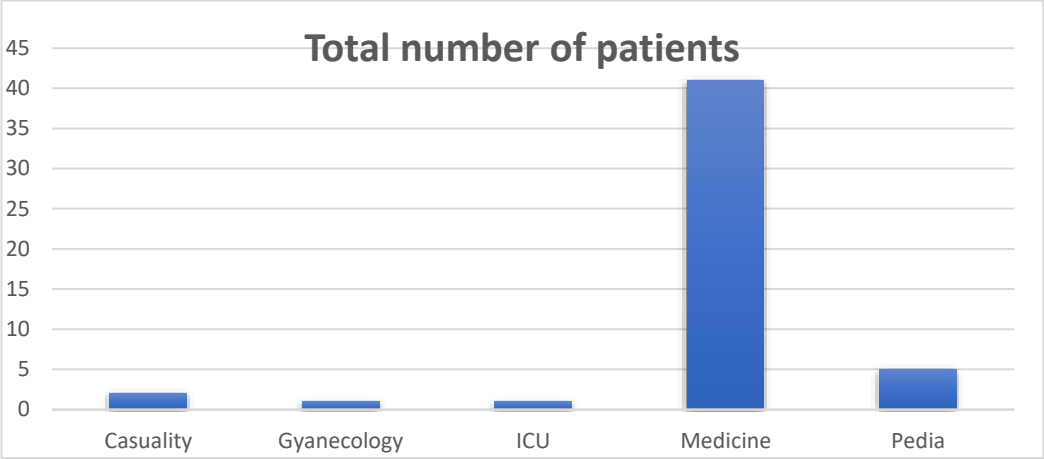
Graph no .1: Age wise distribution of suspected cases of *Leptospira.*

Out of total, maximum suspected cases were male (56%) and female (44 %) . The distribution of suspected cases was done according to sign & symptoms, in which fever is most common followed by headache as shown in graph [2].



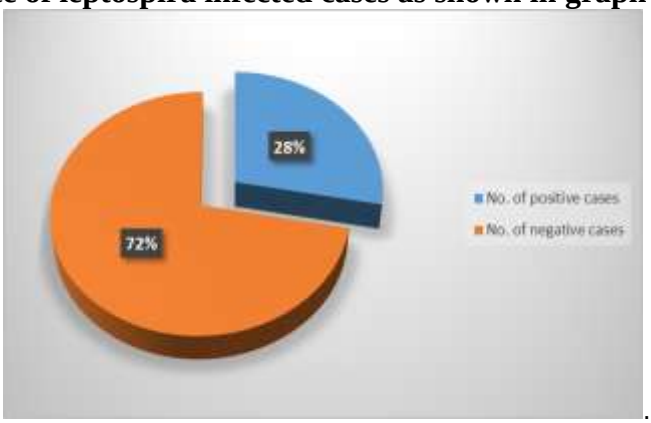
Graph No.2: Distribution of suspected cases according to Signs and symptom.

Among all the suspected cases 64% cases were from rural area and 36% cases were from urban area. Among all the suspected cases maximum number of cases are from Medicine ward followed by Pedia as shown in Graph no: 3



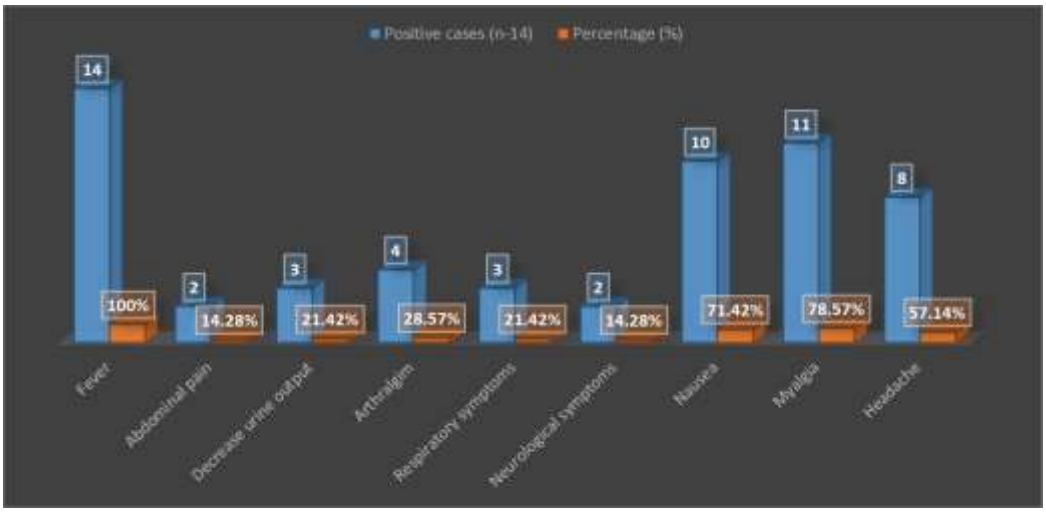
Graphno.3: Ward wise distribution of suspected cases for *Leptospira* .

- **28% is the incidence of leptospira infected cases as shown in graph no 4.**



Graphno.4: Incidence of *Leptospira infected* cases.

The most common age group infected by leptospira is 31-40 years followed by 51-60 years.
In Leptospira infected patients males (64%) are more affected than female (36%).
In ward wise distribution of infected cases medicine ward patients are more commonly infected followed by casuality ward .



Graph no. 5: Distribution of *Leptospira* infected patients according to sign and symptoms.

In this study we have done the comparison between DFM and Ig M ELISA so total no of positive case were 14 by Ig M ELISA and out of that 6 were positive by DFM as shown in Table 1.

DFM	Ig M ELISA(gold standard)		TOTAL
	Positive	Negative	
Positive	6	0	6
Negative	8	36	44
Total	14	36	50

Table 1 : Comaprison of DFM and IgM ELISA in infected cases.

The sensitivity and specificity of Ig M ELISA & DFM is 100%,42.85%; 81.81%, 100% respectively. The PPV and NPV of Ig M ELISA & DFM is 42%, 100%; 100%, 81.1% respectively as shown in table 2.

Test methods	Sensitivity	Specificity	PPV	NPV
Ig M ELISA	100%	81.81%	42%	100%
DFM	42.85	100%	100%	81.1%

Table 2: Sensitivity, specificity, PPV and NPV of Ig M ELISA and DFM.

DISCUSSION

In our study, most of the cases belonged to the agegroup is between 31 to 40 year which is similar to the studies conducted by S*hanmuga Sundaram Rajamani et al* [6] .

There is a difference in the incidence of Leptospirosis in males and females in different regions in the same country. In our study, male (64.28%) was more affected than female (35.71%). The finding was in accordance with Ahmad N et al., reported that males constituted 66.7% of total cases.[7]. Male preponderance can be attributed to increased risk exposure due to outdoor activities and occupation.

In our study, the incidence of Leptospirosis was observed to be 28 % in patients. The finding was in accordance with Sonu Kumari Agrawal et al showed incidience 26.90 % [8] .

The sign and symptoms observed in the present study were – Fever (100%),Myalgia (78.57%,), Nausea (71.42%), which was comparable to the findings of Surabhi Shukla et al., [9] .

In the present study, the sensitivity of dark field microscopy was found to be 42.85% and the specificity was 100% when compared with IgM ELISA, which was much higher when compared to the study done by Kanchan Sharma [10] where the sensitivity and specificity was reported to be only 62%. In this there is difference in sensitivity as it requires technical, experimental and Dark ground microscope, not all resource limited settings has this type of facilities and equipments.

CONCLUSION

In our study IgM ELISA is more effective method when compared to Dark Ground Microscopy with higher sensitivity and specificity than the DGM. Hence, IgM ELISA is rapid and relevant method for detection of Leptospirosis.

LIMITATION

In our study, limited numbers of samples were studied due to cost constraints.

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