

## Original Research Article

# Human leptospirosis in patients with undifferentiated acute febrile illness in a tertiary care hospital in New Delhi

Garima Gautam\*, Manoj B. Jais, Ravinder Kaur, Megh Singh Dhakkad

Department of Microbiology, Lady Hardinge Medical College and Associated Hospitals, New Delhi, India

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### \*Correspondence:

Dr. Garima Gautam,

E-mail: garimagautam1920@gmail.com

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### ABSTRACT

**Background:** Leptospirosis is an important cause of febrile illness with a widespread global distribution. Although endemic in the southern and western states of India, its true incidence in the northern states of the country remains underreported. The main objective of this study was to estimate the seropositivity of infections by *Leptospira* in patients presenting with undifferentiated acute fever, in a tertiary care hospital in New Delhi.

**Methods:** This prospective observational study was conducted from November 2016 to March 2018 in a tertiary care hospital in New Delhi. Blood samples of the patients (n=370) having fever for more than one week and seronegative for typhoid, dengue, chikungunya and malaria were subjected to *Leptospira* IgM ELISA (DRG International Inc., USA). Data was analyzed using statistical software SPSS version 21.

**Results:** Out of 370 patients, 142 (38.4%) were males and 228 (61.6%) were females; the mean age was 32.18 years. The most common symptom was headache (55.5%) and icterus (55.55%) whereas increased ALT/AST was the most common laboratory finding (88.8%) followed by anaemia (77.7%). On employing *Leptospira* IgM ELISA, 2.43% (9 cases, n=370) were strongly reactive (>1.0 OD) whereas 15.1% (56 cases) were weakly reactive (1.15-1.00 OD).

**Conclusions:** There is a need to increase awareness and suspicion regarding diagnosis of leptospirosis. A positive IgM ELISA when correlated with patient's clinical profile and epidemiological factors can aid in the timely diagnosis and treatment of these infections.

**Keywords:** Acute febrile illness, IgM *Leptospira* ELISA, Leptospirosis

### INTRODUCTION

Leptospirosis, a spirochetal zoonosis caused by pathogenic members of the genus *Leptospira*, is an emerging global disease.<sup>1</sup> Although endemic in the southern and western states of India, its true incidence in the northern States of the country remains underreported. This may be accounted by the fact that most of the infections are asymptomatic or mild hence often go unnoticed and also, the lack of awareness of the disease amongst clinicians. This may be explained by the scanty data available on leptospirosis from Delhi.<sup>2-4</sup>

Leptospirosis is known to be endemic in India since the early 20<sup>th</sup> century. The incidence of leptospirosis in developing countries is 10-100/1,00,000 cases per year.

Though less than 10,000 cases are reported, India should report 0.1-1.0 million cases per year by this estimate. More than 500 cases per year are reported from only four states (Kerala, Gujarat, Tamil Nadu and Maharashtra). Cases have also been reported from Andaman, Andhra Pradesh, Assam, Goa, Delhi, Karnataka, Orissa, Pondicherry and Uttar Pradesh. In 1966, human leptospirosis was reported in Delhi.<sup>2</sup> Recently, West Bengal, Punjab, Haryana and Himachal Pradesh have also been affected by leptospirosis.<sup>5</sup>

Diagnosis of leptospirosis may be made by demonstration of leptospire microscopically in blood or urine, by isolating them in culture or by inoculation of guinea pigs, or by serological tests. However, microscopy requires immunofluorescence technique or dark ground

illumination which is not available at every laboratory. Due to high degree of cross reactivity between the large number of serotypes of *Leptospira*, identification of isolates by culture techniques is a complicated procedure and is generally confirmed by one of the WHO/FAO Reference Laboratories.<sup>6</sup>

IgM ELISA has been extensively used among serological tests for diagnosis of leptospirosis in low resource settings. IgM antibody in leptospirosis begins as early as first week of illness and may take months or years to decrease. Using a single acute MAT as a reference standard, IgM-ELISA has a sensitivity of 86.0% and specificity of 84.5%.<sup>7,8</sup> Though some studies have shown that the PPV as well as the sensitivity and specificity of this test to be as low as to be 52.3% and 66.4% respectively.<sup>9,10</sup>

Even though the disease is endemic in India, they are less reported from New Delhi and surrounding regions. Currently the true prevalence of this disease in North India is not known. The aim of the study is to estimate the seropositivity of infections by *Leptospira* in patients presenting with undifferentiated acute fever, in a tertiary care hospital, New Delhi.

## METHODS

This prospective observational study was conducted from November 2016 to March 2018. Blood samples of the patients having fever were submitted to the tertiary care hospital in New Delhi, where serological testing was done with informed consent.

Patients with history of fever for more than one week, from 5 to 60 years of age and seronegative for typhoid, dengue, chikungunya and malaria were included in the study. Samples of HIV positive patients or patients with any other immunodeficiency condition or on cancer chemotherapy were excluded. A total of 370 samples seronegative for typhoid, dengue, chikungunya and malaria were included in the study. Clinical findings were recorded on pre designed proforma.

Blood samples were collected and centrifuged at 1000 rpm for 5 minutes to collect serum and IgM *Leptospira* test (DRG International Inc., USA), a qualitative enzyme immunoassay for the detection of antibodies to *Leptospira biflexa* in samples of human serum or plasma was performed according to the manufacturer's instructions.

Samples interpreted as non-reactive (0.0-0.15 OD) indicate antibody is not present in the sample. Weakly reactive samples (>0.15-1.0 OD) are infrequent in normal population but possible. Samples interpreted as strongly reactive (>1.0 OD) may indicate presence of antibody.

Data analysis was performed using the SPSS windows version 21.0 software. Data obtained from this study was

also analyzed using descriptive statistics such as percentage and proportion.

## RESULTS

Out of 370 patients, 142 (38.4%) were males and 228 (61.6%) were females. Samples were taken from patients aged 5 to 60 years; the mean age was 32.18 years.

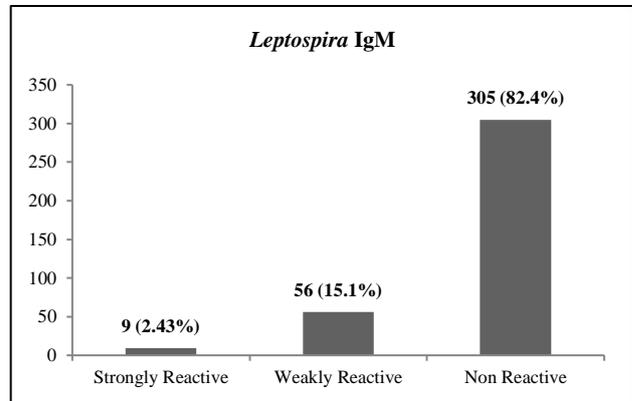


Figure 1: *Leptospira* IgM ELISA results.

Out of total 370 cases, 2.43% (9) cases were strongly reactive by *Leptospira* IgM ELISA, whereas 15.1% cases (56) were found to be weakly reactive (Figure 1).

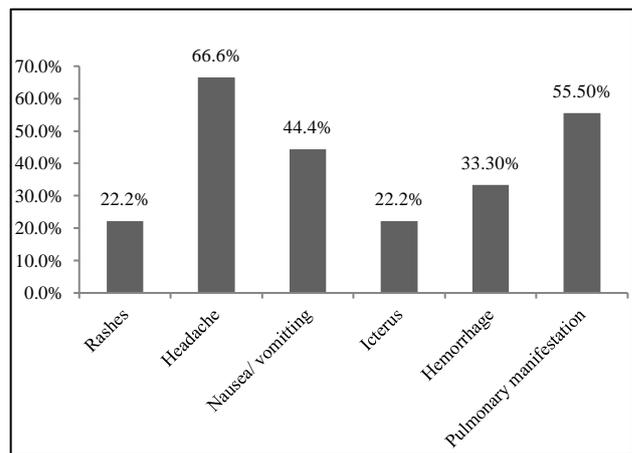
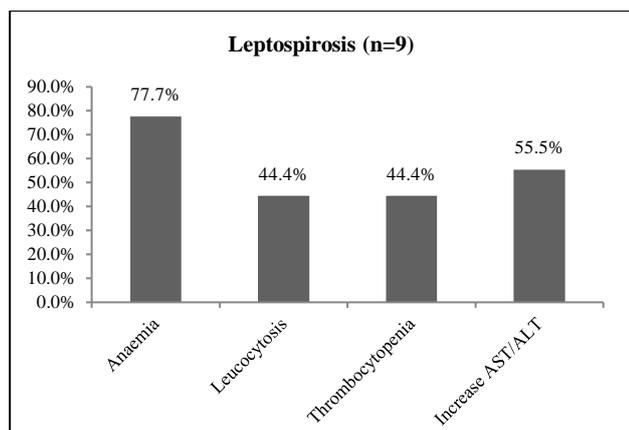


Figure 2: Distribution of signs and symptoms in for *Leptospira* IgM strongly reactive cases (n=9).

For strongly positive cases, duration of fever varied from 7-12 days. The most common symptom was headache (66.6%), pulmonary manifestations (55.5%) and nausea/vomiting (44.4%). Haemorrhage was seen in 33.3% cases whereas icterus and rash were present only in 22.2% cases as shown by Figure 2.

The laboratory parameters among *Leptospira* strongly positive cases, anaemia (77.7%) was the most common finding (88.8%) followed by increased ALT/AST (55.5%), thrombocytopenia (44.4%) and leucocytosis (44.4%) as shown in Figure 3.



**Figure 3: Laboratory manifestations in *Leptospira* IgM strongly reactive cases.**

## DISCUSSION

Leptospirosis is an emerging infectious disease with increasing incidence in both developing and developed countries. Though it is sub-clinical or mild in most cases, severe illness can sometimes end fatally.<sup>10</sup> This disease remains underreported because most of the infections are asymptomatic or mild hence often go unnoticed or there is a lack of awareness on the part of the treating physicians or the lack of diagnostic techniques.<sup>2</sup> There has been a scarcity of data on leptospirosis from Delhi.<sup>4</sup>

The total seropositivity in this study was 2.43% by use of IgM ELISA. In a study conducted by Chaudhary et al, the seropositivity was reported to be 26.90%.<sup>4</sup> The reason for low prevalence could be explained on the basis that previous studies included only clinically suspected cases of *Leptospira* whereas this study included all febrile cases irrespective of other manifestations. Also, weakly positive cases were not considered. The probable reasons for spread of leptospirosis could be that rodents have an enormous ability to excrete large number of leptospire in the urine, which is the main source of contamination incriminating human and animal leptospirosis.<sup>11</sup> Apart from rodents, both wild and domestic animals such as cattle, pigs and dogs also serve as reservoirs of leptospire. In India, it is *Rattus rattus* that has been found reservoir for several serovars. The number of cases is higher during and after heavy rain falls. The peak incidence is seen during the monsoon season; August-September.<sup>2</sup> Our study supports the warning from other researchers regarding the threat of leptospirosis in areas such as northern India. Preventive measures should be initiated and rapid and definitive diagnostic tests must be developed and made available in all laboratories.<sup>2</sup>

The clinical presentation is difficult to distinguish from dengue, malaria, influenza and many other diseases characterized by fever, headache and myalgia. Similar clinical presentation was seen in study conducted by Gupta N et al where headache (50%) and myalgia (50%) are common entities but rash (10%) was uncommonly

seen in leptospirosis. Pulmonary manifestations were present in 50% cases whereas icterus was present in 40% of leptospirosis cases. Among laboratory findings, most common was transaminitis (40%) followed by leucocytosis (30%) and thrombocytosis (20%).<sup>9</sup>

IgM ELISA has extensively been used for diagnosis of leptospirosis in low resource settings. IgM antibody in leptospirosis begins as early as first week of illness and may take months or years to decrease. It is easily available and results can be obtained within an hour. Using a single acute MAT as a reference standard, IgM-ELISA has a sensitivity of 86.0% and specificity of 84.5%.<sup>7,8</sup>

Out of 9 strongly positive *Leptospira* IgM cases, 2 were found to be cross reactive with Weil-Felix OXK antigen. It has been shown in a previous study, the possibility of cross reactivity as a major issue with ELISA.<sup>12</sup> So, a patient diagnosed with leptospirosis on the basis of IgM ELISA may turn out to be falsely positive with antibodies against scrub typhus cross reacting with antigen for leptospirosis and vice versa. Also, rheumatoid factor which is an IgM antibody against the Fc portion of IgG, is known to interfere with indirect ELISA and may cause false positivity. Several cross reactions of leptospirosis has been reported with dengue, hepatitis E, enteric fever etc. in the past.<sup>9</sup> Most of the previous reports of co-infection have also used IgM ELISA. Whether these reported cases are actually co-infection is a point of contention.<sup>9</sup> Hence, in this study there is a strong chance that the serological dual positivity is because of cross reactivity.

The limitations of this study were that microagglutination test (MAT) could not be performed for confirmation of positive cases. Also, in many cases the samples were collected at 7-8 days of febrile illness that could have led to low seropositivity of this infection due to low level of antibodies in the early phases of illness.

## CONCLUSION

Although the results obtained in our study may not be considered conclusive, they probably reflect certain lacunae in the laboratory diagnosis of leptospirosis. Also, it shows that leptospirosis is one of the causes for undifferentiated fever and active surveillance is required to know exact magnitude and distribution of vectors and diseases.

There is a definite need to increase awareness and heighten the suspicion, especially in the light of increasing number of patients presenting with non-specific manifestations.

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