



ORIGINAL RESEARCH PAPER

Clinical Microbiology

A CLINICO-EPIDEMIOLOGICAL STUDY ON LEPTOSPIROSIS IN WEST BENGAL: A NEGLECTED CAUSE OF ACUTE FEBRILE ILLNESS

KEY WORDS: *Leptospira*, ELISA, West Bengal, acute febrile illness.

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ABSTRACT

Introduction: Leptospirosis is a major neglected public health problem and is highly underreported in India. It is a common cause of the acute febrile illness (AFI), but accurate diagnosis is quite challenging and often delayed because of overlapping symptoms with many other infectious diseases, and limited access to laboratory diagnosis. **Aim:** The aim of the study was to assess the proportion of *Leptospira* infection among patients suffering from AFI in West Bengal and to analyse socio-demographic characteristics, clinical features, and laboratory parameters of leptospirosis cases. **Materials and methods:** Serum samples were collected from 350 hospital-admitted patients suffering from AFI (fever >five days ≤ two weeks) but without any identifiable cause and tested for *Leptospira* IgM using PanBio *Leptospira* IgM ELISA kit. Clinical features, laboratory parameters and epidemiological data were collected from each patient and analysed. **Result:** 91 (26%) out of 350 fever cases tested positive for *Leptospira* IgM ELISA. Most of them were male and belonged to the age group of 31–60 years (42.85%) and 16–30 years (30.76%). Common symptoms were fever (100%) myalgia (76.92%), headache (70.32%), icterus (69.23%), vomiting (67.03%) and pain abdomen (65.93%). **Conclusion:** Leptospirosis is a common cause of acute undifferentiated febrile illness in West Bengal. Our findings demonstrated the importance of active surveillance of leptospirosis among cases of acute febrile illness to facilitate its early detection and prevention of complications.

INTRODUCTION:

Leptospirosis is presumed to be one of the most widespread as well as neglected zoonosis in the world¹, caused by thin, helical, Gram negative bacteria, belonging to genus *Leptospira*. Though animals such as rodents, dogs, cattles, pigs etc act as the primary host, humans can acquire infection accidentally through direct or indirect contact with water or soil contaminated by urine of infected animals.² Portals of entry of *Leptospira* include cuts and abrasions or mucous membranes such as the conjunctival, oral, or genital surfaces.

The incidence of leptospirosis is significantly higher in tropical countries than in temperate regions³, mainly due to longer survival of *Leptospira* in the warm, humid environment. Leptospirosis, also known as “the Great Mimicker”, remained under-diagnosed due to its varied clinical presentations.⁴ In humans the disease ranges from sub-clinical infection to severe syndromes of multi-organ dysfunction with high mortality rate. In India, the disease is endemic in Kerala, Tamil Nadu, Gujarat, Maharashtra, Karnataka and Andamans.

It has also been reported from Andhra Pradesh, West Bengal, Orissa, Uttar Pradesh, Delhi and Puducherry.^{5,6} It is one of the most common under reported zoonotic diseases particularly in West Bengal due to paucity of literature describing the actual burden, varied clinical manifestations and subsequent complications of the disease.

Laboratory confirmation of human leptospirosis mainly aimed at the detection of specific antibodies in serum samples. Though the microscopic agglutination test (MAT) is considered as the standard serological test,⁷ this assay is technically demanding and expensive.⁸ On the other hand, Enzyme Linked Immune-Sorbent Assay (ELISA) can detect *Leptospiral* IgM fifth (5th) day onwards from appearance of symptoms using a single serum sample. The aim of this study was to assess the proportion of leptospirosis among acute febrile illness cases in Kolkata and surrounding districts; and to determine the socio-demographic characteristics, clinical features, and laboratory parameters of those patients.

MATERIALS AND METHODS:

Study place and design:

A hospital based retrospective cross-sectional study was conducted at Calcutta School of Tropical Medicine, Kolkata, West Bengal. The study protocol was reviewed and approved by the Institutional Clinical Research and Ethics Committee.

Study population:

350 hospital admitted patients suffering from acute febrile illness (fever >five days but ≤ two weeks) during the period April 2019 to March 2020, were included in the study. All these patients were screened for the common causes of acute febrile illness, such as Dengue, Malaria, Scrub typhus, Typhoid fever and Chikungunya. Other relevant causes as suspected by the treating physician were also ruled out.

METHODS:

Serum separated from clotted blood samples (about three ml) were collected and submitted to the referral Virology laboratory at the Calcutta School of Tropical Medicine. Specimen collection was done according to standard protocol and transported maintaining cold chain. Serum samples were initially stored at 4°C in the refrigerator and tested within 24- 48 hrs. IgM antibody capture (MAC) ELISA was performed using PanBio Leptospira IgM ELISA kit; strictly following the manufacturer’s protocol.

Statistical analysis:

Detailed history regarding socio-demographic characteristics, clinical features, complications and various laboratory parameters was taken from patients/patients parties (in case of minor and seriously ill), recorded and analysed.

RESULTS:

Among 350 AFI cases, 91(26%) tested positive for *Leptospira* IgM by ELISA method. Out of those 91 cases, 58 (63.73%) were male patients [Fig; 1]. Majority of the leptospirosis cases were in the age group of 31-60 years (39, i.e; 42.85%) and 16–30 years (28, i.e; 30.76%), thus affecting mainly the working population [Fig; 2]. Month wise distribution revealed an upsurge of cases during monsoon, post monsoon and early winter. [Fig;3]

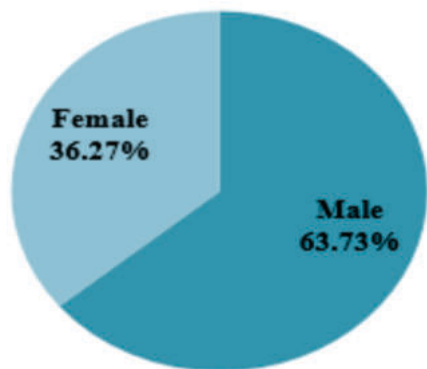


Figure 1: Gender distribution of leptospirosis cases

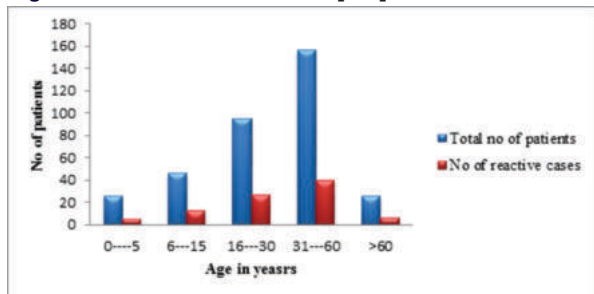


Figure 2: Distribution of leptospirosis specific IgM seropositivity among different age groups.

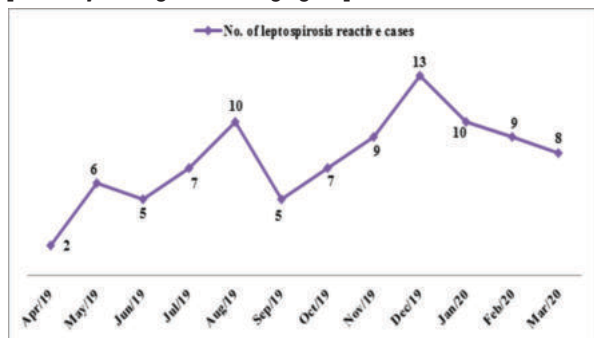


Figure 3: Seasonal distribution of leptospirosis reactive cases.

Water-logging following rainfall, presence of surrounding paddy fields and sugarcane plantation were common associated environmental factors and were noticed in 62(68.13%), 34 (37.36%) and 6 (6.59%) cases respectively. Fifty (50) i.e 54.94% leptospirosis patients resided in kutchas houses. Sixty four (64) i.e 70.33% patients had history of outdoor activities; among whom 17(26.56%) were school students, 14(21.87%) were associated with agricultural works, 8(12.5%) were factory workers, 7(10.94%) were hawkers or street vendors, 4(6.25%) each were water tank mechanics and municipal sewage cleaners. Rest 10(15.63%) patients were found to be engaged in other non-specific manual work.

Apart from fever, other common clinical presentations were myalgia (70, i.e. 76.92%), headache (64, i.e. 70.32%), icterus (63; 69.23%), vomiting (61; 67.03%) and pain abdomen (60; 65.93%). Other two interesting findings; haematuria and calf muscle tenderness were detected in 47 (51.64%) and 29 (31.86%) patients respectively. The prevalence of various symptoms/ signs has been summarized in Table-1.

Table 1: Clinical presentations among the leptospirosis IgM sero-positive patients, included in the study (n=91)

Clinical presentations	No. of patients	Percentage (%)
Fever	91	100
Myalgia	70	76.92
Headache	64	70.32
Jaundice	63	69.23
Vomitting	61	67.03
Pain abdomen	60	65.93
Haematuria	47	51.64
Calf muscle tenderness	29	31.86
Hepato-splenomegaly	29	31.86
Cough	23	25.27
Loose stools	22	24.17
Skin rashes	20	21.97
AES features	19	20.87
Hepatomegaly	17	18.68
Lymphadenopathy	14	15.38
Edema	14	15.38
Arthralgia	12	13.18
Respiratory distress	09	9.89
Malena	02	2.19

Among the laboratory parameters, leukocytosis (>11000/cmm) was common finding (44; 48.35%) while leukopenia (<4000/cmm) was seen only in 7 (7.69%) patients. Rest 43.96% patients had normal leucocyte count (4000-11000/cmm). Fifty (54.94%) out of 91 reactive patients had platelet count within normal limits (1,50,000–4,50,000)/cmm. Thirty six (39.56%) patients had thrombocytopenia (platelet count < 1,50,000/cmm) and only 3 out of these 36 patients had platelet count < 50,000/cmm. Hyperbilirubinaemia was quite common finding in this study and total serum bilirubin level more than 1.2mg/dl was found among 63(69.23%) patients. Twenty three (25.27%) out of 91 *Leptospira* IgM reactive patients had serum bilirubin >5mg/dl. Both serum aspartate transaminase i.e AST (>80 IU/L) & serum alanine transaminase i.e ALT (>80 IU/L) were found raised among 46 (50.54%) and 45 (49.45%) patients respectively. Most of the leptospirosis patients presented with elevated serum lactate dehydrogenase i.e LDH - 27(29.67%) patients had moderate (280–500 IU/L) elevation of LDH, while 58(63.73%) had serum LDH >500 IU/L.

Commonest complication found among the sero-positive cases was renal impairment. 30 (32.96%) patients had elevated levels of serum urea (>40mg/dl) and 23 (25.27%) had serum creatinine level >1.5mg/dl. Routine and microscopic examination of urine revealed albuminuria in 45 (49.45%) patients, presence of RBCs in urine among 36 (39.56%) patients and presence of occult blood in urine

among 11 (12.08%). Another important complication associated with leptospirosis was central nervous system involvement. Nineteen patients developed features of Acute Encephalitic Syndrome (AES), among whom 7 (36.84%) patients had history of convulsion and 6 (31.57%) presented with altered sensorium. More than half of these 19 patients (10; 52.63%) had normal (50-80 mg/dl) CSF glucose level, 14 (73.68%) had CSF LDH level >40 IU/L and 11 (57.89%) had CSF micro-protein level > 60 mg/dl. Fifteen (78.94%) patients had increased CSF cell count (≥ 5 cells/cmm), out of which 3 patients had CSF cell count more than 100 cells/cmm and in majority of the cases there was absolute lymphocytic predominance.

Chest radiogram was routinely performed for all patients, most of them are within normal limits. 12 (13.18%) patients showed presence of pleural fluid in the lung and only one patient had nodular opacity in lung field.

USG whole abdomen was advised in all patients routinely and the findings revealed 29(31.86) patients had hepatosplenomegaly and 17 (18.68%) patients had only hepatomegaly. One patient each presented with complications like acalculous cholecystitis and pancreatitis.

Table 2: Laboratory parameters associated with the Leptospira IgM sero-positive patients, included in the study (n=91)

Laboratory parameters	No of cases	Percentages (%)
Haemoglobin (7-9 gm/dl)	26	28.57
Haemoglobin (> 9 - 11 gm/dl)	40	43.95
Leukocytosis (>11000/cmm)	44	48.35
Leucopenia (<4000/cmm)	07	7.69
Thrombocyte (<1,50,000/cmm)	34	37.36
Thrombocyte (<50,000/cmm)	03	3.29
Bilirubin (>1.2 mg/dl)	63	69.23
Aspartate Transaminase(>80IU/L)	46	50.54
Alanine Transaminase(>80IU/L)	45	49.45
Serum Urea (>40mg/dl)	30	32.96
Serum Creatinine (>1.5mg/dl)	23	25.27
Serum Lactate Dehydrogenase (>500IU/L)	58	63.73
Serum Alkaline Phosphatase (>140IU/L)	40	43.95
Serum Albumin (<3mg/dl)	41	45.05

DISCUSSION:

In our present study, 91 (26%) cases were detected leptospira IgM reactive out of 350 suspected cases from different districts of West Bengal during the period of April 2019 – March 2020. Though there is not enough clinico-demographic studies on leptospirosis in north and eastern India as compared to south⁹ in 2003, Sethi et. al. reported seroprevalence of leptospirosis as 21.7% from Varanasi¹⁰ and another study in 2013 reported 35.64% from West Bengal.¹¹ In our present study, male preponderance was noted (male-63.73%, female-36.27%) which is consistent with other studies^{12,13} which can be explained by more outdoor exposure of males due to their occupational and behavioural habits.¹⁴ In this study, 70% of the patients were outdoor workers and mainly associated with occupations like agriculture, factory work, street vending, water-tank cleaning and sewage cleaning. Other risk factors included wet environmental living conditions, infestation of in-dwelling rodents and lack of personal hygiene. In our study, 62(68.13%) patients resided in area with water-logging problems, and 48 (52.74%)

patients resided in an area with improper waste management facilities. Majority of previous studies from different parts of India showed the maximum numbers of leptospirosis cases during monsoon and post-monsoon period. Our study also showed a similar pattern with an extension into the winter months.

Leptospirosis is an acute febrile illness with various non-specific signs and symptoms. Apart from high grade fever which was the inclusion criteria, the other common clinical presentations were myalgia, headache, vomiting, jaundice, abdominal pain followed by cough, loose stools, hematuria and skin rash. Sethi et al reported 73% cases of leptospirosis with jaundice from a tertiary care centre in Northern India which corroborate with our study, i.e, 69.23%.

Moderate (Hb level 7 to 9 gm%) to mild (Hb level >9 to 11 gm%) anaemia was seen in 26 (28.57%) and 40 (43.95%) cases respectively in the present study which corroborates with the study of Sethi et al where 57% cases of leptospirosis had anaemia (Hb level < 10gm%).¹⁰ Both leukocytosis and leucopenia can be seen in leptospirosis. In this present study 44(48.35%) patients had leukocytosis while only 7(7.69%) showed leucopenia. The studies by Sethi et al and Holla et al reported leukocytosis (>11000/mm³) in 61.6% and 50.6% cases respectively. However Chawla et al in their study encountered leucocytosis (>11000/mm³) in all their patients.¹⁵

Present study revealed 36 (39.56%) patients had thrombocytopenia (< 150000 /cmm) and 3 out of them had platelet count below 50,000/cmm while Ramesh et al in his study found thrombocytopenia in 54% cases.¹⁶

Total serum bilirubin level more than 1.2mg/dl was found among 63(69.23%) patients in our study and this finding corroborated with the study conducted by Holla et al who reported elevated serum bilirubin (>1.2 mg/dl) in 67.7% leptospirosis cases. Chauhan et al reported elevated serum bilirubin in 79% patients.¹⁷

46 (50.54%) patients had raised (>80 IU/L) serum levels of AST (SGOT), 45(49.45%) had raised (>80 IU/L) serum levels of ALT (SGPT) and 40(43.95 %) had raised ALP (>140 IU/L) serum levels in our present study. Holla et al in his study reported raised serum AST (73.4%), serum ALT (61.2%), and serum ALP (50.7%).¹⁸

In this present study, 21(23.07%) patients presented with hypoalbuminaemia (<3g/dl) and 41(45.05%) patients had hypoproteinaemia (<6g/dl). The study of Holla et al reported 60.1% patients with hypoalbuminaemia (<3.2g/dl) and 53.1 %patients had hypoproteinaemia (<6g/dl).¹⁸

Renal dysfunction as indicated by elevated levels of Serum Urea (>40mg/dl) was seen in 32.96% cases and elevated levels of serum creatinine (>1.5mg/dl) in 23.07%. Deodhar et al in his study showed elevated levels of serum creatinine level in 8.5% cases.⁹

Hematuria during the early phase of the illness is quite common in leptospirosis and in our study 47(51.64%) patients were found to have hematuria. Jauréguiberry S. et al.¹⁹ and Daher et al²⁰ reported hematuria in 58% and 43% cases respectively in their studies.

In the present study, 11 (57.89%) out of the 19 leptospirosis patients with AES features had elevated CSF micro-protein level (>60mg/dl) and majority (52.63%) of patients had normal CSF glucose levels. Fifteen (78.94%) patients had increased cell count (5 or more), out of which only 3 had more than 100 cell count in CSF.

Eight (8.79%) patients had hyper-echogenic kidneys with

decreased cortical thickness, which might indicate acute kidney injury. Both serum urea and creatinine level of all these 8 patients were elevated ranging (114–450) mg/dl and (3.62 – 11.3) mg/dl respectively. All of these patients presented with edema. Microscopic examination of urine revealed presence of albumin in all these 8 patients while plenty of RBCs were found in 5 out of them.

One patient each developed pancreatitis and acalculous cholecystitis as major complications similar to the study conducted by Dalamaga et al.²²

The patients were either treated with Doxycycline (100mg BD for 10 days in adult) or Ceftriaxone (1gm IV) and there was prompt recovery.

CONCLUSION:

Leptospirosis is a significant public health problem mainly affecting the young population engaged with outdoor activities. Clinical features of leptospirosis may vary from mild illness to severe syndromes of multi organ dysfunction. Late detection and subsequent delayed initiation of treatment may cause development of life threatening complications with fatal outcome.

So a high index of suspicion among the clinical fraternity, while treating patients presenting with acute febrile illness and subsequent early diagnosis with the help of low cost, easy-to-perform method is necessary to avoid undue complications. More and more multi-centric studies can bring out better insight to epidemiology, clinical profile and laboratory parameters of leptospirosis.

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