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Original Research Article

Clinical profile of leptospirosis with focus on inflammatory biomarkers

Srinath K. M., Adarsh L. S.*, Aboobacker Siddiq, Madhu B., Prasanna Kumar H. R., Mahesh M. G.

Department of General Medicine, JSS Medical College, Mysore, Karnataka, India

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***Correspondence:**
Dr. Adarsh L.S.,
E-mail: adarshls.adi@gmail.com

ABSTRACT

Background: In the last few decades leptospirosis has re-emerged and is on the rise. Infectious diseases like malaria, dengue, infective hepatitis which simulate leptospirosis being rampant, so there is a need to know the clinical profile and biomarkers of leptospirosis. Study aimed to understand the clinical profile, laboratory findings and complications of leptospirosis with focus on inflammatory biomarkers.
Methods: All consecutive inpatients of J.S.S medical college hospital with clinical suspicion of leptospirosis were screened for IgM anti-leptospiral antibody. Twenty-five patients who were positive for IgM antileptospiral antibodies were considered as cases and twenty-five patients who were admitted for fever other than leptospirosis were taken as controls and subjected to detailed history, clinical examination, investigations and followed up till discharge or death.
Results: The age group commonly involved was 18-55 years (84%). Most of the patients were males (76%), with occupation involving outdoor activity (66%). Common symptoms were fever (100%), musculoskeletal pain (92%), headache (80%) and jaundice (64%). Less commonly seen were respiratory symptoms (36%), bleeding (24%), and gastrointestinal symptoms (16%). Important signs seen were conjunctival suffusion, hepatomegaly, splenomegaly and hypotension. Hyperbilirubinemia was predominantly of conjugated type and elevation of transaminases and alkaline phosphatase were mild to moderate. Mean bilirubin was 4.67mg/dl, Mean SGOT and SGPT were 99.23U/L and 96.97U/L respectively. ARF was seen in 48% and six of them needed haemodialysis. Thrombocytopenia was seen in 76% patients. 28% had multi-organ failure (MOF), 8% had aseptic meningitis, 8% had ARDS and 4% had GI haemorrhage.
Conclusions: Musculoskeletal symptoms are most common next to fever. Conjunctival suffusion is a common finding. Liver and kidney are more commonly involved organs. MOF was significantly associated with mortality ($p<.05$). Thrombocytopenia was significantly related to clinical bleeding ($p<.001$). TNF-alpha and Interleukin-12 were significantly elevated in leptospirosis and may correlate with severity.

Keywords: Conjunctival suffusion, IgM ELISA, Interleukin-12, MOF, Thrombocytopenia, TNF-alpha

INTRODUCTION

Leptospirosis, a zoonosis with protean manifestations occurs worldwide and is characterized by great clinical variability, ranging from a mild flu like illness to an acute life-threatening condition.[1] In the last few decades, infectious diseases like leptospirosis have re-emerged and are on the rise. It has hit virtually all parts of urban, semi urban, semi-rural and rural India in last decade.[2] Leptospirosis cases have been reported throughout the year at majority of hospitals in India.[3] It occurs sporadically throughout the year, with a peak seasonal incidence in rainy season in tropical regions and the late summer to early fall in temperate regions.[4] Large epidemics can occur after monsoons and periods of heavy rainfall.[3] The concept that leptospirosis always presents in the form of typical Weil's disease has crept into the minds of the medical professionals due to very low

priority given to the disease in medical education. Extremely variable clinical presentation is common not only in India but in other countries also. The possibility that leptospirosis can present in varied forms is often forgotten while making a clinical diagnosis.[5]

With other infectious diseases like Viral fever, Malaria, Dengue and Infective hepatitis which simulate leptospirosis being rampant, we need to know the clinical profile, complications and biomarkers of leptospirosis and hence there is a need for a study, particularly from this part of the country. The objective of the study was to describe the clinical features, complications, course, outcome and biomarkers in leptospirosis in comparison with other acute febrile illnesses.

METHODS

Source of data

Patients with acute febrile illness admitted to the department of Medicine, JSS Hospital during the study period October 2009 to October 2011 fulfilling the inclusion and exclusion criteria were enrolled for the study.

Method of collection of data

During the study period all consecutive patients admitted with clinical suspicion of leptospirosis were screened for immunoglobulin M (IgM) anti-leptospira antibodies. IgM antibody detection was done by ELISA performed using IVD Leptospiral Igm Microwell ELISA kit from USA detecting genus specific antibodies (Leptospira biflexa). Antibody titres more than 80 U/ml were taken as positive in single sera.

All patients with fever, headache, generalized bodyaches, associated with at least any one of the symptoms and signs jaundice, oliguria, cough, hemoptysis, breathlessness, meningeal signs with altered sensorium, bleeding tendencies including conjunctival suffusion were screened for IgM anti-leptospiral antibody by ELISA technique.

Inclusion criteria

All acutely febrile patients who are above 18 years of age with positive IgM anti-leptospira antibody by ELISA technique were considered as cases. Patients with acute febrile illness (CF features simulating leptospira) other than leptospira were taken as controls.

Exclusion criteria

Patients who are HBsAg positive, below 18 years of age and who had Diabetes mellitus were excluded from the study.

Sample size and study design

Out of 90 patients with clinical suspicion of leptospirosis, 25 patients were found to be positive for IgM anti-leptospira antibody by ELISA technique. These were included as cases and subjected to detailed clinical history, examination and investigations. Patients were followed up till death or discharge. Patients with acute febrile illness other than leptospira were taken as controls. All patients were treated as per protocol.

Patients were subjected to following investigations; complete hemogram, IgM anti-leptospiral antibody by ELISA, peripheral blood smear for malaria parasite, urine for albumin and microscopy, urine for dark ground microscopy, chest radiograph, blood urea, serum creatinine, liver function test, electrocardiogram, USG abdomen, lumbar puncture with CSF analysis in patients with altered sensorium, Biomarkers for IL-6, IL-8, IL-10, Il-12, TNF-Alpha were done among study subjects. The interleukin and TNF Alpha kits used were Diaclone ELISA kits which are solid phase sandwich ELISA for in-vitro qualitative and quantitative determination of interleukins and TNF-alpha in supernatants, buffered solutions or serum and plasma samples.

Clinical definitions used in the study

- Oliguria: urine output less than 400ml in 24hrs;
- Anemia: hemoglobin less than 10gm/dl;
- Leucocytosis: total WBC count more than 11000/µl;
- Thrombocytopenia: platelet count less than 150000/µl;
- Hypotension: systolic blood pressure less than 100 mmHg;
- Hepatic involvement: total bilirubin more than 1mg/dl and elevated enzymes; Elevated transaminases: more than 40u/L; Elevated alkaline phosphatise: more than 125u/L;
- Acute renal failure: creatinine more than 1.5 mg/dl;
- Multi organ failure: simultaneous presence of physiologic dysfunction of two or more organs.

Statistical methods

All the collected data were entered in excel sheet and all statistical analysis were carried out using SPSS for Windows (Version 16.0). Manufactured by IBM, USA. For the categorical data. Percentages are calculated and presented. The data of Cytokines (IL-6, IL-8, IL-10,1L-12 and TNF Alpha) were not normally distributed, hence log10 transformation was done to bring the data to normal distribution.

Independent t-test was applied to find the mean difference in the cytokine levels for cases and controls and a p value of less than 0.05 was considered to be statistically significant.

RESULTS

Majority of the Leptospirosis patients were aged between 18 to 25 years and 36 to 45 years with a mean age of 35.6±12.17 years. Majority of the patients were male patients (60%) and were farmers by occupation (Table 1).

Table 1: Demographic profile of leptospirosis cases (n=25).

Variables	Category	Number	%
Age (In years)	18-25	9	36
	26-35	2	8
	36-45	9	36
	46-55	4	16
	56-65	1	4
Gender	Male	15	60
	Female	10	40
Occupation	Farmer	8	32
	Housewife	8	32
	Student	4	16
	Business	2	8
	Manual labour	2	8
	Cattle rearing	1	4

Table 2: Clinical characteristics of leptospirosis cases (n=25).

Clinical features	Categories	Number	%
Duration of fever (In days)	<5	3	12
	5-7	12	48
	>7	10	40
Symptoms	Musculoskeletal	23	92
	Headache	20	80
	Conjunctival suffusion	17	68
	Jaundice	16	64
	Respiratory symptoms	9	36
	Bleeding manifestations	6	24
	GI symptoms	4	16
Complications	Hepatic involvement	16	64
	Acute renal failure	12	48
	Multiorgan dysfunction	10	40
	Aseptic meningitis	2	8
	Respiratory distress syndrome	2	8

Out of the 25 patients, 48% had fever for 5 to 7 days, 40% had fever for more than 7 days. Common symptoms associated with fever were musculoskeletal (92%), headache (80%), jaundice (64%) and less common symptoms being respiratory symptoms (36%), bleeding manifestations (24%), altered sensorium (22%) and gastrointestinal symptoms (16%). Conjunctival suffusion was the commonest sign that was picked up among 68% of the Leptospirosis patients and 70.5% these patients had low platelet count. Liver (64%) was the commonest organ involved followed by the kidney (48%). Multi organ failure was seen in 40% of patients (Table 2).

Elevated Bilirubin (Conjugated fraction) was a common laboratory finding seen in 72% of patients, mild to moderate elevation of transaminases were seen in 68% of patients, Creatinine was elevated in 48% of patients. Thrombocytopenia was the commonest haematological abnormality observed in 76% of patients. Anaemia (40%) and leukocytosis (16%) were less common (Figure 1).

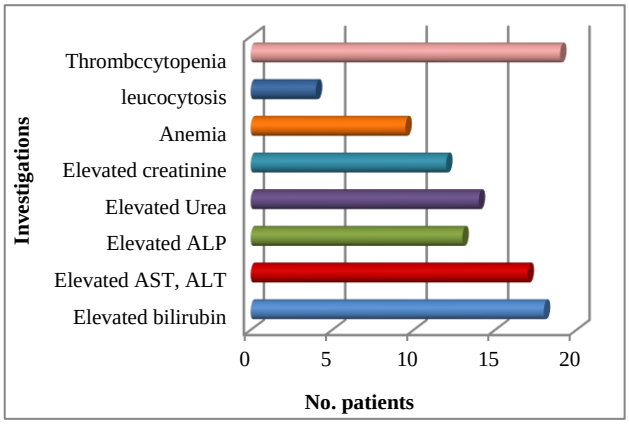

Figure 1: Laboratory findings in leptospirosis cases (n=25).

Acute renal failure was seen as a single complication in 4% of the patients while hepatic involvement was seen in 32% of the patients. Multi organ failure was seen in 40% of the patients and were significantly associated with mortality (Figure 2).

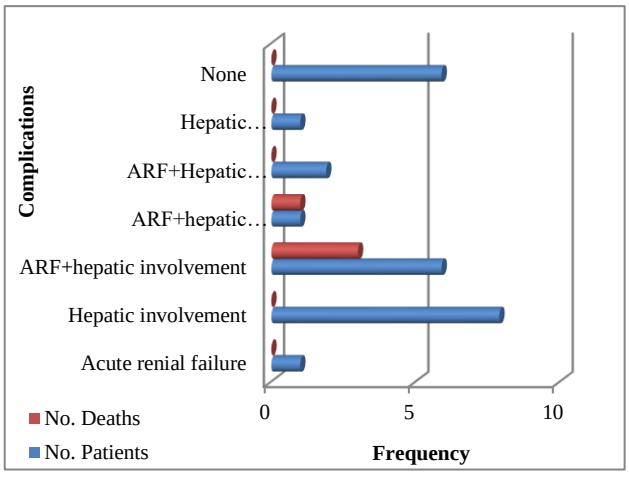

Figure 2: Mortality in relation to complications associated with Leptospirosis (n=25).

Majority of the patients were discharged in 7 to 10 days. Longest stay was 22 days for a patient with multi organ failure. There was a tendency towards increasing mean

hospital stay with the increased number of complications (Figure 3).

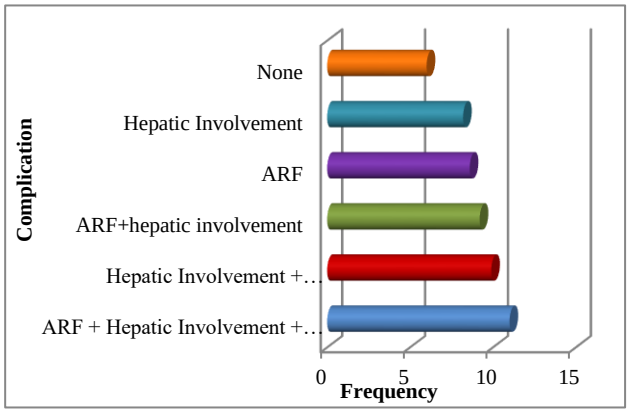

Figure 3: Mean-duration of hospital stay of survivors in relation to complications.

From the table it is observed that, mean interleukin levels of IL-6, IL-8,1L-12 and TNF Alpha were high among Leptospirosis (Cases) except IL-10. However, this difference for IL-12 was statistically significant (Table 3).

Table 3: Comparison of cytokine levels among leptospirosis (cases) and other febrile illness (controls).

Biomarkers	P-value	Cases	Controls
Interleukin 6	0.165	13.523±2.20	10.01±1.774
Interleukin 8	0.455	34.43±1.43	31.47±1.61
Interleukin 10	0.019	10.63±2.67	21.16±2.65
Interleukin 12	0.040	75.56±3.67	39.03±2.36
TNF-alpha	0.667	92.64±2.53	82.35±2.69

DISCUSSION

Leptospirosis, a zoonosis with protean manifestation occurs worldwide and is characterized by great clinical variability, ranging from mild flu-like illness to an acute life-threatening condition.[1] The possibility that leptospirosis can present in varied forms is often forgotten while making a clinical diagnosis.

Thus, the present study was undertaken with an objective to describe the profile, laboratory finding and complications with focus on the inflammatory biomarkers of leptospirosis. In a study conducted in Tamil Nadu, leptospirosis was found to be the cause in 38% of pyrexia of unknown origin and similar observation was found in Surat where 40% of suspected cases showed IgM antileptospiral antibodies during 1997 outbreak.[6,7] Our study is comparable to these studies. Positivity was reported to be 15% in a preliminary survey in Delhi slums.[5] As this study included patients with all febrile illnesses, lower incidence might have been observed.

Age of the patients in our study ranged from 18 -65 years with mean of 36.78 years. Most of our patients were aged between 18 to 55 years (96%).This was comparable to a study by Muthusethupathi et al where mean age of patients was 39.6 yrs with a range of 17 to 72 years and a study by Singh et al, where commonest age group affected was 21 to 40 years.[6,8] Seventy percent of our patients were males, Similar observation were made in other studies where Singh et al documented 86.2 % male patients.[8] And Muthusethpathi et al documented 88% male patients being affected with Leptospirosis, which is attributable to their outdoor activities.[6]

Occupation of patients in our study revealed that occurrence of the disease to be more common in people engaged in outdoor activities like farmers (32%), manual labour (6.25%) and non-manual labour (14%). This was comparable to a study by Muthusethupathi et al where 49% were outdoor workers, 8.8% were outdoor non-manual workers.[6] And according to Alan et al 28% were farmers, 16% were outdoor manual labourers and 31% had no formal employment.[9] Although traditionally considered, occupation as a risk among persons exposed to contaminated water or infected animal urine, leptospirosis is now being recognized as one of the common causes of febrile illness in tropical environments worldwide.

All patients had fever with chills and duration of fever ranged from 4 to 15 days. Other common symptoms were musculoskeletal in the form of myalgia or muscle tenderness (92%), headache (80%) and jaundice (64%). Muthusethupathi et al, have found clinical features of fever (100%), myalgia (82%), jaundice (85%), Oliguria (72%), bleeding (25%) and altered sensorium (49%).[6] Similarly, Viviani-M et al, have found clinical features of fever (98%), Jaundice (40%), myalgia (72%) and bleeding (25%).[10]

Although the incidence of nonspecific constitutional symptoms reported in our study are comparable to these studies, incidence of organ involvement is different. This may be due to different infecting serovars, environment and host factors.

Conjunctival suffusion was seen in most of our patients (68%). It has been noted as being pathognomonic for leptospirosis.[9] It is due to conjunctival haemorrhage. Conjunctival suffusion has been reported by Muthusethupathi et al in 58% of their patients and by Singh et al in 50% of their patients.[6,8] Other findings seen in present study included hepatomegaly (32%), splenomegaly (14%) and hypotension (12%). Singh et al have found clinical features as fever (100%), myalgia (97.9%), jaundice (51.7%), oliguria (20.7%), altered sensorium (12.1%), hepatomegaly (6.9%) and hypotension (40%). The probable cause for hypotension was volume depletion secondary to vomiting, diarrhoea or haemorrhage. Although earlier studies have reported myocarditis among their patients with hypotension, ECG

findings in our patients did not suggest myocarditis to attribute it for hypotension.[11,12] However, hypotension responded to parenteral fluid administration.

Organ involvement

Liver was the most common organ involved among our patients (64%). It was found as part of multiorgan failure in 40% of patients and alone in 24% of patients. Clinical jaundice was seen in 64% of patients.

However, hepatomegaly was seen only in 32% of patients. Majority of patients had conjugated hyperbilirubinemia of mild to moderate degree. Severe hyperbilirubinemia (>6mg/dl) was seen in 20% of patients. Hepatic encephalopathy was not seen in our study patients. Hyperbilirubinemia was accompanied by mild to moderate elevation of transaminases in 68% of the patients (Mean SGOT 99.23U/L, mean SGPT 96.97U/L). Liver function tests returned to normal without leaving any sequelae in survivors.

According to Muthusethupathi et al, Liver was involved in 84% of patients with predominant conjugated hyperbilurubinemia.[6] Clerke et al have shown liver involvement in 71.05% of their patients.[13] Herve Dupont et al have demonstrated liver involvement in 85.29% of patients. All patients who died had MOF including liver involvement.

Kidney was the next common organ involved. Tubulointerstitial nephritis is the main cause of acute renal injury in leptospirosis leading to ARF.[14] Renal involvement was seen in 48% of patients and it was found as part of Multiorgan failure in 40% of patients and alone in 8% of patients. Mild to moderate elevation in creatinine levels was seen in majority while 10% had severe ARF (>6mg/dl).

Six patients in our study required haemodialysis with mean requirement of 5.5 haemodialysis sessions per patient. Muthusethupathi et al also have noted oliguric ARF in all their patients with renal Involvement.[6] According to Muthusethupathi et al, kidney was involved in 71.92% of patients.[15] Clerke et al have observed renal involvement in 63.15% of their patients.[13]

Thrombocytopenia was the most common haematological abnormality in our patients, mild to moderate in (66%) and severe thrombocytopenia was seen in 10%. According to Muthusethupathi et al, thrombocytopenia has been reported in 22%.[6] Edwards et al have shown thrombocytopenia in 81% of patients.[16]

One patient had aseptic meningitis presented with altered sensorium with meningeal signs, without convulsions and neurological abnormalities, with CSF showing elevated protein concentration and lymphocytic pleocytosis. CT scan of brain did not reveal any abnormalities. Singh et al have noted neurological involvement in small proportion of patients but they do not have much prognostic importance as no patient suffered from severe or residual central nervous system sequelae.[8]

Only one patient had pulmonary complication in form of ARDS and needed ventilator support. He succumbed on 8th day after admission.

Pulmonary manifestations were frequently observed in patients with leptospirosis (20% to 70%) in several earlier studies.[12,17-19] Lower incidence of pulmonary involvement in our study may be due to infecting serovars being different. Moreover, mortality tends to occur in the 1st week of disease itself which may lead to underestimation in patients presenting late in their illness.

Multi organ failure was seen in 40% of our patients and was significantly related to mortality (P<.05). As number of complications increased, mortality also increased. However other studies found MOF in 45 to 72% of the patients.[14,15,20]

On Assessing the inflammatory biomarkers in leptospirosis comparing with other fever cases showed that the TNF-alpha was elevated in 60% of the study cases of leptospirosis compared to 24% in other fever cases. The Interleukin-12 was positive in 40% of the leptospirosis cases compared to 24% in other fever cases. Interleukin-6, IL-8 and IL-10 were positive in equal number in both the groups. The TNF-alpha and IL-12 were found to be elevated in 3 out of the 4 patients who expired in our study, which signifies some relation of these inflammatory biomarkers as a prognostic indicator of Leptospirosis.

In a study conducted by Anna papa et al showed that TNF-α levels were linked with pulmonary hemorrhagic implications, while elevated sTNFR1 and IL-10 levels linked with fatal cases. IL-6 and IL-8 did not seem to affect the outcome of the disease. IL-10/TNF-α ratio is proposed as a marker for prognosis.[21]

In another study conducted by Mainho et al showed the presence of TNF-alpha and IL-6 in renal tissues by immunohistochemically examination. Both TNF-α and IL-6 were associated with the immunopathogenesis of leptospirosis.[22]

The limitation of the work was as the study has small sample size, the pattern of cytokines levels in patients may not indicate specific role as a marker of disease severity or progression and the cytokine elevation could be a mediator of pathogenesis of a disease.

Hence, larger prospective study of cytokine evaluation in leptospirosis would be an ideal method for the better understanding of the role of cytokines in the pathogenesis of severe leptospirosis. This may further help in identifying potential therapeutic target intervention.

CONCLUSION

Musculoskeletal symptoms are most common next to fever. Conjunctival suffusion is a common finding. Liver and kidney are more commonly involved organ. MOF was significantly associated with mortality ($p<0.05$). Thrombocytopenia was significantly related to clinical bleeding ($p<0.001$). TNF-alpha and Interleukin-12 were significantly elevated in leptospirosis and may correlate with severity.

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