



Seroprevalence of Leptospirosis at a Tertiary Care Hospital

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ABSTRACT

Background & Objectives: *Leptospirosis is a zoonotic disease worldwide that has emerged as an important cause of morbidity and mortality among impoverished populations. It is a global condition that is found across the world. Rates of leptospirosis are highest in tropical and sub-tropical locations, particularly in rural areas. This study was done to determine the prevalence of leptospirosis infection at Government Medical College, Thiruvananthapuram and to determine the month to month variation. The seroprevalence data are useful to assist preventive strategies. Study was conducted in Government Medical College, Trivandrum during the period March to August of 2015.*

Method: *252 Sera from patients were subjected to IgM Leptospira ELISA test. A total of 13 seropositive samples were analyzed by PCR. 11 positive samples having higher OD values in IgM ELISA were sent to REGIONAL MEDICAL RESEARCH CENTRE for performing Microscopic Agglutination Test (MAT).*

Results: *24 among 252 samples were found to be positive serologically by IgM ELISA. Maximum of 9 leptospirosis cases were found during May. PCR was positive for 3 samples. 7 samples gave positive results in MAT and 2 of them were found to be serovar Lai.*

Interpretation & Conclusions: *The overall seropositivity of leptospirosis was 9.52% with maximum cases during the month of May. Since leptospirosis is a treatable disease, correct and rapid diagnosis may help in effective management of patients.*

Keywords: *Leptospirosis, Seroprevalence, MAT, IgM ELISA, PCR.*

INTRODUCTION

Leptospirosis is an acute bacterial infection caused by spirochetes belonging to the genus *Leptospira* that can lead to multiple organ involvement and fatal complications. It has a wide geographical distribution and occurs in tropical, subtropical and temperate climatic zones¹. In the developed world cases that occur are associated with exposure to contaminated water. The incidence seems to be increasing in developing countries. Some countries, where leptospirosis is

under surveillance, have recorded this increase in incidence². Most countries in the south East Asia region are endemic to leptospirosis. The International Leptospirosis Society (ILS) made an attempt to compile data on occurrence of leptospirosis in various countries and the data showed that tens of thousands of severe cases occur annually world-wide³. This could only be an under-estimate as only a small number of countries participated in the survey and even in those countries; leptospirosis surveillance is far

from complete. Leptospirosis is considered as the most widespread zoonosis in the world⁴. Leptospirosis affects human beings and many other species of vertebrates. It can present in a wide spectrum of clinical manifestations in human beings⁵. The syndrome of icteric leptospirosis with renal involvement is referred to as Weil's disease. Another recognized clinical form is that presenting with severe pulmonary haemorrhage⁶. Other complications include acute respiratory failure⁷, myocarditis⁸, meningitis and renal failure⁹. Uveitis has recently been recognized as a late complication of leptospirosis¹⁰. Pulmonary haemorrhage is perhaps the most fatal complication in leptospirosis. Serovar Lai belonging to serogroup Icterohaemorrhagiae had been incriminated as a cause of leptospirosis with haemoptysis as the predominant symptom in China and Korea¹¹. Serovars Canicola and Pomona were involved in the 1995 outbreak in Nicaragua¹². Serogroup Canicola was responsible for an outbreak of leptospirosis with pulmonary haemorrhage in Orissa, India after the super-cyclone in 1993¹³. In Andaman Islands, a few serovars belonging to the serogroup Grippityphosa including Valbuzzi have been isolated from cases of leptospirosis with pulmonary haemorrhage¹⁴. The other most common fatal complication is renal failure. Leptospirosis, being a zoonotic disease with a large variety of animal species acting as carriers, is difficult to eliminate and perhaps even control in tropical developing countries. The bacteria are adapted to the environment of the tropical region with plenty of rainfall and it is often difficult to avoid exposure of the people to animals or contaminated environment. Because of this, early case detection and prompt treatment and creating awareness about the disease among the people and public health professionals are the steps that could be taken to reduce the magnitude of the problem. Different laboratory methods are available. Serology by IgM ELISA for the detection of leptospirosis is the test used commonly now. Other tests like Polymerase Chain Reaction,

Microscopic Agglutination Tests etc. are time consuming and hence ELISA is placed in topmost priority in Serology

METHODOLOGY

Cross-sectional study conducted in a tertiary care centre ,south India. The study population includes all the clinically suspected cases of leptospirosis from March 2015 to August 2015. All blood samples received from patients of all age group with fever less than 10 days duration with clinical features suggestive of leptospirosis were included in this study. Blood samples were allowed to clot, centrifuged at 2500 rpm for 5 minutes and sera separated were stored at 2 to 8°C . The samples were subjected to IgM ELISA. A total of 13 positive samples were taken to Manipal Centre for Virus Research, Manipal, Karnataka (ICMR Virology Network Laboratory- Grade-I, Ministry of Health and Family Welfare, Government of India) and analyzed by PCR.11 positive sample shaving higher OD values in IgM ELISA were sent to REGIONAL MEDICAL RESEARCH CENTRE (Indian council of Medical Research, Department of Health Research, Ministry of Health & Family Welfare, Govt. of India and WHO Collaborating Centre for diagnosis, Reference, Research and Training in Leptospirosis, PORT BLAIR, ANDAMAN & NICOBAR ISLANDS, INDIA for performing Microscopic Agglutination Test (MAT) .

RESULTS

Table 1 Seropositivity of leptospirosis in SD IgM Leptospira ELISA

IgM ELISA Number Percentage	Number	Percentage
Positive	24	9.52 %
Negative	228	90.48 %
Total	252	100%

Table 2: Age group and Seropositivity

Age group in years	Number of cases
0-15	6
16-30	1
31-45	8
46-60	8
>60	1

Table 3 : Gender wise distribution of seropositive cases

IgM ELISA Number Percentage	Number	Percentage
Males	14	58.30%
Females	10	41.67%
Total	24	100%

Table 4: Month wise analysis

Month	Number of Positive cases	Number of Negative cases
March	2	40
April	8	80
May	9	67
June	3	18
July	1	13
August	1	10

Table 5 : Analysis of various Clinical Features

SI: NO	Clinical features	Positive
1	Myalgia	23(95.83%)
2	Icterus	21(87.50%)
3	Conjunctival Congestion	14(58.33%)
4	Renal Symptoms	13(54.16%)
5	Aseptic Meningitis	1(4.16%)
6	Calf muscle tenderness	1(4.16%)

Table 6: Complications

SI: NO	Complications	Positive
1	Renal Failure	6(25%)
2	Hepatic encephalopathy	2 (8.33%)
3	Myocarditis	1 (4.16%)
4	ARDS	3 (12.5%)
5	Haemorrhage	2 (8.33%)
6	Death	1 (4.16%)

Table 7 Serovars detected by MAT

SI NO	Serovars by MAT	Number of positives
1	L. Australis	0
2	L. Bankiang	0
3	L. Canicola	1
4	L. Grippotyphosa	1
5	L. Hebdomadis	1
6	L. Icterohaemorrhagiae	1
7	L. Lai	2
8	L. Pomona	1
9	L. Pyrogenes	0
10	L. Hardjo	0
11	Unknown	4

DISCUSSION

During the period from March 2015 to August 2015, a total of 252 serum samples were collected from patients fulfilling the inclusion and the exclusion criteria mentioned. These 252 were

tested for the anti leptospira IgM antibody by SD Leptospira IgM ELISA. Among the 252 samples, 24 (9.52%) were found to be positive and 228 were negative (90.48%). A multi-centric study in India by Sehgal SC et al showed that leptospirosis accounts for about 12.7% of cases of acute febrile illness attending hospitals¹⁵. Among those 24 positive cases, 14 were males and 10 were females. Sex wise distribution of seropositive cases shows 58.30% in males and 41.70% females. Males contribute to predominance among the total number of participants as well as among the number of positives. In a study “Seroprevalence of Leptospirosis and Evaluation of three diagnostic kits” conducted at Government Medical College Thiruvananthapuram during 2003-2004 showed 60.90% positivity for males and 39.10% for females.

Age wise classification of seropositive cases showed maximum number of cases (8) both in age groups 31-45 and 46-60 indicating most cases occurred in people of working age group. 6 cases were in the below 15 age group. Between the ages 16 and 30, one case was there. Only 1 case was found to be positive above 60 years. A similar study conducted at Vellore by G. Vimala et al mentioned that leptospirosis mostly occurs in working age groups¹⁶. Month wise analysis of cases shows maximum of occurrence of leptospirosis during the month of May followed by April. Minimum cases were found to occur during July and August. Heavy rainfall and exposure were considered as an important correlation in diagnosing patients with leptospirosis in India. Since Kerala experienced inconsistent and less rainfall this year, it would have resulted in the minimal dissemination of the disease in human beings during the monsoon and postmonsoon season. If the rainfall had been heavy, the percentage would have been more. In a study conducted in Government Medical College Thiruvananthapuram during 2003-2004, the maximum leptospirosis cases were in the month of October which was 22 and there were 13 cases during the month of May. Most of the cases were

in the coastal belt. Inadequate care, scarcity of water, improper waste disposal and increase in rodent population may be increased seroprevalence especially in rural population in Thiruvananthapuram.

The protean clinical manifestations of leptospirosis are very well illustrated in the study. The non-specific symptoms often confuse the clinician and thus delaying diagnosis and therefore treatment, which has to be given early in the course of the disease. According to this study the most common clinical presentation was fever with myalgia (95.83%). Calf muscle tenderness was present in 4 of the positive cases (16.66%). Icterus was present in 87.5% cases and conjunctival congestion in 58.33% cases. Rise in serum bilirubin was detected in these cases and among these; a maximum value of 16.3 mg% of serum total bilirubin was detected. The maximum value obtained for Aspartate transaminase (AST) was 198 IU/L and for Alanine transaminase (ALT) was 100 IU/L. Renal symptoms include oliguria and anuria. Abnormalities in renal functional tests were shown by 54.15% cases. The maximum level of blood urea obtained was 309 mg% and serum creatinine 12.5 mg% for a patient. Peritoneal dialysis was done for this patient and was recovered.

This data represents the varied symptomatology of leptospirosis, the common clinical presentation being fever accompanied by myalgia, icterus and conjunctival congestion. Other nonspecific symptoms were also detected such as rash, cough, vomiting, diarrhoea, cervical lymphadenopathy, hepatomegaly, and localized hemorrhagic manifestations such as sub conjunctival hemorrhage, epistaxis, haemoptysis and malaena.

Leptospirosis is notorious for its fatal complications. In this study, the common complications encountered were renal failure, myocarditis, ARDS and localized hemorrhagic manifestations. One among the 24 leptospirosis cases died of complications. Death was due to renal failure. ARDS was present in 3 cases and haemorrhage in 2 cases. 1 case experienced

Myocarditis and Hepatic encephalopathy was present in 2 cases. Renal failure was found to occur in 6 cases among those one expired and one was recovered through peritoneal dialysis.

Most recognized complication in leptospirosis is severe pulmonary haemorrhage. In a study by Segura et al, the clinical spectrum of pulmonary involvement in leptospirosis was well studied¹⁷. Among 321 seropositives, 7 had severe pulmonary manifestations and 5 died. Among the expired cases, 4 were caused by pulmonary haemorrhage and remaining one was due to acute respiratory distress syndrome and multi-organ failure. Pulmonary haemorrhage is perhaps the most fatal complication in leptospirosis. Presumptive treatment should be given immediately in the appropriate epidemiological and clinical context.

Other complications include acute respiratory failure, myocarditis, meningitis and renal failure. Uveitis has recently been recognized as a late complication of leptospirosis. Renal failure can be a fatal complication but in most cases it can be reversed with conservative measures such as maintaining fluid and electrolyte balance and symptomatic therapy. Other complications such as meningitis rarely become fatal. Myocarditis may sometimes cause intractable hypotension and cardiac arrhythmias and might become fatal. In a study in Thailand, out of 121 seropositives, which included mainly male farmers, 17 died of complications leptospirosis. Causes of death included pulmonary haemorrhage in 8 (87.1%), renal failure in 3 (17.6%), multi-organ failure in 3 (17.6%), ARDS in 2 (11.8%), irreversible shock in 1 (5.9%)¹⁸. This indicates the spectrum of fatal complications associated with leptospirosis.

Microscopic Agglutination Test (MAT) was performed for 11 IgM Elisa positive samples at REGIONAL MEDICAL RESEARCH CENTRE (PORT BLAIR, ANDAMAN & NICOBAR ISLANDS, INDIA). MAT is the reference test described for leptospirosis. It detects both IgM and IgG antibodies against Genus *Leptospira*. It is laborious test that is very difficult to interpret. It utilizes a battery of live cultures of leptospira as

antigen, the maintenance of which are only possible in reference laboratories and also there is risk to lab personnel. MAT needs an assistance of Dark Field Microscopy, the interpretation of which may be subjective. Ideally MAT should be performed on paired sera collected during acute and convalescent stage of the disease to find out sero-conversion or four-fold rise in titre, which is the evidence of current or recent infection. However, collection of convalescent serum sample is difficult in routine practice. The cut-off titre for single MAT depends on the baseline titre in the community in a particular geographical region. The antibodies usually develop within 2-12 days after the onset of illness. IgM antibody starts appearing early in the course of the disease and reaches detectable levels within one week or as early as on third or fourth day of illness. MAT titres equal to or above 1 in 80 were taken as positive. A titre of 1 in 160 and above is considered as cutoff titre for endemic settings and a titre of 1 in 80 is considered as cutoff titre for non-endemic settings. A panel of 10 serovars were used while performing MAT. 7 samples gave positive results and 2 of them were having titre value 1 in 40. A very high titre of 1 in 5120 was observed for serovar Icterohaemorrhagiae. 2 of them were found to be serovar Lai. Rest of the 4 samples gave positive for serovars Canicola, Grippityphosa, Hebdomadis and Pomona. Two samples gave a titre of 1 in 40 for Pomona and Australis. In a study conducted at Government Medical College Thiruvananthapuram during 2003-2004, the most common serovar prevalent was found to be L. Icterohaemorrhagiae.

Polymerase Chain Reaction was performed at Manipal Centre for Virus Research for 13 samples. PCR is a rapid, sensitive and specific means of diagnosing leptospiral infection, especially during the first few days of the disease when antibodies are not fully detectable in serological tests. Conventional PCR was done in Applied Biosystems Veriti™ Thermal Cycler machine. Among these 13 samples, 3 gave positive results for *Leptospira* DNA. Rest of the

10 samples was negative for the presence of *Leptospira* DNA. This may be because these samples were collected in the immune phase of the disease and these were positive for *Leptospira* IgM antibody.

CONCLUSION

The seroprevalence of leptospirosis, common clinical presentation and complications were highlighted in the study. Seroprevalence of leptospirosis among the clinically suspected cases of fever is 9.52%. The common clinical presentation is fever accompanied by myalgia, icterus, and conjunctival congestion. Less commonly vomiting, diarrhea, malaena, cough and muscle tenderness are seen. Rare manifestations are rash, lymphadenopathy and sub conjunctival haemorrhage. Maximum number of cases occurred during the month of May. Expensive lab tests like PCR may not be available in many developing countries and remote areas. Microscopic Agglutination Test Procedure is very complex and time consuming and also requires experienced personnel. MAT requires 14–21 strains to be maintained in culture, which is often very difficult. *Leptospira* IgM detection ELISA can be used for general screening and diagnostic purposes. This study will help the authorities to undertake therapeutic as well as preventive measures to prevent the morbidity and mortality of leptospirosis.

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