Journal of Academia and Industrial Research (JAIR)
Volume 3, Issue 10 March 2015

ISSN: 2278-5213

RESEARCH ARTICLE

Seroprevalence of Leptospirosis among Febrile Patients–A Hospital Based Study

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Abstract

Leptospirosis is one of the most widespread and globally important zoonotic diseases. In India, it is emerged as an important cause of febrile illness with seasonal and geographical variation and affects humans on all continents, in both urban and rural area and in tropical and temperate climates. Most outbreaks of leptospirosis are reported in coastal regions. Leptospirosis has been recognized as an occupational disease. It is a frequent cause of undifferentiated febrile illness in developing countries. In order to understand the clinical spectrum, risk factors associated with leptospirosis and its prevalence, a retrospective hospital-based cross-sectional study was conducted in patients admitted with acute febrile illness in Govt. Medical College, Thiruvananthapuram over the last 1 year. Serum samples were screened for leptospirosis by IgM enzyme-linked immunosorbent assay (IgM ELISA). About 1924 patients who presented with an acute febrile illness in the year 2014 were screened for leptospirosis. Of these 220(11.4%) patients, were positive for *Leptospira* by SD-IgM ELISA. The overall seroprevalence of leptospirosis was 11.4%. Males were affected more than females in this study. The main age group affected was 41-50 years. There were antigenic cross reaction with IgM Dengue for 7 cases and IgM scrub typhus for 12 cases. This study revealed a high seroprevalence of leptospirosis among febrile cases, indicating the importance of doing leptospirosis investigation of febrile illness.

Keywords: Leptospirosis, prevalence, cross-sectional study, ELISA, febrile illness, Leptospira.

Introduction

Leptospirosis is now identified as one of the emerging infectious diseases. It has worldwide distribution, with a higher incidence in tropical climates. It is mainly seen after heavy rainfall (Levett *et al*., 2000). It is an infectious disease caused by bacteria of the *Leptospira* genus. *Leptospira* are thin coiled spirochaetes having hooked ends. It can be observed under dark field microscopy and its serovar specificity is conferred by its lipopolysaccharide surface antigen. The semisolid culture media for its cultivation include EMJH (Ellinghausen, McCullough, Johnson, Harris) medium, Korthof's medium, Fletchers medium etc. The genus *Leptospira* is divided into two species, *L. interrogans*, comprising all pathogenic strains and *L. biflexa*, containing the saprophytic strains. Both *L. interrogans* and *L. biflexa* are divided into several serovars. Based on their antigenic characteristics, over 60 serovars of *L. biflexa* and 250 serovars of *L. interrogans* are recognized. Large number of animals like rodents and other wild and domestic animals act as carrier. *Leptospira* can survive in the renal tubule of the infected animal for long period (Vijayachari *et al.,* 2008). Human infection results from accidental contact either directly or via water or soil contaminated with the bacteria shed in the urine of these carrier animals. These bacteria can survive in the environment for prolonged period. Infection occurs through abrasions or cuts in the skin or through the conjunctiva and mucous membranes.

Incidence of leptospirosis is influenced by occupational and environmental factors. It mainly occurs in agriculture workers, farmers and cattle workers. The spectrum of human disease caused by leptospirosis is extremely wide, ranging from subclinical infection to multi-organ failure with high mortality rate. Symptoms include acute febrile illness with headache, myalgia, abdominal pain, jaundice, conjunctival congestion and rarely skin rash. In severe cases renal failure, pulmonary hemorrhage, myocarditis, liver failure and bleeding manifestation can occur. Severe leptospirosis characterized by jaundice, renal failure and other haemorrhagic manifestation is termed as Weil's disease. Thrombocytopenia is due to *Leptospiral vasculitis* or due to the presence of undetectable antiplatelet antibody. Pathogenesis of leptospirosis is not understood completely, it may be due to vasculitis resulting in damage to endothelial cells of small blood vessel (Greenwood *et al.,* 1973). The clinical presentation of leptospirosis is biphasic with the acute phase lasting about a week, followed by the immune phase, characterized by antibody production and excretion of Leptospires in the urine. Without treatment, leptospirosis can lead to liver failure, kidney failure, meningitis and even death (Farr, 1995). Definite diagnosis of leptospirosis is based on laboratory finding. Most cases of leptospirosis are diagnosed by serology. IgM-ELISA is useful for detecting leptospirosis in the first week of fever which is the genus specific test.

Antibodies are detectable in the blood 5 to 7 days after the onset of symptoms. Immunity to leptospirosis is serovar-specific and conferred by specific antibodies consisting of IgM and IgG (Kuriakose *et al.,* 2008). Specific serovar is detected by microscopic agglutination test (MAT) and culture. Microscopic agglutination (MAT) is the reference method for serological diagnosis of leptospirosis, in which patients sera are reacted with live antigenic suspensions of 23 leptospiral serovars. After incubation, the serum-antigen mixtures are examined microscopically for agglutination and the titers are determined. A fourfold rise in titre between acute and convalescent sera with any of the antigen confirms the diagnosis. It is done only in reference laboratories. A rapid test to detect antibody is Lepto Tek Dri-Dot test which is a latex agglutination test. Most widely used molecular diagnostic method is PCR. Other laboratory parameters like urea, creatinine, bilirubin, SGOT and SGPT will be elevated and platelet count will be lowered. Several outbreaks of leptospira have been reported in recent years. Hence a study was conducted on prevalence of leptospirosis in patients presenting with an acute febrile illness in the tertiary care hospital.

Materials and methods

Study population: This study was done over a period of 1 year at the Dept. of Microbiology, Govt. Medical College and Sree Avittom Thirunal Hospital, Thiruvananthapuram who presented with an acute febrile illness.

Experimental design: Serum samples were screened for leptospirosis by IgM enzyme-linked immunosorbent assay test (IgM-ELISA). Clinical history of patients was also collected. The data were collected from case sheets maintained in the medical records department. The SD *Leptospira* IgM-ELISA test which is an indirect sandwich ELISA was used for the qualitative detection of IgM antibodies. The ELISA test is reported to have a sensitivity of 97% and a specificity of 98%. *Leptospira* negative samples were screened for Dengue, scrub typhus, Enteric fever, hepatitis A and E viruses for the diagnosis of PUO.

Result

There were 1924 patients who presented with an acute febrile illness in the year 2014. Of these, 220(11.4%) patients were positive by IgM-ELISA for *Leptospira.* Table 1 shows the month-wise analysis of the cases. It showed that *leptospira* positive cases were more in Nov and Dec [59(26.8%)]. More febrile cases were also in the month of Nov and Dec. In the month of Aug when there was heavy rainy fall in Thiruvananthapuram, the febrile cases were more and *leptospira* positive cases were also more. Table 2 shows that out of the 220 *leptospira* positive cases, there were 146(66.4%) males and 74(33.6%) females. The ratio is 2:1. Adult males were mainly affected. Table 3 shows the age-wise distribution of *leptospira* positive cases.

Table 1. Month-wise analysis of prevalence of *Leptospira* positive cases.

Month	Total number of sample tested	Number of positives
January	133	9
February	135	4
March	150	13
April	132	14
May	164	21
June	189	16
July	160	20
August	180	27
September	150	20
October	149	17
November	170	29
December	212	30
Total	1924	220

Table 2. Sex-wise distribution of *Leptospira* patients.

Sex	No. of positives
Male	146(66.3%)
Female	74(33.6%)
Total	220

Table 3. Age-wise distribution of *Leptospira* patients.

Age group	No. of positives
0-10	23
11-20	21
21-30	24
31-40	38
41-50	52
51-60	41
>61	21

Most common age group affected was 41-50(23.6%), followed by the age group of 41-50(18.6%) years. Children in the age group of 0-10 years also affected more. Table 4 shows the symptoms of leptospirosis associated with fever. Clinical symptoms associated with leptospirosis are mainly headache [189(85.9%)], Jaundice [185(84.1%)], decreased urine output [103(46.8%))], myalgia [124(56.3%)], nausea, vomiting [148(67.2%)] and abdominal pain [98(44.55%)]. Other symptoms like conjunctival congestion [26(11.8%)], loss of appetite, weakness, dyspnoea, bleeding manifestation were also seen rarely. Table 5 shows the complications that occurred in *Leptospira* positive patients. Complications like renal failure 63(28.6%), Hepatic failure 48(21.8%), ARDS 7(3.1%), myocarditis 3(1.3%), developed in some patients. Out of the 220 *leptospira* positive cases, five patients expired due to complications mainly hepatorenal failure, ARDS and myocardits. Complications were mainly seen in elderly adults. Hemodialysis was done in these patients and they were ventilated. Other investigations like liver function test (LFT), renal function test (RFT), blood routine, Platelet count were done. About 185(84.1%) *leptospira* patients had increased bilirubin, 63(28.6%) had elevated urea and creatinine. Platelet count was decreased in 149(68%) cases.

Table 4. Associated symptoms with *Leptospira* patients.

Symptom	No. of cases
Headache	189(85.9 %)
Myalgia	124 (56.3%)
Abdominal pain	98(44.55%)
Conjunctival congestion	26(11.8%)
Nausea, vomiting	148(67.2%)
Jaundice	185(84.1%)
Oliguria	103(46.8%)

Table 5. Complications.

Complication	No. of cases	Percentage
Acute renal failure	63	28.6%
Hepatic failure	48	21.8%
Acute respiratory distress syndrome	7	3.1%
Myocarditis	3	1.3%

Discussion

A hospital-based cross-sectional study was conducted among febrile patients to study the prevalence and the complications and other clinical features. About 1924 patients presented with an acute febrile illness in the year 2014 were screened for IgM-ELISA. Of these, 220(11.4%) patients were positive for *leptospira* antibody. A multi-centric study in India showed that leptospirosis accounts for about 12.7% of cases of acute febrile illness responsible for attendance at hospitals (Victoriano *et al.,* 2009). It is emerging as an important public health problem in India. Leptospirosis is endemic in many areas of Kerala (Shivakumar, 2008). Leptospirosis appears to be on the increase in Kerala, Tamil Nadu and Andamans during the last two decades, probably due to increased farming and inadequate rodent control (John, 1996). Prevalence of leptospirosis in this study was 11.4%. In the previous year it was 9%. This shows that the prevalence is increasing. According to this study, *leptospira* positive cases were more in Nov [29(13.1%)] and Dec [30(13.6%)] and also in Aug [27(12.2%)]. Statistical analysis in this hospital for the year of 2013 also showed that *leptospira* positive cases were more at the time of heavy rain fall (Jul and Aug). It is correlated by the study conducted by Vimala *et al.* (2014). Rainfall is having an important correlation in diagnosing leptospirosis in India. Out of the 220 *Leptospira* positive cases, there were 146(66.4%) males and 74(33.6%) females and the ratio is 2:1. It is correlated by the study conducted by Antony *et al*. (2012) in Dept. of Community Medicine, M.O.S.C Medical College, Kolenchery (Male: Female ratio 1.96:1). The study conducted by Kar *et al.* (2013) in Dept. of Microbiology, MGM Medical College, Mumbai had the similar finding. High incidence of males may be due to their work in high potential infection areas like farm, sewage and mines. In this study, maximum number of *Leptospira* positive patients was in 41-50 age groups. It is correlated with the studies of Vimala *et al.* (2014) indicating a higher probability of occupational exposure being responsible for the increase of leptospirosis in this age group. Children in the age group of 0-10 years were also affected more. Children can be at risk by playing in infected water or having contact with infected animals (CDC, 2005). All (100%) *Leptospira* positive patients had fever. Other clinical symptoms and signs were mainly headache [189(85.9%)], Jaundice [185(84.1%)], nausea and vomiting [148(67.2%)], myalgia [124(56.3%)], Oliguria [103(46.8%)] and abdominal pain [98(44.55%)]. Other symptoms like conjunctival congestion [26(11.8%)], loss of appetite, weakness, dyspnoea, bleeding manifestation are also seen rarely. The clinical manifestations were similar in other studies on leptospirosis like Myrna *et al.* (2013) and Sehgal (2006).

Complications like renal failure 63(28.6%), hepatic failure 48(21.8%), ARDS 7(3.1%), myocarditis 3(1.3%), developed in some patients. Similar complications reported in other studies. In the study on leptospirosis in Calicut by Shivakumar and Krishnakumar (2006) recorded hepatic (69.8%), renal (56.3%) involvement and the study on leptospirosis from Kottayam indicated hepatic (80%), renal failure (59%) and hypotension (20%). Similar complications were by Maroun *et al*. (2011). Leptospirosis can become considerably dangerous if not treated, potentially leading to kidney damage, meningitis, liver failure and respiratory problem. Thrombocytopenia was reported in 149(68%) *Leptospira* positive cases. Thrombocytopenia was reported in the studies by Maroun *et al.* (2011) in New York and study conducted by Singh and Tiwari (2012). Out of the 220 Leptospira positive cases, five patients expired; three were adult females and two adult males. Death occurred due to complications mainly hepatorenal failure, ARDS and myocardits. The highest mortality rates were seen among the elderly and those with Weil's syndrome.

The *Leptospira* positive patients were treated with inj. Crystalline Penicillin and cap. Doxycyclin (100 mg) orally twice daily for 7 days. Patients with severe leptospirosis were treated as inpatients. In hepatorenal failure cases, inj. thiamine, Inj. VitK, Lactulose, bowel wash, correction of hypokalemia were done. In patients having severe bleeding manifestations, platelet transfusion was also done. In severe renal failure cases, hemodialysis was needed. In children along with fever, other clinical manifestations were abdominal pain, hepatosplenomegaly, nausea, vomiting etc. Hepatic and renal failure was not reported in paediatric age group as the complications of leptospirosis. They were treated with inj. Crystalline Penicillin and supportive care. CDC recommends chemoprophylaxis with Doxycycline (200 mg orally, weekly), begun 1-2 days before and continuing through the period of exposure for people at high risk of leptospirosis.

Doxycycline is not recommended for pregnant women or children aged <8 years. Doxycycline is effective in decreasing the severity and duration of leptospirosis and should be initiated early in the course of the disease if leptospirosis is suspected. Intravenous Penicillin is a drug of choice for patients with severe leptospirosis. Patients with severe leptospirosis may require hospitalization, supportive therapy and close monitoring. Prevention is by effective rodent control, avoiding exposure to urine from infected animal, avoiding taking bath in contaminated water etc. For person at occupational risk, he should wear protective clothing, especially footwear and covering cuts and abrasions with occlusive dressings (Rao, 2006).

Conclusion

Leptospirosis is emerging as an important public health problem in India. It is endemic in many parts of Kerala and considered an occupational disease of persons engaged in agriculture, sewage works, forestry, and animal slaughtering. The presentation may range from a subclinical infection to a severe syndrome of multi-organ dysfunction. Serodiagnosis by a microagglutination test (MAT) is the gold standard, but is not universally available. Leptospirosis can be easily diagnosed using a latex agglutination test and IgM-ELISA. IgG antibodies persist in the blood for many years**.** Variable degrees of thrombocytopenia have been reported with leptospirosis. The pathogenesis of thrombocytopenia in leptospirosis is not well understood. Acute renal failure is one of the most common complications of severe leptospirosis. Renal leptospirosis is a combination of acute tubular damage and interstitial nephritis. A serious type of lung involvement called severe pulmonary hemorrhagic syndrome is considered to be a major cause of death in patients with Weil's disease in developing countries. In Weil's syndrome mortality rate is 5-10%. Important causes of death include renal failure, hepatic failure, cardiopulmonary failure and widespread hemorrhage. Antibiotic treatment should be started as soon as possible on clinical suspicion of leptospirosis because, organ damage usually develops by the second half of first week and late antibiotic treatment does not influence the outcome.

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