ORIGINAL RESEARCH PAPER

Volume-7 | Issue-2 | February-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A SEROLOGICAL STUDY OF LEPTOSPIROSIS IN JSS HOSPITAL,MYSURU

Microbiology

Dr. Tejashree. A* Professor, JSS Medical college & Hospital, Mysuru *Corresponding Author

Pooja P. B Research scholar, JSS Medical college & Hospital, Mysuru

Bhoomolla Rajitha PG student, JSS Medical college & Hospital, Mysuru

ABSTRACT

Leptospirosis, the most widespread zoonosis, is emerging as a major public health problem. The clinical manifestations of human leptospirosis are diverse, ranging from mild, flu-like illness to a severe disease form known as Weil's syndrome

Materials and Methods: A total number of 100 suspected cases were included in the study during the period January 2015 to December 2015. All the samples were subjected to ELISA and ICT.

Results: Out of the 100 samples 39 were positive with ELISA, 01 was positive with ICT. All the leptospirosis positive cases had fever 39(100%) followed by, head ache 34(87.17%), fever followed by chills and rigors 18(46.15%),vomiting 23(58.97%),fever followed by jaundice 10(25.64%) and hepatomegaly 3(7.69%).

Conclusion: We strongly suggest suspicion of leptospirosis by the physicians is possible even during summer season as this disease occurs throughout the year. This forces medical professionals to perform continuous surveillance programme for leptospirosis.

KEYWORDS

ICT-Immuno chromatographic Test, ELISA - enzyme linked immunosorbent assay

Introduction

Leptospirosis, is a zoo anthroponosis of worldwide distribution, is an acute febrile illness caused by spirochetes of the pathogenic Leptospira species. Leptospira are actively motile, delicate spirochetes, possessing a large number of closely wound spirals and characteristic hooked ends .They are too thin to be seen under light microscope. Human hosts commonly acquire infection through skin abrasions and mucosal surfaces following contact with water or soil contaminated with urine of infected rodents or other mammals. Leptospirosis has a wide range of clinical manifestations, from a simple febrile illness to a severe and potentially fatal illness characterized by acute kidney injury, liver derangement, pulmonary haemorrhage, bleeding, and cardiac involvement. In most clinical settings, there is limited availability of specific diagnostic tests, and treating physicians often rely on clinical features to make a probable diagnosis of leptospirosis. This is indeed a problem in areas of high incidence of other infections with similar clinical picture, such as dengue, rickettsial infection, malaria and hantavirus infections[1].

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 [2]. Since the 1980s, outbreaks are being increasingly reported especially from the states of Tamil Nadu, Kerala, and Karnataka [3]. Laboratory diagnosis of leptospirosis is based on several methods: the microscopic agglutination test (MAT), detection of organism DNA by polymerase chain reaction (PCR), isolation of the organism through culture methods, or detection of antibodies to the organism[4]. Isolation of Leptospira spp. from clinical samples has low diagnostic sensitivity, requires specialized expertise, and most importantly takes too long to be of use to the treating team[5].

Several rapid serological diagnostic tests have been developed as alternatives to MAT, of which a commercial immunoglobulin M enzyme-linked immunosorbent assay (IgM ELISA) is promising because it can be performed in a greater number of laboratories throughout the tropics, and is inexpensive compared with MAT. The IgM ELISA has been recommended by the World Health Organization (WHO) as a diagnostic test for the serodiagnosis of leptospirosis where healthcare resources are limited,[6].Although its reported accuracy is variable. A number of studies have reported that IgM ELISA has high sensitivity and specificity for the diagnosis of acute leptospirosis.[7-9]

Materials and methods

A prospective based study was conducted in the department of microbiology JSS hospital Mysuru for a period of one year January 2015-December 2015, A total of 100 consecutive patients who came to this hospital during the above period from various places in and around Mysuru, Southern India were included. This study was approved by our Institutional Human Ethical Committee (IHEC). Written informed consent was obtained from all patients prior to collection of blood samples. About 3ml blood was collected in Clot activator tube and the serum was separated and kept frozen at -20°C till the time of testing.

Inclusion criteria

High grade fever with or without chills and rigors; fever with rash/ eschar/hepatosplenomegaly/jaundice/lymphadenopathy/thrombocyto penia; fever with constitutional symptoms like malaise, myalgia, nausea, vomiting

Exclusion criteria

Samples which were positive for Widal ,weil felix or Mal Card were excluded from the study.

Serum samples collected from subjects were tested serologically for anti-Leptospira IgM antibodies by a quantitative enzyme linked immunosorbent assay (ELISA) from PanBio, Brisbane, Australia and by Immuno chromatographic Test (ICT) from Bio Standard Diagnostics, Haryana. The test procedure was performed according to the protocol provided along with the kit.

Leptospira species was determined using a commercially available Leptospira IgM ELISA (Panbio Pty., Ltd., Queensland, Australia). The assay was performed according to the manufacturer's instructions. Briefly, test sera, cutoff calibrator, and positive and negative control sera were diluted 1:100 in serum diluent, and 100 µL added to Leptospira antigen-coated microwells and incubated for 30 minutes at 37°C. After washing with phosphate-buffered saline containing 0.05% Tween 20, 100 µL of HRP conjugated anti-human IgM was added and incubated for another 30 minutes at 37°C plates covered with parafilm . After further washing, 100 µL of tetramethylbenzidine substrate was added and incubated at room temperature for 10 minutes, after which the reaction was stopped with 100 µL of 1 M phosphoric acid. The absorbance of each well was read at a wavelength of 450 nm with a reference filter of 600-650nm. The results were expressed as Panbio units calculated by the ratio of sample absorbance to the mean cutoff absorbance multiplied by 10. The recommended cutoff for a positive result is a value of > 11 Panbio units, and is interpreted by the manufacturer to indicate recent infection of leptospirosis.

Immunochromatography test for Leptospira IgM was carried out by commercially available SD BIOLINE LEPTOSPIRA IgG/IgM kit. The assay was performed according to the manufacturer's instructions. Using capillary pipette 5 µL of sera is added to the square sample well marked as “S” and 4 drops of assay diluents to the well and the result is interpreted after 20 min .The positive result is the control line (C) and IgM line (M) ,IgG line (G) will be visible on the test device .

Results :

A total of 100 blood samples were collected from the clinically

suspected cases of leptospirosis ICT and IgM ELISA were performed on 100 serum samples obtained from patients with signs and symptoms of leptospirosis from January 2015 to December 2015. Out of the 100 samples 39 were positive with ELISA, 01 was positive with ICT. Comparison of ICT and IgM ELISA was done. Results of ICT and IgM ELISA are presented in table 1.

Table 1: Showing comparison between ICT and ELISA

Test	Positive	Negative
ICT	01(1%)	99(99%)
ELISA	39(39%)	52(52%)

Leptospirosis was found more common among male 32(82.05%) than female 7(17.94%). Patients in the age group of 31-40 years 15 (38.46%) were found predominantly infected due to leptospirosis followed by the age group 21-30 9 (23.07%), 51-60 years (15.38%) and 11-20 years 71-80 years 2(5.12%).

In the month of June 2015, we noticed number of Leptospirosis positive cases more than compared to April and May months but the suspected numbers of cases were higher during the month of May 2015 this may be due to the increased rainfall.

All the leptospirosis positive cases had fever 39(100%) followed by, head ache 34(87.17%), fever followed by chills and rigors 18 (46.15%), vomiting 23(58.97%),fever followed by jaundice 10 (25.64%) and hepatomegaly 3(7.69%).

Table 2 showing clinical features of leptospira positive patients

Clinical features	Number	Percentage %
Fever	39	100
Head ache	34	87.17
Chills and rigors	18	46.15
Vomiting	23	58.97
Loose motion	17	43.58
Abdominal pain	13	33.33
Nausea	12	30.76
Jaundice	10	25.64
Hepatomegaly	03	7.69

Discussion

In the present study, we found 39(39%) IgM ELISA positive where as that of ICT was only (1)1% positive. This was similar in the study conducted by R. Chaudhry et al[10], with 42%IgM ELISA ,37% Smitha B. Shekatkar [11]. Comparatively high incidence percentage was seen in many other studies like Krishna Kanchan Sharma et al [12] (72% positive sensitivity), L. Suresh Babu el al"[13](62.6% IgG and IgM ELISA positive). 76-90% in study conducted by Sunil Shethi el al [14] and in Daniel H. Libraty et al [15] .,and 83% sensitivity was seen in study done by Safar Ali Alizadeh et al., [16] and the less incidence was seen in Ambrose James et al. [17] 9.7% and 15% in Kaur I R et al.,[18] respectively. Similar to our study but slightly high positive were seen in immunochromatograhic test Linda et al., with 11.8%. But several other studies showed discrepancy in which they have summated in terms of results and utility that both ICT and ELISA were not much sufficiently accurate for the diagnosis of primary Leptospirosis as per Sehgal et al., [19] and Blacksell et al.,[20]

Mansoon rainfall plays very important role in spreading of the disease to humans Ko et al, [21] ,Vimala et al.,[22] In our study, the highest incidence rate occurred in April to June. In our study maximum number of positive result came in June with 23(58.97%). Similar results were obtained in the study of Sunil Shethi et al [14'] with 60% and in R. Chaudhry et al.,[10]with 50-60% in the month of July to October.

The predominant symptom among the patients in our study was found to be fever. The commonest symptoms of those who were confirmed as having leptospirosis were fever, headache, myalgia and vomiting. These finding are agreeable with the findings of the study by Davol (2006).[23]

In this study we evaluated two rapid assays for early diagnosis of leptospirosis in a hospital based population. Elisa IgM was highly sensitive and specific compared to ICT . IgM ELISA is suitable for early and definitive diagnosis of acute leptospirosis. Neither of them requires specialized equipment and could be performed in peripheral laboratories with relatively little expertise. We conclude that there has been a dramatic increase in the number of leptospirosis cases reported in our laboratory . This study shows leptospirosis was found more common among male than female, probably due to occupational status of leptospirosis.

We strongly suggest suspicion of leptospirosis by the physicians is possible even during summer season as this disease occurs throughout the year. This forces medical professionals to perform continuous surveillance programme for leptospirosis. Hence all the suspected cases of leptospirosis with fever for >7 days should be screened for leptospirosis with IgM ELISA it can serve as a very good alternative to the unaffordable tests like MAT and PCR .

Reference

1. Izurieta R, Galwankar S, Clem A. Leptospirosis: The "mysterious" mimic. Journal of emergencies, trauma, and shock. 2008;1(1):21–33. pmid:19561939; PubMed Central PMCID: PMC2700559.
2. John TJ. Emerging & re-emerging bacterial pathogens in India. Indian Journal of Medical Research. 1996;103:4–18. [PubMed]
3. Muthusethupathi MA, Shivakumar S, Suguna R, et al. Leptospirosis in Madras—a clinical and serological study. The Journal of the Association of Physicians of India. 1995;43(7):456–458. [PubMed
4. Musso D LSB. Laboratory diagnosis of leptospirosis: A challenge. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi. 2013;46(4):245–52. doi: 10.1016/j.jmii.2013.03.001 . [PubMed]
5. Wuthiekanun V, Chierakul W, Limmathurotsakul D, Smythe LD, Symonds ML, Dohnt MF, et al. Optimization of culture of Leptospira from humans with leptospirosis. Journal of clinical microbiology. 2007;45(4):1363–5. doi: 10.1128/JCM.02430-06 ; PubMed Central PMCID: PMC1865830. [PMC free article] [PubMed]
6. World Health Organization/International Leptospirosis Society, 2003. Human leptospirosis: guidance for diagnosis, surveillance and control
7. Ooteman MC, Vago AR, Koury MC, 2006. Evaluation of MAT, IgM ELISA and PCR methods for the diagnosis of human leptospirosis. J Microbiol Methods 65: 247–257.
8. Winslow WE, Merry DJ, Pirc ML, Devine PL, 1997. Evaluation of a commercial enzyme-linked immunosorbent assay for detection of immunoglobulin M antibody in diagnosis of human leptospiral infection. J Clin Microbiol 35: 1938–1942.
9. Zochowski WJ, Palmer MF, Coleman TJ, 2001. An evaluation of three commercial kits for use as screening methods for the detection of leptospiral antibodies in the UK. J Clin Pathol 54: 25–30.
10. Chaudhry .R, A.Das, M.M Premlatha, A . Choudhary, B.K, Chourasia ,D .S Chandel & A.B Dey.Serological and molecular approaches for the diagnosis of leptospirosis in a tertiary care hospital in North India ,A 10 year study , Indian J Med Res 137, Apr 2013, 785-790.
11. Smita B. Shekatkar, Belgode N. Harish, Godfred A. Menezes and Subhash C. Parija, Clinical and serological evaluation of leptospirosis in Puducherry, India, J Infect Dev Ctries 2010; 4(3):139-143.
12. Krishna Kanchan Sharma, Usha Kalawat, Early diagnosis of leptospirosis by conventional methods: One-year prospective study, 2008,Vol: 51(2) : 209-211
13. L. Suresh Babu, Isolation and Serological Evidences in Suspected Leptospirosis Cases in and Around Chennai, American Journal of Clinical and Experimental Medicine ,2014; 2(4): 70-73.
14. Sunil Sethi, Navneet Sharma, Nandita Kakkar , Juhi Taneja , Shiv Sekhar Chatterjee , Surinder Singh Banga , Meera Sharma, Increasing Trends of Leptospirosis in Northern India: A Clinico-Epidemiological Study, PLoS Negl Trop Dis 4(1): e579.
15. Libraty DH, Myint KSA, Murray CK, Gibbons RV, Mammen MP, et al. (2007) A Comparative Study of Leptospirosis and Dengue in Thai Children. PLoS Neg,Trop Dis 1(3): e111.
16. Safar Ali Alizadeh, Seyyed Saeed Eshraghi, Mohammad Reza Pourmand, Taghi Naserpour, Gholamreza Abdollahpour, Abbas Rahimiforoshani, Reza Najafipour, Diagnostic Efficacy of Lsa63 Antigen for Human Leptospirosis ,Iran Red Crescent Med J. 2014 Mar; 16(3): e14753.
17. Ambrose James, Kingsley Siele, Neeka Harry, Sharianne Suepaul, Alva Stewart-Johnson, Abiodun Adesiyun, Serological Evidence of Exposure to Leptospira spp. in Veterinary Students and Other University Students in Trinidad and Tobago, Interdiscip Perspect Infect Dis. 2013; 2013: 719049
18. Kaur, I.R., Sachdeva, R., Arora, V. and Talwar, V. 2003. Preliminary survey of leptospirosis amongst febrile patients from urban slums of East Delhi. J. Assoc. Physicians India, 51: 249-51.
19. Sehgal S C. Epidemiological patterns of leptospirosis. Indian J Med Microbiol 2006;24:310-1
20. Stuart D. Blacksell, Lee Smythe, Rattanaphone Phetsouvanh, Michael Dohnt, Rudy Hartskeerl, Meegan Symonds, Andrew Slack, Manivanh Vongsouvath, Viengmone Davong, Olay Lattana, Simmaly Phongmany, Valy Keolouangkot, Nicholas J. White, Nicholas P. J. Day, Paul N. Newton, Limited Diagnostic Capacities of Two Commercial Assays for the Detection of Leptospira Immunoglobulin M Antibodies in Laos ,Clin Vaccine Immunol. 2006 Oct; 13(10): 1166–1169.
21. Albert I. Ko, Cyrille Goarant, Mathieu Picardeau, Leptospira: The Dawn of the Molecular Genetics Era for an Emerging Zoonotic Pathogen ,Nat Rev Microbiol, 2009 Oct; 7(10): 736–747.
22. G. Vimala, A. Mary Josephine Rani, and V. Raja Gopal, "Leptospirosis in Vellore: A Clinical and Serological Study," International Journal of Microbiology, vol. 2014, 5 pages.
23. Davol PA. Canine leptospirosis: current issues on infection and vaccination. 2006.