Serological Evaluation of Clinically Suspected Leptospirosis Cases in a Tertiary Care Hospital

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ABSTRACT

BACKGROUND

Leptospirosis is a zoonotic infection caused by the spirochaete Leptospira, which has worldwide distribution. Laboratory diagnosis is routinely performed by serological tests like dipstick assay, lateral flow assay and latex agglutination which are rapid tests recommended for screening the disease. Microscopic Agglutination Test (MAT) is the standard test for serological diagnosis of leptospiral infection which is not included in the test panel in most of the peripheral laboratories as the procedure is laborious and it requires to maintain live leptospira. Therefore, one of the rapid tests is routinely employed for demonstration of leptospiral antibodies. Our objective was to screen the acute cases of leptospirosis by Leptochek WB IgM and PanBio IgM Elisa and compare the findings with the MAT and correlate the clinical findings with the serological tests.

METHODS

This study was conducted in a tertiary care hospital in Mangalore from August 2010 to September 2013. A total of 314 cases of clinically suspected leptospirosis were included based on the Faine's criteria. Patients' serum was screened for leptospiral antibodies by Leptochek WB IgM, PanBio IgM ELISA and Microscopic Agglutination Test (MAT).

RESULTS

Out of 314 clinically suspected cases screened, seropositivity for leptospirosis by Leptochek WB-IgM, PanBio IgM ELISA and MAT was found to be 49 (15.6%), 65 (20.7%) and 78 (24.8%) respectively. Thus, an overall prevalence rate of leptospirosis was 24.8% (78/314) based on the MAT test. Sensitivity, specificity, positive predictive value and negative predictive value of Leptocheck WB IgM was 53.8%, 97%, 85.7 and 86.4% with MAT while the comparative values of PanBio-ELISA IgM with respect to MAT test was 74.5%, 97 %, 89.2% and 92% respectively. Common clinical features among MAT positive cases were fever, chills and rigors, oliguria, vomiting, jaundice and headache.

CONCLUSIONS

MAT is a standard serological test for Leptospirosis. This test is not always available for peripheral health centres, as the test is time consuming and cumbersome. Thus, screening tests are now being employed for screening the patients. Rapid tests like Leptocheck -WB can be supplemented with an ELISA test for screening of clinically suspected cases of Leptospirosis and later confirmed with the MAT at reference centres.

KEY WORDS

Diagnosis, Leptospirosis, Microscopic Agglutination Tests, Serological Tests, Zoonotic Disease

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BACKGROUND

Leptospirosis is a zoonotic infection widely distributed among humans globally. It is caused by the genus Leptospira that includes the pathogenic species *Leptospira interrogans* that manifests with varying degrees of severity. The clinical manifestation of the disease range from fever with chills to jaundice with renal failure and/or haemorrhagic disorders.[1,2,3] The disease is reported to be endemic in the states of Kerala, Tamil Nadu, Gujarat, Andaman, Karnataka and Maharashtra.[4] Only a few published reports are available from Dakshina Kannada, Karnataka. Of which, in one of the study conducted in Manipal, 26.93% seropositivity by ELISA was reported.[5] A study involving 100 serum samples reported presence of Leptospiral IgM antibodies in 35% of the cases by ELISA, 34% of the cases positive by PCR and 28% of the cases were positive both by ELISA and PCR.[6] In a retrospective study conducted in a tertiary care hospital during the year 2012, 135 suspected cases of Leptospirosis were seropositive by ELISA.[7]

The laboratory diagnosis of the disease is by Dark Ground microscopy (DGM), culture of Leptospira and serological tests. DGM is one of the diagnostic tools for early diagnosis but has its own limitations. It requires an expertise to report the findings and further has a low sensitivity rate. Though isolation of Leptospira by culture contributes to the definitive diagnosis of Leptospirosis, is not useful for its early diagnosis. The reason is that the culture reports are available only after 13 weeks of incubation as the organism has a long generation time. Secondly the sensitivity of culture is low. Due to these limitations, the laboratories routinely employ serological tests for the diagnosis of Leptospirosis. The various tests include the rapid tests like latex agglutination, haemagglutination assay, lateral flow assay or immuno-chromatography based methods, indirect immuno-fluorescence and ELISA tests. Microscopic agglutination test (MAT) is a conventional serological test which is considered as the gold standard test for diagnosis of Leptospirosis because it employs specific serogroup/serovars for the detection of leptospiral antibodies in a region.[1] But MAT is available only in reference centres as it requires maintenance and use of live strains for the test. There is also a risk of cross infection with the strains. Due to these limitations of MAT, various rapid tests were introduced. Most of these tests are genus-specific tests that are recommended for screening of the disease.[8, 9]

The objective of the study was to screen the acute cases of leptospirosis using Immunochromatography based Leptochek -WB IgM and PanBio ELISA IgM in our setup and to determine the sensitivity and specificity of the tests with the MAT for routine use and also to correlate the serological findings with the clinical features.

METHODS

This prospective cross-sectional and diagnostic based study was carried out in a tertiary care hospital in Mangalore, Karnataka during the period August 2010 to September 2013. The study was carried out after obtaining the ethical clearance from K. S. Hegde Medical Academy, NITTE (deemed to be university). A total of 314 clinically suspected leptospirosis cases were included. The Modified Faine's criteria were followed for selecting the cases.[2] Patients reported positive for Dengue, Hepatitis, Enteric fever and Malaria were excluded from the study. The details of each patient were recorded into a prepared proforma after obtaining their consent for inclusion in the study. The details included demographic facts, occupation and clinical findings.

Serological Tests

1. Leptochek-WB IgM: A volume of 3 mL blood was collected and centrifuged later to obtain the serum. The test was carried out as per the manufacturer's manual insert (Zephyr Biomedicals India). A positive test was identified following the appearance of a band in the test area along with a control band. No colour band meant the test was negative. The formation of a control band showed that the test result was valid.[10,11]

2. PanBio ELISA IgM: Serum from clinically suspected cases and asymptomatic cases were processed as per the manufacturer's instructions in the test kit (PanBio ELISA IgM, Queensland Australia).[12,13] Case and control sera (10 µL) were diluted 1:100 and tested according to the manufacturer's instruction. Each set of tests was run with positive control, negative control and cut-off calibrator in duplicate. The test was valid when the absorbance reading of the above met the specification of the PanBio ELISA instructions. The results were interpreted according to the manufacturer's recommendation. Specimens having an absorbent ratio greater than that of the cut-off calibrator were defined as positive. PanBio units of <9: a negative result interpreted as no evidence of recent infection. A PanBio unit of 9- 11: low positive or borderline result and may suggest a recent infection. PanBio units of >11: positive result suggestive of a recent or current infection. Sample with equivocal values was repeated with the second sample after 5-7 days.

3. Microscopic Agglutination Test (MAT): Sera were examined by the MAT at Zoonosis Research Laboratory, TANUVAS, at Chennai. A battery of 13 serovars was employed to establish seroconversion or a rise in titer.[13,14] Five-seven-day old Leptospira culture grown in liquid EMJH medium was used as an antigen. Serogroups/serovars of Leptospira used as antigens were Australis, Autumnalis, Ballum, Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, Hardjo, Tarassovi and Semaranga Patoc.

Procedure- Two mL fresh venous blood was centrifuged at 5000 rpm for 5 minutes. The serum was then transferred to a sterile 2 mL microcentrifuge tube. The microtiter plates and the dilution tray were UV-sterilized before use. The wells of the dilution plate and the microtiter plates were cleaned using a cotton swab moistened with 70% ethyl alcohol. A volume of 980 µL of PBS and 20 µL serums were dispensed to the wells of a dilution plate. The diluted serum was mixed well and 50 µL was transferred into a microtiter plate using a multichannel pipette. A control culture diluted in phosphate-buffered saline was used as the negative control. Twenty µL of antigen was added to the wells of the microtiter plate. The plate was covered with a lid and incubated at 37º C for 2 hours. Five µL of incubated serum mixture was spotted from each well onto a clean microscopic slide. The slide was observed under a dark field microscope at 200x magnification. The highest dilution showing 50% agglutination was recorded as the titer.

Reporting and Interpretation of Results

The endpoint was defined as the highest dilution of serum that shows 50% agglutination, compared with a control culture diluted in phosphate-buffered saline. The result of the test is reported as the endpoint dilution of serum (>1/100).

Modified Faine's Criteria

Criteria for confirmed cases of Leptospirosis: A case that was clinically suspected as leptospirosis following Modified Faine's criteria and with MAT titer of >1: 100 or seroconversion or four-fold rise in titer by second sera collected after >14 days of onset of infection.

Statistical Analysis

Standard Deviation, Mean and percentages were determined for the variables. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the serological tests were determined using SPSS 16.

RESULTS

Out of 314 clinically suspected cases, 221 (70.4%) were males and 93 (29.6%) females. The age of the subjects ranged from 13 years to 86 years with a mean of 42. Seropositivity for leptospirosis by Leptocheck WB-IgM, PanBio IgM ELISA and MAT was found to be 49 (15.6%), 65 (20.7%) and 78 (24.8%) respectively. Thus, an overall prevalence rate of leptospirosis was 24.8% (78/314) based on the MAT test (a titer of >1 in 100) as shown in Figure 1. Among the 78 MAT positive cases, 60 (76.9%) were males and 18 (23.1%) were females. Among 78 MAT seropositive, 42/78 (62.8%) were positive by Leptocheck IgM only, 58/78 (74.35%) are positive by PanBio IgM only and total seropositive combined by Leptocheck and PanBio was 60/78 (76.9%) as shown in Table 1. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Leptocheck WB IgM and PanBio ELISA IgM with respect to MAT test was 53.8%, 97%, 85.7 and 86.4% and 74.5%, 97 %, 89.2% and 92% respectively as illustrated in Table 2.

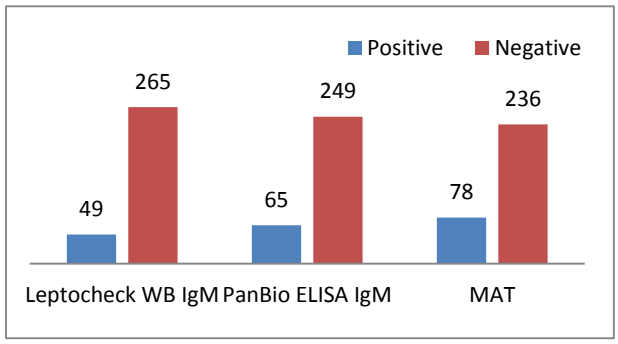

Figure 1. Serological Analysis of Suspected Cases of Leptospirosis. (314)

Tests	Total
Leptocheck+/ PanBio+/ MAT+	40
Leptocheck+/ PanBio-/ MAT+	2
Leptocheck-/ PanBio+/ MAT+	18
Leptocheck-/ PanBio-/ MAT+	18
Total	**78**

Table 1. Seropositivity among Serological Tests

*Leptocheck and PanBio ELISA- 40+2+18=60/78(76.9%)

- Leptocheck WB only-42/78 = 62.8%
- PanBio ELISA only- 58/78 = 74.35%

	Leptocheck WB IgM (%)	Pan Bio- ELISA (%)
Sensitivity	53.8	74.4
Specificity	97	97
PPV	85.7	89.2
NPV	86.4	92

Table 2. Evaluation of the Leptocheck WB IgM Test and PanBio ELISA with MAT

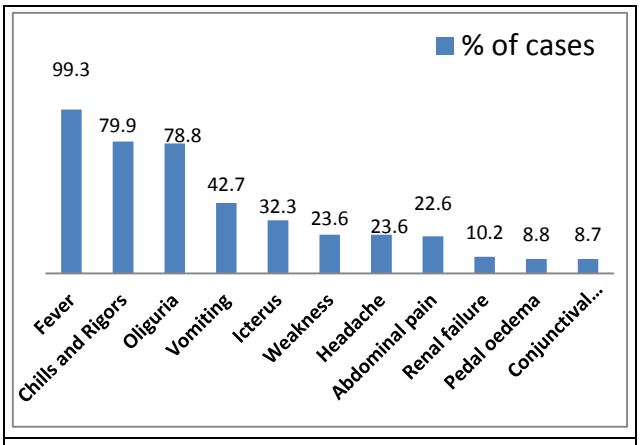

Figure 2. Common Clinical Features Presented among MAT Positives (78)

Common clinical features among MAT positives cases were fever, chills and rigors, decreased urine output, vomiting, jaundice, and headache as shown in Figure 2. The remaining 236 clinically suspected cases were diagnosed with an alternative disease that included viral fever, acute pancreatitis, acute renal failure, acute respiratory distress syndrome and cirrhosis due to alcohol abuse.

DISCUSSION

Laboratory diagnosis of acute leptospirosis is routinely done by a serological test. In our laboratory, patients with clinical suspicion with leptospirosis were subjected to testing by Leptocheck -WB IgM, PanBio-IgM ELISA and MAT. Our study recorded seropositivity of 15.6%, 20.7% and 24.8% by Leptocheck WB IgM, PanBio-IgM ELISA, and MAT respectively. Previous research works cited a comparatively higher positive rate of infection.[15-18] In contrast, two studies reported a relatively lower rate of positivity by Leptocheck -WB IgM.[7,19] Similar seropositivity by PanBio IgM was also reported by one of the studies[20]. The low positive rate by Leptocheck -WB and ELISA in our study may be due to patients treated by local physicians before getting admitted in the tertiary care centre. In comparison with the gold standard MAT test, the seropositivity of single test Leptocheck -WB or PanBio ELISA was 62.8% and 74.35% respectively when tested alone. When the positivity of both the kits was combined, it increased the sensitivity of the screening tests to 76.9% which was in agreement with one study.[19]

In our study, the sensitivity and specificity of Leptocheck -WB with MAT were 53.8% and 97% respectively. According to some of the previous reports, a lower rate of specificity rate was observed when compared to the present study.[16-18] The specificity rate of PanBio ELISA in our study (97%), was found to be comparatively higher from previous reports.[16, 18] The analysis of MAT reports of 314 cases, showed 78 MAT

seropositive with a titer ranging from 1 in 100 to 1 in 6200 (24.8%). There was variability in MAT reports from different parts of India and Sri Lanka ranging from 36% to 48%.[16,18,21,22] Among the MAT positives, the gender distribution of cases was male to female ratio (3.3:1) which agreed with other studies.[15,23,24] The high incidence rate was among the age group 40-50, which is suggestive of occupation risks and working outdoors.[2,20] The common clinical features in our study were fever with chills, decreased urine output, abdominal pain, headache and vomiting which correlated with one study. Jaundice was also seen among 32% of the cases, while other studies reported relatively higher rates ranging from 40-43%.[22,18,23] Renal failure (10.2%) and pedal oedema (8.8%) were the other distinctive clinical features observed in our study.

CONCLUSIONS

Our study records a prevalence rate of 24.8% based on the MAT test with male predominance. Serological tests are routine tools in the laboratory diagnosis of leptospirosis among which MAT is the standard test. This test is not always available for peripheral health centres, as the test requires skilled personnel to carry out the tedious procedure and maintain live strains for the tests. Therefore, screening tests are employed for diagnosis in peripheral centres. Rapid tests like Leptocheck WB IgM may be supplemented with an ELISA test for screening of clinically suspected cases of Leptospirosis followed by confirmation with MAT.

REFERENCES

[1] Levett PN. Leptospirosis. Clin Microbiol Rev 2001;14(2):296-326.

[2] Faine S. Guidelines for the control of leptospirosis. Vol. 67. Geneva: WHO offset Publication 1998: p. 67-71.

[3] Ben A, Faine S. Leptospira. In: Borriello PS, Patrick MR, Funke G, edrs. Topley and Wilson's Microbiology and microbial infections: systemic bacteriology. Vol. 2. 10th edn. London: Hodder Arnold 1998: p. 1865-81.

[4] Mathur M, Anuradha De, Turbadkar D. Leptospirosis outbreak in 2005: L.T.M.G. hospital experiences. Indian J Med Microbiol 2009;27(2):153-5.

[5] Rao PS, Sridhar VS. Serological evidence in suspected cases of leptospirosis in and around Manipal. Indian J Med Microbiol 2001;19(2):32-5.

[6] Khalid P, Suman VB, Nayanatara AK, et al. Leptospirosis in febrile patients: diagnosis by serology and polymerase chain reaction. Journal of Biology & Life Science (JBLS) 2011;2(1):1-3.

[7] Ramesh HK, Dias A, Dias M. A clinicoepidemiological analysis of seropositive cases of leptospirosis in a tertiary care hospital in Mangalore. Tropical Journal of Medical Research 2015;18(2):100-3.

[8] Toyokawa T, Ohnishi M, Koizumi N. Diagnosis of acute leptospirosis. Expert Rev Anti Infect Ther 2011;9(1):111-21.

[9] Budihal SV, Perwez K. Leptospirosis diagnosis: competency of various laboratory tests. Journal of Clin and Diag Research 2014;8(1):199-202.

[10] Sehgal SC, Vijayachari P, Sugunan AP, et al. Field application of lepto lateral-flow for rapid diagnosis of leptospirosis. Journal of Medical Microbiology 2003;52(Pt 10):897-901.

[11] Gussenhoven GC, Van der Hoorn MA, Goris MG, et al. LEPTO dipstick, a dipstick assay for detection of leptospira-specific immunoglobulin m antibodies in human sera. Journal of Clinical Microbiology 1997;35(1):92-7.

[12] Winslow WE, Merry DJ, Pirc ML, et al. Evaluation of a commercial enzyme-linked immunosorbent assay for detection of immunoglobulin m antibody in diagnosis of human leptospiral infection. Journal of Clinical Microbiology 1997;35(8):1938-42.

[13] Vijayachari P, Sugunan AP, Sehgal SC. Role of Microscopic Agglutination Test (MAT) as a diagnostic tool during acute stage of leptospirosis in low and high endemic areas. Indian J Med Res 2001;114:99-106.

[14] Kar H, Urhekar A, Pai (Bhat) C, et al. A clinico microbiological study of leptospirosis. International Journal of Research in Pharmaceutical and Biomedical Sciences 2013;4(2):412-16.

[15] Niloofa R, Fernando N, de Silva NL, et al. Diagnosis of leptospirosis: comparison between microscopic agglutination test, igm-elisa and igm rapid immunochromatography test. PLoS One 2015;10(6):e0129236.

[16] Panwala T, Mulla S, Patel P. Sero-prevalence of leptospirosis in south Gujarat region by evaluating the two rapid commercial diagnostic kits against the mat test for detection of antibodies to leptospira interrogans. National Journal of Community Medicine 2011;2(1):64-70.

[17] Eugene EJ, Handunnetti SM, Wickramasinghe SA, et al. Evaluation of two immunodiagnostic tests for early rapid diagnosis of leptospirosis in Sri Lanka: a preliminary study. BMC Infectious Diseases 2015;15:319.

[18] Jose LR, Sumana MN. Early diagnosis of leptospirosis using conventional techniques: a study in a tertiary care hospital, Mysore, South Karnataka, India. International Journal of Advanced Research 2014;2(12):344-8.

[19] Bhatia M, Umapathy BL, Navaneeth BV. An evaluation of dark field microscopy, culture and commercial serological kits in the diagnosis of leptospirosis. Indian J Med Microbiol 2015;33(3):416-21.

[20] Majumdar M, Bhaduri A, Mandal M, et al. A study on serological correlation of clinically suspected leptospirosis cases in West Bengal, India. Ann Trop Med Public Health 2013;6(1):47-9. http://www.atmph.org/text.asp?2013/6/1/47/115186.

[21] Shekatkar SB, Harish BN, Menezes GA, et al. Clinical and serological evaluation of leptospirosis in Puducherry, India. J Infect Dev Ctries 2010;4(3):139-43.

[22] Kamath S. Leptospirosis. In: Das S, edr. Mumbai: API Medicine Update, 2003;13:1008-11.

[23] Natarajasreenivasan K, Prabhu N, Selvanayaki K, et al. Human leptospirosis in Erode, South India: serology, isolation and characterization of the isolates by Randomly Amplified Polymorphic DNA (RAPD) fingerprinting. Jpn J Infect Dis 2004;57(5):193-7.

[24] Jeyakumar N, Khan AA, Dhasarathan P, et al. Seroprevalence of leptospirosis in clinically suspected cases in Chennai city. Indian J Med Microbiol 2004;22(1):67.