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 leptospirosis 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 Leptochek-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
**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.\(^1\) 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 20% 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 Leptospirosis 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 Leptospirosis 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.

**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, Pyrogens, Hardjo, Tarassovi and Semaranga Patoc.

**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.
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.

Common clinical features among MAT positives 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 studies20. 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 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%. Among the MAT positives, the gender distribution of cases was male to female ratio (3.31) which agreed with other studies. The high incidence rate was among the age group 40-50, which is suggestive of occupation risks and working outdoors. 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%. 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
