Seroprevalence of leptospirosis among subjects with fever in Chennai: A cross sectional study.

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Abstract

Aim: This study was undertaken to estimate the seroprevalence of leptospirosis among subjects with fever and to compare three diagnostic methods used in leptospirosis among Chennai population.

Materials and methodology: Two hundred and sixty seven patients were included in the study. All the cases had history of fever for five or more days of the total study entrants, all were tested for Leptospira antibodies by MSAT, ELISA as well as MAT and the antigen detection was done using Dark Field Microscopy. The common clinical conditions with which the patients presented were 267 cases of fever, 89 cases of myalgia, headache in 63 cases, jaundice in 10 cases, conjunctival suffusion in 9 cases, diarrhea in 3 cases and nausea/vomiting in 35 cases.

Results: Comparison of efficacy of MSAT and MAT was done. 92 cases were positive on Microscopic agglutination test (MAT) and 116 were positive on MSAT. All the MAT positive cases were positive on DFM. Though DFM alone is not a reliable test, it was compared with gold standard ELISA test and the findings shows that all DFM positive cases were also positive for MAT. However the number of positive cases detected using MSAT was higher than that of MAT. On comparing the results of MSAT and ELISA with MAT, there was 82% agreement between the tests.

Introduction

Leptospirosis is a zoonotic disease caused by the spirochete of the genus Leptospira. It affects humans worldwide, in both urban and rural areas, in temperate as well as tropical climates. It is important to distinguish leptospirosis from other conditions like dengue and viral hemorrhagic fevers. Leptospirosis is potentially serious but treatable disease. Its symptoms may mimic those of any other unrelated infections such as Dengue, Viral hemorrhagic fevers, Influenza, Meningitis or Hepatitis. Cases of leptospirosis are very common in country like India, but the disease is undiagnosed due to lack of diagnostic facilities [1].

This study aims to find out the seroprevalence of leptospirosis among fever cases in Chennai and to compare three diagnostic methods to detect anti leptospiral antibody. This study will emphasize the importance of early diagnosis of leptospirosis with patients who come with symptoms of fever with more

than 5 days. A presumptive bedside diagnosis with serological confirmation may be made, only if a high index of suspicion is maintained. Failure to identify leptospirosis in these patients will result in high mortality rates. Early diagnosis and appropriate treatment will halt the progression of the disease and reduces the mortality and morbidity of this zoonotic infection.

Methodology

A Cross Sectional Study was conducted to study the seroprevalence among fever cases attending the outpatient department of government primary hospitals in Chennai from May 2011 to January 2012. Ten corporation hospitals in Chennai (One Hospital each from 10 Zones of Chennai) were selected and Cases with the symptom of fever for more than five days, attending OPD were included. Purposive sampling method was used to select cases. After obtaining informed consent, a pre coded semi structured questionnaire was

administered to the patients to collect their socio demographic details and their clinical symptoms. Two hundred and sixty patients were included in the study. The patients included were with clinical symptoms of fever for more than five days and not under antibiotics. Patients were selected from Primary Health Centre and from each centre 25 samples were enrolled. From each patient two to three ml of blood was collected and serum was separated and tested for antibody by MSAT, MAT and antigen detection was done by Dark Field Microscopy. Statistical analysis was done using SPSS software version 18.Demographic and clinical variables were given in frequencies and percentages, Prevalence is given in proportion with 95% confidence interval.

Results

A total of 267 patients were recruited for this study. Respondents were in the age group ranging between 4 to 75 years. Among the total subjects, 81.3% had metro water (drinking water supplied by the corporation of Chennai through tankers) as their major source of drinking water, 14.6% used mineral water which were supplied through cans. Among the metro water users only 13.4% drank boiled water. Among the 114 positives cases, 27% belonged to the age group of 11-20 years, followed by age group of 31-40 and 41-50 which were equal in percentage i.e 21% Minimum number of cases were seen in the age group of 71-80 years which accounted to a mere 0.8%. Among the total cases, male (55%) and female (45%) are almost equally infected with Leptospirosis.

Among the total sample population recruited from 10 Chennai corporation zones, 43% were confirmed cases of Leptospirosis. Out of 43% of cases, the prevalence was high in Zone V - 19% and Zone I- 18% followed by Zone II and Zone IX which had equal number of cases (14%), however Leptospirosis cases were detected in other zones also. This infers that Zone V, I, II and IX, of Chennai had more number of detected cases or the epidemic was high in those regions (Figure 1).

This may be attributed to poor hygienic practices or contamination of drinking water in those regions. However, the epidemic was seen in almost all the zones, which infers that every area in Chennai had drinking water contamination and rain water stagnation to certain extent. Among the 141 males 63(56.5%) were positives and among the 126 females
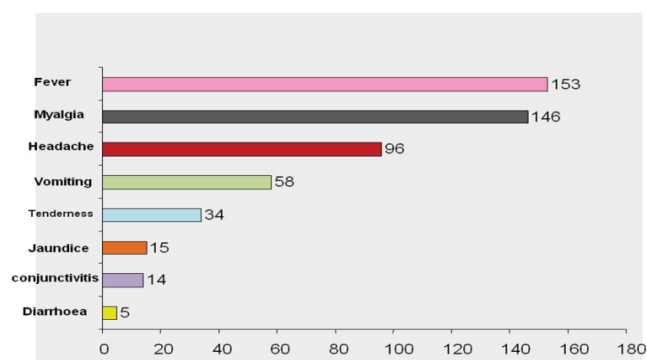
51 (43.50%) were leptospirosis positives irrespective of their age group (Figure 2).

Among the total cases, 34.5% (N=92) were positive for Microscopic Agglutination Test (MAT) which is the Serological test that is considered gold standard in the diagnosis of Leptospirosis (Figure 3).

This figure shows the comparison of serovars and Chennai zones. *L. australis* was present in all the zones except zone VI and VIII. *L. Canicola* was also present in all the zones except zone VIII and X. *L. grippotyphosa* was seen in all the zones. *L. autumalis* was present in zone I, III, V and IX. But only one case of *L. icterohemorrhagia* was observed in zone V and only one case of L. louisina was observed in Zone VIII. This infers that *L. australis* and *L. grippotyphosa* were almost spread in all the zones. Sensitivity and specificity of MSAT compared with gold standard MAT was 100% and 87%. Measure of agreement between MSAT and MAT was estimated by kappa and its value was 0.827 (p<0.01) which indicates that there is a good measure of agreement between MSAT and MAT (Figure 4).

Among the total positive cases, 38% of them were infected with *L. australis*, 27% were infected with *L. canicola* and 25% with *L. grippotyphosa* followed by *L. autumnalis* (8%). And the persons infected with the serovars *L. icterohemorrhagia*
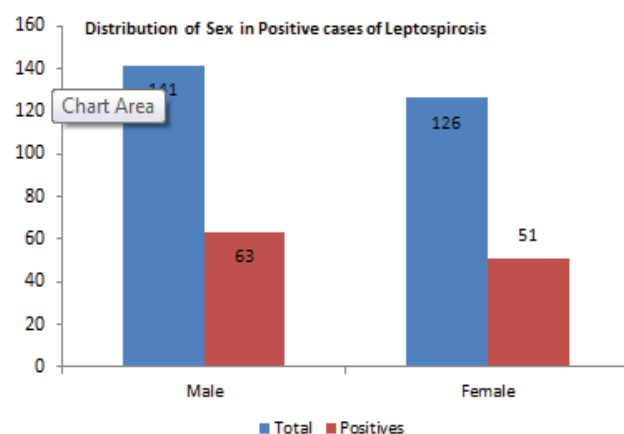

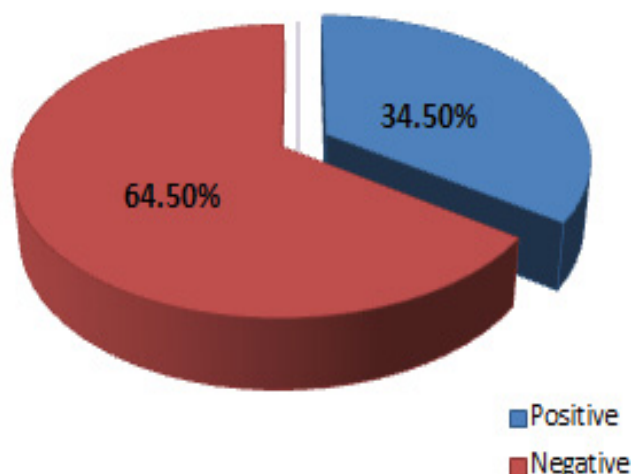*Figure 2. Distribution of Sex among leptospirosis positive cases.*
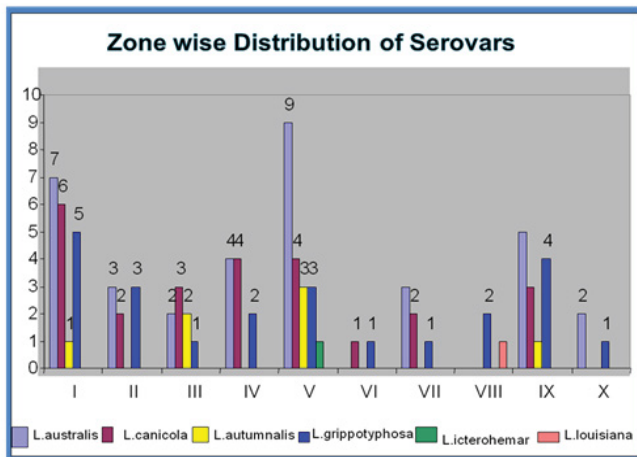

Figure 3. Seroprevalence of Leptospirosis.

Figure 1. Distribution of signs and symptoms in Leptospirosis.

Figure 4. *Zone wise distribution of Leptospira serovars in Chennai. Grippotyphosa.*
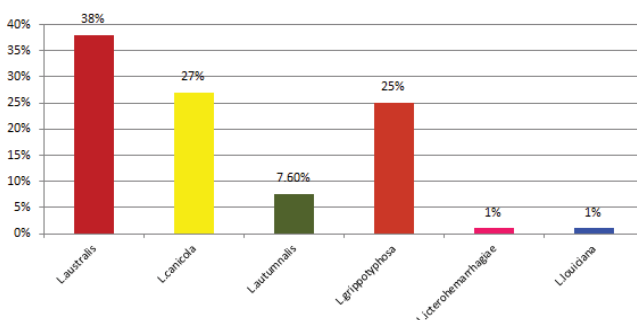
Figure 5. *Serovars distribution in Chennai city.*

Table 1. *Measure of agreement between MSAT and MAT.*

MSAT	MAT				Kappa value	p-Value
		Positive	Negative	Total	0.827	0
	Positive	92	22	114		
	Negative	0	153	153		
	Total	92	175	267		

(1%) and L. *luiciana* (1%) was very less compare to other serovars. This infers that the common serovars present in Chennai was L. *austalis* and L. *canicola* followed by L. *grippotyphosa* (Figure 5).

Agreement between MSAT and MAT estimated by kappa and its value is 0.827 (p<0.01) which indicates that there is a good measure of agreement between MSAT and MAT (Table 1).

Discussion

Leptospirosis is a public health threat due to its morbidity and mortality. The detailed knowledge of its clinical features and other laboratory parameters is necessary for early clinical diagnosis which is then confirmed by serological tests. Seroprevalence of the present study is twofold more than the seroprevalence rate of a study conducted by Ganesan arumugam et al. in which Seroprevalence rate was 20% [2]. A sero survey in Chennai conducted by Rathnam et al. reported the prevalence rate as 32.9% [3]. In the present study Fever, Headache, Myalgia was the common clinical manifestation. Jaundice occurred in 13% of positive cases. Anicteric leptospirosis was the common clinical presentation. This

is consistent with the study done at Chennai [4]. Milder forms (Anicteric) of Leptospirosis are more common (90%) than severe Leptospirosis (10%) similar to studies done at Vietnam [5]. This may be due to the ever changing distribution of the serovars and the association between serovars and clinical features (For example L. *autumnalis*, and L. *icterohemarrhagiae* are virulent pathogens and they play a role in the severe form of leptospirosis. In the present study both of the serovars were very less.

Among the 267 samples, MSAT was positive in 114 (43%). From this study, we have found that MSAT is a very sensitive and specific test for diagnosis of leptospirosis. We have utilized this as a screening test for all the patients with fever. This is adequate for diagnosis of current infection. In single samples, positive MSAT (with negative and positive MAT) confirms current leptospiral infection. It is preferable to do MAT in samples which are positive by MSAT, as during epidemics, large number of samples may have to be tested and it is not possible to do MAT alone as it is a complicated test [6,7]. MAT is regarded the gold standard in diagnosis of leptospirosis, however the sensitivity is low compared to MSAT. This is because antibody titers rise and peak only in 2nd or 3rd week. High titers of past infection persist for a long time (1 to 5 years) and therefore interfere with diagnosis of current infection. A single positive declining titer may represent a rising titer of current infection or declining titer of past infection. The cut off titer for diagnosis depends on whether the area is endemic or non-endemic. Therefore a 2nd sample is definitely required to demonstrate seroconversion or a 4 fold rise in titer to diagnose current infection [8]. In the present study, out of 267 samples, 92 were positive (34.5%), by using MAT, which is a serovar specific. The following 6 serovars were included in the MAT panel. i.e., L. *icterohemorrhagia*, L. *australis*, L. *canicola*, L. *autumnalis*, L. *grippotyphosa*, L. *louicina*.

In present study, the predominant serovar was L. *australis* (38%) followed by L. *canicola* (27%), L. *grippotyphosa* (25%), L. *autumnalis* (8%), L. *icteroheamorrhagiae* and L. *louiciana* were each 1%. The findings of the present study was not consistent with the study done by Sumathi et al. who observed that L. *ictero* (48%) was the predominant serovar followed by L. *australis* (37%) and L. *grippotyphosa* (26%) in a study done at Chennai. During 1990-1991 L. *autumnalis* was the most common serovar in Chennai according to their study [7]. A similar study done at Chennai by Ganesan et al. observed the common serovars to be L. *australis* (37%), L. *canicola* (30%), L. *autumnalis* (14.5%) [9,10]. The study results were in accordance with our study.

Conclusion

In the present study, the screening test MSAT has 100% sensitivity and 87% specificity, when comparing with the gold standard MAT. This value indicates that the MSAT will be a good test for the screening. Specific preventive measures should be adopted to prevent any contact with infected animals, and animal immunization should also be implemented. There

is need of an epidemiological surveillance system to allow proper diagnosis. As recommendation, all patients admitted with fever to the hospital are screened for leptospirosis, as milder disease (anicteric) was the common presentation. Though it's a seasonal disease, clinicians should think of leptospirosis in rainy season. To get correct epidemiological data on leptospirosis, all government hospitals should have leptospirosis diagnostic laboratory.

References

1. Laras K, Cao BV, Bounlu K, Nguyen TK, Olson JG, Thongchanh S, Tran N V, Hoang KL, John WS, Paul DM, Punjabi N, Khiem HB, An US, Insisiengmay S, Campbell JR, Corwin AL. The importance of Leptospirosis in Southeast Asia. Am J Trop Med and Hygn 2002; 67: 278-286.

2. Arumugam G, Jacob SM, Anitha D, Rajappa SM. Occurrence of leptospirosis among suspected cases in Chennai, Tamil Nadu. Indian J Pathol Microbiol 2011; 54: 100.

3. Ratnam S, Everard CO, Alex JC, Suresh B, Thangaraju P. Prevalence of Leptospiral agglutinins among conservancy workers in Madras city. J Trop Med Hyg 1993; 96: 41-45.

4. Muthusethupathi MA, Shivakumar S, Suguna R, Jayakumar M, Vijayakumar R, Everard CO, Carrington DG. Leptospirosis in Chennai. A clinical and serological study. J Assoc Phys India 1995; 43: 450-458.

5. Berman SJ, Tsai MDC. Sporadic Anicteric leptospirosis in South Vietnam. Ann Intern Med 1973; 79: 167-173.

6. Shivakumar S, Shareek PS. Diagnosis of Leptospirosis: Utilizing modified Faine's Criteria. J Assoc Physicians India 2004; 52: 678-679.

7. Sumathi G, Narayanan R, Shivakumar S. Leptospirosis Laboratory, Madras Medical College: Review of Our Experience (2004–2006). Indian J Med Microbiol 2008; 26: 206-207.

8. Shivakumar S, Krishnakumar B. Diagnosis of leptospirosis: Role of MAT. Jn Assoc Phys India 2006; 54: 338.

9. Budihal SV, Perwez K. Leptospirosis Diagnosis: Competancy of Various Laboratory Tests. J Clin Diagn Res 2014; 8: 199-202.

10. Sethi S, Navneet S, Nandita K, Juhi T, Shiv Sekhar C, Surinder Singh B, Meera S. Increasing Trends of Leptospirosis in Northern India: A Clinico-Epidemiological Study. PLoS Negl Trop Dis 2010; 4: e579.

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