Research Article

Changing trends of leptospirosis in Chennai city

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Abstract

Objective: Leptospirosis, a direct zoonotic disease caused by spirochetes of different pathogenic species of the genus leptospira, is an emerging global health problem. Many outbreaks have been reported from Chennai in the past mainly due to rain and floods. Majority of the leptospiral infections are either subclinical or result in mild illness and recover without any complications. However, some develop serious complications due to involvement of multiple organ systems. In such patients, the clinical presentation depends upon the organs involved and the case fatality rate could be about 40-45% or more. Acute Febrile illness with muscle pain, febrile illness with pulmonary haemorrhage with haemoptysis, jaundice with pulmonary haemorrhage and haematuria, meningitis with subconjunctival haemorrhage and febrile illness with cardiac arrhythmias with or without haemorrhages are some of the syndromes. Since, published data on all the three tests together are inadequate, this study has been done to evaluate the efficacy in diagnosing human leptospirosis by assessing the changing serovar pattern of leptospirosis among the febrile cases in Chennai city. **Method:** Sera from patients with clinical suspicion of leptospirosis during September 2010 - February 2011 were examined by i) Microscopic agglutination test (MAT) ii)Macroscopic slide agglutination test (MSAT) and iii)Enzyme linked immunosorbent assay (IgM ELISA). The diagnosis of leptospirosis was observed by i) an initial titre of $\geq$1in 80 or a fourfold rise in the titre of MAT, ii) a 3+ agglutination titre by MSAT was taken as positives iii) In IgM ELISA $\geq$11.0 panbio units were taken as positives. **Results:** 73 of 257 patients were found to be confirmed positives by MAT method. 20 showed a significant MAT titre 1 in 80, 51 cases were reactive with more than 1 in 80 titre.2 cases were reactive at 1 in 40 dilution.65 cases (89%) were found to be positive by MSAT. Only 60 cases were IgM ELISA positives. **Conclusion:** MSAT is a very sensitive and specific test for diagnosis of leptospirosis.

Keywords: Leptospirosis, Microscopic agglutination test, Macroscopic slide agglutination test.

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INTRODUCTION

Leptospirosis is an acute bacterial infection caused by spirochetes belonging to the genus Leptospira[1]. It is considered as the most widespread direct zoonotic disease in the world.[2] Leptospirosis affects human beings and many other species of vertebrates. It can present in a wide spectrum of clinical manifestations in human beings.[3] In the endemic areas, leptospirosis is a major cause of various clinical syndromes such as jaundice, renal failure, myocarditis and atypical pneumonia (Muthusethupathi *et al* 1995). In 1886, Adolf Weil described a clinical syndrome characterized by splenomegaly, jaundice, haemorrhages and nephritis.[4] This syndrome is usually referred to as Weils disease and this has become synonymous with leptospirosis. Leptospira were first identified as the cause of Weil's disease in Japan where it was common among coal miners.[5] The Japanese workers who discovered the organisms responsible for Weil's disease named it *Spirochaeta Icterohaemorrhagiae*. It has probably been under reported and under diagnosed in other parts of the country due to lack of diagnostic facilities. Laboratory diagnosis involved the isolation of

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leptospira in culture of blood and urine and serological tests. The common serological tests are genus specific tests (MSAT and ELISA) and serovar specific test (MAT). The presence of leptospiral antibodies from previous infections (usually mild and subclinical) in large proportion of people living in endemic areas complicates laboratory diagnosis of current illness. Genus specific tests tend to become positive early and are of value in diagnosing current infection. As published data on these tests together are inadequate, this study has been done to evaluate their efficacy in the diagnosis of leptospirosis.

MATERIALS AND METHODS

Two hundred and fifty seven serum samples obtained from patients with clinical features suggestive of leptospirosis received in the Microbiology department, Madras medical college during September 2010 – February 2011 were subjected to MAT, MSAT and IgM ELISA. MAT test was performed with 12 live serovars of leptospira (Australis, Autumnalis, Bataviae, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Louisiana, Pyrogenes, Pomona, Canicola, Sejroe and Patoc) using standard Micro titre methodology.[6-8] The highest dilution of serum which agglutinated 50% or more of leptospires under dark field microscope was presumed to represent the titre of antibody specific for the particular serogroup used, when two or more serogroups reacted at the same (highest) titre, the result was recorded as mixed equal. Controls were put up for each one of the batteries of antigens used in the test. MSAT antigen was prepared using local prevalent serovars. Eleven pathogenic and one non pathogenic serovars were included in the antigen preparation. The antigen was prepared using standard methods.[6] MSAT was done by mixing a drop of twelve pooled suspension of leptospires with a drop of serum on a slide and rotator (120/minute). IgM ELISA test was done by using the Pan bio kit. Leptospira IgM ELISA has been demonstrated to detect infections caused by a number of L.interrogans serovars including: copenhagani, madanesis, szwajizak, diasiman and tarassovi.[8] Detection of antibody of the IgM class Leptospira genus antigen by ELISA is a screening procedure for the diagnosis of acute infection.[9-10] Serum containing antibody to leptospira antigen when present combine with leptospira antigen attached to the polystyrene surface of the microwells. Residual serum is removed by washing and peroxidise conjugated antihuman IgM is added. The microwells are washed and a colourless substrate system, tetramethyl benzidine (TMB) / hydrogen peroxide (TMC chromogen) is added. The substrate is hydrolyzed by the enzyme and the chromogen changes to blue colour. After stopping the reaction with acid the TMB becomes yellow colour. This development is indicative of the presence of IgM antibody. Negative and positive controls were put up. The results were observed. Above 11 pan bio units were considered as positives and below 11 units were considered as negatives. The following were considered as significant parameters for the diagnosis of leptospiral infection.

i) An initial titre of $\geq$ 80 or a fourfold rise in titre of MAT
ii) A 3+ agglutination titre by MSAT
iii) A titre of > 11 units IgM ELISA

RESULTS

All the 257 samples were subjected to Microscopic Agglutination Test (MAT), Macroscopic slide aggluitination test (MSAT) and IgM ELISA (Pan bio). Antibody titres suggestive of current leptospirosis was obtained in 73 of the 257 patients and of these 60 patients provided single serum samples and 13 provided paired sera. The clinical symptoms are shown on Table -1. Twenty patients had MAT titres of 1 in 80, Fifty one patients had MAT titres of $\geq$ 1 in 160 (Table-2) and 2 patients had a MAT titre of 1 in 40 to any one of the antigens in the panel. (20.5%). Patoc was noted in 2 patients and mixed equal in 2 patients. Of the 20 repeated samples 15 showed fourfold rise in titre, 2 showed two fold rise in titre, one showed the same titre while the other showed a decline in titre. Sixty five cases were found to be positive by MSAT. IgM ELISA was positive among 60 patients. All patients with MAT titres of $\geq$ 1 in 80 had positive. IgM ELISA confirming that it was current infection. In addition the patients with MAT titre of 1 in 40 had positive IgM ELISA.

Table 1: Clinical Spectrum in positive patients (n=73)

Clinical features	Number	Percentage
Fever	73	100
Anicteric	61	83
Myalgia	58	79
Jaundice	12	17
Renal Failure	25	34
Conjunctival Suffusion	17	23
Pulmonary haemorrhage	12	16
Haematuria	9	12

DISCUSSION

All patients presented with febrile illness. Icteric leptospirosis was noted in 43% of patients. Shivakumar *et al* from Chennai has stated that acute renal failure (ARF) due to leptospirosis in Chennai has significantly declined from 31% during 1987-1991 to 7.5% in 1995-2004. ARF during the period of 1987-1991, the highest number of

cases of 45 were reported during 1990.[12] Our experience suggest that although severe leptospirosis has declined, anicteric type of leptospirosis has increased. In the analysis of 73 positive cases in this study during 2010-11 jaundice occurred in 17% of cases and renal failure occurred in 34% of the patients. Serogroup Icterohaemorrhagiae was the most common serogroup encountered. 8 patients were dialysis patients.[13] Fever, headache and myalgia were the common presentations, only two patients were dialysed and there were no deaths. Contaminated environment (95%) and rainfall (50%) were the important epidemiological risk factors. Icterohaemorrhagiae was the most common serogroup and Autumnalis was not detected. The original Faine's criteria have utilized MAT for diagnosis of leptospirosis. Though MAT is a specific test the sensitivity is low compared to ELISA. This is because antibody titres rise to a peak by about 2nd and 3rd week. The high titres of past infection persist for a long time (1-5 years) and therefore interfere with diagnosis of current infection. A single positive titre may represent a rising titre or current infection or declining titre of past infection. Previous studies from Chennai revealed that jaundice and renal failure are important clinical features.[14] The cut off titre for diagnosis depends on whether the area is endemic or non-endemic. Therefore a 2nd sample is definitely required to demonstrate sero conversion at a fourfold rise in titre to diagnose current infection. The test is also complicated requiring dark field microscopy and cultures of various live serovars which is usually not available in small laboratories.[15] In our experience 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 suspected for leptospirosis. In single samples positive MSAT confirms current leptospiral infection and it plays a vital role in the diagnosis. It is preferable to do MAT in samples which are positive by MSAT as during epidemic large number of sample may have to be tested and it is not possible to do MAT alone as it is a complicated test.[16] IgM ELISA is the choice for the diagnosis of current infection, to become positive early in the disease (5th day) and also reverts early (60 days). This test cannot determine the infecting serogroup. A high titre in a single sample can be diagnostic though repeated samples may be necessary if initial titres are low. It is preferable to have repeat serum samples for diagnosis of leptospirosis. If only single sample is available it is essential to do both ELISA (IgM) and MAT. MSAT will help in the diagnosis of current infection and MAT will help in identifying the infecting serogroup. To conclude anicteric leptospirosis was the common presentation (83%) and renal failure (34%) followed by jaundice (17%) were important complications. Contaminated environment and rainfall were found to be the most important epidemiological factors.

CONCLUSION

This study concludes that MSAT is very sensitive and specific test for diagnosis of leptospirosis among all the available three tests.

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