Diagnosis of Leptospirosis - Role of MAT

Sir,

We read the article by Dutta et al on “Leptospirosis - An Overview” with interest. The problem in utilizing microscopic agglutination test (MAT) has been highlighted and we shall discuss briefly the current status of MAT.

MAT is considered the gold standard test for diagnosis of leptospirosis. It has unsurpassed specificity, but its sensitivity is low compared to ELISA/SAT (Slide agglutination test). Angelo P Brendo et al from Brazil in their study of 108 cases of leptospirosis have stated that 65% of first sample where positive by SAT compared to 44% by MAT.

A four-fold rise in titer or seroconversion is the most definitive criteria for diagnosis of leptospirosis. Therefore a second sample is mandatory, which is difficult to obtain. In such circumstances, a single high titer in MAT can be taken as diagnostic criteria. As MAT titers peak and persist for a long time (5-10 yrs), they would interfere with current diagnosis. Therefore many workers use different criteria. A titer of 1:100 is taken as significant criteria, but there is controversy on the single diagnostic titer as they depend on endemicity (Table 1). In endemic areas, a titer of 1/100 or 1/200 is considered low; while high titer is usually > 1/400 (some consider 1/800 or 1/1600 as diagnostic criteria). In non-endemic areas, 1/100 titer is taken as diagnostic criteria. It is preferable to do SAT/ELISA along with single high titers. Positive SAT/ELISA with high titers suggest current infection, while negative SAT/ELISA is probably due to past infection (Table 2). Therefore in the modified Faine’s criteria, a four-fold rise in MAT has been given 25 points, while single high titer has been given 15 points along with SAT/ELISA. In addition, low titers based on endemicity in the original Faine’s criteria has been excluded as they complicate diagnosis. Serosurvey in the asymptomatic high risk group should be done with MAT only and a titer of > 1/50 can be taken as cut off titer.

During an epidemic, the microbiology laboratories would be burdened with large number of samples (about 25 or more). It would be impossible to do MAT as it is complicated test. In addition the laboratories need to have all the 24 serogroups; otherwise, a negative MAT does not exclude current leptospirosis if the considered serogroup is not available. Therefore ELISA/SAT are adequate for current diagnosis. If facilities for MAT are available, then the test should be done to confirm the diagnosis and identify serovars (Table 3).

To conclude there is an urgent need to study the prevalence and incidence of leptospirosis in India. This can be done by
1. Sero-survey of asymptomatic high risk groups utilizing MAT
2. Diagnosis of current infection utilizing ELISA/SAT
3. Evaluating the cut-off titers of single high titer and determine the serogroups utilizing MAT in samples with positive ELISA/SAT.

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Reply from the Authors

Sir,

We thank Drs. S Shivakumar and B Krishnakumar for reacting to our review paper. The current status of microagglutination test (MAT) in leptospirosis brought about by them in the letter is appreciated.

The tabular approach and interpretation of results, as mentioned, according to varying MAT titres is a standard practice in any laboratory situated in a leptospirosis endemic area including at ours at JIPMER, Pondicherry. We too take single high titre of 1:400 as positive.

Nevertheless, the problem with MAT will be one of availability of facility, as mentioned in our original article. Thus, ELISA IgM will continue to play a positive role in the present setting (in India).

We have however, some reservation in equating slide agglutination test (SAT) with ELISA IgM in interpreting the results since ELISA IgM as test is definitely superior to SAT.

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REFERENCE


Polymerase Chain Reaction in the Diagnosis of Leptospiral Infection

Sir,

The aims of our study were to identify cases of leptospiral infection in patients admitted with fever, myalgia and jaundice and to institute treatment before complications set in. We have used Polymerase Chain Reaction (PCR) as a laboratory method for the early diagnosis of leptospirosis infection.

A total of 200 patients who presented with fever, myalgia, jaundice and conjunctival congestion were studied over one year (2002-03). Detailed histories were taken from all patients and a screening protocol was prepared using available medical data and clinical examination findings. These patients were also screened for malaria, dengue and typhoid fever.

In 50 patients the clinical picture and abrupt presentation were suggestive of leptospiral infection and in 34 cases (68%) polymerase chain reaction for leptospira was found positive within 5 days of the illness. 16(32%) patients presented in the second week of the illness and had complications of renal failure, neuroparalysis, hepatic failure and bleeding tendencies. In them, polymerase chain reaction for leptospira was negative, however, IgM antibody assay was found positive confirming the diagnosis of leptospiral infection.

Polymerase chain reaction: The technique employed in our study was a simple, rapid and flexible one. In cases where the clinical picture was suggestive of leptospiral infection, the patient's serum was collected in EMJH medium and centrifuged. The sample was added to a master mix and the leptospiral DNA was fixed to the PCR primers covering all leptospiral strains. The amplified DNA was subjected to electrophoresis and identified as a golden fluorescence by ultraviolet light on agar plates. This technique is 100% specific for pathogenic leptospira. A positive test leads to a definite diagnosis of infection, early treatment and prevention of fatal complications like hepatorenal syndrome and renal failure. Polymerase chain reaction by DNA amplification has a 100% specificity and sensitivity for the diagnosis of leptospiral infection.

Leptospiral infection is a zoonotic disease rampant in our population during the rainy season. The clinical manifestations range from a mild asymptomatic illness to a fulminant hepatorenal failure which may be fatal if the diagnosis has been delayed. Hence the early diagnosis of this potentially dangerous disease is imperative in preventing fatalities caused by leptospirosis. The organism responds readily to antibiotics like crystalline penicillin, doxycycline and third generation cephalosporins. In complicated cases life supporting measures like dialysis, hydrocortisone and inotropic drugs may be included in the treatment schedule.