Two Test Strategy for the Diagnosis of Leptospirosis

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Abstract

***Background*: Leptospirosis is Spirochaetal zoonosis with protean manifestation. Rapid diagnosis is important as if appropriate therapy is instituted on time, clinical response is dramatic. The Lepto Dri-Dot test is being extensively used for rapid diagnosis especially during outbreaks.**

***Aims*: To evaluate the results of Dri-Dot test with IgM ELISA and microscopic agglutination test (MAT).**

***Material and Methods* : Acute phase serum samples from 175 cases of suspected leptospirosis were screened by Dri Dot test and later confirmed by IgM ELISA test.**

***Result*: In comparison with IgM ELISA, the sensitivity and specificity of the Dri Dot was 96.46% and 72.58% respectively. Vis a Vis the MAT, the sensitivity and specificity of Dri Dot was 96.67% and 50% respectively.**

***Conclusion*: Since the specificity of Dri Dot test is low; it is useful for diagnosis of outbreaks only at the periphery where facility for ELISA are not available. In a hospital scenario, laboratory diagnosis should be done by ELISA.**

Introduction

Leptospirosis is a Spirochaetal zoonosis of worldwide distribution.[1] The disease shows protean clinical manifestations, so laboratory confirmation is a must. As isolation of leptospires from clinical samples is time consuming serology remains the mainstay of diagnosis. The 'gold standard' serological test is microscopic agglutination test (MAT). MAT test is very tedious and requires the maintenance of several leptospiral serovars in the laboratory. Also the test requires the expertise personnel to read the results.

Therefore rapid and easy to perform tests have emerged in recent years for the diagnosis of leptospirosis. IgM ELISA is one of such test, which is popularly done for the diagnosis of acute leptospirosis. The cost of the test and requirement of specialized equipments still restricts the use of IgM ELISA in Reference laboratories only.

Screening tests in the form of 'one step' diagnostic test, not requiring any instrumentation, emerge every year as mushrooms during the post monsoon epidemic season of leptospirosis.[1-5] Majority of these rapid tests are immuno-chromatographic or particle agglutination tests.

Introduction of such tests in the market as diagnostic tests needs their evaluation by comparing their results with the 'gold standard' test, MAT and a widely used diagnostic test, IgM ELISA.

The present work was done with the aim to evaluate a screening test, lepto Dri-Dot, manufactured by The Dutch Royal Tropical Institute (KIT) in Amsterdam. Also a

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diagnostic strategy for the diagnosis of leptospirosis was thought based on the results obtained in the present study.

Material and Methods

A total of 175 serum samples of clinically suspected leptospirosis from Pune and outside Pune were included in the study. Ten negative control sera were collected. The study was conducted at Maharashtra State Leptospirosis Diagnostic Center, B.J. Medical College Pune during January 2004 to December 2004.

All sera were screened by lepto Dri-dot test. Lepto Dri-dot test is a new card agglutination test developed by the Dutch Royal Tropical Institute in Amsterdam[6] for the rapid diagnosis of leptospirosis. It consists of coloured latex particles activated with a broadly reactive leptospira antigen that is dried onto an agglutination card. 10 µl serum was added to the card next to the blue dot and within area marked by the black circle. It was mixed with blue dot using flat end of spatula to get homogeneous suspension. Card was then rotated slowly in a circular motion. Results were recorded within 30 seconds after start of mixing.

IgM ELISA test from Panbio, Australia was performed on all samples as per the manufacturers' instructions.

The serovar specific MAT was done on 50 sera using 10 serovars.[7]

The results of the Dri-dot and IgM ELISA were compared to calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Results

Results of different tests conducted on serum samples from suspected cases of Leptospirosis were shown in Tables 1 and 2.

The Dri-Dot test detected 126 (72%) cases of Leptospirosis. Of this, 109 (62.28%) were positive by both Dri-Dot and IgM ELISA; while only 4 Dri-Dot negative cases were positive by IgM ELISA. Thus when the Dri-Dot was compared with IgM ELISA it gave a sensitivity of 96.46% and specificity of 72.58% (Table 3) .

50 serum samples were tested by MAT. In comparison with MAT, 39 (78%) samples were positive by Dri-Dot and 29(58%) by both; Dri-Dot and MAT. So vis a vis the MAT, sensitivity and specificity of Dri-Dot was 96.67% and 50% respectively (Table 3).

Discussion

Positive diagnosis of leptospirosis in humans is tedious and time consuming.

Leptospires can be isolated from blood in the first days of illness or from urine after

Table 1 : Comparision of the results of Dri dot and IgM ELISA

	ELISA Positive	ELISA Negative	Total
Dri-Dot positive	109	17	126
Dri-Dot negative	4	45	49
Total	113	62	175

Table 2 : Comparision of results of Dri dot and MAT

	MAT Positive	MAT Negative	Total
Dri-Dot positive	29	10	39
Dri-Dot negative	1	10	11
Total	30	20	50

Table 3 : Evaluation of Dri-dot test vis a vis ELISA and MAT

Dri-Dot Test	ELISA (%)	MAT (%)
Sensitivity	96.46	96.67
Specificity	72.58	50.00
PPV	86.5	74.36
NPV	91.83	90.9

about 14 days for direct microscopic viewing but this procedure lacks sensitivity except in the early phase of disease. Isolation using inoculated growth medium is tedious and slow, usually requiring 6 to 14 days and in some cases up to 5 weeks for positive diagnosis. Repeated culture attempts are often necessary, especially in patients given antibiotics. Thus serological demonstration of infection is now commonly performed.

A sensitive, specific and rapid diagnostic method is of vital importance for the physician, the patient and effective epidemiological surveillance. The Microscopic Agglutination Test (MAT) considered to be a classical test for leptospirosis, has some disadvantages such as technical complexity, high cost and the need to maintain live strains of different serovars with an associated risk of infection to the professionals performing the test. The MAT is reactive relatively late in the infection when compared with the immunoenzymatic ELISA. Therefore MAT has become a test of choice only in reference laboratories.

Several rapid tests are evolved for the diagnosis of leptospirosis. They are easy to perform and read, although needs to be scientifically evaluated with respect to sensitivity and specificity.

Dri-Dot test, a card agglutination test recently developed by The Dutch Royal Tropical Institute (KIT) in Amsterdam, was used in the present study. The assay is based on the binding of leptospira specific antibodies in patient's serum to the broadly reactive antigen coated on the latex particles leading to a fine agglutination.

175 sera of suspected leptospirosis patients were processed by IgM ELISA and Dri-Dot test. From 175 sera, 50 sera were tested by Microscopic Agglutination Test (MAT) along with Dri-Dot test and IgM ELISA.

When compared with IgM ELISA, the sensitivity of Dri-Dot was obtained to be 96.4% and specificity 72.5%. Positive predictive value (PPV) and Negative predictive value (NPV) was found to be 86.55 and 91.45 respectively. When compared with MAT the sensitivity and specificity of Dri-Dot test was found to be 96.6% and 50% respectively.

P.Vijayachari *et al*[6] evaluated lepto Dri-Dot test as a rapid test for the diagnosis of leptospirosis. In the first week of illness, they found the test to be 67.7% sensitive and 66% specific. In contrast IgM ELISA was 48.6% sensitive and 78% specific. After 7 days, lepto Dri-Dot showed the sensitivity of 85% and specificity 80%, while IgM ELISA was 89.1% sensitive and 84% specific. Their results clearly indicate the efficiency of the Dri-Dot test after one week as a “single test” diagnosis.

In the present study, after comparison with MAT and IgM ELISA, Dri-Dot test was found to be more sensitive. Being a screening test, high sensitivity is well justified. Low specificity of the Dri-Dot test might be due to the crude nature of antigen.

The sensitivity and specificity of IgM ELISA was found to be 90% and 89.3% respectively, when compared with MAT.

Based on only one test, confirmed diagnosis of leptospirosis is not achieved. This may be because of either low sensitivity or low specificity of the particular test. In the present study only Dri-Dot or IgM ELISA alone could not confirm the diagnosis due to low specificity of Dri-Dot test and inability to detect past/ chronic infection (IgG antibodies) by IgM ELISA.

Therefore, we strongly recommend a “Two Test Positive” phenomenon for the confirmed diagnosis of leptospirosis. The two tests must be based on different principles. The “Two Test Positive” phenomenon should include a

screening test along with IgM ELISA. A well-evaluated screening should be selected for the diagnosis. The above mentioned results obtained by P.Vijayachari *et al*[6] also supports this phenomenon even if used in the first week of the disease. In the first week of the infection, according to their study, Dri-Dot test gave considerable sensitivity (67.7%) along with good specificity (78%) by IgM ELISA.

As MAT is out of reach for most of the diagnostic laboratories, the above said "Two Test Positive" phenomenon will be useful to diagnose most of the leptospirosis cases with high precision.

References

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CGRP - RECEPTOR ANTAGONISM IN MIGRAINE TREATMENT

in The Lancet today, Tony Ho and colleagues present the first phase III trial of a novel oral calcitonin-gene-related peptide (CGRP) receptor antagonist telcagepant, which they tested against one of the currently most effective triptans, zolmitriptan, and against placebo. In the study telcagepant had an antimigraine effect that was not significantly different from that of zolmitriptan but with fewer side-effects.

These findings were then followed by the demonstration that CGRP is released during migraine and cluster-headache attacks.

However, telcagepant is associated with a lower incidence of side-effects than the triptan. This result marks a new era in migraine therapy.

Lars Edvinsson; The Lancet; 2008; 372 : 2089-90.