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Human Leptospirosis; an emerging problem in WestBengal, India

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I. Introduction:

Leptospirosis remains one of the most common and most dreaded zoonotic infections worldwide.[1] Traditionally related to certain socioeconomic or climatic conditions that favorendemicity in animal vectors and human exposure, it is generally confined to the developing parts of the world, being randomly reported from industrialized countries[2] often as an imported disease following international travel to exotic destinations.[3]

Underreporting is a major problem in evaluating the presence and the actual incidence of leptospirosis in many Asian countries. In India, leptospirosis is a major health problem obviously related both to the monsoons and poor sanitary conditions, with multiple epidemics reported in recent years.[4,5,6,7,8] The Andaman and Nicobar Islands top the list of the most endemic areas of the world, yet no official incidence data on most provinces of India exist. Similar environmental and sanitary conditions, with the added burden of overcrowding, apply to neighboring Bangladesh and Nepal, where the disease was recently recognized as an important alternative diagnosis in patients with suspected dengue and febrile diseases in general, respectively.[9,10]
IgM antibodies are detectable after about the fifth day of illness.[11]For serological diagnosis of Leptospirosis, Leptospiral IgM ELISA is an accepted method.[12]

Lateral flow immunochromatography to detect IgM against Leptospira is rapid, convenient, commercially available serological test with good sensitivity and specificity.[13]

Objectives:

Objective of our study was to find out the seroprevalence of human Leptospirosis among patients attending a tertiary care hospital in Kolkata in patients presenting with fever along with icterus or haemorrhagic pulmonary and/or renal failure manifestations.

II. Materials & Methods:

The study was conducted in the Microbiology Dept. of School of Tropical Medicine, Kolkata for six months from January 2012 to December2012 with 83 suspected cases fulfilling the inclusion criteria as follows:

1. Patients with fever (for more than 7 days).
2. With or without jaundice.
3. With or without renal failure.
4. Patients giving informed consent.

Cases of Fever, Icterus and Renal failure caused by microorganisms other than Leptospira were excluded from the study.

Blood samples aseptically collected from the patients were sent to the laboratory for Immunochromatography and ELISA test to detect antibody against Leptospira.
Lateral flow Immunochromatography was done by using On Site Leptospira IgG/IgM Combo Rapid Test-Cassette (serum/plasma/whole blood) [CTK Biotech, Inc].
1 drop (40-50µL) whole blood or 30-45µL serum was poured into the sample well by keeping the dropper vertically.
35-50µl of diluent was poured immediately. Results could be read in 15 minutes. Positive results could be visible in as short as one minute.
Readings were taken strictly before 15 minutes. Presence of Control bands validated the results.
Abovesaid rapid lateral flow immunochromatography could detect presence of both IgM and IgG.

IVD Leptospira IgM Microwell ELISA test kit (USA) was used to confirm the result of the Immunochromatography kit. Microwells were coated with purified Leptospira Patoc 1 antigen; 140 μl of patient's serum (1:40 dilution) was transferred to test well and incubated at room temperature for 10 minutes. After washing two times with wash buffer, 2 drops of enzyme conjugate were added to each well and then incubated at room temperature for 10 minutes again. Then, after washing three times with wash buffer, two drops of chromogen were added and incubated again for 5 minutes. Two drops of stop solution were then added

and final reading was taken in an ELISA reader. Positive and negative controls were kept in each test. Samples with O.D. 0 to 0.3 O.D. was considered negative. Samples having O.D. of >1 were considered as strongly reactive. Patients with weak reaction (0.5-<1) were tested 2-3 weeks later to detect seroconversion.

III. Results:

Out of total 83 test samples from January 2012 to December 2012, 12 were found to be seropositive for Leptospira IgM antibody by IgM Microwell ELISA technique (14.45%). Out of these 12 Leptospiral IgM seropositive patients, 10 patients were detected to be reactive by IgM/IgG Combo Immunochromatography.

Out of 12 IgM Leptospira reactive fever cases, 5 suffered from jaundice (41.66%). None of the patients presented with renal failure or haemorrhagic manifestations. Only one patient developed maculopapular erythematous skin rash in face and extremities along with fever and jaundice. No mortality was reported.

Out of 12 IgM seropositive cases, 8 were males (66.67%).

Out of total 12 acute Leptospirosis cases (serologically diagnosed), 5 cases (41.66%) were under the age of 15 years whereas rest 7 were above 15 years among which, 6 (50%) were above 35 years.

All the Leptospira IgM seroreactive cases belonged to poor socioeconomic strata and semiurban& rural communities. All of the IgM seroreactive cases were reported during the months of September (3 cases), October (7 cases)and November (2 cases).

Majority of the acute Leptospirosis cases (7 out of 12) were farmer by profession (58.33%).

IV. Discussion:

IgM ELISA, which uses Leptospira Patoc 1 strain, is a standard serological test for early diagnosis of leptospirosis. In our study, IgM ELISA shows a positivity of 14.45%. An outbreak of Leptospirosis in Mumbai in 2002 showed a positivity of IgM ELISA of 36.27%. [14] In the studies of Chandrasekhar *etal*. [15] and Babu*etal*. [16] IgM ELISA positivity were 41.77% and 88.9%, respectively.

Microscopic AgglutinationTest is a widely used reference test for leptospirosis but is inadequate for rapid case identification as it requires analysis of paired sera.[17] Moreover, the prevalent serovars in a particular geographic area must be known as it is cumbersome to test for all 200 serovars of *L. interrogans.* In a study conducted in Chennai in 1988 during the peak monsoon season, out of 40 patients 33 (82.5%) had specific leptospiral antibodies. [18]In 1987, a seroprevalence of 25% was reported in patients hospitalized in Karachi, Pakistan.[19]Studies from different parts of India have revealed a seroprevalence ranging from 17.8% to 40.5% (Ratnam*etal*.). [20] Fever(100%) was the commonest presenting symptom followed by Jaundice(41.66%) in our study keeping accordance with a study conducted by Muthusethupati*etal*. in 1995, [21] fever and jaundice were the most common presentation. Male preponderance (66.6%) has been found in our study and 58.3% of the cases has been found in adults >15 years of age. The higher prevalence in males can be attributed to more frequent outdoor activities; [22]All reactive cases were noted during the months of September-November (monsoon and post-monsoon season) in our study.

According to other workers, he sensitivity of diferent tests for diagnosisof Leptospirosis varies from 88% to 100% and specificity from 95% to 99%. [23] Similarly, in our study,rapid test could detect 10 (83.33%) seropositive cases (out of 12 detected by IgM ELISA).

Further epidemiological studies should be carried out for proper evaluation of the scenario of endemicity of leptospirosis in this part of the country.

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