LEPTOSPIROSIS IN NORTHERN INDIA: A CLINICAL AND SEROLOGICAL STUDY

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Abstract. A total of 400 serum samples collected from patients, clinically suspected of leptospirosis, were evaluated for antibodies by LEPTO dipstick and microscopic agglutination test (MAT). Twenty of these patients (5%) had serological evidence of leptospirosis. *Leptospira interrogans* serovars Autumnalis and Icterohaemorrhagiae, Canicola and Javanica were serogroups recorded serologically. Fever and jaundice were the most common clinical presentations. Male preponderance was seen in the leptospirosis cases. Outdoor activities, agricultural activities, contact with animals were significantly associated with seropositivity for *Leptospira.* This study highlights that leptospirosis is a significant health problem in northern India, though grossly under reported due to the absence of routine laboratory diagnostic facilities for this disease.

INTRODUCTION

Leptospirosis is a well-known, worldwide zoonotic disease with a much greater incidence in tropical countries. Until recently it was considered an uncommon disease in most countries. Recent epidemics and case series from various places have indicated the significance of the disease in the human population (Machang'u, 1992; John, 1996; Sehgal, 1996; Everard and Everard, 1993). In India, leptospirosis is a grossly under reported disease, probably due to the lack of diagnostic modalities and lack of awareness of the disease among physicians. Though social, environmental and occupational factors offer ideal conditions for successful transmission of leptospirosis, the disease was considered infrequent in occurrence until early 1980, after which it has been reported sporadically or as epidemics from different parts of the country (Sehgal, 2000). There is no accurate estimate of the problem of leptospirosis in non-endemic areas like North India.

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A definitive diagnosis is made by isolation of the organism from blood or urine, but it takes time for the organism to develop in cultures and growth is unreliable. Diagnosis usually depends upon clinical assessment and serological tests, combining these can dramatically increase the recognition of patients with leptospirosis.

MATERIALS AND METHODS

A total of 400 patients with acute febrile illness with headache and severe myalgia and one or more of the following clinical features were included in the study group: a) presence of jaundice, b) oliguria and features of renal failure, c) cough, hemoptysis and breathlessness, d) hemorrhagic tendencies, e) signs of meningeal irritation and convulsions and f) conjunctival suffusion and hemorrhage.

Single and paired sera (taken at an interval of 10 days) were collected from each patient. Serum was separated and stored at -20°C until tested. The LEPTO dipstick (kit, Royal Tropical Institute, Amsterdam, Netherlands) for the detection of IgM antibodies was used on these serum samples according to manufacturers' instructions (Gussenhoven *et al*, 1997). The LEPTO dipstick kit contains one vial of lyophilized detection reagent (vial B), one vial of reconstitution fluid (vial A), one vial of dipstick fluid (vial C), dipsticks,

test tubes and a test tube holder. The dipsticks have two bands, a lower band of heat stable antigen prepared from a culture of *Leptospira biflexa* and an upper internal control band. The test was performed following the prescribed procedure. Five ml of reconstitution fluid was added to the detection reagent and 250 µl of the reconstituted reagent was transferred to the test tubes. The test serum (5 µl) was added to the test tubes containing reconstituted detection reagent and the contents were mixed. Dipsticks were incubated in the tubes containing the serum-reagent mixture at room temperature for 3 hours. The strips were read by three independent readers. Depending upon their unanimous observations, the results were recored as 1+, 2+, 3+ or 4+ based on the intensity of color of the antigen band. If there was a difference of opinion about the development of the color band, the result was taken as equivocal and test was repeated. All the positive samples by LEPTO dipstick were sent to the National Leptospirosis Reference Center, Port Blair, for a microscopic agglutination test (MAT), performed following the standard procedure (Wolff, 1954) using 8 live leptospiral strains as antigens. The strains belonged to serogroups, Grippotyphosa, Australis, Icterohaemorrhagiae, Pomona, Autumnalis, Canicola, Javanica and Sejroe.

RESULTS

Of 400 human sera, 20 samples (5%) were positive for IgM leptospira antibodies by the LEPTO dipstick assay. The MAT could be done on only some of the samples, the most common serogroups were Autumnalis (2), Icterohaemorrhagae (2), Javanica (1) and Canicola (1).

The mean duration of the disease was 12 days and the mean age of the *Leptospira* positive patients was 33.5 years (range 26-41 years). The male to female ratio was 4:1. The occupation varied and most of the patients were agricultural workers 10 (50%) (Table 1). The patients were from different parts of Northern India and no geographical clustering was seen. Fourteen patients (70%) had a history of rat infestation in their houses and 50% of patients had contact with animals, particularly cattle. Six patients (30%) had wet house surroundings.

Table 1
Occupation of 20 cases of leptospirosis in Northern India.

Work category	Number (%)
Outdoor manual workers	
Agricultural workers	10 (50)
Laborers	4 (20)
Indoor non-manual workers	1 (5)
House wives	2 (10)
Unemployed	2 (10)
Students	1 (5)

Table 2
Clinical features of leptospirosis patients.

Clinical features	Number (%)
Fever	20 (100)
Headache	19 (95)
Myalgia	18 (90)
Jaundice	16 (80)
Subconjunctival suffusion	10 (50)
Oliguria	9 (45)
Lymph node enlargement	6 (30)
Abdominal pain	5 (25)
Vomiting	6 (30)
Diarrhea	5 (25)
Altered sensorium	4 (20)
Neck stiffness	4 (20)
Hematuria	1 (5)
Breathlessness	1 (5)
Epistaxis	1 (5)
Petechial hemorrhage	1 (5)

The most important clinical presentations are shown in Table 2. All patients had fever. Headache and myalgia were present in 95% and 90% patients, respectively. Jaundice was present in 16 (80%). Of 20 patients, 9 patients (45%) were oliguric. Subconjuctival suffusion was present in 50% of patients. Other clinical features, which were more commonly seen, were lymph node enlargement (30%), diarrhea (25%), abdominal pain (25%), altered sensorium (20%), neck stiffness (sign of meningeal irritation) (20%), epistaxis

(5%) and petechial hemorrhages (5%).

DISCUSSION

Due to the lack of diagnostic tools, the diagnosis of leptospirosis cannot be easily made in many laboratories. Leptospirosis is often not recognized or is erroneously mistaken for other diseases with similar symptoms (Faine, 1987). In this study we have included patients with clinical criteria for leptospirosis, 5% of cases were seropositive for leptospirosis. Studies from other parts of the country showed a seroprevalence ranging from 17.8 to 40.5% (Ratnam *et al*, 1993, 1994, Muthusethupati *et al*, 1995). The low prevalence (5%) in this study could be due to the fact that this is the first study of its kind in this region. Further evaluation by clinicians and better awareness of the disease in conjunction with the availability of a rapid screening test like the IgM dipstick are required for early diagnosis and institution of therapy. MAT could not be performed in some cases, as some samples were contaminated in transit to the reference laboratory. Some studies (Sehgal *et al*, 1999; Smits *et al*, 1999) have shown that the LEPTO dipstick has a good level of agreement with MAT. The standardization of MAT is difficult as there are more than 250 serovars of *Leptospira* and it is not possible to look for each serovar in an area where the prevalence of *Leptospira* is not known (Cole *et al*, 1973; Arimitsu *et al*, 1994; Muthusethupathi *et al*, 1995). The IgM LEPTO dipstick uses a broadly reactive antigen for detecting IgM antibodies and it does not provide information about the serotype involved in the infection. Knowledge of the serotype is not essential for treatment of these patients. The test is simple to perform, not requiring special equipment or technical expertise and has sensitivity of 86.8-87.4% and specificity of 92.7-94.11% (Gussenhoven *et al*, 1997; Smits *et al*, 1999), thus is a good screening test for the diagnosis of leptospirosis.

Fever with or without rigors was the commonest presentation in our study, followed by jaundice in 80% of patients. This is in concordance with a study conducted in Chennai (Muthusethupathi *et al*, 1995) where fever and jaundice were the most common presentation, in contrast to the Barbados hospital admission, where 97% of patients had jaundice (Everard and Everard, 1989). A clinico-epidemiological study (Barua *et al*, 1999) carried out in the North Eastern states of India reported headache as predominant symptoms (84.21%) followed by fever (73%).

Symptoms and signs like extreme muscle tenderness and suffusion of conjunctiva, which are considered as cardinal signs of leptospirosis, occurred in a proportion of patients and were helpful in making a provisional clinical diagnosis.

Though neck stiffness and altered sensorium occurred in 4 patients indicating central nervous system involvement, none had convulsions or the complication of meningitis.

Leptospirosis prevails among a wide range of occupational groups. General environmental contamination is particularly likely in developing countries. In our study, 50% of leptospirosis patients gave a history of contact with animals, particularly cattle, and 70% had a history of rat infestation in their houses. The majority of them were agriculturists. All these environmental and household factors increase the risk of leptospiral infection (Murhekar *et al*, 1998). A seropositivity of 32.9% was seen in conservancy workers in Chennai (Ratnam *et al*, 1993). Male preponderance was seen in leptospirosis infected cases in our study and mean age group affected was 26-41 years. The higher prevalence in young males is a universal phenomenon (Everard and Everard, 1990) and is generally attributed to their more frequent outdoor activities and with the age group that are most active in agricultural activities. These findings indicate that leptospirosis is a significant health problem in Northern India. Increased awareness among clinicians in areas of increased prevalence and community-based studies are required to understand the actual morbidity and mortality due to leptospirosis.

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