



LEPTOSPIROSIS - AN EXPERIENCE IN A TERTIARY CARE HOSPITAL AT KOLKATA

Clinical Microbiology

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ABSTRACT

Background & Objective: Leptospirosis is a zoonotic disease caused by a pathogenic Spirochetes of Genus *Leptospira* generally remains under diagnosed due to lack of proper diagnostic facility & setup in peripheral Health facilities. As the clinical manifestations of Leptospirosis are nonspecific, proper Laboratory Diagnostic method is needed for definitive diagnosis. **Material & Methods:** This is a cross-sectional study conducted in the Dept. Of Microbiology, Calcutta National Medical College from July 2021 to June 2022, in patients with clinical features of febrile illness. A total of 252 blood samples were received for leptospira diagnostic assay. With centrifuged serum samples specific antileptospira IgM antibodies detected by using MAC ELISA method. **Results:** Amongst 252 received samples 48 samples (19%) were serologically reactive. Maximum positive cases were clustered around July 2021 (18%) & August 2021 (25%). Amongst the positive cases male (58%) female (42%). Most common symptoms fever (100%), followed by myalgia (81%), & headache (72%). Highest prevalence of sero positivity found in 31-40 Yrs (28%). **Conclusions:** In this study we used MAC ELISA to study sero prevalence of Leptospirosis. This gives an idea about the epidemiological background of Leptospirosis in our locality. Highest seroprevalance was found in rainy season. It is an occupational disease so improvement in hand hygiene & sanitation should be used as a preventive measure to control the disease burden.

KEYWORDS

INTRODUCTION:

Leptospirosis is a worldwide common zoonosis mostly prevalent in tropical & subtropical areas with high rain fall. (1) It is caused by pathogenic spirochetes of genus *Leptospira*. This genus comprises at least 22 species grouped into three categories pathogenic, intermediate & saprophytes. Currently there are more than 250 pathogenic serovars (3)

It has been reported in India since 1931 by an extensive survey of the disease outbreak in the Andaman Islands and researcher's isolated *L. andamans* and *L. grippityphosa*.

In recent years incidence has been reported from Southern, Central, Eastern & Western India following heavy monsoon, animal rearing practices & unplanned urbanisation. (2) Humans are the accidental host & usually infected through direct contact with urine from infected animals like rodents, dogs, cattle & pigs or through water and soil contaminated with their urine (1). Organism enters the body through cuts & abrasions of the skin or mucus membrane. Infections therefore related to occupational activities like sewage workers, agricultural labourers, animal handlers etc are at high risk for the disease. Person to person transmission rare.

Traditionally this zoonosis was considered mostly in rural population amongst farmers work barefooted in land during monsoon, in places where cattle & pigs are reared up. Gradually there was increased recognition that there is an additional risk of infections in urban areas as they are exposed to domestic rats, moreover canine leptospirosis is also a potentially infectious veterinary source of pathogenic serovars of *Leptospira*. (NIH PUBLIC ACCESS)

After an incubation period of 2-20 days the disease manifest as biphasic illness consisting of initial leptospiremic phase 3-7 days, followed by immune phase 4-30 days. The mild anicteric form of disease characterised by non specific symptoms like fever, headache, chills, myalgia, nausea, vomiting is more common than icteric form of Leptospirosis (Weil's Disease) characterised by Jaundice, renal dysfunction & bleeding diathesis (4) Severe complications like Hepato – Renal failure or central nervous system involvement (Meningitis) may arise particularly if the diagnosis is delayed. The accuracy of clinical diagnosis of Leptospirosis is poor because clinical features are similar to those other causes of tropical febrile illness like Malaria, Dengue, Typhoid, scrub typhus, Chikungunya & other viral fever. In Indian scenario, leptospirosis accounts for 12.7% of acute febrile illness. Therefore the diagnostic testing of *Leptospira* should be included in the differential diagnosis of pyrexia of unknown origin.

Isolation of the organism is poor due to prior indiscriminate usage of antibiotic usage and also difficult and expensive technique. Hence serological diagnosis remains the main cornerstone in the diagnosis of Leptospirosis. IgM Antibodies against *Leptospira* appear after 5 days & attains high titre during 3rd to 7th week & persist for near about 5 months or more. But only tertiary centres has the diagnostic facilities by ELISA method, so till now mostly the disease is under reported. So an attempt was made to evaluate the seroprevalence of Leptospirosis amongst patients with acute febrile illness in our hospital.

The objective of this study is to determine the sero prevalence of Leptospirosis amongst the patients of acute febrile illness attended OPD & IPD of Calcutta National Medical College during the year July 2021- June 2022.

MATERIALS & METHODS:

The present study is an observational study conducted in the Dept. of microbiology, Calcutta National Medical College & Hospital for a period of one year from July 2021 to June 2022. Samples were collected from patients with high fever > 39°C for more than 5 days in both IPD & OPD. As standard literature search revealed that *Leptospira* antibody takes at least 5 days to appear in peripheral blood & persist for 5 month. So the residents of West Bengal with fever for less than 3 days & past H/O of *Leptospira* within 5 month were excluded. A standard data entry form was provided to document demographic and epidemiological data (age, sex, socio-economic status, residential address, contact with flood water etc) & clinical information from each patient.

5 ml blood collected in plain vial by aseptic venipuncture method from all patients. During these period we received 252 samples for specific Antileptospira antibody testing in our serology lab. All samples are centrifuged at 3000 rpm for 5 min to separate the serum. Then centrifuged serum samples were tested for specific anti *Leptospira* IgM antibodies by MAC Elisa method using IgM.MAC ELISA DIAGNOSTIC KIT. MAC ELISA Kit is for quantitative detection of anti *Leptospira* Antibody in serum. The ELISA procedure was performed according to the protocol provided with the diagnostic Kit & interpreted as per manufacturers instructions i.e

Values less than
<9 units were considered Negative
9-11 s equivocal
> 11 Positive

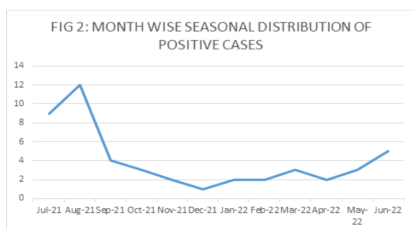
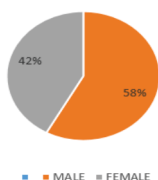
For samples showing equivocal results another blood samples were drawn after a period of 10 days & the test was repeated.

RESULTS:**Table 1: Symptom Wise Distribution**

SIGN & SYMPTOM	FREQUENCY	PERCENTAGE
FEVER	48	100%
MYALGIA	38	81%
HEADACHE	34	72%
CONJUNCTIVAL SUFFUSION	37	78%
NAUSEA	31	65.5%
PAIN ABDOMEN	33	70.8%
JAUNDICE	21	45%
OLIGURIA	11	25%
ARTHRALGIA	36	75%
PALLOR	14	30%

Table 2: Age Wise Distribution

AGE IN YEAR	NUMBER	PERCENTAGE
11-20	4	8.3%
21-30	8	16%
31-40	17	28%
41-50	11	23%
51-60	5	10.4%
61-70	2	4%
71-80	1	2%

FIG 1: PIE CHART SHOWING DISTRIBUTUION OF SEX

- (i) During the study period from July 2021 to June 2022 (FIG 2) amongst 252 cases of acute febrile illness 48 samples (19%) were serologically reactive by quantitative IgM ELISA test for Leptospirosis .
- (ii) Amongst the positive cases (FIG 1) 28 (58%) were male & 20 (42%) were female.

Occupationally more than 50% of the affected patients were outdoor manual workers.

Most of the positive cases presented with (TABLE 1) Fever (100%) ,followed by myalgia (81%), Headache (72%), conjunctival suffusion (78%) respectively.

Maximum clustering of cases were seen during the month of July (18.7%) to August (25%) 2021 .

Highest prevalence of seropositivity was found in 31-40 Yrs age group (28%)(TABLE 2)

Maximum cases were clustered around nearby urban slum dwellers (Rural 30% & urban 70%)

DISCUSSION:-

In our study sero prevalence of *Leptospira* in febrile patients attending both out patient & in patient Dept: during the year 2021-22 was 19%. In another review article (11) as India is endemic for *Leptospira* seroprevalence in healthy population showed 24%.(8) Since in early phase of Leptospirosis fever, myalgia & head ache are the only identifiable symptoms we restricted our inclusion criteria as to those three symptoms for our study. Three other studies conducted in Thailand, Trinidad & Nicaragua the percentage of patients diagnosed

as having Leptospirosis was 15%, 4.9%, 6% respectively. In a study restricted to paediatric age group conducted in Mumbai 34% were diagnosed to have Leptospirosis (4)

In our study the mean age group mostly affected was 31-40 year, mean age 36.4 years are young adults working population, actually involved in outdoor activities & more chances of contact with animals & rainwater etc , other studies by Gangadhar et al (12) mean age group affected was 36 years, Shiv Kumar et al(5) recorded 39.6 years co-related with our study.

Gender wise male preponderance noted 58% than female 42% correlates with other studies like pappaeahan et al. (59.8)% (6), Shiv Kumar et al. (77%)(5) due to outdoor activities and high risk of occupational exposures.

Regarding occupational areas data revealed from epidemiological survey that 52% of the patients were outdoor manual workers, butchers, meat slaughters, poultry workers & municipal sewage workers or plumbers. Other studies like Shivkumar et al(5) in 1993 49% , Sharma et al (7) 2006 revealed 59.6% affected were outdoor manual workers which co-relates with present study. Probably these workplaces are associated with lack of hygiene and provide abundant food sources for rodents and also dogs, cats & other urban reservoirs of this disease. None of them used protective measures like gloves during working & lack of hand hygiene habits. Repeated cut injury in hands increases the chance of entry of *Leptospira* & constantly producing antibodies thus may be giving protection from re infection by the same (Morocco study) (8).

Temporal association between high rainfall & human Leptospirosis has been noted in India from Chennai, Orissa, Mumbai & other tropical countries (4). In our study also sudden spurt of cases were noticed in monsoon during (July- September) quarter. Intermittent flooding after a heavy rainfall during July to September (monsoon) have flushed the rodent burrows & contaminated all water resources like ponds, rivers, streams, canals etc with rat urine . Only one Moroccan study conferred that the seasonal peak was detected in summer & autumn.(Morocco study) (8)

So following monsoon there was chance of outbreaks as water logged roads may be contaminated with rat urine & people will come in contact with dirty water. This was co-related with Mumbai study following flood in 2002.(4) The low lying urban slums in vicinity of our hospital noticed highest caseloads . These slums remains water logged for 6-8 days in rainy season. Slum dwellers waded through contaminated water with rat urine provides an opportunity to invade Leptospirosis. Isolated cases of Leptospirosis in adults due to exposure to rat urine have been reported from Mumbai. In urban areas of Sao Paulo Brazil the source of infection through contaminated rain water during flood (88%) has been reported (4) . So it can be inferred that poor urban slums with lack of educational knowledge about possible risk of transmission like contact with contaminated flood water with rat urine may be possible mode of transmission .

In our study most common symptom were high fever (>39°C) (100%), followed by myalgia, headache & conjunctival suffusion co-related with other studies CharitaThalva et al(9). Jaundice as a complication has been described in many studies.(7) Due to lack of follow up facilities complications & treatment outcome cannot be interpreted by us.

Nowadays, most common and easiest method to diagnose acute Leptospirosis to by IgM *Leptospira* Antibody testing by with MAC-ELISA method.

It has a highest sensitivity, specificity & positive prediction value, our study also relied on this method.(4) *Leptospira* Antibody becomes detectable in serum after 1 week of illness and in later stages, after 2 weeks may appear in urine.

Study of Charita Thalva et al . rural 31% & urban 68% as majority of samples in their study received from rural farmers who actively engage themselves in farming during monsoon .In our study cases were mostly from urban location (70%) than rural (30%).as in catchment areas of our hospital many urban slums are located, unhygienic habits & frequent water logging during monsoon may be explanation of this facts.

Availability of this test eliminated the shortenings of reference standard sero diagnostic test-MAT (Macroscopic agglutination test) which is not sensitive enough but time consuming, , required high expertise , required paired sera & maintenance of live strain. This facility is available only in National Reference Centre of *Leptospira* , in Port Blair Andaman . Since ELISA can detect only genus specific antibody but unable to identify serovars or serogroups . Only MAT could have been given this epidemiological data of identifying the serovars . Unfortunately sending the samples to Port Blair could not be resolved by us ,so this is the limitation of our study.

Overall, Leptospirosis was found to be the most important cause of acute febrile illness in India therefore necessary screening testing of this disease along with other causes of PUO should be included in differential diagnosis of acute febrile patients to decrease the morbidity & mortality associated with this disease.

To prevent future outbreaks proper rodent control measures & improvement in sewage & drainage facilities are necessary. Reservoir host plays an important role in transmission dynamics of *Leptospira*. In India rodents are the most widespread & famous carries of Leptospirosis (particularly domestic rats (*Rattus norvegicus*) Urban stray dogs also plays an important role as reservoir to maintain the transmission chain in nature by sub clinical infection & thus plays a significant source to spread Infection. (10) More & more research studies should be conducted in future to identify the reservoirs of infection & prevalent serovars amongst them which will assess the risk of transmission and apply preventive measures to control the burden of human leptospirosis .

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