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Seroepidemiological trends of human leptospirosis in Coimbatore, India between 2007 and 2009

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ABSTRACT

For patient management and therapeutic initiation, the rapid method of diagnosing leptospirosis is important. The microscopic agglutination test (MAT) is the gold standard serological test used in the reference laboratories with high sensitive and specific. In this study, the survey was done in order to understand the leptospiral epidemic in Coimbatore. One hundred and ten serum samples were included in this study from patients with suspected leptospirosis and were subjected to IgM Enzyme Linked Immuno Sorbent Assay (ELISA), Microscopic Agglutination Test (MAT). A battery of 10 predominant infecting serovars was included as MAT antigen. Among 240 cases, 213 serum samples showed positive results to MAT and predominant serogroup was Grippotyphosa (69%), followed by Autumnalis (17%), Australis (10%) and Patoc (4%). IgM ELISA showed a sensitivity of 97.3% and specificity of 100% compared to MAT. Among the serovar included in this serological study, Grippotyphosa was observed predominant and provide 100% specificity in diagnostics.

Keywords: Leptospirosis, Microscopic Agglutination Test, IgM Enzyme Linked Immuno Sorbent Assay, Grippotyphosa.

INTRODUCTION

Leptospirosis is an anthrozoönotic disease caused by genus *Leptospira*, human gets infection with multi systemic involvement globally in both rural and urban areas [1]. This is worldwide in

both urban and in rural areas, similarly in temperate and in tropical countries [2,3]. Infection is being accidental; usually occur after direct or indirect contact with urine of leptospiruric animals, risk factors mainly due to acute renal failure through acute tubular necrosis [4]. Individuals at risk include farmers, abattoir workers, sewage workers, miners and occupation related to handling of animals are at a great risk [5]. Leptospirosis is more common in southern parts of India and large number of outbreaks has been noticed during the period of October to December, every year in Tamilnadu [6,7,8].

The morbidity and uncontrolled mortality was witnessed in the recent flood in Mumbai and reports showed that there is no systemic prevention and control programme for leptospirosis in India and it has not been identified as priority under the national health policy. The true incidence of human infection in India is not known either because of lack of awareness on the part of the treating physicians or the lack of diagnostic techniques [9]. Very few reports of leptospirosis were noticed in India till 1980s [10,11]. Isolation of the causative agent was first reported as early as 1931 from Andaman and Nicobar Islands [12]. In 1992, Seroprevalent rate more than 55% was observed in the population of Andaman [13]. In south India reports of leptospirosis was observed in several places like Tamilnadu, Kerala and Karnataka [5-8,14] whereas in Andhrapradesh there is no systemic study on human leptospirosis and the disease remains largely under reported [5].

Due to protean clinical manifestations and lack of simple diagnostic methodology, lot of positive cases was unrecognized and piloted to increase in mortality rate. The symptomology of this disease ranges from mild flu like infections to chronic multiple organ failure (CMOF). To overcome this problem, need the construction of simple diagnostic tool for the early detection and need standard prevention programme [5]. The detection of leptospira from clinical specimens by Direct Dark Field Microscopy (DDFM) providing a presumptive proof and rapid diagnosis whereas it requires expensive laboratory facilities [15].

Epidemiological investigation of leptospirosis is often hampered by the difficulty of making a definitive microbiologic diagnosis. Isolation of leptospire from clinical samples provides a definitive diagnosis; however, the value of culture is limited because samples have to be collected before the administration of antibiotics and culturing requires prolonged incubation. The most applicable and practical method for the diagnosis of leptospirosis is based on detection of antibodies by serological test. Among the identification techniques, the microscopic agglutination test (MAT) remains gold standard because of its unsurpassed specificity compared with other available tests.

MATERIALS AND METHODS

Study Area and Design

The study area covered the patients admitted to Kovai Medical Centre and Hospital, Coimbatore which portrays most of the patients from the town of Coimbatore and surrounding villages. The climate is tropical and mostly equable; average rainfall is 1,800 to 2500 mm per year, the temperature ranges from 24°C to 32°C, and the humidity ranges from 75 to 84%. Population based surveillance was conducted between October 2007 to December 2009. Clinical (according to the data observed in OP ward and their major clinical signs with suspected leptospirosis) and

epidemiological data were collected using a detailed notification form [16] included personal characteristics of 13 variables; Symptoms of before admission of 14 variables; signs after admission of 12 variables and laboratory test results of 30 variables including total, differential blood counts (RBC indices and Total leukocyte count), hemoglobin estimation, ESR and PCV analysis, blood pressure, bleeding time, clotting time, blood urea and major biochemical estimations, ELISA, MAT and so on.

Leptospiral serovars

A battery of 10 leptospiral serovars (according to the prevalence of serovars in this endemic area) was used as antigen in the Microscopic Agglutination Test (MAT) of this study. They were as follows, 1. Australis (strain Ballico), 2. Autumnalis (strain Bangkinang), 3. Grippotyphosa (strain Moskva V), 4. Canicola (strain Hond Utrech IV), 5. Javanica (strain Poi), 6. Icterohaemorrhagiae (strain RGA), 7. Hebdomadis (strain Hebdomadis), 8. Pomona (strain Pomona), 9. Pyrogens (strain Salinem) and 10. Patoc (strain Patoc I). Leptospiral serovars were obtained from WHO collaborating centre for diagnosis, reference, research and training in Leptospirosis – Indian Council of Medical Research, Port Blair, Andaman & Nicobar Islands. The strains were maintained in semisolid EMJH medium supplemented with 1% BSA and vitamin solutions (Thiamine, Nicotinic acid and Cyanocobalamine) in screw capped test tubes [5].

Serum samples

Serum samples were separated from blood collected from the patients suspected with leptospirosis. The serum samples were then stored in polyvinyl vials at -20°C till its use. Samples were subjected to standard serological tests including IgM specific ELISA and Microscopic Agglutination Test.

IgM ELISA

IgM ELISA was performed using micro titer plates prepared in house [6]. The plates were coated with heat killed whole cell antigen prepared from Patoc I strain. 10µl of test serum was added to all wells and incubated for 30 minutes at room temperature. Then all the steps were followed according to standard procedure [17]. A titer of 1:80 is considered as indicative of the presence of leptospiral antibodies.

Microscopic Agglutination Test (MAT)

MAT was performed on the samples using ten live leptospiral strains as antigens which are grown in EMJH liquid medium and the growth density was compared with McFarland standard 1.0. Care was taken to ensure that no culture with clumping or auto agglutination was observed. Doubling dilution of serum was performed in phosphate buffered saline from 1:20 and positive samples were titrated up to end titers. To all this dilutions, 50µl of specific serovars was added and control included in this study have live antigen alone without test serum. Then the plates were closed with aluminium foil to provide dark condition and incubated at room temperature for four hours. After incubation one drop from each well was taken by micro diluter and a thick wet smear was prepared on microscopic slides for examining the agglutination under dark field microscopy [18]. A titer of 1:80 or above was considered as positive.

RESULTS AND DISCUSSION

During the period of study (October 2007 to December 2009), 240 cases suspected of having leptospirosis based on clinical criteria described above were observed to Kovai Medical Center and Hospital, Coimbatore. The age of the patients ranged from 15 – 70 years where 196 (81.7%) of 240 were males. Most of the patients were agricultural laborers. All the patients had fever and head ache followed by myalgia and icterus. The prevalence of various symptoms/ signs is summarized in Table 1. Acute blood samples were collected from all the patients and follow up samples could be obtained in the case of 195 patients.

Table 1: Prevalence of signs/symptoms observed among the patients

Signs/Symptoms	%
Fever	100
Headache	100
Myalgia	87
Arthralgia	56
Conjunctival Suffusion	41
Jaundice	35
Renal Failure	15
Splenomegaly	15
Hepatomegaly	10
Nausea	15
Vomiting	15

Among the 195 patients who had paired samples, 36 showed four fold rises in titer. However, MAT negative patient had a positive IgM ELISA test. Seven patients had a titer of 1:20 (which is not considered) in the first sample, and 1:80 and 1:160 respectively in the second sample. All others had titers above 1:160 in both the samples. Thus 188 out of 195 patients with paired samples fulfilled the MAT diagnostic criteria. All supported the results of IgM ELISA also, thus these 195 patient's samples satisfied the criteria of diagnosis of leptospirosis. Remaining 45 patients with single sample yield negative result of 4 numbers by both MAT and ELISA. Thirty Three patients had MAT titers of 1:80 and above and all of them showed positive IgM ELISA too and fulfilled the diagnostic criteria.

Among the 240 serum samples, 213 samples supported the presence of leptospiral antibodies by MAT analysis and the predominant serogroup is Grippopyphosa (87%, 186/213) followed by Autumnalis (22% 46/213), Australis (11%, 24/213) and non pathogenic serovar Patoc (5%, 11/213). There was no reaction observed against other serovars included in this study. All the positive samples were titrated up to the end titers and a titer of 1:80 and above are considered as positive. However these MAT negative patients had a positive IgM ELISA test. 76 patients of 213 had MAT titers of 1:80 and remaining all had the range from 1:160 – 1:5120.

The distribution of antibody titers among these 213 patients as determined by MAT and IgM ELISA is shown in Table 2. A comparison of ELISA with MAT showed relatively high sensitivity 97.2%, a specificity of 100% and positive predictive value of 100%. One hundred and six of this 240 subjected to blood culturing and preliminarily 12 samples showed positive based on Dinger's ring on the medium. Further it was sent to Reference center for confirmation.

Table 2: Distribution of antibody titers as determined by MAT and IgM ELISA

Titer	MAT (+ve 213/240)		IgM ELISA (+ve 215/240)	
	Number	%	Number	%
1:80	70	32.86	71	33.02
1:160	42	19.72	42	19.53
1:320	22	10.33	22	10.24
1:640	23	10.80	24	11.17
1:1280	33	15.49	33	15.35
1:2560	14	6.57	14	6.52
1:5120	5	2.35	5	2.32
1:10240	3	1.41	3	1.39
1:20480	1	0.47	1	0.46

Microscopic agglutination test was able to identify 213 cases as positive which can be attributed for the detection of higher level antileptospiral antibodies in these patients. This probably would not be the case if MAT was used in a prospective study for screening a population at risk, as the titers of antibodies would be low and may not be found positive in this test [5]. The leptospirosis cases occurred frequently in various places on the river basin during the north east monsoon [6], considering the environmental conditions and occupational habits of the population, this is expected.

Due to unawareness and improper diagnostic tools, the presence of leptospirosis has never been detected and hence most of the physicians were not alert to observe this infection [6]. The clinical criteria for screening patients had a strong predictive value. This is very important to screen all the PUO cases for leptospirosis and follow them up for the development of signs/symptoms of leptospirosis to confirm unequivocally the infection. The clinical presentation of leptospirosis in Coimbatore was similar to findings in other reports [6,19,20,21]. Noticeably the frequency of symptoms, signs and laboratory findings was not different in patients with definite leptospirosis compared with patients with unconfirmed leptospirosis [16] with the exception of myalgia and icterus which were more frequent in patients with confirmed leptospirosis.

Though some early diagnostic test like kit methods will be designed in future is favorable, easy to perform and time consuming compared with MAT helps to identify the predominant serogroups in the specific geographical region. In this investigation, we identified Grippotyphosa as the major infective serogroup which has not been identified in Tamilnadu frequently as also observed in Andamans [22], Kerala [23] and Andhrapradesh [5]. In other studies in Tamilnadu, Autumnalis dominate Chennai, Cumbum and Tirunelveli; Panama in Madurai and Icterohaemorrhagiae in Bodi [24]. Some other data highlighted the serogroup Autumnalis and Australis are the dominant one in Tamilnadu [6]. In contrast the present study depicted Grippotyphosa was the dominant one.

CONCLUSION

Thus this study showed the leptospirosis is a potential health hazard of people who are close proximity with sewage, stagnant rain water in roads and rice fields. There is a need of etiological diagnosing leptospirosis in all hospitals as this would increase public awareness and help to deal

with situations of outbreaks, which was recently witnessed in Mumbai floods. Consequently, since a substantial number of cases are clinically missed and considering the severity of the disease, it seems justified to start effective antibiotherapy along with awareness programme to the public. Further studies needed to investigate the factors associated with the frequency and severity of human disease for effective and sustained prevention and control programs.

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