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Research Article

Changing Trends in the Seroprevalence of Leptospirosis in Kelambakkam: A Ten-Year Retrospective Study

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A B S T R A C T

Introduction: Leptospirosis is a zoonotic infection affecting humans. The main sources of infection are animal reservoir hosts and man is the accidental host in the disease transmission process. The diagnosis is usually made by microscopy, culture, molecular techniques, and serological tests like ELISA, MAT (Microscopic Agglutination Test) and MSAT (Macroscopic Slide Agglutination Test). The ELISA method to detect IgM antibodies is used as a good cost-effective testing method. An increasing titre of IgM antibody is a sign of active leptospirosis.

Aims and Objectives: The study aimed to evaluate the seroprevalence of Leptospirainfection over a 10-year period in a tertiary care hospital located in Kelambakkam village in Chengalpattu district.

Material and Method: The samples were tested for the presence of specific Leptospira IgM antibodies in the patient's serum using the Panbio Leptospira IgM ELISA kit. The samples were reported as positive/ negative/ equivocal accordingly.

Results: This retrospective study included a total of 2035 patients, clinically suspected of leptospirosis, over a 10-year period from 2011 to 2021. 186 patients tested positive for specific IgM antibodies by ELISA method,giving an overall prevalence rate of 9.14%.

Conclusion: The seroprevalence of leptospirosis over a time period of more than 10 years is highlighted in our study. Clinical suspicion of leptospirosis should be kept in mind at all times, especially now during the COVID-19 pandemic. The Panbio Leptospira IgM ELISA test kit used in our study proves to be a very useful method for diagnostic purposes, especially in limited-resource settings.

Keywords: Leptospira IgM Antibody, ELISA, Seroprevalence, Acute Febrile Illness

Introduction

Leptospirosis is a zoonosis which affects humans. It is caused by a spirochete belonging to the genus Leptospira comprising two important species - L. interrogans (pathogenic) and L. biflexa (saprophytic). There are more than 22 serotypes with more than 250 pathogenic serovars of the species, L. interrogans, at present.[1]

The clinical range of infection caused by this organism varies from subclinical to severe. The disease onset is usually sudden. Symptoms initially include prodromal symptoms like fever, headache, myalgia, and conjunctival suffusion. Later worsening complications like hepatorenal failure, severe abdominal pain, meningitis, and other central nervous system symptoms such as aseptic meningitis, depression, and irritability can arise, especially if treatment and diagnosis are delayed.[2]

The main source of infection is infected animals, especially livestock and also soil and water contaminated with the diseased animal's urine and tissues. Man becomes the accidental host in this disease transmission process, being infected by contaminated animal products. The main source of entry of the pathogen into the human body is via minor abrasions or cuts in the skin or mucosal membranes. The spread of infection can occur via occupational or recreational activities. Man-to-man transmission is very rarely reported.[2]

Leptospira are thin, delicate spirochetes that are actively motile. They possess characteristic closely wound spirals with hooked ends. These organisms are very thin and delicate to be viewed under the light microscope and hence best viewed under dark field microscopy. They can be cultured in several liquid and semisolid media such as Korthof's, Stuart's and Fletcher's media. Commonly used media is the EMJH (Ellinghausen, McCullough, Johnson, Harris) media.[1]

The organism is present in the blood during the first week of illness and disappears after the first week. Hence blood tests to demonstrate the organism by microscopy or isolation of the organism by culture remain significant only during the early phases of infection before antibiotics are started. The organism is shed in urine from the second week onwards, and thereafter for up to 4-6 weeks. But the sample should be examined immediately, as the spirochete undergoes rapid lysis in an acidic environment.[1]

The diagnosis is usually made by microscopy, culture and serology-based tests like ELISA, MAT (Microscopic Agglutination Test) and MSAT (Macroscopic Slide Agglutination Test). MAT is the gold standard test which is serovar specific. However, since this test requires a reference laboratory and also live Leptospira organisms, the ELISA method to detect IgM antibodies is used as a good alternative testing method. IgM ELISA is genus-specific and is the most cost-effective serological testing method. Initially, after 3 to 5 days of infection, IgM antibodies appear and persist for around 5 months in the human blood. IgG appears by the tenth day of illness and persists for a long duration. The detection of Leptospira species-specific antibody in blood is an important and valuable screening procedure. This is done by the IgM ELISA method. A diagnosis of acute leptospirosis can be made by this test, in comparison with other agglutination and complement fixation tests. An increasing titre of Leptospira IgM antibody is a sign of active leptospirosis.[3]

This study was aimed to evaluate the seroprevalence of leptospira infection over a 10-year period in a tertiary care hospital located in Kelambakkam, Chengalpattu district. The changing trends in seroprevalence rates were also estimated. This facilitated the understanding of the caseload and endemic nature of the disease. This also helped to determine whether the control measures had been appropriate andadequate.

Materials and Methodology

This retrospective observational study was carried out in the Department of Microbiology, Chettinad Hospital and Research Institute. A total of 2035 patients comprising both adults and children with clinical features suggestive of leptospirosis such as fever, myalgia, conjunctival suffusion and who had been tested for Leptospira IgM ELISA were included in the study. Adults and children with symptoms non-suggestive for leptospirosis and not tested themselves for Leptospira IgM ELISA were excluded from the study. This study was initiated after obtaining Institutional Human Ethics Committee approval.

Patient Selection

The details of the patients, both adults and children with fever and other clinical features suggestive of Leptospirosis, for whom Leptospira IgM ELISA was performed were collected from the hospital information system. The demographic details such as age, gender and the results of Leptospira IgM ELISA were tabulated. The details were collected for a decade long time period, from January 2011 to December 2021.

Specimen Collection and Preparation

Venous blood samples were collected from patients with clinical suspicion of Leptospirosis by venepuncture under aseptic techniques. This was allowed to clot at room temperature (20-25^{o}C) and then centrifuged according to the CLSI guidelines - Approved Standard Procedures for the Collection of Diagnostic Blood Specimens by Venepuncture, H3. The serum was separated by centrifugation and stored at 2-8 ^{o}C in the refrigerator or frozen at -20^{o}C, if not tested

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within 2 days. Icteric/haemolytic/lipemic sera or those having signs of bacterial growth were not included for testing.[4]

The samples were tested for the qualitative presence of specific Leptospira IgM antibodies using the Panbio Leptospira IgM ELISA test kit. It is used for the detection of many Leptospira serovars including icterohemorrhagiae, pomona, hardjo, canicola, grippotyphosa, copenhageni, nokolaevo, madanesis, celledoni, etc.[4]

Principle and Procedure

Positive serum samples containing Leptospira IgM antibodies bind to the Leptospira antigen coated over the polystyrene-containing microwells. The residual serum was washed off and then, peroxidase-conjugated anti-human IgM antibodies was added. The microwells were washed again, after which a colourless substrate, tetramethyl benzidine (TMB) chromogen, was added. This substrate was hydrolysed by the enzyme, after which it changed colour to blue. The reaction was finally stopped with acid, after which the TMB chromogen colour changed to yellow. The colour development was suggestive of the presence of Leptospira IgM antibodies in the patient's blood. The tests were performed in the Microbiology and Serology section of the Clinical Laboratory at our tertiary care hospital. Each batch of ELISA was processed with Reactive, Negative and Calibrator Control in addition to patient samples. The acceptable values for each of these have been stated in the specification sheet provided with the kit. If the absorbance readings of any of the 3 controls mentioned did not meet the specifications, the test was considered invalid and was repeated.[5]

Test Interpretation

The test was considered positive if the sample contained more than 11 Panbio units, equivocal if it contained 9 to 11 Panbio units and negative if it contained less than 9 Panbio units. A negative result implied that no detectable IgM antibody was present in the patient sample, but if a patient was still suspected of a recent infection, it could be confirmed by testing a repeat sample 7-14 days later. An equivocal result meant that those samples should be repeated for testing again. Samples which remained equivocal even after repeat testing were tested by an alternate method or another fresh sample was collected. A positive result implied the presence of detectable IgM antibodies. The magnitude of the result obtained is not indicative of the total amount of antibodies present in the patient's blood sample.[6]

Results

This retrospective study included a total of 2035 patients, clinically suspected of leptospirosis, over a 10-year time period from 2011 to 2021 (Figure 1). Out of these 2035 patients, 1331 (65.41%) patients were male, while 704 (34.59%) patients were female (Table 1).

Serum samples with more than 11 Panbiounits were taken as positive. 186 (9.14%) patients tested positive for specific IgM antibodies by ELISA method (Figures 2 and 3). Out of these 186 patients, 120 (64.52%) patients were male while 66 (35.48%) patients were female.

With males showing overall predominance, females showed increased seropositivity during the years 2020 and 2021 i.e. during the period of the COVID-19 pandemic (Table 2).

The patients included in our study were categorised into different age groups (Table 3 and Figure 4). Of these, the age group that showed maximum positivity over the period of 10 years included in our study, was 21-30 years.This age group had a total positivity of 62 patients, over the 10-year period. This accounted for around one-third (33.33%) of the total positives (186) obtained (Tables 4, 5, and 6).

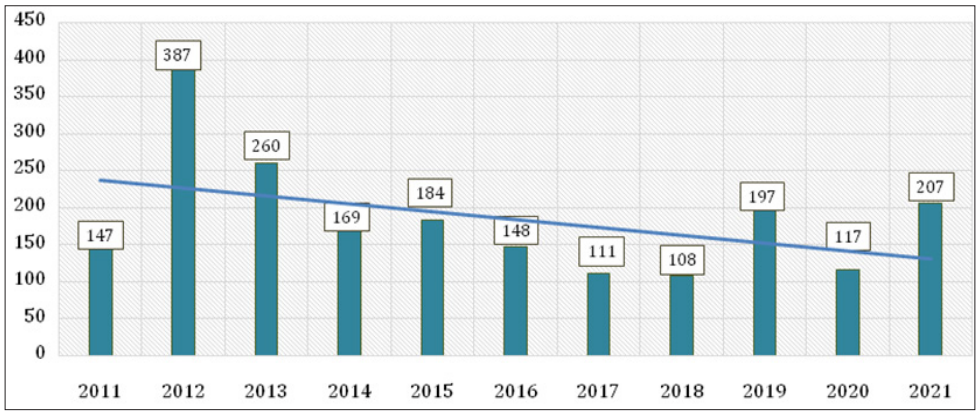

Figure 1.Yearwise Distribution of the Total Number of Samples Tested for Leptospira IgM ELISA over the 10-yearPeriod

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The overall 10-years eropositivity of patients obtained in other age groups were as follows - <10 years: 2%, 11-20 years: 14%, 21-30 years: 34%, 31-40 years: 19%, 41-50 years: 11%, 51-60 years: 10%, 61-70 years: 7%, 71-80 years: 2% and >80 years: 1%. Thus, followed by the 21-30 years age group (34% positivity), the next age group with increased positivity is 31-40 years age group (19% positivity) followed by 11-20 years age group (14% positivity). Increased seropositivity was noted in the younger age group patients (Figure 5).

Table 1.Genderwise Distribution of the Patients Tested

Year	Total Tested	Male	Female
2011	147	113	34
2012	387	254	133
2013	260	178	82
2014	169	111	58
2015	184	132	52
2016	148	94	54
2017	111	72	39
2018	108	67	41
2019	197	124	73
2020	117	74	43
2021	207	112	95
Total	2035	1331	704
Percentage	100	65.41	34.59

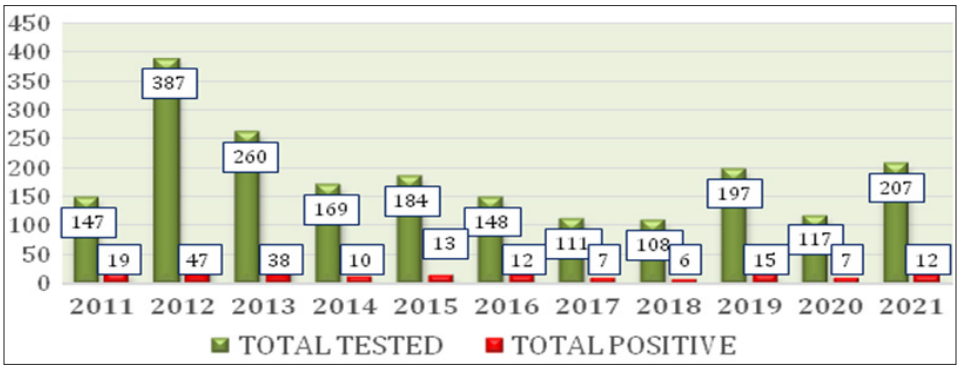

Figure 2.Yearwise Distribution of the Total Number of Samples Tested vs Total Number of Samples Positive for Leptospira IgM ELISA over the 10-year Period

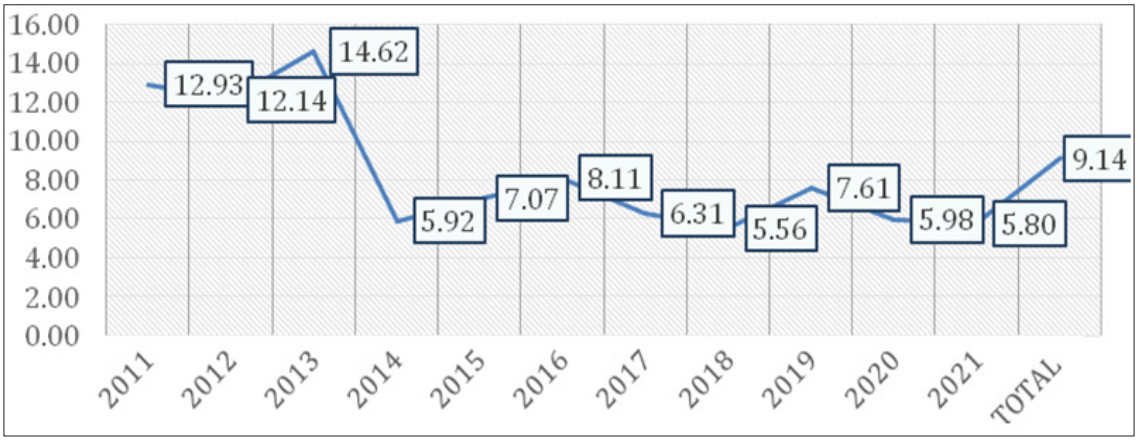

Figure 3.Trend of Yearwise Positivity of Leptospira IgM ELISA over the 10-year Period

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Table 2.Genderwise Distribution of the Positives

Year	Total Positive Cases	Male	Female
2011	19	15	4
2012	47	27	20
2013	38	30	8
2014	10	4	6
2015	13	11	2
2016	12	11	1
2017	7	5	2
2018	6	3	3
2019	15	9	6
2020	7	3	4
2021	12	2	10
Total positives	186	120	66
Total positives %	9.14%	5.90%	3.24%
	Overall, 10-year positivity %	64.52%	35.48%

Table 3.Agewise Distribution of the Patients Tested

Age Group (Years)	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Total
<10	2	15	18	11	18	5	6	3	4	2	1	85
11-20	15	58	41	12	23	9	15	8	18	8	9	216
21-30	57	130	77	55	53	39	20	24	52	25	37	569
31-40	33	65	47	33	26	33	20	17	40	18	47	379
41-50	17	40	27	17	25	24	17	16	26	24	25	258
51-60	14	52	28	17	22	17	21	18	29	14	30	262
61-70	6	13	16	18	10	14	8	13	20	19	34	171
71-80	3	9	6	4	8	3	3	8	4	10	19	77
> 80	0	3	0	2	3	0	1	1	4	3	5	22
Total Tested	147	385	260	169	188	144	111	108	197	123	207	2039

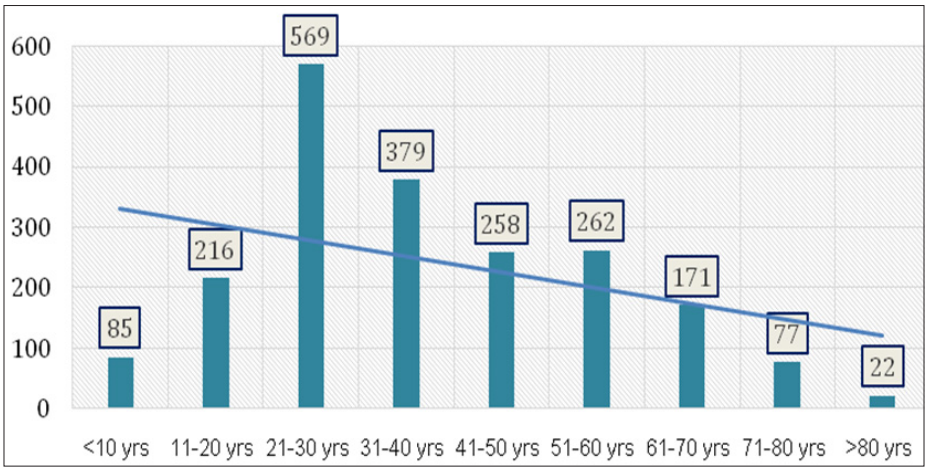

Figure 4.Agewise Distribution of Patients Tested for Leptospira IgM ELISA over the 10-year Period

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Table 4.Agewise and Yearwise Distribution of Leptospira IgM ELISA Positive Patients

Age Group (Years)	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Total
< 10	0	1	1	0	1	0	0	0	0	0	0	3
11-20	1	9	11	1	2	1	0	0	0	0	1	26
21-30	11	19	11	4	2	5	2	0	4	2	2	62
31-40	2	6	6	4	3	2	3	1	3	0	5	35
41-50	1	4	3	0	3	2	0	3	2	2	1	21
51-60	3	4	3	0	1	1	2	1	2	1	1	19
61-70	1	1	2	1	0	1	0	1	3	2	1	13
71-80	0	0	1	0	1	0	0	0	1	0	1	4
> 80	0	1	0	0	0	0	0	0	0	0	0	1
Total Positive Cases	19	45	38	10	13	12	7	6	15	7	12	184

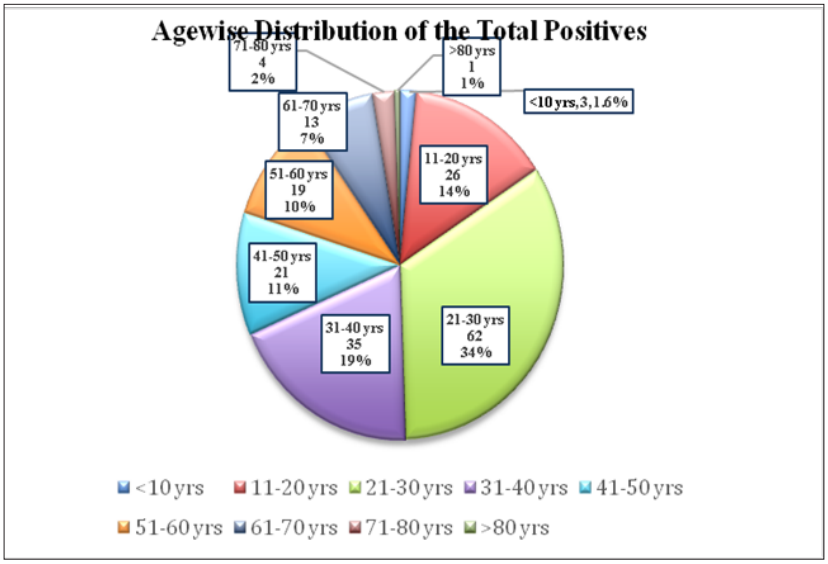

Figure 5.Agewise Distribution of Total Cases Positive for Leptospira IgM ELISA over the 10-year Period

Table 5.Yearwise and Agewise Details of the Males Tested and Found Positive

Age Group (Years)	2011		2012		2013		2014		2015		2016		2017		2018		2019		2020		2021	
	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P
<10	1	0	9	0	6	1	4	0	12	0	1	0	4	0	1	0	1	0	1	0	1	0
11-20	11	0	42	7	33	8	57	1	21	2	8	1	12	0	5	0	10	0	7	0	5	1
21-30	47	10	97	11	63	11	40	2	41	2	29	5	17	2	19	0	30	0	17	2	19	0
31-40	29	2	37	2	29	4	22	0	15	3	24	2	15	2	8	1	29	2	12	0	29	0
41-50	12	1	19	3	14	2	9	0	15	3	13	1	9	0	7	1	15	2	13	0	15	0
51-60	6	1	30	1	22	3	13	0	13	1	6	1	10	1	15	1	18	2	10	0	18	1
61-70	2	1	11	0	6	1	12	1	9	0	10	1	2	0	8	0	15	2	13	1	12	0
71-80	3	0	6	0	5	0	2	0	7	0	3	0	2	0	4	0	4	1	4	0	11	0
>80	0	0	2	0	0	0	2	0	3	0	0	0	1	0	0	0	2	0	1	0	5	0

T: Tested, P: Positive

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Table 6.Yearwise and Agewise Details of the Females Tested and Found Positive

Age Group (Years)	2011		2012		2013		2014		2015		2016		2017		2018		2019		2020		2021	
	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P
< 10	1	0	6	1	12	0	7	0	6	1	4	0	2	0	2	0	3	0	1	0	0	0
11-20	4	1	16	2	8	3	5	0	2	0	1	0	3	0	3	0	8	0	1	0	4	0
21-30	10	1	33	8	14	0	15	2	12	0	10	0	3	0	5	0	22	4	8	0	18	2
31-40	4	0	28	4	18	2	11	4	11	0	9	0	5	1	9	0	11	1	6	0	18	5
41-50	5	0	21	1	13	1	8	0	10	0	11	1	8	0	9	2	11	0	11	2	10	1
51-60	8	2	22	3	6	0	4	0	9	0	11	0	11	1	3	0	11	0	4	1	12	0
61-70	4	0	2	1	10	1	6	0	1	0	4	0	6	0	5	1	5	1	6	1	22	1
71-80	0	0	3	0	1	1	2	0	1	1	0	0	1	0	4	0	0	0	6	0	8	1
> 80	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	2	0	2	0	0	0

Discussion

The total Leptospira IgM positivity rate in our study is 9.14%. This rate was calculated over a period of more than 10 years, from 2011-2021, among a total of 2035 patients. This positivity rate is similar to the positivity rate obtained in arecent study by Muhsin PV et al., but this study had a shorter study duration of 6 months.[2] Other studies carried out by Patel SS et al. and Agrawal SK et al. showed lower seroprevalence rates of 5.1% and 6.4% respectively when compared to our study.[3,7] However, studies carried out by Sethi S et al. and Chaudhry R et al. showed positivity ranging from 18% to 38%, which is quite high when compared with our study.[8,9]

This comparative decrease in seroprevalence in our study could be attributed to a number of factors such as increased awareness, better hygienic practices, consumption of clean drinking water, better socio-economic standards etc. However, the persistence of leptospirosis suggests that environmental risk factors like contaminated rodents and domestic animals, rainfall and contaminated environment continue to play a major role in the continuous spread and occurrence of leptospirosis.[2]

Overall, males showed increased seropositivity (64.52%) when compared to females (35.48%). This can be attributed to the increased occupational exposure to infected animals and contaminated environment for males ascompared tofemales.[3] However, in our study, females showed increased seropositivity than males during the years 2020 and 2021, which is apparently the period of the COVID-19 pandemic. During the year 2020, the seropositivity among the females was 4 out of 43 (9.30%) while in males it was 3 out of 74 (4.05%). During the year 2021, seropositivity in females was 10 out of 95 (10.53%) while among males,it was 2 out of 112 (1.79%). This comparative increase in seropositivity in females, especially during the COVID-19 pandemic, can be attributed to increased exposure to occupational hazards and contaminated environment even for females, in view of the changing times.

In our study, increased positivity rates were predominantly seen in the younger age groups;21-30 years age group had 34% positivity, 31-40 years age group had 19% positivity, and 11-20 years age group had 14% positivity. This could be attributed to the increased exposure to infections and contaminated environment among the younger age groups.[10] The total number of patients tested over the 10-yearperiod was also high in these age groups; 569 patients (28%) in 21-30 years age group, 379 patients (19%) in 31-40 years age group, and 216 patients (11%) in 11-20 years age group.

The clinical symptoms of leptospirosis can range from mild symptoms like fever, headache, and myalgia to severe symptoms like renal failure and jaundice. Severe ARDS can also occur which is rather a rare complication of leptospirosis. With a background of the COVID-19 pandemic now, a clinical picture of ARDS makes all clinicians suspicious of SARS-CoV-2 only. Thus, a clinical diagnosis of Leptospirosis can be often missed, especially during this pandemic period. Also, the clinical picture of Leptospirosis can be most commonly mistaken for infectious conditions like dengue and typhoid. Many case reports portraying co-infections of leptospirosis, dengue, typhoid and COVID-19 have also been reported. Thus, the treating physician should keep in mind all these rare complications and infectious conditions, which will ultimately help him in reaching the correct diagnosis.[11,12]

The IgM antibody usually appears in the serum of infected individuals around the fifth to tenth day of the onset of Leptospirosis. The presence of a specific IgM antibody in a patient's sample indicates a recent infection.[1] A positive test result which presents along with clinical evidence,

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can be taken as a positive diagnosis. Serological evidence of recent infection is confirmed by rising levels of specific antibodies in paired sera. The failure to demonstrate any rise or fall in specific IgM antibody titres in consecutively collected patient samples excludes the possibility of a recent leptospira infection.[6]

The Panbio Leptospira IgM ELISA test kit used in our study primarily serves as a screening test, although a small proportion of patients with other acute infections, e.g.,Q fever can produce a positive result. The performance characteristics of the test gave impressive results with increased values of specificity and sensitivity and minimal cross-reactivity. The clinical diagnosis must be correlated with the clinical signs and symptoms of the patient. The results of an immunosuppressed patient should be dealt with caution.[13]

Consecutively, all positive serum samples should be referred to a standard reference laboratory for the confirmation of specific IgM antibodies using standard confirmatory tests and also for epidemiological recording purposes. Different geographical regions may exhibit different population seroepidemiology overtime. Therefore, the final cut-off may require adjustments based on local studies.[13,14]

Conclusion

The seroprevalence of leptospirosis over a time period of more than 10 years is highlighted in our study. The patients were categorised sex-wise and age-group-wise, and appropriate positivity rates were estimated. This helped us to understand the changing prevalence in the trends of Leptospirosis disease. Also, clinical suspicion of Leptospirosis should be kept in mind at all times, especially now during the COVID-19 pandemic and should never be missed out.

The Panbio Leptospira IgM ELISA test kit, used in our study, proves to be a very useful method for diagnostic purposes, especially in limited-resource settings, when compared with other diagnostic methods like MAT and MSAT. Thus, this study gives a good idea ofthe seroprevalence rates of leptospirosis in a tertiary care hospital in Kelambakkam, Chengalpattu district. This highly neglected, tropical infectious disease should always be handled with a high degree of suspicion and care, for both diagnosis and treatment, keeping in mind the dangerous complications it can lead to.[15]

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Conflict of Interest: None

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