Clinical profile of leptospirosis and role of various diagnostic methods, a hospital based prospective observational study

K Prakash¹,*

¹ Dept. of Microbiology, Government Medical College (old RIMS), Prakasham, Andhra Pradesh, India

ABSTRACT

Objectives: Leptospirosis is a potentially life-threatening zoonotic disease of worldwide distribution. Accurate diagnosis and prompt treatment are essential to minimize morbidity and mortality. The current study was conducted to analyse the clinical profile of leptospirosis and the diagnostic yield of various diagnostic methods.

Materials and Methods: The present study was a cross-sectional study. A total of 60 patients who were suspected of leptospirosis were enrolled in the study. Direct examination of blood was done using darkground microscopy, the culture was done by inoculation of the blood sample into the EMJH medium, antibodies against Leptospira was demonstrated using Panbio IgM ELISA kit, and antigen products were demonstrated using polymerase reaction (PCR) with primers G1 and G2.

Results: The study population included 63% of males and 37% females. A majority of 38% of the study subjects were farmers. Pallor and icterus are the predominant clinical signs found among 96% of the study population. IgM ELISA has labelled the highest number i.e. 55 (91.66%) of subjects as positive. The number of subjects diagnosed positive by PCR, culture and Dark ground microscopy (DGM) were 33 (55.00%), 22 (36.66%) and 12 (20%) subjects respectively.

Conclusion: Leptospirosis proves to be an important health concern. Prominent clinical conditions were observed. IgM-ELISA proved superior to Dark ground microscopy (DGM), Culture and Polymerase chain reaction (PCR) and suitable for early diagnosis of leptospirosis.

© 2020 Published by Innovative Publication. This is an open access article under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/)

1. Introduction

Leptospirosis is one of the most widely prevalent zoonotic diseases globally. It is caused by spirochetes of the genus Leptospira.¹ The disease is acquired through contact of abraded skin with the water or soil which is contaminated with infected urine. Hence humans are accidental hosts. Once in the soil, the bacteria can survive for prolonged periods if the soil is damp.²

In India, outbreaks of leptospirosis occur during monsoon seasons due to flooding. In South-India, the peak incidence of leptospirosis occur between June and October.³ Bacteraemia heralds the onset of clinical illness. Manifestations may range from subclinical infection to as severe as multi-organ dysfunction, that is associated with a high case fatality rate. In advanced stages, liver failure, pulmonary haemorrhage, acute kidney injury and bleeding manifestations may occur. Hence a high index of suspicion and timely diagnosis is vital in preventing serious morbidity and mortality.

Despite common occurrence and the possibility of serious adverse consequences, the diagnosis is often missed by clinicians. This is due to varied manifestations and the majority of cases presenting as undifferentiated febrile illnesses.⁴ It is often misdiagnosed as influenza, fever of unknown origin or aseptic meningitis.⁵ Also, the misconception that it is predominantly a rural disease, contributes to delayed diagnosis. Along with a high index of suspicion, choosing an appropriate laboratory test is of paramount importance.
Lab diagnosis includes methods such as microscopy, culture, cerology and molecular diagnostic tests. Dark field microscopy can visualize leptospirosis, but it requires a minimum of 10⁴ organisms/mL to be visible in microscopy. IG ELISA is widely used, but it can give false-positive results. PCR can detect leptospira DNA in the serum and urine samples of patients. But it requires a large amount of DNA in the sample to give a positive result.

The current study was conducted to analyse the clinical profile of leptospirosis and the diagnostic yield of various diagnostic methods.

2. Materials and Methods

The present study was a cross-sectional study, conducted at Govt. Medical College (old RIMS), Ongole in the department of Microbiology. The study was conducted between January 2017 to December 2018. A total of 60 patients who were suspected of leptospirosis were enrolled in the study. Those who did not fulfill the inclusion criteria were subjected for exclusion. The study was approved by the institutional human ethics committee. Informed written consent was obtained in the local language from all study participants. The confidentiality of the study participants was maintained throughout the study. The study has thus been conducted in compliance with the ethical standards required by the 1964 Declaration of Helsinki and its subsequent amendments.

Direct examination of blood was done using dark-ground microscopy, the culture was done by inoculation of the blood sample into the EMJH medium, antibodies against Leptospira was demonstrated using Panbio IgM ELISA kit, and antigen products were demonstrated using polymerase reaction (PCR) with primers G1and G2.

3. Results

The study population included a total of 60 subjects with 63% males and 37% females. Among the study population, 11 individuals (18%) belonged to the paediatric age group of fewer than 18 years, and the remaining 42 individuals (82%) were adults. The age distribution of the study subjects based on gender is given in Table 1.

| Age in year | Male | Female |
|------------|------|--------|
| 0-10 yrs   | 3(5%)| 0(0%)  |
| 10-20 yrs  | 5(8%)| 3(5%)  |
| 20-30 yrs  | 7(12%)| 2(3%)  |
| 30-40 yrs  | 8(13%)| 2(3%)  |
| 40-50 yrs  | 10(17%)| 12(20%)|
| >50 yrs    | 5(850)| 3(5%)  |
| Total      | 38(63%)| 22(37%)|

A majority of 38% of the study subjects were farmers. There were 17% each working as poultry workers or in animal rearing. Among the remaining study subjects, there 8% of the subjects each reported working in sewers, gardening, and sedentary jobs. Only 4% were involved in the veterinary occupation. With regards to contact with infected animals, almost 47% of the study population had a history of contact with dogs. A little less than one-fourth, 23% had a history of contact with cattle. Only 14% had a history of contact with rodents. Among the rest, history of contact with cats and hens were present among 12% and 4% of the study population respectively.

With regards to water being the source of infection, 36% of the study population had a history of contact with a public source of water. History of contact with ponds or sewage was present among 18% of each of the study population. Almost one-fifth (21%) of the study population had a history of contact with canal water. Only 7% had a history of contact with river water (Table 2).

Table 1: Age and sex distribution of subjects in the study (N=60)

| Parameter | Number | Percentage |
|-----------|--------|------------|
| Occupation (N=60) |        |            |
| Farmer | 23 | 38% |
| Poultry worker | 10 | 17% |
| Animal rearing | 10 | 17% |
| Sewer | 5 | 8% |
| Gardner | 5 | 8% |
| Sedentary | 5 | 8% |
| Veterinary | 2 | 4% |
| History of contact with the animal (N=43) |        |            |
| Dog | 20 | 47% |
| Cattle | 10 | 23% |
| Rodents | 6 | 14% |
| Cat | 5 | 12% |
| Hen | 2 | 4% |
| Source of water (N=28) |        |            |
| Public source | 10 | 36% |
| Canal | 6 | 21% |
| Pond | 5 | 18% |
| Sewage | 5 | 18% |
| River | 2 | 7% |

Table 2: Distribution of subjects based on occupation

| Clinical signs | Frequency (%) |
|----------------|---------------|
| Pallor | 58(96%) |
| Icterus | 58(96%) |
| Hepatomegaly | 40(67%) |
| Hypochondrium tenderness | 30(50%) |
| Splenomegaly | 20(33%) |
| Lymphadenopathy | 10(17%) |
| Edema | 2(3%) |
| Purpura | 5(8%) |
| Meningeal signs | 2(3%) |
With regards to clinical signs, fever, conjunctival congestion and nausea were present in all the study subjects. The other most common clinical symptoms were pallor and icterus seen in 58 (96%) of subjects. Hepatomegaly was present in 67% and splenomegaly was present in 33% of cases. Meningeal signs were present in 3% of subjects (Table 3).

Table 4: Comparison of positive results of dark-ground microscopy, blood culture, IgM ELISA and PCR. (N=60)

| Test                      | No of positives | Percentage |
|---------------------------|-----------------|------------|
| IgM ELISA                 | 55              | 91.66%     |
| PCR                       | 33              | 55.00%     |
| Culture                   | 22              | 36.66%     |
| Dark ground microscopy (DGM) | 12          | 20.0%      |

IgM ELISA has labelled the highest number i.e. 55 (91.66%) of subjects as positive. The number of subjects diagnosed positive by PCR, culture and Dark ground microscopy (DGM) were 33 (55.00%), 22 (36.66%) and 12 (20%) subjects respectively.

Table 5: Comparison of dark-ground microscopy (DGM), blood culture, IgM ELISA with PCR

| Parameters       | PCR Positive (n=33) | PCR Negative (n=28) | Total |
|------------------|---------------------|---------------------|-------|
| DGM              | Positive            | Negative            |       |
| Positive         | 12 (37.5%)          | 0 (0%)              | 12    |
| Negative         | 20 (62.5%)          | 28 (100%)           | 48    |
| Blood Culture    | Positive            | Negative            |       |
| Positive         | 22 (68.7%)          | 0 (0%)              | 22    |
| Negative         | 10 (31.2%)          | 28 (100%)           | 38    |
| IgM ELISA        | Positive            | Negative            |       |
| Positive         | 30 (93.7%)          | 25 (89.2%)          | 55    |
| Negative         | 2 (6.25%)           | 3 (10.7%)           | 5     |

Among the 33 PCR positive Leptospira cases, 30 (93.7%) were identified by IgM ELisa as positive. Blood culture and Diagnostic microscopy (DGM) had diagnosed 22 (68.7%) and 12 (37.5%) respectively. Among the 28 cases diagnosed as negative by PCR all of them were labelled as negative by DGM and Blood culture, but only 3 (10.7%) were labelled as negative by IgM ELISA.

4. Discussion

Leptospirosis is a worldwide public health problem. The magnitude of the problem in tropical and subtropical regions can be largely attributed to climatic and environmental conditions. Despite this knowledge, the information about the existing status of the disease in the country is lacking and we do not have an accurate estimate of disease burden in the country. Probably the disease is under reported in humans. All available evidences suggest that the Leptospirosis is now emerging in India as important public health problem.9–11

The source of infection among the study population is either in direct contact with animals and poultry or direct contact such as farmers. Among the occupational group, farmers were majorly affected. A study by Patil VC et al.12 had of total 23 patients among which 18 (78.26%) were farmers. This is concurrence with the review by Levett PN et al.,13 where the authors mention that farmers, veterinarians and abattoir workers are at risk for infection with leptospirosis through direct contact. Indirect modes of transmission can occur in sewage workers or canal workers. Also, similar to the present study, other studies,14,15 have reported outbreaks of leptospirosis after recreational exposure to water such as swimming, exposure to public water sources, ponds, and canals.

With regards to the clinical features of the patients, 96% had icterus in the present study, and all had a fever and conjunctival suffusion. This proportion of icterus in concurrence with the study by Edwards CN et al.,16 where 95% had jaundice. But a lesser proportion of the study population had a fever (76%) and conjunctival suffusion (54%) when compared to the present study. In a study by Holla R.,17 majority of the patients presented with fever (92.1%). In a prospective study, the most common organs involved were liver (27, 71.05%).18 In a study done by Ibrahim SK et al.19 Hepatomegaly was found in 88% of the total population. The extent of respiratory involvement is different among various studies on leptospirosis. In the current study around 8% of the study, the population had respiratory symptoms. In the study by Yersin C et al.,20 12% of the study population had hemoptysis, and pulmonary infiltrates on chest X-ray which is higher compared to the present study.

The present study releveled that IgM ELISA showed the highest number of positive subjects. Similarly in a study done by Niloofa R et al.21 IgM-ELISA positivity was 45.8% which was greater than MAT and Leptocheck-WB. Khan F et al22 in their results found that thirty-one (14.9%) patients were found positive for specific anti-leptospira IgM antibodies by ELISA.

5. Conclusion

Leptospirosis remains a significant public health issue that mainly affects the population of the productive age group. Current study results depict the role of occupation and development of leptospirosis. Also, the source of water had a significant part to play. Febrile and hepatic conditions were most common. As this disease is of endemic nature leading to a fatal outcome, it should raise a high index of suspicion among the medical practitioners when they come across a person suffering with fever and jaundice. A well planned multicentric study done at different geographical locations should be carried out to bring out better insight to the epidemiology of leptospirosis.
6. Acknowledgements
We acknowledge the technical support in data entry, analysis and manuscript editing by “Evidencian Research Associates.”

7. Source of Funding
Self-funded.

8. Conflict of Interest
None.

References
1. Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA. Peru-United States Leptospirosis Consortium. Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis.* 2003;3(12):757–71.
2. Plank R, Dean D. Overview of the epidemiology, microbiology, and pathogenesis of Leptospira spp. in humans. *Microbes Infect.* 2000;2(10):1265–76.
3. John TJ. Emerging & re-emerging bacterial pathogens in India. *Indian J Med Res.* 1996;103:4–18.
4. Budihal SV. Leptospirosis Diagnosis: Competancy of Various Laboratory Tests. *J Clin Diagn Res.* 2014;8(1):199–202.
5. Turner LH. Leptospirosis. *Trans R Soc Trop Med Hyg.* 1967;61(6):842–55.
6. Turner LH. Leptospirosis. 3. Maintenance, isolation and demonstration of leptospires. *Trans R Soc Trop Med Hyg.* 1970;64(4):623–46.
7. Johnson RC, Harris VG. Differentiation of Pathogenic and Saprophytic Leptospira I. Growth at Low Temperatures. *J Bacteriol.* 1967;94(1):27–31.
8. Bal AE, Gravekamp C, Hartskeerl RA, Meza-Brewster JD, Korver H, Terpstra WJ. Detection of leptospires in urine by PCR for early diagnosis of leptospirosis. *J Clin Microbiol.* 1994;32(8):1894–8.
9. Pappas G, Papadimitriou P, Siozopoulou V, Christou L, Akritidis N. The globalization of leptospirosis: worldwide incidence trends. *Int J Infect Dis.* 2008;12(4):351–7.
10. Sethi S, Sharma N, Kakkar N, Taneja J, Chatterjee SS, Banga SS, et al. Increasing Trends of Leptospirosis in Northern India: A Clinico-Epidemiological Study. *PloS Negl Trop Dis.* 2010;4(1):e579.
11. Shekarkar SB, Harish BN, Menezes GA, Parija SC. Clinical and serological evaluation of Leptospirosis in Paducherry, India. *J Infect Dev Ctries.* 2010;4(03):139–43.
12. Patil VC, Patil HV, Agrawal V. Clinical profile and outcome of leptospirosis at tertiary care centre in western Maharashtra. *J Acad Med Sci.* 2012;2(1):30.
13. Levett PN. Leptospirosis. *Clin Microbiol Rev.* 2001;14(2):296–326.
14. Fuortes L, Nettleman M. Leptospirosis: a consequence of the Iowa flood. *Iowa Med.* 1994;84(10):449–50.
15. Oliveira VJC, Rocha JMB, Silva GB, Cabral CLN. Observations on a new epidemic outbreak of leptospirosis in greater Recife, Brazil, in 1975. *Rev Inst Adolfo Lutz.* 1977:33–6.
16. Edwards CN, Nicholson GD, Hassell TA, Co E, Callender J. Leptospirosis in Barbados. A clinical study. *West Indian Med J.* 1990;39(1):27–34.
17. Holla R, Darshan B, Pandey L, Unnikrishnan B, Kumar N, Thapar R, et al. Leptospirosis in Coastal South India: A Facility Based Study. *Biol Med Res Int.* 2018;2018. doi:10.1155/2018/719128.
18. Clerke AM, Leuva AC, Joshi C, Trivedi SV. Clinical profile of leptospirosis in South gujarat. *J Postgrad Med.* 2002;48(2):117–8.
19. Ibrahim SK. Clinical Profile of Leptospirosis with Special Mention to its Multiorgan Involvement in Kilpauk Medical College Hospital. Kilpauk Medical College, Chennai; 2016.
20. Yersin C, Bovet P, Merien F, Clement J, Lalille M, Ranst MV, et al. Pulmonary haemorrhage as a predominant cause of death in leptospirosis in Seychelles. *Trans R Soc Trop Med Hyg.* 2000;94(1):71–6.
21. Nilooofa R, Fernando N, de Silva NL, Karunanyake L, Wickramasinghe H, Dikmadugoda N, et al. Diagnosis of Leptospirosis: Comparison between Microscopic Agglutination Test, IgM-ELISA and IgM Rapid Immunochromatography Test. *PloS One.* 2015;10(6):e0129236.
22. Khan F, Mahtab M, Ahmad N, Shakla I, Rizvi M, Azam M. Rapid Diagnosis of Leptospirosis by IgM ELISA in Resource Poor Settings. *Int J Health Sci Res.* 2016;6(3):73–9.

Author biography

K Prakash Associate Professor

Cite this article: Prakash K. Clinical profile of leptospirosis and role of various diagnostic methods, a hospital based prospective observational study. *Indian J Microbiol Res* 2020;7(2):195-198.