Case report :

Leptospirosis- A Physician's dilemma or diagnostic enigma?

Dr. Mohit Bhatia[1] , Dr. Umapathy B.L[2]

[1] Senior Resident, Department of Microbiology, Govind Ballabh Pant Institute of Post Graduate Medical Education and Research, Jawahar Lal Nehru Marg, New Delhi-110002.

[2] Professor, Department of Microbiology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Kanyakumari District, Tamil Nadu (India) -629161

Corresponding author: Dr. Mohit Bhatia

Abstract:

A two years old female child was admitted in a tertiary care hospital in June 2012 with history of high grade fever of two weeks duration. On examination, no systemic abnormality was detected. Acute and convalescent sera of this patient were positive by widal test and rapid leptospira serological tests like Macroscopic Slide Agglutination Test (MSAT), IgM Enzyme Linked Immunosorbent Assay (IgM ELISA) and immunochromatographic card test (IgM Leptocheck). However, both of these serum samples were negative by Microsopic Agglutination Test (MAT). Blood culture was sterile. Leptospires were isolated from urine sample of this patient and identified as *Leptospira inadai* by Polymerase Chain Reaction (PCR). This patient was treated successfully with Amoxicillin/Clavulanic acid syrup and discharged after one week of admission.

Key-words: *Leptospira inadai,* widal, MSAT, IgM ELISA, IgM Leptocheck, MAT, PCR

Introduction:

Leptospirosis is an emerging infectious disease which is often missed clinically. [1] The signs & symptoms of leptospirosis resemble a wide range of infectious diseases.[2] A high index of suspicion is needed in endemic areas & leptospirosis must be considered when a patient presents with acute onset of fever, headache & myalgia. The diagnosis of leptospirosis in humans is almost entirely dependent on laboratory findings. The most frequently used diagnostic approach for leptospirosis has been that of serology.[3,4] We hereby present a case of human infection caused by a rare species *Leptospira inadai*.

Case History:

A two years old female child was admitted in a tertiary care hospital during monsoon season in June, 2012, with history of high grade, intermittent fever of insidious onset of two weeks duration along with headache and myalgia. The patient belonged to an economically backward family living under poor sanitary conditions. Further interrogation revealed the presence of numerous rats in their house, with many open drains around their residence and history of barefoot walking. Many similar cases of febrile illness had been reported in their locality during that season. However, they were unaware of the diagnosis of those cases. On examination, the patient was febrile (Temperature-101.5˚F). No systemic abnormality was detected. Laboratory investigations showed

(1) Hemoglobin: 9.3g/dl
(2) Total Leucocyte Count: 9700/Cu.mm of blood
(3) Differential Leucocyte Count: Polymorphs-34%; Lymphocytes-61%; Monocytes-5%
(4) Platelet count: 2.3 lakhs/Cu.mm of blood

(5) Peripheral smear for Malaria parasite: Negative

(6) Urine Routine and Microscopy: No abnormality detected

(7) Blood culture : Sterile

(8) Widal test: Two serum samples of this patient were collected one week apart and labeled as acute and convalescent respectively. The results obtained were as follows: 'STO' 1:80, 'STH':1:320, 'SPAH' < 1:20 & 'SPBH' <1:20 (acute sample) and 'STO' 1:160, 'STH' 1:1280, 'SPAH' <1:20 & 'SPBH' < 1:20 (convalescent sample).

As per the requisition received from pediatrician, blood and urine samples of this patient were also evaluated for leptospirosis as per standard procedures:[5]

(1) Dark Field Microscopy (DFM) of blood and urine samples: Negative

(2) Blood culture for leptospirosis using commercially available Ellinghausen-McCullough-Johnson-Harris (EMJH) semisolid medium (BD-Difco): Sterile

(3) Urine culture for leptospirosis using EMJH medium containing 100µg/ml 5-Fluoro Uracil (Rolex Chemical Industries) as selective agent: Leptospires were grown after 96 hours of incubation as indicated by Dinger's ring (ring of growth present on sub-surface) and confirmed by DFM. (**Fig 1)**

The following rapid leptospira serological tests were also performed on acute and convalescent sera of this patient: Macroscopic Slide Agglutination Test (MSAT; Bio-Rad), immunochromatographic card test (IgM Leptocheck; Zephyr Biomedicals), IgM Enzyme Linked Immuno Sorbent Assay (IgM ELISA; J. Mitra & Co. Pvt. Ltd.). Both serum samples were positive by all the aforementioned tests.

Since the acute and convalescent sera of this patient were tested positive by widal and all aforementioned leptospira serological tests, the Microscopic Agglutination Test (MAT) was performed on these sera upon receiving a special requisition from the department of Pediatrics. MAT was performed at Regional Medical Research Centre (Indian Council of Medical Research), WHO collaborating centre for diagnosis, reference, research & training in leptospirosis, Port Blair, Andaman and Nicobar islands (India) using the following serovars: *Australis, Bankinang, Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Pomona, Pyrogenes* & *Hardjo*. Both serum samples were negative by MAT.

The urine culture leptospira isolate was sent to Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS), Bengaluru for confirmation by PCR. This isolate was characterized at species level as Leptospira inadai by using partial RNA polymerase β-subunit (rpoB) gene sequences.

Based on these results, this patient was treated with Amoxicillin/Clavulanic acid syrup (125/31.25 mg) 2.5 ml thrice a day for seven days. The patient recovered and was discharged after one week of admission.

Discussion:

Leptospirosis is considered as the most common zoonotic infection in the world with higher incidence in the tropics than temperate regions.[6] Though it is sub-clinical or mild in most cases, severe illness can sometimes end fatally.[1] The clinical presentation is difficult to distinguish from dengue, malaria,

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

influenza & many other diseases characterized by fever, headache & myalgia. The differential diagnosis of leptospirosis depends on the epidemiology of acute febrile illnesses in the particular area. The mainstay of diagnosis is microbiological which has various shortcomings.

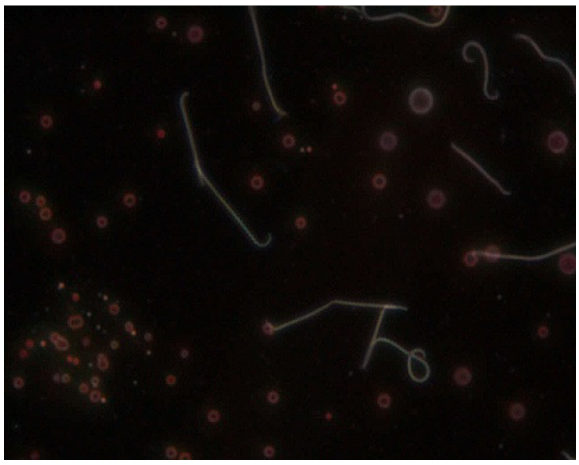
Both blood and urine samples of this patient were tested negative by DFM which has not been accepted for diagnostic purposes as it is considered insensitive and the results are non specific.[1] IgM Leptocheck, MSAT and Leptospira IgM ELISA are rapid sensitive serological diagnostic tests for leptospirosis. Many studies have shown that these tests have very high Positive Predictive Values (PPV).[4,7,8] Widal test was positive on paired sera of this patient with rising titres of both 'STO' and 'STH' antigens. This may be inferred as co-infection or serological cross-reactivity. Dual infections with leptospires & other etiologic agents like Dengue virus, Human Immunodeficiency Virus, Hepatitis B & E viruses etc. have rarely been reported. Serological cross-reactivity between leptospirosis and other infectious diseases has also been reported.[1,3,4]

However, both serum samples were negative by MAT which is considered as serological gold standard for the diagnosis of leptospirosis.[9] It is imperative to know the circulating *Leptospira* species/serovars in animals and humans in different geographical locations in order to investigate the prevalence of *Leptospira* species during monitoring of the leptospirosis. This helps in appropriate use of panel of leptospira serovars in the MAT for providing proper diagnosis without false negative results.[10] Hence, due to non-inclusion of *Leptospira inadai* in the panel of serovars used for MAT, negative result was obtained.

Leptospira inadai was isolated from urine sample of this patient. Phylogenetic analysis based on 16S rRNA gene sequences have identified three clades of *Leptospira spp.* containing branches that, with few exceptions, reflect species designations based on the pathogenicity status (pathogenic, saprophytic & intermediate strains of unclear pathogenicity) and *Leptospira inadai* belongs to intermediate branch of unclear pathogenicity.[11] In India, earlier sporadic human case reports with *Leptospira inadai* infection and circulation of *Leptospira inadai* in reservoir hosts have been reported.[12,13]

Given the clinico-epidemiological background and results of various laboratory tests, this patient was successfully treated with amoxicillin/clavulanic acid and discharged subsequently. This case report affirms the long held belief that diagnosis of leptospirosis (both laboratory & clinical) is an uphill task. It is an enigmatic disease which presents with various challenges for both clinicians & laboratory physicians.

Fig 1: Leptospires under Dark Field Microscope (x1000)



www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

Acknowledgements:

Dr. Pushpalatha K, Professor & HOD, Department of Pediatrics, ESIC-MC & PGIMSR, Rajajinagar, Bengaluru, Dr.P.Vijayachari, Director, Regional Medical Research Centre, Port Blair, Andaman & Nicobar Islands, India, Dr. Vimal Raj, Regional Medical Research Centre, Port Blair, Andaman & Nicobar Islands, India, Dr.K. Prabhudas, Dr.H.Rahman, Dr.M.R.Gajendragad, Dr.V.Balamurugan, Dr.Nagalingam, Mrs.Sushma and K Assadi, (Ex-Director, Director, Principal Scientist, Senior Scientist, Scientist and Trainee respectively at Leptospira Lab, PD_ADMAS Bengaluru

References:

1. Prasad SR, Rajini M. Leptospirosis: An Overview. The Journal of the Academy of Clinical Microbiologists 2008;10(2):89-97

2.Sharma KK, Kalawat U. Early diagnosis of leptospirosis by conventional methods: One year prospective study. Indian J Pathol Microbiol 2008; 5:209-11

3. Mary DB, David AA, Sandra LB, Christopher WW, Tin A, Richard AS et al. Evaluation of Four Commercially Available Rapid Serological Tests for Diagnosis of Leptospirosis. J Clin Microbiol 2003 Feb;41(2):803-09

4. Pimjai N, Piyada W, Wimol P, Ornnalin L, Paijit W. A Comparitive Evaluation of Different Methods for the Serological Diagnosis of Leptospirosis. J Trop Med Parasitol 2000 December;23(2):59-65

5. Vijayachari P, Sameer Sharma & Natarajaseenivasan K. Laboratory Diagnosis. In: Leptospirosis Laboratory Manual. Regional Medical Research centre, Port Blair ,WHO Country office for India: WHO;2007.p.27-45

6. Vijayachari P. Introduction. In: Leptospirosis Laboratory Manual. Regional Medical Research centre, Port Blair, WHO Country office for India:WHO;2007. p.1-3

7. Brandao AP, Camargo ED, Silva ED, Silva MV, Abrao RV. Macroscopic Agglutination Test for rapid diagnosis of human Leptospirosis. J Clin Microbiol 1998 Nov;36(11):3138-42

8. Tanvi P, Summaiya M, Parul P. Seroprevalance of leptospirosis in south Gujarat region by evaluating the two rapid commercial diagnostic kits against the MAT test for detection of antibodies to Leptospira interrogans. National Journal of Community Medicine 2011;2(1):64-70

9. Sambasiva RR, Naveen G, Bhalla P, Agarwal SK. Leptospirosis in India and the rest of the world. Braz J Infect Dis 2003;7(3):178-93

10. Balamurugan et al.: Characterization of leptospira isolates from animals and humans: phylogenetic analysis identifies the prevalence of intermediate species in India. Springer Plus 2013; 2:362

11. Gustavo MC, Mathieu P. A century of Leptospira strain typing. Infection, Genetics and Evolution 2009; 9:760-68

12. Gangadhar NL, Rajasekhar M, Smythe LD, Norris MA, Symonds ML, Dohnt MF. Reservoir hosts of *Leptospira* inadai in India. Rev Sci Tech OIE 2000;19(3):793-799

13. Gangadhar NL, Prabhudas K, Bhushan S, Sulthana M, Barbuddhe SB, Rhaman H. *Leptospira* infection in animals and humans: a potential public health risk in India. Rev Sci Tech OIE 2008; 27(3):885-892