JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2012. Vol. **6**(Spl. Edn.), p. 01-07

Assessment of Interleukin-10 in Leptospira Associated Acute Hepatic Syndrome

Meher Rizvi[1]*#, M. Azam[1]#, Asfia Sultan[1], Fatima Khan[1], Indu Shukla[1], Abida Malik[1] and M.R. Ajmal[2]

[1]Department of Microbiology, [2]Department of Medicine,
Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India.

(Received: 04 March 2012; accepted: 10 June 2012)

Leptospirosis is a worldwide zoonosis caused by pathogenic Leptospira and is characterized by a broad spectrum of clinical manifestations of varying severity ranging from inapparent infection to fulminant, fatal disease. Although several components of this organism have been identified, the molecular mechanisms underlying pathogenesis of this infectious disease are still poorly understood. Besides, direct injury by microbial factors, cytokines produced in response to infection have been proposed to be involved in pathogenesis of leptospirosis. The present study was done to document the prevalence of leptospira induced acute hepatitis and to estimate the levels of Interleukin-10 in these patients. 247 consecutive cases with symptoms of acute hepatitis and 30 healthy controls were enrolled in the study and detailed clinical history was elicited from them. ELISA for HAV, HBV, HCV and HEV were performed to rule out common viral etiology of hepatitis. IgM antibodies to leptospira were detected by ELISA. IL-10 levels were estimated in leptospira positive cases by ELISA. Out of 247 cases of acute hepatitis 46 (18.62%) were observed to be positive for IgM antibodies for leptospira. ALT, AST, ASP was raised in majority of patients. IL-10 was found to be elevated in a large majority of cases 56.52% (26/46). Patients with more severe symptoms were associated with higher levels of IL-10.

Key words: Leptospirosis, IL-10, Hepatitis, ELISA.

Leptospirosis, a zooanthroponosis of worldwide distribution is an acute febrile illness, the severity of which varies from mild to rapidly fatal.[1] Leptospirosis is caused by bacteria from genus leptospira, which comprises of 12 species (*L.alexanderi, L.biflexa, L.borgpetersenii, L.fainei, L.inadai, L.interrogans, L.kirschneri, L.noguchii, L.santarosai, L.weilli, L.meyeri, L.wolbachii*). To date only *L.interrogans* and L.fainei have been reported to be pathogenic in humans.[2,3] Common symptoms of leptospirosis in humans are sudden onset of fever, headache, chills, severe myalgia, and conjunctival suffusion.[3]

Southeast Asia is an endemic area for leptospirosis and infection in humans has been reported throughout the region.[4-10] In India, leptospirosis has been reported to be endemic in South India such as Kerala, Tamil Nadu, Pondicherry and Andamans.[1]

Leptospirosis is being underreported in India due to lack of awareness of disease, inadequate epidemiological data and unavailability of appropriate laboratory diagnostic facilities in most parts of the country.[11] The non specificity of signs and symptoms and limited availability of

* Corresponding author: Meher Rizvi
E-mail: rizvimeher@gmail.com
# Note: These authors contributed equally to this work

laboratory confirmation in endemic areas probably have contributed to significant under reporting. This occurs most notably in association with jaundiced disease.[7] The timely diagnosis of leptospirosis is usually based on demonstration of antibodies by serological tests such as microscopic agglutination test (MAT) and enzyme linked immunosorbent assay (ELISA).[12]

For this disease, study of immune response does not only provide information for proper vaccine development but also for further elucidating mechanisms of pathogenesis. An improved understanding of host immune response in leptospirosis will lead to development of more effective treatment and prevention of disease.[13] Expression of IL-2, IL-4, Il-10, IL-12p40, TNF-α, IFN-γ, TGF-β in peripheral blood mononuclear cells of hamsters infected with L.interrogans serovar icterohaemorrhagiae were analysed using real-time PCR.[14] The increase in TNF-α, IFN-γ and IL-2 expression could be detected since a few hours post-infection, however, levels of IL-4 and IL-10, anti-inflammatory cytokines were prominent in delayed samples from 1 to 4 days post infection.[13]

The present study was done to document the prevalence of leptospira induced acute hepatitis and to estimate the levels of Interleukin-10 in these patients

MATERIALS AND METHODS

Study subjects

247 consecutive cases [160 male (65%) and 87 female (35%); mean age: 31.99±14.02 years] with symptoms of acute hepatitis attending the Medicine Outpatient Department or admitted in the Medicine Wards of J.N.MedicalCollege over a 10-month period from June 2009 to March 2010 were enrolled in the study. A written informed consent was obtained from each patient. Detailed clinical history was elicited from them and details of their liver function tests were recorded. Patients with autoimmune hepatitis, alcoholic hepatitis and drug-induced hepatitis, renal, pulmonary disorders, other acute or chronic inflammatory diseases or patients who had history of surgery, trauma within the preceding 2 months were excluded from the study. ELISA for HAV, HBV, HCV and HEV were performed to rule out common viral etiology of hepatitis. IgM antibodies to leptospira were detected by ELISA (DRG, USA).

Healthy blood donors

30 Healthy blood donors who had no history of fever in the last 6 months [22 male (73.34%) and 8 female (26.66%); mean age: 37 years] were selected from the blood bank and included in the study. They had no serological markers of HAV-HEV, CMV, EBV infection and liver functions were normal.

Collection and Transport of Sample

Blood was collected aseptically from all patients with acute liver disease within 1 week ± 3 days of onset of symptoms. Serum was separated by centrifugation, aliquoted and stored at -20°C till further tests were performed. A battery of biochemical and haematological investigations were conducted.

Serological Test for Leptospira

Patients negative for all the hepatitis viruses and 30 healthy controls were tested for evidence of recent leptospiral infections by specific leptospira IgM antibody using the commercially available ELISA kit (DRG International, Inc.). The test procedure was performed according to the protocol provided along with the kit and absorbance was read at 450 nm. The results for leptospira IgM ELISA were interpreted according to the manufacturer's instructions, i.e. values, 0.0–0.3 optical density (OD) units (DRG ELISA) were considered negative, 0.5-1.0 OD units were equivocal and .1.0 OD units were positive. For samples showing equivocal results, another blood sample was drawn after a period of 10 or more days, and the test was repeated. Negative and positive control sera were provided by the manufacturer and their absorbance were used for the calculation of the cut-off and for determining the validity of the test.

Statistical Analysis

Chi-square test and Student's t-test were used for leptospira prevalence. The level of significance in all cases was set at a two-tailed $P<0.05$.

Detection of IL-10

IL-10 levels were estimated in the serum of all leptospira positive cases and healthy blood donors by ELISA (Orgenium, Finland).

RESULTS

Out of 247 cases of acute hepatitis 46 (18.62%) were observed to be positive for IgM antibodies for leptospira. Mean age of these patients was 31.99 yrs±0.28 (Males were 25 & females were 21). Fig. 1 shows distribution of Leptospiral and Viral Hepatitis (A-E) positive cases.

The most important clinical presentations are shown in Table 2. All patients had fever. Jaundice and myalgia were present in 76% and 34.7% patients, respectively. Calf muscle pain was present in 37 (80%). Of 46 patients, 26 patients (57.2%) were oliguric. Subconjuctival suffusion was present in 13% of patients. Other clinical features, which were more commonly seen, were tachycardia (64.2%), diarrhoea (8.6%), renal tenderness (14.8%), tachypnoea (55.4%). Hepatomegaly (36.9%) and spleenomegaly (10.86%) were less common findings in leptospira patients while these findings were common in viral Hepatitis.

The biochemical profile of patients with leptospirosis and viral hepatitis shown in Table 2. All the parameters were mildly raised in leptospira patients.

Table 1. Comparision of clinical features of leptospirosis and acute viral hepatitis (AVH)

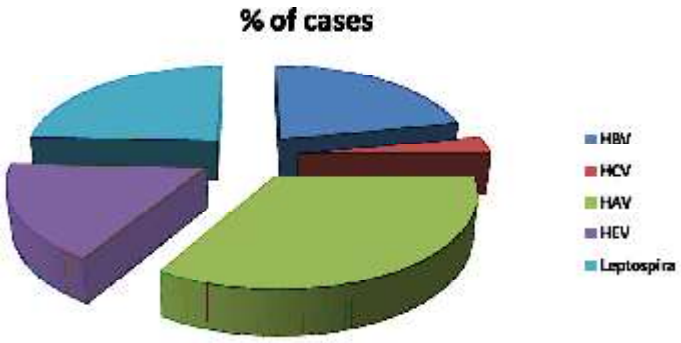
Clinical features	Leptospirosis (%) (n=46)	AVH (A-E) (%) (n=142)	p value
Jaundice	35(76)	134 (94.36)	
Fever	45(97.8)	88(61.97)	
Nausea/vomiting	17(36.9)	120(84.5)	P<0.05
Myalgia	16(34.7)	39(27.46)	
Hepatomegaly	17(36.9)	142(100)	P<0.001
Splenomegaly	5(10.86)	82(57.7)	P<0.01
Conjunctival suffusion	6(13)	-	P<0.001
Diarrhoea	4(8.6)	-	
Respiratory symptoms	9(19.5)	-	
Oliguria	26(57.2)	-	P<0.01
Calf muscle pain	37(80.5)	-	P<0.001
Arthralgia	3(7.2)	15(10.56)	
Tachycardia	30(64.2)	-	P<0.001
Tachypnea	26(55.4)	-	P<0.001
Renal tenderness	7(14.8)	-	

All patients had fever. Jaundice and myalgia were common in leptospira patients, respectively. Subconjuctival suffusion was present in 13% of patients. Hepatomegaly (36.9%) and spleenomegaly (10.86%) were less common findings in leptospira patients while these findings were common in viral Hepatitis.

Table 2. Biochemical profile of leptospirosis and acute viral hepatitis

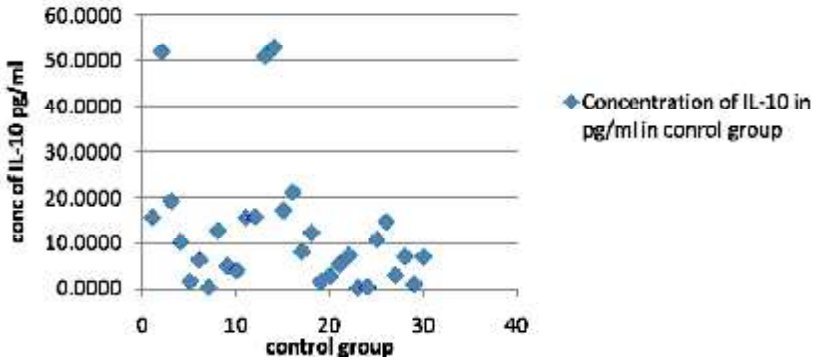
Parameters	Leptospirosis (mean values)	Acute viral hepatitis (mean values)	Reference range
SGOT	31.3	160.33	2-20 IU/L
SGPT	32.61	127.16	2-15 IU/L
ALP	16.46	19.91	3-13 KAU/100ml
Serum Bilirubin	2.84	13.66	0.2-1.0 mg/100ml
Albumin	2.75	3.06	3.4-5.4 g/dl
INR	1.64	2.48	0.8-1.2
ESR	44	18	M 12-19F 18-21

All the parameters were mildly raised in leptospira patients



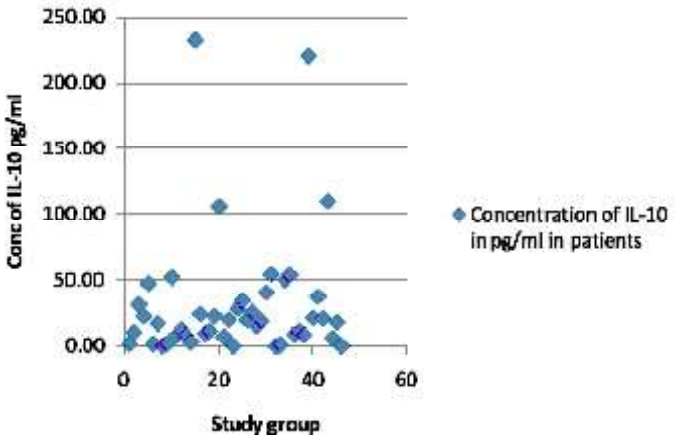
Out of 247 cases of acute hepatitis 46 (18.62%) were observed to be positive for IgM antibodies for leptospira

Fig. 1. Distribution of Leptospiral and Viral Hepatitis (A-E) positive cases



The mean value of IL-10 was found to be 12.79 pg/ml in control group. Majority of individuals showed IL-10 values near about 15 pg/ml with few values occurring around 50 pg/ml. Lowest values of IL-10 was found to be 0.2890 pg/ml while highest value was 52.978 pg/ml

Fig. 2. Distribution of IL-10 in control group



IL-10 was found to be elevated in 56.52% (26/46) ($p<0.001$) patients as compared to healthy controls. The mean value of IL-10 in patients was 32.94 pg/ml. Majority of patients 30% (14/46) showed values of IL-10 clustering between 20 – 50 pg/ml. The lowest value was found to be 0.19 pg/ml with 232.44 pg/ml being the highest value in our study group

Fig. 3. Distribution of IL-10 in patients with leptospirosis

Fig 2. Show distribution of IL-10 in healthy control group. The mean value of IL-10 was found to be 12.79 pg/ml. Majority of individuals showed IL-10 values near about 15 pg/ml with few values occurring around 50 pg/ml. Lowest values of IL-10 was found to be 0.2890 pg/ml while highest value was 52.978 pg/ml. Fig 3. Shows the concentration of IL-10 in pg/ml in patients positive for Leptospira by IgM ELISA. IL-10 was found to be elevated in 56.52% (26/46) ($p<0.001$) patients as compared to healthy controls. The mean value of IL-10 in patients was found to be 32.94 pg/ml. Majority of patients 30% (14/46) showed values of IL-10 clustering between 20 – 50 pg/ml with few patients having values as high as 220.44 pg.ml and 232.44 pg/ml. The lowest value was found to be 0.19 pg/ml with 232.44 pg/ml being the highest value in our study group. One mortality was observed due to leptospira.

DISCUSSION

Leptospirosis is a common cause of acute febrile illness in India. Early diagnosis is essential. If untreated the illness can progress rapidly and mortality rates are high in severe cases. It is therefore important to differentiate leptospirosis from other causes of acute febrile illnesses. In the present study, the prevalence of leptospirosis was 18.6%, which is higher than previously published data from Uttar Pradesh in 2004.[15] The prevalence of leptospirosis in our study is also far higher than other studies published from North India.[16] This increase may be due to increased awareness of the disease among the clinicians, which in turn leads to early diagnosis and appropriate treatment of patients. Another reason for higher prevalence can be increased use of fertilizers, which makes the pH of the water and soil alkaline, thereby allowing Leptospira to survive for longer duration and thus facilitating its transmission.[17] However, prevalence of leptospirosis in North India is lower than in South India.[1] It could be because this disease is more endemic in southern India due to certain environmental factors. Leptospirosis can be considered to be an emerging problem in northern part of the country also. When compared with other Asian studies the prevalence of leptospirosis in our study was found to be higher than in Italy[18] while lower than Pakistan[19]. Lower prevalence of 8% has been reported from Cambodia (Kanti et al., 2002).The preponderance of cases in males between 15 to 44 years of age shows that disease is common in working population who are most likely to be exposed to this organism. Since males are more involved in outdoor activities they are at more risk of acquiring the infection. The IgM ELISA kit used in our study does not provide information about the serotype involved in the infection. Knowledge of the serotype is not essential for treatment of these patients.

Fever (97.6%) was the commonest presentation in our study, followed by jaundice in 76.4% of patients. This is in concordance with a study conducted in Maharashtra (Bharadwaj *et al*, 2002) and Chandigarh (Sethi *et al*, 2003) where fever and jaundice were the most common presentation, in contrast to the Barbados hospital admission, where 97% of patients had jaundice (Everard and Everard, 1993). A clinico-epidemiological study (Barua *et al*, 1999) carried out in the North Eastern states of India reported headache as predominant symptoms (84.21%) followed by fever (73%).

Symptoms and signs like extreme muscle tenderness and suffusion of conjunctiva, which are considered as cardinal signs of leptospirosis, occurred in a proportion of patients and were helpful in making a provisional clinical diagnosis. Conjunctival suffusion was found to be low (13%) in our study as compared to Bharadwaj *et al*. and Sethi *et al*. but higher than that reported by Manocha *et al.* Frequency of oliguria, muscle pain, tachypnoea, tachycardia was also high in our study compared to previously published studies.[16] Only one mortality was observed which was very low as compared to another study.[17]

Among the biochemical profile of patients with leptospirosis all the parameters were mildly deranged as compared to patients with viral hepatitis. Leptospirosis should be considered in patients having clinical features similar to viral hepatitis but with mildly deranged liver function tests.

Detection of IgM antibodies to leptospira by ELISA is now widely used in diagnosis of leptospirosis, which is more sensitive than microscopic agglutination test (MAT) and most laboratories prefer IgM ELISA formats for the diagnosis of leptospirosis (Sambasiva *et al.,* 2003).

Furthermore, this test is reactive even in early cases of leptospirosis when MAT may be negative (Sambasiva *et al.,* 2003).

At present, limited data are available on potential biomarkers that may aid the clinician in monitoring disease progression and identify severe disease at an early stage which might reduce mortality. In this study, we examined the ability of IL-10 to delineate disease severity. This is a preliminary report on the role of IL-10 in patients with hepatitis induced by leptospirosis. The concentration of IL-10 in pg/ml in patients was higher than in healthy controls. IL-10 was found to be elevated in majority 56.52% (26/46) patients as compared to 2% healthy controls. The mean value of IL-10 in patients was found to be 32.94 pg/ml. Majority of patients 30% (14/46) showed values of IL-10 clustering between 20 – 50 pg/ml. The highest was found to be 232.44 pg/ml. The mean value of IL-10 in healthy control group was found to be 12.34 pg/ml. One patient died after developing severe hepatitis and acute renal disease. IL-10 levels were highly elevated in this patient. These findings suggest that IL-10 is generally raised in patients with leptospirosis and unusually high IL-10 levels are associated with severe disease. This relation between IL-10 and severity of disease is in concordance with findings of Kyriakidis et al. (2011). This is a baseline study and it will be useful to assess the levels of cytokines in both mild and severe leptospiral infections. A single point evaluation of IL-10 was carried out in our study which may represent a limitation. We recommend that a high index of suspicion for leptospirosis should be maintained in patients presenting with acute hepatitis syndrome, especially so in the presence of history of rural background, animal contact with water contaminated with urine. Early diagnosis of this potentially dangerous disease is imperative in preventing fatalities caused by leptospirosis.

CONCLUSION

In conclusion, leptospirosis is an important etiology that should be considered and investigated in patient negative for A-E viral hepatitis with mildly deranged liver functions. Interleukins play an important role in pathogenesis of different diseases and more studies should be done on establishing their role in disease severity and their impact on cure of disease. This was preliminary study to established role of IL-10 in leptospirosis, other interleukins should also be considered and studied.

ACKNOWLEDGMENTS

This work was supported by grants from Department of Science and Technology (DST), Ministry of Science and Technology, INDIA.

REFERENCES

1. Smita B. Shekatkar, Belgode N. Harish, Godfred A. Menezes and Subhash C. Parija; Clinical and serological evaluation of leptospirosis in Puducherry, India. *J Infect Dev Ctries* 2010; 4(3):139-143.
2. Yersin C, Bovet P, Merien F, Wong T, Panowsky J, Perolat P. Human leptospirosis in the Seychelles (Indian Ocean): a population-based study. *Am J Trop Med Hyg* 1998; **59***:* 933–940.
3. Chin J, ed, 2000. Leptospirosis. In: *Control of Communicable Diseases Manual* 17th ed. American Public Health Association, Washington, 293–296.
4. Van CB, Thuy NTT, San NG, Hien TH, Baranton G, Perolat P, Human leptospirosis in the Mekong delta, Viet Nam. *Trans R Soc Trop Med Hyg* 1998; **92***:* 625–628.
5. Bahaman AR, Ibrahim AL, A review of leptospirosis in Malaysia, *Vet Rec Commun.* 1998; **12***:* 179–189.
6. Heisey GB, Nimmanitya S, Karnchanachetanee C, Tingpalapong M, Samransamruajkit S, Hansukjariya P, Elwell MR, Ward GS. Epidemiology and characterization of leptospirosis at an urban and provincial site in Thailand. *Southeast Asian J Trop Med Public Health.* 1988; **19***:* 317–322.
7. Bounlu K, Insisiengmay S, Vanthanouvong K, Saykham, Widjaja S, Iinuma K, Matsubayashi K, Laras K, Putri MP, Endy TP, Vaughn DW, Raengsakulrach B, Hyams KC, Hayden M, Scheffel C, Corwin AL. Acute jaundice in Vientiane, Lao People's Democratic Republic. *Clin Infect Dis* 1998; **27**: 717–721.
8. Easton A, Leptospirosis in Philippine floods. *BMJ* 1999; **319**: 212.
9. Chan OY, Paul DR, Sng EH. Leptospirosis among abattoir workers—a serological survey. *Singapore Med J* 1987; **28**: 293–296.
10. Light RH, Nasution R, Van Peenen PFD.

Leptospirosis in febrile hospital patients in Djakarta. *Southeast Asian J Trop Med Public Health* 1971; **2**: 493–495.

11. Muthusethupathi MA, Shivkumar S, Suguna R, Jayakumar M, Vijaykumar R, Everard COR, *et al*. Leptospirosis in Madras: a clinical and serological study. *JAPI* 1995; **43**: 456-58.
12. Terpstra WJ, Ligthart GS, Schoone GJ. ELISA for the detection of specific IgM and IgG in human leptospirosis. *J Gen Microbiol* 1985; **131**: 377–85.
13. Lowanichapat A, Payungporn S, sereemaspun A, Ekpo P, Phulsuksombati, Poovorawan Y, Chirathworn C. Eypression of TNF-α, TGF-α, IP-10 and IL-10 mRNA in kidneys of hamsters infected with pathogenic Leptospira. *Comp Immunol Microbiol Infect Dis* (2009).
14. Vernal-Pauillac F, Merien F. Proinflammatory and immunomodulatory cytokine mRNA time course profiles in hamsters infected with a virulent variant of *Leptospira interrogans. Infect Immun* 2006; **74**: 4172-9.
15. Manocha H, Ghoshal U, Singh SK, Kishore J, Ayyagari A. Frequency of Leptospirosis in Patients of Acute Febrile Illness in Uttar Pradesh. *JAPI*, **52**; 2004.
16. Sethi S, Sood A, Pooja, Sharma S, Sengupta C, Sharma M. Leptospirosis in northern India: a clinical and serological study. The Southeast *Asian journal of tropical medicine and public health* 2003; **34**(4):822-825.
17. Bharadwaj RS, Bal AM, Joshi SA, Kagal AS, Pol SS, Garad G, *et al*. An urban outbreak of leptospirosis in Mumbai, India. *Jpn J Infect Dis* 2002; **55**: 194-96.
18. Cacciapuoti B., Vellucci A., Ciceroni L., et al. Prevalence of Leptospirosis in man: A pilot survey. *Eur J Epidemiol* 1987; **3**(2):137-42.
19. Ahmed IP. Serological studies on Leptospirosis in Pakistan. *J Pak Med Asso* 1987; **37**: 233-6.
20. Kyriakidis, I., Samara, P. & Papa, A., Serum TNF-a, sTNFR1, IL-6, IL-8 and IL-10 levels in Weil's syndrome. *Cytokine*, 2011; **54**: 117–120.
21. Kanti L, Cao BV, Khanthong B, Nguyen TKT, James GO, Sisouk T, Tran NVA, Hoang KL, Narain P, Ha BK, Ung SA, Sithat I, Douglas MW, Beecham HJ & Andrew LC., The importance of leptospirosis in Southeast Asia. *American Journal of Tropical Medicine and Hygiene,* 2002; **67**: 278–286.
22. Sambasiva RR, Naveen G, Bhalla P & Agarwal SK., Leptospirosis in India and rest of the world. *Brazilian Journal of Infectious Diseases*, 2003; **7**: 178–193.
23. Barua HC, Biswas D, Mahanta J. Regional Medical Research Centre, NE Region (ICMR), Assam. Clinico-epidemiological study on leptospirosis in certain parts of north-eastern region. *J Commun Dis* 1999; **31**: 201-2.
24. Everard JD, Everard COR. Leptospirosis in the Caribbean. *Rev Med Microbiol* 1993; **4**: 114-22.