Evaluation of Cytokine Levels in Human Leptospirosis as Prognostic Indicator

Khan Fatima[1*],Mahtab Md[2], Ahmed Shariq[3], Sultan Asfia[4,5],Azam Mohd[5], Rizvi Meher[4,6], Raafiah Izhar[7]

[1] *Department of Microbiology, J.N Medical College & Hospital, AMU, Aligarh, India.*

[2] *Department of Microbiology, J.N Medical College & Hospital, AMU, Aligarh, India.*

[3] *Department of Microbiology, J.N Medical College & Hospital, AMU, Aligarh, India.*

[4] *Department of Microbiology, J.N Medical College & Hospital, AMU, Aligarh, India.*

[5] *College of Applied Medical Sciences, Qassim University, Buraydah, Saudi Arabia.*

[7] *Department of Bio Science, Faculty of Natural Sciences, Jamia Millia Islamia, New Delhi..*

*E-mail: fatimasalmanshah@gmail.com

Received: 17/12/2021
Revised: 16/06/2022
Accepted: 28/06/2022

Abstract

Purpose: Leptospirosis is a tropical zoonotic illness, in which the role of immune response in the pathogenesis is proven but poorly understood. Response of cytokines is said to play a key role in disease progression and pathogenesis. There are proven studies on pro-inflammatory cytokines like TNFα, IL-6 and anti-inflammatory like IL-10 in human leptospirosis however, the role of IL-2, IL-4, IL-15, GCSF and MCP-2 needs more comprehensive studies. Present study was conducted to evaluate the role of IL-2, IL-4, IL-15, GCSF and MCP-2 in human leptospirosis as prognostic indicator.

Methods: Blood samples from patients meeting the inclusion criteria for leptospirosis were included in the study. PCR and IgM ELISA were carried out for diagnosis. Serum cytokine levels in Leptospira positive patients and in controls were estimated by ELISA. Statistical analysis was done using IBM SPSS Statistics 20 and Med Calc 16.1. software.

Results: Out of 270, 45(16.7%) patients were confirmed as cases of leptospirosis. The mean level of the cytokines (IL-15, MCP-2, G-CSF) differed significantly between the patients and the control group ($p < 0.001$). GCSF, MCP-2, IL-15 and IL-4 were elevated in most cases. IL-2 level was depressed in 34 out of 45 cases. The AUCs for IL-2, IL-15, MCP-2 and GCSF were 0.906 (95% CI 0.341 to 0.665), 0.929 (95% CI 0.837 to 0.978), 0.909 (95% CI 0.812-0.966) and 0.881 (95% CI 0.777 to 0.948) respectively. On spearman rank correlation, GCSF level showed correlation with MCP-2 (rho = 0.415, $p < 0.01$).

Conclusions: The study provided an understanding of cytokine patterns in leptospirosis, and concluded that IL-15, MCP-2 and GCSF can be used as an effective biomarker for leptospirosis and indicators of disease progressions.

Keywords: *Biomarkers. Cytokines, Leptospira*.

Introduction

Leptospirosis is a widely distributed but scantly diagnosed zoonotic disease mainly of impoverished populations lacking proper sanitation facilities. The Indian subcontinent provides many opportunities for Leptospira to flourish with hot humid climate during major part of the year and with poor sanitation practices in majority of urban slums and in rural areas. Disease manifestations are varied. Leptospirosis may range from mild influenza like illness to severe fatal manifestations like weil's disease (Gouveia et al., 2008; Khan et al., 2016; Martínez García et al., 2000; McBride et al., 2005; Vijayachari et al., 200)

Severity varies according to the infecting serovars of Leptospira, and the age, health and immunological competence of the patient (Adler & de la Peña Moctezuma, 2010). Leptospires enter the body through small cuts or abrasions, via mucous membranes such as the conjunctiva or through wet skin. They circulate in the blood stream, with the bacteremic phase lasting for up to 7 days (Adler & de la Peña Moctezuma, 2010). The second stage of acute leptospirosis is referred as the immune phase, in which the disappearance of the organism from the bloodstream coincides with the appearance of antibodies (Levett, 2001) .

The Leptospiral infection is a perpetual battle between host and bacterium. On the host side, an array of inflammatory cytokines and chemokines, is used to stimulate cells to resist infection and kill the bacteria. The bacterium presents an array of molecules to the host immune system that may initiate a destructive immune response leading to tissue damage, sepsis and death (Zuerner, 2015). Pathogenesis of the disease initiates the adhesion of leptospires to host tissues. Several infectious agents have been reported to be implicated in the pathogenesis of the disease, like the lipopolysaccharide (LPS), hemolysins, the outer membrane proteins and the glycolipoprotein (Dorigatti et al., 2005; Evangelista & Coburn, 2010). Besides, direct injury by microbial factors, cytokines produced in response to infection have been anticipated to be involved in pathogenesis of leptospirosis (Rizvi et al., 2014). The innate immune system constitutes the first line of host defense, playing a crucial role in early recognition and elimination of leptospires. The activation of the alternative pathway of the complement system is one of the most important effector mechanisms during the first hours of infection (Barbosa et al., 2009; Meri et al., 2005). It has been demonstrated that the secretion of pro-inflammatory cytokines is induced by hemolysins of Leptospira interrogans in human macrophages (Wang et al., 2012). Anti-inflammatory effectors also play an important role in counter regulating the effects of pro-inflammatory cytokines (Rizvi et al., 2014)

There are proven studies on pro-inflammatory cytokines like TNFα, IL-6 (Tajiki & Salomão, 1996; Wagenaar et al., 2009) and anti-inflammatory like IL-10 in human leptospirosis. The present study was planned with the objective to study the expression of un-explored or lesser explored circulatory cytokines in patients with leptospirosis.

Materials and Methods

The prospective study was conducted in the Department of Microbiology, JNMCH over a period of four years from July 2014 to August 2018. All patients meeting the inclusion criteria, presenting in medicine OPD or admitted in medicine ward with acute febrile illness, acute hepatitis and acute renal failure were included in the study. Utilizing clinical, epidemiological, and laboratory parameters modified Faine's criterion was scored and assessed (Shivakumar & Shareek, 2004).

Inclusion Criteria/Case Definition:

Three groups of patients were included in the study

1. Acute febrile illness (AFI): A case of AFI was defined as an individual with history of fever (temperature > 38°C) for 3 days or more.

2. Acute hepatitis: Any individual presenting with signs and symptoms of acute jaundice (bilirubin > 1mg/dl) was defined as a case of acute hepatitis.

3. Acute renal failure: A case of acute renal failure (ARF) was defined as any individual with rapid deterioration in kidney function (within 48 hours). Deterioration in kidney function was assessed by rise in serum creatinine (absolute increase in serum

creatinine of ≥0.3mg/dl or percentage increase in serum creatinine of ≥50%) or in those patients who presented with a reduction in urine output (defined as <0.5 ml/kg/hr for more than 6 hours).

An informed consent was taken from patients before their inclusion in the study. The study was approved by the Institute ethical committee, J.N.M.C.H., A.M.U.

Exclusion criteria:

Following patients were excluded from the study

1) Patients with obvious clinical signs for diseases such as diarrhoea, pneumonia, UTI, typhoid fever, malaria, or established fever of unknown origin (FUO).

2) Patients found positive for Hepatitis B, Hepatitis C, Chikungunya, Malaria or Dengue.

3) Patients with ARF with a known underlying etiology were also excluded from the study.

Controls

Thirty age and sex matched controls were taken from the blood bank, department of Pathology, JNMCH, AMU.

Collection of Specimen for diagnosis and cytokine analysis:

Seven-milliliter blood was collected with all aseptic precautions, from patients suspected of leptospirosis for PCR.

The lepto immunoglobulin M (IgM) ELISA (PANBIO IgM ELISA, Standard Diagnostics, Korea):

ELISA was done for all the serum samples using commercially available kits according to the manufacturer's instructions. The results were interpreted according to manufacturer's instructions, i.e., values < 9 PANBIO ELISA units were considered negative, 9–11 equivocal, and >11 positive. For samples showing equivocal results, another blood sample was drawn after a period of 10 days, and the test was repeated.

Polymerase chain reaction:

DNA isolation: DNA was extracted from serum samples using QIA amp DNA blood mini kit (Qiagen, Germany).

Amplification of DNA (Bio-Rad Laboratories India Pvt. Ltd): The amplification of DNA was performed in a total volume of 25 µl. The primers used for PCR (Gravekamp et al., 1993) were:

G1 5'- CTG AAT CGC TGT ATA AAA GT-3' &

G2 5'- GGA AAA CAA ATG GTC GGA AG-3'

The program for amplification included 35 cycles of 94°C (denaturation) for 1 min, 55°C (annealing) for 1 min and 72°C (extension) for 2 min and a final extension step at 72°C for 7 min. The PCR was performed in a final reaction volume of 25 µl containing 5 µl of 10x assay buffer [10 mM Tris HCl (pH 9.0), 1.5 mM MgCl2, 50 mM KCl and 0.01% Gelatin], 200 µM each dNTPs, 20 pM of each primer, 0.5 U of Taq DNA Polymerase, and template DNA (10 µl). The PCR products were loaded in 1% wt/vol agarose gel prepared in TAE (tris base, acetic acid and EDTA- pH 8.0) buffer and detected by ethidium bromide staining after electrophoresis (BioRad, USA).

Amplification of 285 base pair DNA fragment was considered as positive for Leptospiral DNA.

Cytokine analysis:

Serum cytokine levels in Leptospira positive patients and in controls were estimated by ELISA (IL-2, IL-4, IL-15 ELISA Daiclone, France; MCP-2 and GCSF ELISA Sigma Life Sciences, USA). A standard curve of optical densities versus concentrations of different cytokines was generated to determine their concentrations in serum samples.

Statistical analysis:

Statistical analysis was performed with the IBM SPSS Statistics 20 (IBM Inc. Armonk, New York, USA) and Med Calc 16.1. software. The evaluation of differences in cytokine levels between two groups was performed using non-parametric Mann-Whitney U test. For descriptive statistics, mean and IQRs (inter-quartile range) are reported. Spearman's rank correlation test was used for the assessment of correlation. A p-value of < 0.05 was considered significant. Med Calc 16.1 used receiver operating characteristic (ROC) curve to assess the diagnostic value, sensitivity and specificity of IL-2, IL-4, IL-15, MCP-1 and G-CSF in leptospirosis. The comparisons of area under curve (AUCs) were performed using z test. The optimal diagnosis threshold was determined according to Youden index J and relative sensitivity, and specificity were calculated.

Results

Patient Characteristics:

Out of 270 patients clinically suspected of leptospirosis %16.7)45), were confirmed as cases of leptopirosis by IgM ELISA (PanBio, Korea) and detection of 285 base pair DNA fragment by PCR (Fig 1). The median age of the patients was ± 29.7 13.8 years (range 3.5 to 65 years) and male to female ratio was 16/29) 1.81). Thirty healthy controls were included in the study with mean age 6.4 ± 30.7 years with a male to female ratio of 13/17) 1.31).

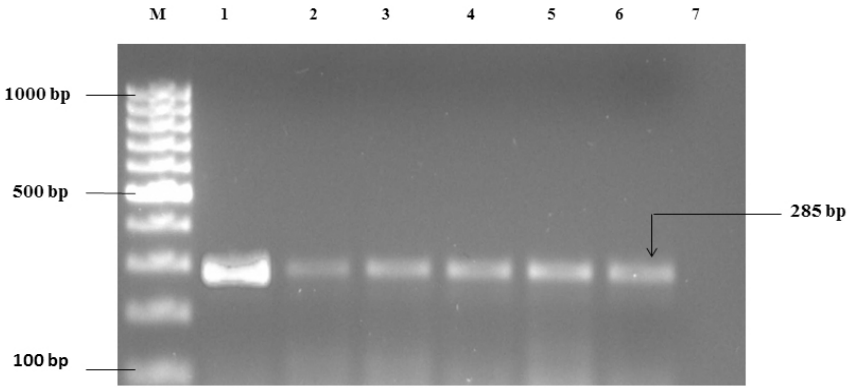
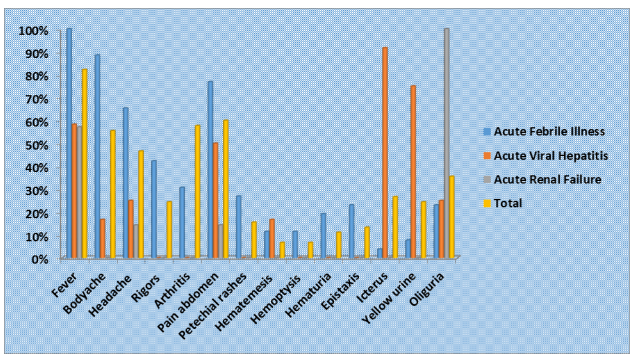


Figure 1. Lane M showing DNA ladder, Lane 1 and 7 showing positive & negative controls respectively, DNA Lane 2-6 showing 285 bp amplified PCR product of Leptospira

Clinical characteristics:

Out of 45 patients confirmed of leptospirosis, 26 (57.7%) were cases of AFI, 12 (26.6%) had manifestations of acute hepatitis and 7 (15.5%) patients presented with symptoms of ARF. Fever and pain in abdomen were the most common manifestations of leptospirosis among all the groups taken together. Headache, rigors, arthritis, and vomiting were more common in patients with AFI. Bleeding manifestations in various forms (epistaxis, hematemesis, hemoptysis, hematuria, and petechial rashes) were seen in 16(35.5%) out of 45 patients (Graph 1).



Graph 1. Clinical presentation of leptospirosis patients with acute febrile illness, acute viral hepatitis, and acute renal failure

Cytokine expression:

The mean level of the cytokines (IL-15, MCP-2, G-CSF) differed significantly between the patients and the control group ($p < 0.001$) (Table 1, Fig 2). IL-2 level was lower in the cases than in controls, while IL- 4 was elevated in cases, but these were not significant in controls.

It was found that age and sex did not affect the cytokine levels. GCSF, MCP-2, IL-15 and IL-4 were elevated in most cases (39/45, 43/45, 41/45 and 35/45 respectively).

depressed in 34 out of 45 cases. Compared to the controls, the patients with leptospirosis had higher concentration of IL-15 (33.87 ± 8.83 vs 48.10 ± 5.6, $p < 0.001$, Mann Whitney U test U: 25.00); MCP-2 (1.88 ± 2.69 vs 15.4 ± 18.07, $p < 0.001$, Mann Whitney U test U:82.50) and GCSF (10.68 ± 1.71 vs 22.11 ± 31.81, $p < 0.001$, Mann Whitney U test U: 91.50). IL-4 levels were also higher (2.99 ± 6.22) as compared to controls (1.40 ± 0.63) but were not significant (p=0.2932, Mann Whitney U test U: 159.0). Levels of IL-2 were higher in controls as compared to the patients (11.33 ± 2.98 vs 10.16 ± 3.31, p=0.967 NS, Mann Whitney U test U: 189.5).

Table 1. Elemental composition by Fluorescence X of the two algae

Cytokines	Healthy Controls	Leptospirosis patients	U*-values	P* values
IL-2	11.33 ± 2.98 (8.70-22.40)	10.16 ± 3.31 (0.80-13.70)	189.5	(NS)0.9676
IL-4	1.40V ± 0.63 (0.60-3.00)	2.99 ± 6.22 (0.85-29.30)	159.0	(NS)0.2932
IL-15	33.87±8.83 (14.65-53.18)	48.10 ± 5.61 (38.53-67.29)	25.0	0.001>
MCP-2	1.88 ± 2.69 (0.14-8.80)	15.40± 18.07 (0.25-79.95)	82.50	0.001>
GCSF	10.68 ± 1.71 (6.76-14.70)	6.83± 9.09 (9.33-50.20)	91.50	0.001>

*Mann-Whitney U test (two tail) was used to calculate significant differences (NS- not significant) Independent sample T-test was used to evaluate cytokine level differences between healthy controls and leptospirosis patients

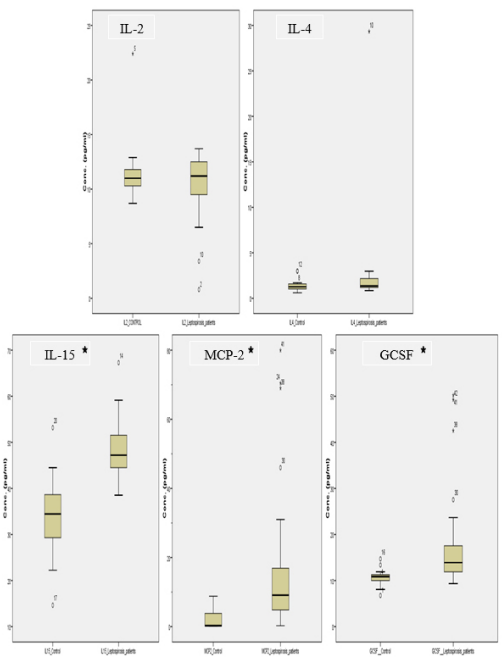


Figure 2. Comparison of serum cytokine concentration between control group and leptospirosis patients: Box plots of serum cytokine concentrations among studied patients with leptospirosis and in controls. The bottom, median and top lines of the box mark the 25th, 50th and 75th percentiles respectively. The vertical line with whiskers shows the range of values. Dots show individual data points. *p < .001

As analyzed by ROC (Table 2, Fig 3), the cytokines IL-2, IL-15, MCP-2 and G-CSF were differentially related to leptospirosis. The AUCs for IL-2, IL-15, MCP-2 and GCSF were 0.906 (95% CI 0.341 to 0.665), 0.929 (95% CI 0.837 to 0.978), 0.909 (95% CI 0.812-0.966) and 0.881 (95% CI 0.777 to 0.948) respectively, indicating that IL-2, IL-15, MCP-2 and GCSF were effective biomarkers for leptospirosis. A serum IL-15 level of more than 39.07 pg/ml had a sensitivity of 97.83% and a specificity 78.95% (youden index J: 0.7677; z statistics 9.579); a serum MCP-2 level of more than 0.42 pg/ml had a sensitivity of 97.81% and a specificity 69.42% (youden index J: 0.6625; z statistics 10.587); a serum GCSF level of more than 11.39 pg/ml had a sensitivity of 86.96% and a specificity 84.21% (youden index J: 0.6537; z statistics 8.102); a serum IL-2 level 9.9 pg/ml or lower had a sensitivity of 40% and a specificity 80% (youden index J: 0.2000; z statistics 0.0394). The AUC for IL-4 for diagnostic criterion of leptospirosis was 0.598. On spearman rank correlation (Table 3), GCSF showed correlation with MCP-2 (rho = 0.415, $p < 0.01$) in patients with leptospirosis while among the controls IL-2 showed correlation with MCP-2 (rho = 0.472, $p < 0.05$). None of the other cytokines tested showed correlation in patients or in controls.

Table 2. Predictive value of cytokines in patients with leptospirosis

Diagnostic Indicators	AUC	SE	95 % CI	Associated Criterion (pg/ml)	Yoden Index J	Z-Statistics	Sensitivity (%)	Specificity (%)	+LR	LR-
IL-2	0.906	0.0952	0.341 to 0.66	≤9.9	0.2000	0.0394	40	80	2.00	0.75
IL-4	0.598	0.0928	0.431 to 0.749	>1.71	0.2500	1.051	35	90	3.50	0.72
IL-15	0.929	0.0448	0.837 to 0.978	>39.07	0.7677	9.579	97.83	78.95	4.65	0.028
MCP-2	0.909	0.0386	0.812 to 0.966	>0.42	0.6625	10.587	97.81	69.42	3.10	0.032
GCSF	0.881	0.0470	0.777 to 0.948	>11.39	0.6537	8.102	86.96	84.21	5.51	0.15

Med Calc 16.1 used receiver operating characteristic (ROC) curve to assess the diagnostic value, sensitivity and specificity of IL-2, IL-4, IL-15, MCP-1 and G-CSF in leptospirosis.

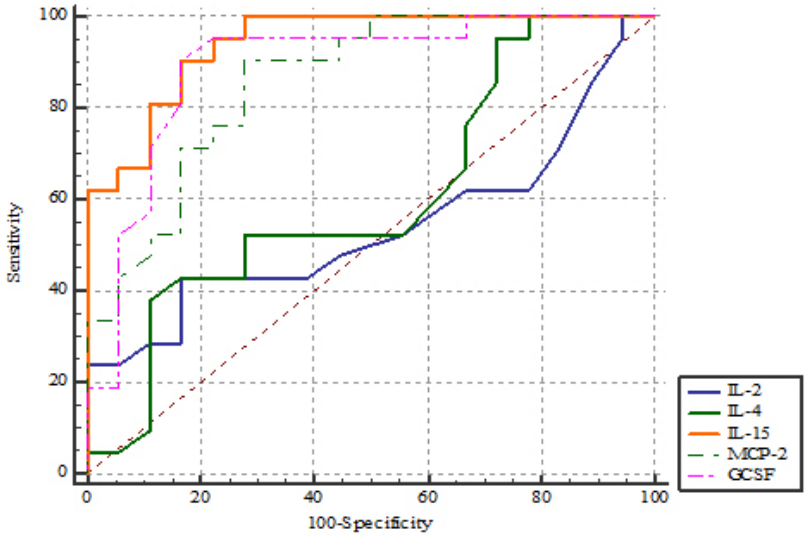


Figure 3. ROC curve for predictive value of cytokines in patients with leptospirosis

Table 2. Predictive value of cytokines in patients with leptospirosis

Cytokines	Statistical values	IL-2	IL-4	IL-15	MCP-2	GCSF
IL-2	**R**	-	0.005	-0.320	-0.021	-0.114
	P	-	0.982	0.169	0.930	0.632
IL-4	**R**	-	-	-0.110	-0.357	0.163
	P	-	-	0.644	0.123	0.491
IL-15	**R**	-	-	-	-0.188	0.147
	P	-	-	-	0.216	0.335
MCP-2	**R**	-	-	-	-	0.415**
	P	-	-	-	-	0.005
GCSF	**R**	-	-	-	-	-
	P	-	-	-	-	-

[B]

Cytokines	Statistical values	IL-2	IL-4	IL-15	MCP-2	GCSF
IL-2	**R**	-	-0.040	-0.462	0.472*	-0.012
	P	-	0.876	0.054	0.048	0.961
IL-4	**R**	-	-	0.344	0.122	0.200
	P	-	-	0.137	0.619	0.411
IL-15	**R**	-	-	-	-0.388	0.257
	P	-	-	-	0.157	0.289
MCP-2	**R**	-	-	-	-	-0.354
	p	-	-	-	-	0.138
GCSF	**r**	-	-	-	-	-
	p	-	-	-	-	-

***Correlation is significant at the 0.01 level (2-tailed).*

** Correlation is significant at the 0.05 level (2-tailed).*

Discussion

Out of 270 clinically suspected cases 45 (16.67%) were confirmed of leptospirosis based on IgM ELISA and detection of 285 base pair DNA fragment by PCR. Fever and abdominal pain were the most consistent symptoms with bleeding manifestations in a total of 35.5% patients. In regions where malaria, enteric fever and dengue are common, clinicians can easily miss leptospirosis in such cases unless they have index of suspicion.

As there is varied array of clinical manifestations in leptospirosis, it would be helpful to determine the parameters that could predict the disease outcome. Increasing evidences indicate that immune response could enhance tissue damage observed in leptospirosis (Chirathaworn & Kongpan, 2014). Cytokines produced in response to infection have been proposed to be involved in the pathogenesis of leptospirosis (Rizvi et al., 2011). Therefore, this study was planned to evaluate the expression of cytokines (IL2, IL-4, IL-15) and other chemo-attractant proteins (MCP-2, GCSF). We found that in patients with leptospirosis, there was an elevation of both anti-inflammatory and pro-inflammatory cytokines. This broad activation of pro and anti-inflammatory cytokines generally termed as “cytokine storm” more commonly seen in patients with sepsis has been described in patients with severe leptospirosis (Reis et al., 2013).

Although there was a generalized elevation cytokine in these patients, we noticed pikes in G-CSF, IL-15 and MCP-2 levels in patients with a triad of jaundice, oliguria and hematemesis along with fever. Elevated levels of G-CSF, IL-15 and MCP-2 levels in patients with a triad of jaundice, oliguria and hematemesis signals their role in disease progression. In patients with peak GSCF levels neutrophil count was also noted to be elevated. High neutrophil count in these patients may be since G-CSF promotes proliferation of neutrophil precursors and the release of neutrophils into the circulation from the bone marrow (Kaushansky, 2006). It is a growth factor for the proliferation, differentiation, effector function and survival of neutrophils.

MCPs are produced by a variety of cells on stimulation with cytokines (interleukin-1, tumor necrosis factor-alpha, interferon-gamma), bacterial and viral products or mitogens. MCP-1 levels are enhanced during infection and inflammation, which are characterized by leukocyte infiltration. In vitro, MCPs are chemotactic for a distinct spectrum of target cells and show different specific biological activities depending on the cell type and the chemokine tested. MCP-3 has the broadest range in that it activates monocytes, dendritic cells, lymphocytes, natural killer cells, eosinophils, basophils, and neutrophils. The most sensitive cells to all three MCPs are lymphocytes and monocytes. MCP-1 is a potent basophil activator but does not attract eosinophils, whereas at higher concentrations, MCP-2 stimulates both eosinophils and basophils. A study on mice showed increased expression of MCP-1 in severe leptospirosis (da Silva et al., 2009). However, the role of MCP-2 in such patients is not well studied. Elevated levels of MCP-2 along with G-CSF and IL-15 were noted in patients with severe leptospirosis manifestations. Correlation was also noted between MCP-2 and G-CSF levels in patients with leptospirosis (rho = 0.415, $p < 0.01$). MCP-2 is a relatively unexplored cytokine which needs to be studied in more detail, particularly its correlation with MCP-2 as a predictor of leptospirosis and its prognosis.

Along with GCSF and MCP-2, IL-15 levels were significantly elevated in patients than in controls ($p < 0.001$).

biological functions in many diverse cell types. It plays a major role in the development of inflammatory and protective immune responses to microbial invaders and parasites by modulating immune cells of both the innate and adaptive immune systems (Perera et al., 2012). A special feature of IL-15 is that it shares important functional attributes with IL-2, including enhanced proliferation, survival and differentiation of many distinct cell types as NK, T and B cells (Armitage et al., 1995; Budagian et al., 2006). Several studies suggest that IL-15 is involved in the immunological control of infections with a variety of bacterial, viral and fungal agents (Takano et al., 1998; Winn et al., 2003). Exact role of IL-15 in leptospirosis is yet to be studied in detail.

IL-4 levels were raised in patients, but it was not significant. IL-2 is a known pro-inflammatory cytokine and acts as the major growth factor for T lymphocytes, and the binding of IL-2 to its specific receptors on TH cells stimulates the proliferation of these cells and the release of a number of cytokines from these cells (M. M. Khan, 2016) In the present study, a decrease in the level of IL-2 was observed in patients with leptospirosis (p = NS). In an animal study, a low-level basal expression of IL-2 was detected in uninfected hamsters but no increase was noted in response to infection with L interrogans (Vernel-Pauillac & Merien, 2006). Studies have shown that T-cell suppression is one of the important factors of infection (Sciuca et al., 2013). Treg-mediated IL-2 deprivation is a reason of T-cell suppression during infection (Jenabian et al., 2013). Therefore, IL-2 often decreases when infection occurs (Ye et al., 2014).

Conclusion

The present study provides an understanding of cytokine pattern in leptospirosis. Accordingly, G-CSF, IL-15 and MCP-2 may be used as indicators of disease progression. However, this study cannot predict that the elevated cytokine patterns in leptospirosis reflect a mechanistic role as mediators of pathogenesis or if they are only markers of disease progression. Further studies are needed to expound the role of cytokines in the immune-pathogenesis of leptospirosis that could form the basis for immune-therapeutic models for treating the disease.

Credit Author Statement

Fatima Khan: Conceptualization, Methodology, Writing- Original draft preparation, Supervision, experimental analysis, data handling and statistical analysis

Md Mahtab: Data curation, Investigation, experimental analysis, data handling and statistical analysis

Shariq Ahmed: Supervision, data handling, Writing- Reviewing and Editing

Asfia Sultan: Supervision, experimental analysis, Writing- Reviewing and Editing

Mohd Azam: Data curation, Investigation, experimental analysis

Meher Rizvi: Conceptualization, Methodology

Raafiah Izhar: Methodology

All authors critically revised the manuscript for important intellectual content and read and approved the final manuscript.

Sources of support in the terms of grant: DST-SERB/LS-281/2013 - Young Scientist Scheme, India

Ethical approval: The study was approved by the Institutional Ethics Committee, Faculty of Medicine, Aligarh Muslim University, Aligarh.

Conflicts of interest: There are no conflicts of interest.

Acknowledgements

Dr. Vijayachari P, Dr. Vimal Raj and Mr. Vinod, Regional Centre for Leptospirosis, ICMR, Port Blair, for providing training and other technical support on Leptospirosis..

Prof. S H Arif, Blood Bank In-Charge, JNMCH, AMU for providing samples for controls.

Dr. Mohd. Maroof, Department of Community Medicine, for helping in statistical analysis.

References

Adler, B., & de la Peña Moctezuma, A. (2010). Leptospira and leptospirosis. *Veterinary Microbiology*, 140(3), 287–296. https://doi.org/10.1016/j.vetmic.2009.03.012

Armitage, R. J., Macduff, B. M., Eisenman, J., Paxton, R., & Grabstein, K. H. (1995). IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation. *Journal of Immunology* (Baltimore, Md.: 1950), 154(2), 483–490.

Barbosa, A. S., Abreu, P. A. E., Vasconcellos, S. A., Morais, Z. M., Gonçales, A. P., Silva, A. S., Daha, M. R., & Isaac, L. (2009). Immune Evasion of Leptospira Species by Acquisition of Human Complement Regulator C4BP. *Infection and Immunity*, 77(3), 1137–1143. https://doi.org/10.1128/IAI.01310-08

Budagian, V., Bulanova, E., Paus, R., & Bulfone-Paus, S. (2006). IL-15/IL-15 receptor biology: A guided tour through an expanding universe. *Cytokine & Growth Factor Reviews*, 17(4), 259–280. https://doi.org/10.1016/j.cytogfr.2006.05.001

Chirathaworn, C., & Kongpan, S. (2014). Immune responses to Leptospira infection: Roles as biomarkers for disease severity. *The Brazilian Journal of Infectious Diseases: An Official Publication of the Brazilian Society of Infectious Diseases*, 18(1), 77–81. https://doi.org/10.1016/j.bjid.2013.08.002

da Silva, J. B., Ramos, T. M. V., de Franco, M., Paiva, D., Ho, P. L., Martins, E. A. L., & Pereira, M. M. (2009). Chemokines expression during Leptospira interrogans serovar Copenhageni infection in resistant BALB/c and susceptible C3H/HeJ mice. *Microbial Pathogenesis*, 47(2), 87–93. https://doi.org/10.1016/j.micpath.2009.05.002

Dorigatti, F., Brunialti, M. K. C., Romero, E. C., Kallas, E. G., & Salomão, R. (2005). Leptospira interrogans activation of peripheral blood monocyte glycolipoprotein demonstrated in whole blood by the release of IL-6. *Brazilian Journal of Medical and Biological Research = Revista Brasileira De Pesquisas Medicas E Biologicas*, 38(6), 909–914. https://doi.org/10.1590/s0100-879x2005000600013

Evangelista, K. V., & Coburn, J. (2010). Leptospira as an emerging pathogen: A review of its biology, pathogenesis, and host immune responses. *Future Microbiology*, 5(9), 1413–1425. https://doi.org/10.2217/fmb.10.102

Gouveia, E. L., Metcalfe, J., de Carvalho, A. L. F., Aires, T. S. F., Villasboas-Bisneto, J. C., Queirroz, A., Santos, A. C., Salgado, K., Reis, M. G., & Ko, A. I. (2008). Leptospirosis-associated Severe Pulmonary Hemorrhagic Syndrome, Salvador, Brazil. *Emerging Infectious Diseases*, 14(3), 505–508. https://doi.org/10.3201/eid1403.071064

Gravekamp, C., Van de Kemp, H., Franzen, M., Carrington, D., Schoone, G. J., Van Eys, G. J., Everard, C. O., Hartskeerl, R. A., & Terpstra, W. J. (1993). Detection of seven species of pathogenic leptospires by PCR using two sets of primers. *Journal of General Microbiology*, 139(8), 1691–1700. https://doi.org/10.1099/00221287-139-8-1691

Jenabian, M.-A., Seddiki, N., Yatim, A., Carriere, M., Hulin, A., Younas, M., Ghadimi, E., Kök, A., Routy, J.-P., Tremblay, A., Sévigny, J., Lelievre, J.-D., & Levy, Y. (2013). Regulatory T cells negatively affect IL-2 production of effector T cells through CD39/adenosine pathway in HIV infection. *PLoS Pathogens*, 9(4), e1003319. https://doi.org/10.1371/journal.ppat.1003319

Kaushansky, K. (2006). Lineage-specific hematopoietic growth factors. The New England *Journal of Medicine,* 354(19), 2034–2045. https://doi.org/10.1056/NEJMra052706

Khan, F., Mahtab, Ahmad, N., Shukla, I., Rizvi, M., Azam, M., Sultan, A., Quaiser, S., Resident, & Scholar. (2016). Rapid Diagnosis of Leptospirosis by IgM ELISA in Resource Poor Settings. P. *Undefined*. https://www.semanticscholar.org/paper/Rapid-Diagnosis-of-Leptospirosis-by-IgM-ELISA-in-P.-Khan-Mahtab/5d4329e928f4695e50906ab95ca83b4480c1fd22

Khan, M. M. (2016). *Immunopharmacology*. Springer International Publishing. https://doi.org/10.1007/978-3-319-30273-7

Levett, P. N. (2001). Leptospirosis. *Clinical Microbiology Reviews*, 14(2), 296–326. https://doi.org/10.1128/CMR.14.2.296-326.2001

Martínez García, M. A., de Diego Damiá, A., Menéndez Villanueva, R., & López Hontagas, J. L. (2000). Pulmonary Involvement in Leptospirosis. *European Journal of Clinical Microbiology and Infectious Diseases*, 19(6), 471–474. https://doi.org/10.1007/s100960000294

McBride, A. J. A., Athanazio, D. A., Reis, M. G., & Ko, A. I. (2005). Leptospirosis. *Current Opinion in Infectious Diseases*, 18(5), 376–386. https://doi.org/10.1097/01.qco.0000178824.05715.2c

Meri, T., Murgia, R., Stefanel, P., Meri, S., & Cinco, M. (2005). Regulation of complement activation at the C3-level by serum resistant leptospires. *Microbial Pathogenesis*, 39(4), 139–147. https://doi.org/10.1016/j.micpath.2005.07.003

Perera, P.-Y., Lichy, J. H., Waldmann, T. A., & Perera, L. P. (2012). The role of interleukin-15 in inflammation and immune responses to infection: Implications for its therapeutic use. *Microbes and Infection*, 14(3), 247–261. https://doi.org/10.1016/j.micinf.2011.10.006

Reis, E. A. G., Hagan, J. E., Ribeiro, G. S., Teixeira-Carvalho, A., Martins-Filho, O. A., Montgomery, R. R., Shaw, A. C., Ko, A. I., & Reis, M. G. (2013). Cytokine Response Signatures in Disease Progression and Development of Severe Clinical Outcomes for Leptospirosis. *PLOS Neglected Tropical Diseases*, 7(9), e2457. https://doi.org/10.1371/journal.pntd.0002457

Rizvi, M., Azam, M., Ajmal, M. R., Shukla, I., & Malik, A. (2011). Prevalence of leptospira in acute hepatitis syndrome and assessment of IL-8 and TNF-alpha level in leptospiral hepatitis. *Annals of Tropical Medicine and Parasitology*, 105(7), 499–506. https://doi.org/10.1179/136485941 1Y.0000000041

Rizvi, M., Azam, M., Sultan, A., Khan, F., Shukla, I., Malik, A., & Ajmal, M. (2014). Role of IL-8, IL-10 and TNF-α levels in pathogenesis of Leptospiral acute hepatitis syndrome. *Annals of Pathology and Laboratory Medicine*, 1, A10–A17.

Sciuca, S., Neamtu, L., & Mangalu, V. (2013, June 24). Evaluation of levels of IL-2 in *Mycoplasma pneumoniae infection in children with respiratory tract deseases.*

Tajiki, M. H., & Salomão, R. (1996). Association of Plasma Levels of Tumor Necrosis Factor α with Severity of Disease and Mortality Among Patients with Leptospirosis. *Clinical Infectious Diseases,* 23(5), 1177–1178. https://doi.org/10.1093/clinids/23.5.1177

Takano, M., Nishimura, H., Kimura, Y., Mokuno, Y., Washizu, J., Itohara, S., Nimura, Y., & Yoshikai, Y. (1998). Protective Roles of γδ T Cells and Interleukin-15 in Escherichia coli Infection in Mice. *Infection and Immunity,* 66(7), 3270–3278.

Vernel-Pauillac, F., & Merien, F. (2006). Proinflammatory and immunomodulatory cytokine mRNA time course profiles in hamsters infected with a virulent variant of Leptospira interrogans. *Infection and Immunity*, 74(7), 4172–4179. https://doi.org/10.1128/IAI.00447-06

Vijayachari, P., Sugunan, A. P., & Shriram, A. N. (2008). Leptospirosis: An emerging global public health problem. *Journal of Biosciences*, 33(4), 557–569. https://doi.org/10.1007/s12038-008-0074-z

Wagenaar, J. F. P., Gasem, M. H., Goris, M. G. A., Leeflang, M., Hartskeerl, R. A., van der Poll, T., van 't Veer, C., & van Gorp, E. C. M. (2009). Soluble ST2 levels are associated with bleeding in patients with severe Leptospirosis. *PLoS Neglected Tropical Diseases*, 3(6), e453. https://doi.org/10.1371/journal.pntd.0000453

Wang, H., Wu, Y., Ojcius, D. M., Yang, X. F., Zhang, C., Ding, S., Lin, X., & Yan, J. (2012). Leptospiral hemolysins induce proinflammatory cytokines through Toll-like receptor 2-and 4-mediated JNK and NF-κB signaling pathways. *PloS One*, 7(8), e42266. https://doi.org/10.1371/journal.pone.0042266

Wagenaar, J. F. P., Gasem, M. H., Goris, M. G. A., Leeflang, M., Hartskeerl, R. A., van der Poll, T., van 't Veer, C., & van Gorp, E. C. M. (2009). Soluble ST2 levels are associated with bleeding in patients with severe Leptospirosis. PLoS Neglected Tropical Diseases, 3(6), e453. https://doi.org/10.1371/journal.pntd.0000453

Winn, R. M., Gil-Lamaignere, C., Roilides, E., Simitsopoulou, M., Lyman, C. A., Maloukou, A., & Walsh, T. J. (2003). Selective Effects of Interleukin (IL)–15 on Antifungal Activity and IL-8 Release by Polymorphonuclear Leukocytes in Response to Hyphae of Aspergillus Species. *The Journal of Infectious Diseases,* 188(4), 585–590. https://doi.org/10.1086/377099

Ye, Q., Shao, W.-X., Xu, X.-J., & Yang, Y. (2014). The Clinical Application Value of Cytokines in Treating Infectious Diseases. *PLOS ONE*, 9(6), e98745. https://doi.org/10.1371/journal.pone.0098745

Zuerner, R. L. (2015). Host Response to Leptospira Infection. In B. Adler (Ed.), *Leptospira and Leptospirosis* (pp. 223–250). Springer. https://doi.org/10.1007/978-3-662-45059-8_9

تقييم مستويات السيتوكين في داء البريميات البشرية كمؤشر تنبؤي

خان فاطمة [1*] ، **مهتاب** [2] ، **أحمد شريق** [3] ، **سلطان أصفيا** [4] ، **عزام محمد** [4، 5] ، **رضوي مهير** [4، 6] ، **رافيه إزهار** [7]

[1] قسم الأحياء الدقيقة ، كلية ومستشفى JN الطبية ، AMU ، عليكرة ، الهند.
[2] قسم الأحياء الدقيقة ، كلية ومستشفى JN الطبية ، AMU ، عليكرة ، الهند.
[3] قسم الأحياء الدقيقة ، كلية ومستشفى JN الطبية ، AMU ، عليكرة ، الهند.
[4] قسم الأحياء الدقيقة ، كلية ومستشفى JN الطبية ، AMU ، عليكرة ، الهند.
[5] كلية العلوم الطبية التطبيقية، جامعة القصيم ، بريدة ، المملكة العربية السعودية.
[6] كلية الطب والعلوم الصحية ، جامعة السلطان قابوس ، مسقط ، سلطنة عمان.
[7] قسم العلوم الحيوية ، كلية العلوم الطبيعية ، جامعة الملة الإسلامية ، نيودلهي.

* البريد الإلكتروني: ifatimasalmanshah@gmail.com

المُستَخلَص

تاريخ استلام البحث: 2021/12/17
تاريخ تعديل البحث: 2022/06/16
تاريخ قبول البحث: 2022/06/28

الهدف: داء اللولبية النحيفة هو مرض حيواني استوائي المنشأ ، حيث تم إثبات دور الاستجابة المناعية في التسبب في المرض، ولكن لم يتم فهمه بشكل جيد. يقال إن استجابة السيتوكينات تلعب دورًا رئيسيًا في تطور المرض والتسبب فيه. هناك دراسات مثبتة على السيتوكينات المؤيدة للالتهابات مثل TNFα و IL-6 ومضادات الالتهاب مثل IL-10 في داء البريميات البشرية ، ولكن دور IL-2 و IL-4 و IL-15 و GCSF و MCP-2 يحتاج إلى المزيد من الدراسات الشاملة. أجريت الدراسة الحالية لتقييم دور IL-2 و IL-4 و IL-15 و GCSF و MCP-2 في داء البريميات البشرية كمؤشر تنبؤي.

منهجية الدراسة: تم تضمين عينات الدم من المرضى الذين يستوفون معايير إدراج داء البريميات في الدراسة. ثم تم إجراء PCR و IgM ELISA للتشخيص. تم تقدير مستويات السيتوكينات في الدم في المرضى الموجودين في Leptospira وفي الضوابط بواسطة ELISA. كما أجرينا التحليل الإحصائي باستخدام برمجيات IBM SPSS Statistics 20 و Med Calc 16.1.

النتائج: من أصل 270 ، تم تأكيد 45 (16.7٪) من المرضى على أنهم حالات من داء البريميات. اختلف متوسط مستوى السيتوكينات (G-CSF ، MCP-2 ، IL-15) اختلافًا كبيرًا بين المرضى والمجموعة الضابطة ($p < 0.001$). تم رفع GCSF و MCP-2 و IL-15 و IL-4 في معظم الحالات. انخفض مستوى IL-2 في 34 حالة من أصل 45 حالة. كانت AUCs لـ IL-2 و IL-15 و MCP-2 و GCSF 0.906 (95٪ CI 0.341 إلى 0.665) و 0.929 (95٪ CI 0.837 إلى 0.978) و 0.909 (95٪ CI 0.812-0.966) و 0.881 (95) ٪ CI 0.777 حتى 0.948) على التوالي. على ارتباط رتبة سبيرمان ،، أظهر مستوى GCSF ارتباطًا مع MCP-2 ($rho = 0.415, p < 0.01$).

الاستنتاج: قدمت الدراسة فهمًا لأنماط السيتوكين في داء البريميات ، وتوصلت إلى أنه يمكن استخدام IL-15 و MCP-2 و GCSF كمؤشر حيوي فعال لداء البريميات ومؤشرات لتطور المرض.

الكلمات المفتاحية: المؤشرات الحيوية. السيتوكينات ، اللبتوسبيرا.