INTRODUCTION

Leptospirosis is an acute bacterial disease caused by the genus Leptospira. Members of the Leptospira are grouped into serovars on the basis of their antigenic relatedness where more than 260 serovars have been recognized. The Leptospira cytoplasmic membrane is closely associated with a peptidoglycan cell-wall, which is overlaid by an outer-membrane, phospholipid, outer-membrane proteins (OMPs). Several Leptospiral OMPs have been shown to attach the host extracellular matrix which are responsible for adhesion of Leptospira to host tissue.

This study has been undertaken to find the immune reaction of the OMPs to the patient's sera of Leptospirosis by estimating the cytokine profile and antibody levels.

MATERIALS AND METHODS

A total of 120 blood samples were collected from patients (90 male/30 female) with febrile illness and fulfilling the criteria of clinical diagnosis of Leptospirosis. All the 120 samples & 40 healthy controls were subjected to ELISA (IgM serion), MAT and Leptospira interrogans serovar tarassovi IgM ELISA and also to detect TNFα and IL-6 levels using (BD Opt EIA Human IL-6 & TNFα ELISA kits).

RESULTS: Sera which were found to be reactive in L. interrogans serovar tarassovi IgM ELISA and also to detect TNFα and IL-6 levels using (BD Opt EIA Human IL-6 & TNFα ELISA kits).

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CONCLUSIONS: Patients from Group I had significant levels of TNFα in the serum i.e., p = 0.02 which indicates the pro-inflammatory Th1 response as TNFα are macrophage derived cytokine mediators. The IL-6 level of Group I, II, III were significantly higher than the Group IV (p = 0.02) suggesting that Th2 anti-inflammatory response seen in majority of these cases which has a protective role in leptospirosis.

Key words: OMPs, Leptospira, TNFα and IL-6
groups in microscopic agglutination test

• Criteria for presumptive diagnosis: (Group II) A suspected case of Leptospirosis positive by MAT (1:100)
• Clinically suspected case of leptospirosis but serologically negative (ELISA, MAT and Dridot) (Group - III)
• Healthy Controls: (Group IV).

IgM ELISA: Performed according to the manufacturer’s instructions. Evaluation of the test was done by serion ELISA classic Leptospira (Institute virion, serion GmbH, Würzburg, Germany).

Microscopic agglutination test (MAT): Performed using Faine’s methodology1 according to WHO guidelines.

L. tarassovi OMP IgM ELISA: Performed according to the method of Premlatha et al.2

Estimation of IL-6 and TNFα: Using (BD Opt EIA™ HUMAN IL-6, TNFα ELISA kits) was performed according to manufacturer instructions.

RESULTS

The level of TNFα and IL-6 were estimated from the four different Groups each having 40 samples. The patients from Group I had significant levels of TNFα in the serum (i.e., p=0.02). The mean of IL-6 level of Group I and Group II were significantly higher than the Group III. Sera which were found to be reactive in L.tarassovi OMP IgM ELISA, had showed significant levels of TNFα and IL-6 and had antibody titre in IgM ELISA and MAT.

DISCUSSION

Pathogenic Leptospira causes Intercellular infection. The immunological reaction of host to intercellular infection is unique. The potential immunogen of expressed protein should be degraded by antigen presenting cells in the hosts, and should have antigenic epitope which recognizes MHC II protein and T-cell receptor.

A total of 120 serum samples and 40 healthy controls were subjected to L. tarassovi OMP IgM ELISA, IgM ELISA and MAT, and estimation of IL-6 and TNFα was done, using (BD Opt EIA™ HUMAN IL-6, TNFα ELISA kits).

In this study the patients from Group I had significant levels of TNFα in their serum (i.e., p=0.02) which indicates pro-inflammatory Th1 response as TNFα are macrophage derived cytokine mediators. Most of these patients had high morbidity. In a study Tajiki and Salomao circulating level of TNFα has been detected in patients with Leptospirosis which associated with severity of the disease.3

The mean of IL-6 level of Group I and Group II were significantly higher than the Group VI p=0.02 suggesting that Th2 anti inflammatory response seen in majority of these cases.

Studies by Doğgatt et al4 has shown that OMP of pathogenic Leptospira when inoculated to tubular cells in culture activated nuclear NF Kappa B binding and stimulated downstream inducible nitric oxide (INOS), monocyte chemoattractant protein-1 (MOP-1) and tumor necrosis factor. Another study reported by Magrinho et al5 detected TNFα and IL-6 in rat renal experimentally infected with L.canicola.

In this study sera which were found to be reactive with L. tarassovi OMP had significant levels of TNFα and IL-6 and also reacted positive in MAT and ELISA (Table 1). The detection of TNFα and IL-6 in the sera suggests that OMPs play an important role in the interaction of host cells with Leptospira during infection.

CONCLUSION

Leptospires enter the body through small cuts, via mucous membranes such as the conjunctiva or skin. They circulate in the blood stream, with the bacteremic phase lasting for up to 7 days The second stage of acute leptospirosis is immune phase, with the disappearance of the organism from the bloodstream and the appearance of antibodies .It was observed that the extracted OMP of L.tarassovi reacted to the patient sera to produce cytokines i.e TNF alpha and IL-6 and also produced antibodies during the course of Infection. Proteins play a major role in binding to various components of the extra cellular matrix and thereby mediate host pathogen interaction and the proteins which are expressed during infection play an important role in immuno-pathogenesis.

<table>
<thead>
<tr>
<th>L. tarassovi OMP IgM ELISA</th>
<th>TNFα</th>
<th>IL-6</th>
<th>ELISA (IgM)</th>
<th>MAT</th>
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<tbody>
<tr>
<td>Group I 29/40</td>
<td>27</td>
<td>24</td>
<td>20</td>
<td>27</td>
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<tr>
<td>Group II 19/40</td>
<td>9</td>
<td>12</td>
<td>10</td>
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<td>Group III 8/40</td>
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<td>Healthy control 40</td>
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Table 1: Profile of cytokine and antibody production during human Leptospirosis
REFERENCES


Authors Contribution:
IRK, RA, ABD, RC: concept. MMPL: concept and design, data collection and analysis, manuscript preparation, editing and review.

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