ABSTRACT
Leishmaniasis is a parasitic disease caused by different species of genus *Leishmania*, a unicellular kinetoplastid protozoan flagellate. It manifests mainly in three clinical forms; visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL), of which VL is the most severe form of the disease. The pathogenesis caused by *Leishmania* is associated with depressed cellular immunity and significant elevation of antileishmanial antibodies. The present study was aimed to know the prevalence of *Leishmania* spp. as well as elucidated the pathogenicity caused by *Leishmania donovani* and *Leishmania tropica* strains prevalent in Sutlej river valley, Rampur Bushahr and Kinnaur district of H.P. A total of 100 samples were collected from individual found positive for *Leishmania* spp. from different clinics and Hospital situated at Rampur town. 5ml blood was collected, and divided in to two aliquots. One aliquot was utilized for serum and plasma for immunological analysis. The second aliquot of blood was processed for parasite culture. Simultaneously skin scraping from CL patient was also taken in NNN medium. We observed promastigote forms in culture medium, which were found to be fast swimming, motile forms, and spindle shaped bodies with a long anterior flagellum. We have detected the antileishmanial IgG and its subclasses, IgG1, IgG2, IgG3 and IgG4 antibodies in serum samples by indirect ELISA method. The whole IgG antibodies was detect in sera of 42% individuals. Further these IgG positive samples were analyzed for IgG subclasses which revealed that the presence of IgG1, IgG3 and IgG4 in the serum samples. The highest frequency of IgG1 (38.1%) subclass was detected among the individuals followed by IgG3 26.2% and IgG4 9.52% respectively. However IgG2 subclass was not detected in any of the samples. We have observed the predominance of IgG1 and IgG3 subclasses while IgG4 was observed in low level. Our findings also determined the prevalence of high frequency IgG antibodies among young age group i.e. between 21-40 years old, suggested that this age group is more susceptible to *Leishmania* parasite infections and are on high risk. The flowcytometric analysis of cytokines by using Cytometric Bead Array (BD Biosciences) method to know the expression level of anti and pro-inflammatory cytokines i.e. IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α and IFN-γ. We found that, among all the pro-inflammatory cytokines, IL-2 cytokine showed highest level of expression followed by TNF-α, IFN-γ, IL-6 while IL-17A has shown minimum level of expression. IL-2 was expressed significantly very high in males similarly IL-6 and IFN-γ have also showed high expression level in males then females. While IL-17A cytokine expression was found to be lowest among all the cytokines.
The frequency of IL-10 anti-inflammatory cytokine was very high among the study population, while IL-4 could not detected or diluted in very low in any of the subject. IL-10 among an anti-inflammatory cytokines showed the high expression level. The expression level in males was observed significantly high in comparison to those of females. The PCR sequencing analysis of ITS-1 region was performed to know the species of strain prevalent in this region. The Bioinformatic analysis of sequences revealed that the species are belongs to *L. donovani* and *L. tropica*. Hence the study suggested the *L. donovani* and *L. tropica* have coexistence in this region. The presence of *L. donovani* in Sutlej river valley of Rampur, Himachal Pradesh may be migrated from other part of the country with the migrated population came for developmental projects on Sutlej valley. Cytokines analysis revealed that the parasites are more virulent as pro-inflammatory cytokine was found significantly more suppressed which help in progression of disease expression.

**KEY WORDS:** Leishmaniasis, Visceral leishmaniasis, Cutaneous leishmanisis, ELISA, IgG. Cytokines, ITS-1.