Abstract

Leishmaniasis is a tropical disease caused by an intracellular, protozoan parasite from over 20 *Leishmania* species. Clinical manifestations range from a life-threatening systemic infection (visceral, VL) to self-limiting or chronic skin sores (cutaneous, CL), or dreaded metastatic complications that can cause facial disfigurement (mucosal, MCL). After recovery, VL patients may present with a secondary form called Post Kala-azar Dermal leishmaniasis (PKDL). Current diagnostic methods for PKDL include parasite detection (stained smears, culture or histopathology) but are invasive and have poor sensitivity, while immunological methods (Direct Agglutination Test, enzyme-linked immunosorbent assay, rK39 strip test etc.) have limited specificity, fail to distinguish between past and present infections. A proper diagnostic and prognostic method for PKDL as well as for VL is urgently required.

All patients suspected to be suffering from VL/PKDL (n = 72) were diagnosed by PCR, rK39 strip test and ELISA. A PCR assay was standardized being specific for the Internal transcribed spacer 1 (ITS1) region of *Leishmania* sp.; the specificity and sensitivity of PCR was 100% in both VL and PKDL cases. rK39 strip test failed to diagnose 1 patient with VL and 2 patients with PKDL, but none were immunocompromised. The rK39 strip test is undoubtedly useful in a field setting, but if the rK39 is negative, yet clinical suspicion is high, one may consider performing a DNA-based test.

Parasite species typing was performed by amplifying various regions of *Leishmania* genome (ribosomal ITS1 and hsp70) followed by *Hae*III digestion. In 4/60 patients (VL and PKDL) and 2/10 isolates, an unusual RFLP band pattern was evident. Sequencing of the ITS1-PCR product and ClustalW alignment showed several mismatches and deletions (score, 73), indicating significant differences. Furthermore, two sequences were BLAST searched independently, where the aberrant sequence showed a strong match with *Leptomonas seymouri* (accession no. EU623433.1), received GenBank accession no. JN848802.

Along with diagnosis, parasite load was also measured in patients with VL and PKDL by amplifying a small region of kDNA by qPCR. Since VL is a systemic disease and PKDL a dermal variant, parasite load was quantified in blood and dermal skin biopsies of patients with VL and PKDL respectively. Among the clinical parameters studied, in both diseases, only disease duration was associated with parasite load. Among immunological markers, in both diseases no correlation was found between humoral markers and parasite load. However, during VL, to a large extent, it represented the cell mediated immune response in terms of chemokines and cytokines.

Since, immune response and disease severity varied among individuals and did not proportionally change with parasite load, some host specific genetic factors might play a role in disease pathogenesis. Therefore, a polymorphism analysis was undertaken in VL/PKDL patients, wherein a Gly-Ile haplotype of TLR4 gene was present in a higher proportion, while the Gly-Thr haplotype was absent in Indian Leishmaniasis. This was in contrast to the African population (according to HapMap), suggesting that further studies are needed to understand the importance of these haplotypes, in view of the diverse disease manifestations of PKDL observed in India vs. Sudan.

In a 30 days post-infection hamster model of VL, the spleen was primarily affected as compared to the liver. The levels of Th1 cytokine IFNγ was significantly increased in infected animals whereas Th2 cytokines IL-10 and TGFβ were unaltered. An increased expression of arginase1, mannose receptor, CC chemokine ligand 17 (CCL17), CCL22, and chemokine receptor CCR2 by real time RT-PCR suggested a phenotype of alternatively activated macrophages. Additionally, correlation of CCL17 and CCL22 with parasite load in infected spleen suggests the involvement of alternatively activated macrophages in parasite survival and disease persistence.