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Title of the Thesis: Molecular Characterization and Genomics Study of the Clinical Isolates of *Leishmania sp.* From Indian Kala-azar and Para-KDL Patients.

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Visceral leishmaniasis (VL) or Kala-azar (KA) is a neglected tropical parasitic disease caused by *Leishmania donovani*, though recent reports confirmed the association of *L. tropica* with the disease. About 10%-20% cases of apparently cured VL patients may develop Post Kala-azar Dermal Leishmaniasis (PKDL). Currently, the sustained presence of active VL in PKDL patients is observed where co-association of PKDL manifestation in active VL is recorded. These cases are referred to as Para Kala-azar Dermal Leishmaniasis (para-KDL). In the Indian subcontinent, 70% of cases reported being unresponsive to the clinically available first-line drug Sodium stibogluconate (SSG). Thus, the emergence of drug-resistant parasites towards the applicable drugs is an alarming signal for future VL elimination. Prior to opt for any drug regimen, the taxonomical identification and detailed characterization of the parasite is a prerequisite for epidemic surveillance and eradication of any disease. The present study has been carried out to examine the structural and functional genomic attributes of the recently collected clinical isolates (n=15) from Indian KA and para-KDL patients. Different PCR based methods and computational tools are used to analyse the parasite genome with restriction analysis of the amplified ITS and hsp70 locus (RFLP), Multilocus Sequence Typing (MLST) of some enzyme coding genes, genome-wide comparison by Whole Genome Sequencing (WGS) and *in silico* structural analysis of some novel mutated genes. The introduction of ten new RFLP markers suggests that only six markers have restriction sites in the ITS amplicon. Among them, Fsp I and Mse I markers unambiguously differentiated *L. donovani* from *L. tropica*. While RFLP patterns of the hsp70 and ITS1 region have confirmed that only one isolate (T5) was *L. tropica*, which is collected from a confirmed KA patient corroborating our earlier observation. To understand the current phylodynamics of the *Leishmania* parasite, a potential molecular approach, namely MLST, has been carried out with fifteen housekeeping gene loci in the genus *Leishmania* at the interspecies and intraspecies level. The results indicate that the genus *Leishmania* displays high nucleotide diversity among the sequenced housekeeping genes coding regions. Notably, only two housekeeping gene loci *enol* and *alat*, were seen amplified in the Indian clinical isolate of *L. tropica*, T5. For the locus *alat*, a unique sequence type ST3 has been found in the T5 isolate. This is reported here for the first time. Neighbour Joining (NJ) phylogeny analysis of the concatenated sequences of *aco, alat, enol, pgm, spdsyn* and *hgprt* genes for 11 Indian *L. donovani* isolates and 24 retrieved sequences of other *Leishmania* species from GenBank could differentiate *Leishmania* species complexes and subgenus level with high bootstrap support. In contrast, rooted phylogenetic analysis of individual locus isocitrate dehydrogenase (*icd*) sequence for 38 isolates could demonstrate the sub-continental origin of the *Leishmania donovani* complex.

Interestingly, the intraspecies phylogenetic analysis based on the concatenated sequence of seven housekeeping genes loci (*aco, alat, asat, enol, gpi, nh1, pgm*) form four distinct clusters in the Indian clinical isolates of *L. donovani* studied here. It is the first instance where intraspecies allelic profile analysis has been taken into account to observe any specific sequence type among the Indian *L. donovani* population. One gene, *spermidine synthase* (*spdsyn*), was conserved among all the Indian clinical isolates of *L. donovani* collected for the study.

The other part of the study dealt with the identification and comparison of genome-wide variation by whole genome sequencing of the SSG-sensitive VL and para-KDL, SSG resistant and MIL resistant Indian clinical isolates of *Leishmania sp.* Chromosome copy numbers were estimated using normalized whole chromosome median read depths along with the analyses of non-synonymous SNVs and InDels to identify the possible association of aneuploidy and the contribution of genome-wide mutation profile of the protein coding genes in the development of PKDL and the drug resistance in the Indian VL patients. In the para-KDL isolates, a unique set of 13 genes and in MIL resistant VL isolate (T9), 56 gene mutations homologous to genes with previously known cellular functions have been reported. In the next part, we highlighted the *in silico* structural analyses of the five novel mutated genes (out of 13) in the para-KDL isolates to examine the role of observed mutations in the protein secondary structure, whose functions have previously been observed as a surface protein and transporter. The structural analyses of the observed single nucleotide polymorphisms that lead to a frameshift mutation in the transmembrane domain of encoded surface proteins, including neutral sphingomyelinase activation associated factor-like protein, beta galactofuranosyl transferase and transporters like calcium-translocating P-type ATPase and amino acid transporter aATP11(putative) in the SSG sensitive para-KDL isolates confer the major secondary structure changes in their mutated position. Only one protein, p-glycoprotein e (partial) acting as a surface and a transporter protein showed transversion mutation. The observed mutation present in the ABC transporter transmembrane type-1 domain leads to the replacement of the coil at positions 1238 and 1239 with a helix in the ABC transporter 3rd domain. The study revealed a strong correlation between the development of drug resistance and the clinical manifestation of para-KDL, with special emphasis on aneuploidy and existing mutations.

Overall, this clinical isolates-based study is very useful to enhance the epidemiological surveillance of the country and provides detailed information for para-KDL for the first time.

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