

Assessment of the varieties between hybrid and non-hybrid *Leishmania* strains isolated from cutaneous leishmaniasis patients in Turkey using genotypic and proteomic methods and mouse model

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OBJECTIVES: Leishmaniasis is a significant vector-borne infection in Turkey, where almost 15,000 cutaneous leishmaniasis (CL) and only 207 visceral leishmaniasis (VL) cases have been reported between 2005 and 2012. Application of molecular methods in epidemiologic studies of *Leishmania* spp. unveiled the unique genomic and proteomic composition of parasites, as well. Exchange of genetic material has long been known within *Leishmania* species that may create hybrid strains in nature. Initially, we reported the first hybrid *Leishmania* strains in Turkey using Real Time PCR (RT-PCR), MALDI-TOF and isoenzyme analyses. Here, we report the proteomic differences between the hybrid and non-hybrid *Leishmania* strains, together with their genotypic and clinical manifestations on mice.

METHODS: Twenty lesion samples of autochthonous CL cases, 10 from Sanliurfa and 10 from Hatay provinces were obtained. All isolates were stained with Giemsa and inoculated in NNN medium. Real time PCR that targeted ITS-1 region of *Leishmania* spp. was applied to all samples, followed by sequence analysis. Promastigotes obtained from the culture were used for three purposes: Inoculation in Balb-C mice; MALDI-TOF assessment and two-dimensional electrophoresis of proteins. Reference strains of four *Leishmania* species (*L. tropica*, *L. infantum*, *L. donovani* and *L. major*) were used for the comparisons of all results.

RESULTS: All isolates from Sanliurfa were found to be *L. tropica* with RT-PCR and MALDI-TOF; their proteomic compositions were concordant with *L. tropica*, while they caused only cutaneous lesions on mice. Isolates from Hatay showed distinct variations; one sample was found to be *L. major* while one other was found to be *L. tropica* which acted as the samples from Sanliurfa. Four samples were found to be *L. tropica*, but with 6 different proteins compared to the reference strain. The remaining four samples showed two peaks in RT-PCR that were concordant with both *L. infantum* and *L. tropica*. Sequence analyses indicated them as *L. infantum*. Comparison of the proteomic content of these samples with the reference strain showed that 7 different proteins were present in hybrid strains. The Hatay isolates with different proteins showed not only dermal but also visceral tropism on mice.

CONCLUSION: Genetic and proteomic varieties within *Leishmania* species in Turkey are significant. Despite the isolates from Hatay showed total similarity to the reference strain of *L. tropica*, Hatay isolates showed obvious varieties in terms of species, RT-PCR, MALDI-TOF and sequence analyses and clinical manifestations in vivo. Proteomic analyses showed distinct differences, which may cause emergence of hybridization or clinical cases irresponsive to regular treatments. Proteomics studies will no doubt continue to unveil the secrets of *Leishmania* species. [The study was supported by TUBITAK (Project No: 111S179). All samples are kept as cryopreserved in the Parasite Bank located in Celal Bayar University in Manisa, Turkey.]