Leishmaniasis in Turkey: first demonstration of the proteins identified only in hybrid Leishmania isolates in autochthonous cases of cutaneous leishmaniasis

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Background: Leishmania species have long been considered to reproduce asexually; however, there is now a huge pile of data that indicate the occurrence of genetic material exchange between Leishmania species, by which they could better adapt to changing environmental conditions. We previously reported the first confirmed hybrid Leishmania isolates from Turkey, which showed visceralization in laboratory mice after inoculation into footpads. Recent clinical reports of cutaneous leishmaniasis (CL) cases from different provinces of Anatolia that are culture-negative and got no benefit from antimonial therapy may show that hybrids are not uncommon. We aimed to assess the genetic and proteomic diversity of autochthonous Leishmania isolates from two groups of CL patients.

Material/methods: A total of 20 CL patients from two provinces, 10 from Sanliurfa (Group 1) where only L. tropica is isolated in CL cases, and Hatay (Group 2) where both L. tropica and L. infantum are isolated, were enrolled to the study. Giemsa-stained smears of lesions were initially prepared, followed by inoculation to NNN medium and enriched medium, which was specially designed for the culture of Leishmania species. A Real Time PCR protocol that targeted the ITS-1 region of Leishmania spp. was applied using both amastigotes and promastigotes, followed by DNA sequence analysis and isoenzyme analysis (for 1 sample each). Proteomic profiles of two groups were compared with MALDI-TOF and 2-dimensional electrophoresis (2DE). All isolates were inoculated into the right footpads of mice to assess their in vivo activities.
**Results:** All isolates in Group 1 were found as *L. tropica* with Real-Time PCR, and confirmed by sequence analyses and MALDI-TOF. They caused only cutaneous lesions in mice, just as two isolates from Group 2 which were shown to be *L. tropica* and *L. major*. Four of the 8 remaining isolates from Hatay showed two peaks in RT-PCR concordant with *L. tropica* and *L. infantum*, and confirmed as *L. infantum* with sequence analyses. Comparison of their proteomic profiles with the reference *L. infantum* strain using 2DE identified seven different proteins, after which they were named as *L. infantum/L. tropica* hybrids. Others were found as *L. tropica* but they had six different proteins compared to the reference *L. tropica* strain in 2DE, and named as *L. tropica/L. infantum* hybrids. All these eight isolates caused life-threatening visceralization in mice.

**Conclusions:** This is the first demonstrations of proteomic differences between the hybrid and non-hybrid isolates of *Leishmania spp.* from Turkish CL patients. These different proteins may be involved in significant biochemical pathways and associated with visceralization in mice. Further analyses are needed to unveil their roles in hybridization and pathogenesis of leishmaniasis in vivo. [This project was supported by TUBITAK (Project no: 111S179) and Parasite Bank of Celal Bayar University]