

Genetic polymorphism within the leishmania major in two hyper endemic areas in Iran

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Background and Aims: In this study restriction fragment length polymorphism (RFLP) analysis of amplified Internal Transcribed Spacer (ITS) in the ribosomal operon and primed Intergenic Polymorphic-Polymerase Chain Reaction (PIIP-PCR) was used to investigate the genetic variations among *L. major* isolates and correlate the findings with the clinical manifestations of ZCL in two hyperendemic areas of Iran, (Isfahan & Ahwaz).

Methods: The leishmania promastigotes were isolated with the use of NNN medium from skin lesions of 120 patients with typical and atypical lesions, then subcultured in RPMI-1640 medium supplemented with 15% fetal bovine serum. Identification was based on PIIP-PCR. PCR amplification was performed in volume 50 microliter with specific leishmania primer (2B). Amplification products were separated in a 1% agarose gel and visualized under ultraviolet light after staining with ethidium bromide. *Leishmania major* and *L. tropica* were used as positive control and distilled water instead of DNA was used as negative control.

Results: ITS-rDNA-RELP analysis revealed five and PIIP-PCR revealed nine polymorphic profiles. These different patterns of PIIP-PCR were classified as I, II, III, IV and V groups. The isolates group I and II subdivided in A, A1, A2 and B, B1, B2. Strain A1 was more in Isfahan, and B2 strain in Ahwaz.

Conclusions: The results of this research detect the genetic and clinical polymorphism of *L. major* and showed that strain A is more frequency than other strains. Although clinical manifestation of disease has been attributed both to differences in the host response and to genomic heterogeneity of the parasites, the results presented here are in favour of an important role of the genetic constitution of *L. major* in determining the clinical characteristics of ZCL. To consolidate these findings, additional *L. major* isolates from patients, reservoir host and vectors from different locations need to be collected to further investigation.

Keywords: *Leishmania major*; PIIP-PCR; ITS