ITS-rDNA Gene Sequencing, RFLP, and Phylogenetic Analysis Methods for Firmly Identification of Leishmania Parasites in Human, Vectors and Reservoirs of Leishmaniasis.

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Background & Objectives: Visceral Leishmaniasis, urban and rural Cutaneous Leishmaniasis are parasites born disease, with a great distribution all over the world, (66 countries in the old world and 22 in the new world) including Iran. Different types of Leishmania parasites are the agents, the vectors are Phlebotomus sandflies, the reservoirs are rodents and canine, and human is the accidental reservoir. Since firmly identification of Leishmania species is impossible by conventional methods, new molecular tools are needed to identify, cure and manage controlling process of the diseases.

Methods: Sampling was conducted from suspected lesions of patients, abdomen and thorax of sandflies, rodents' ears, and dogs and foxes' serum. DNA extraction, PCR and Nested PCR were done for amplifying ITS-rDNA gene. Positive samples were used in RFLP by BsuRI enzyme for species determination and were proved by sequencing, molecular and phylogenetic analyzes.

Results: *Leishmania infantum*, *L. tropica* and *L. major* parasites were found in sandflies, *L. infantum* and *L. tropica* were identified from foxes and dogs, and only *L. infantum* was found in human in Visceral Leishmaniasis endemic areas. We found *Leishmania turanica* and *L. gerbilli*, in sandflies, *L. major* and L. turanica in rodent reservoirs. And also *L. major* and *L. turanica* were detected for the first time in human from cutaneous Leishmaniasis endemic regions. More new haplotypes of *L. major* was found especially in sandflies.

Conclusion: Comparison between Leishmania sequences of our results and those registered in GenBank were done. *L. infantum, L .torpica, L. turanica* and *L. gerbilli*, were accurately detected and proved by sequencing ITS-rDNA and RFLP and phylogenic analysis, which was impossible with conventional Methods.

Keywords: ITS-rDNA Gene; Sequencing; RFLP; Phylogenetic Analysis; Leishmaniasis; Identification