

## Original Paper

# Identification of *Leishmania* using microscopic and molecular methods in suspected patients of Cutaneous Leishmaniasis by targeting ITS-rDNA gene, Golestan province, Iran (2009-10)

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## Abstract

**Background and Objective:** Zoonotic Cutaneous Leishmaniasis (ZCL) is a parasitic disease which caused by a protozoan belongs to the genus *Leishmania*. ZCL is of great public health importance in many countries and also in endemic parts of Iran. *Leishmania major* is the causative agent, *Phlebotomus papatasi* as the main vector and *Rhombomys opimus* is the most important reservoir of the disease. Species identification of *Leishmania* in a large scale of human samples is necessary to conduct a useful program for controlling the disease outspread. This study was done to identify the *Leishmania* using microscopic and molecular methods in suspected patients of Cutaneous Leishmaniasis by targeting ITS-rDNA gene, Golestan province, Iran.

**Materials and Methods:** 121 smears collected from suspected patients of ZCL, in Eastern region of Golestan province, Iran during 2009-10, stained and examined under a light microscope. DNA of parasites extracted directly from smears and ITS-rDNA gene amplified. Positive samples digested with BsuRI restriction enzyme, according to RFLP method and subsequently the parasite was identified. After sequencing the ITS-rDNA gene, Molecular software was applied for verification of RFLP results. The achieved results were definitely approved by this procedure.

**Results:** 113 out of 121 and 92 out of 121 samples detected as *Leishmania* positive using microscopic examination and molecular method respectively. All 92 molecular positive samples digested with BsuRI endonuclease and 90 individuals identified as *Leishmania major*. In order to final verification, 8 samples of *Leishmania major* sequenced and confirmed by molecular software analysis. Unfortunately, sequences of two samples which were not *Leishmania major* were not readable, and consequently, these could not be identified.

**Conclusion:** Comparison of obtained sequences of current study with Gene Bank sequences confirmed *L. major* in human from Northern Iran. Other species of *Leishmania* were not identified in this investigation but detection of two other samples, which were not *L. major*, could indicate the role of other *Leishmania* species causing infection in human in Eastern region of Golestan province, northern Iran. These findings should be considered to improve the disease control programs, which can be led to increase the rate of public health in Golestan province.

**Keywords:** *Leishmania major*, ITS-rDNA, clinical samples, Golestan, Iran

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