

Original Article

Identification of Cutaneous Leishmaniasis Agents by Nested Polymerase Chain Reaction (Nested-PCR) in Shush City, Khuzestan Province, Iran

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Abstract

Background: Leishmaniasis is a common parasitic disease world wide. *Leishmania tropica* and *L. major* are two common cause of cutaneous leishmaniasis in Iran. The aim of this study was determination of the cause of cutaneous leishmaniasis in Shush city, Khuzestan Province, Southwest Iran

Methods: One hundred samples were collected from patients at the age of 1-80 year with documented cutaneous leishmaniasis referred to the health centre and a private medical diagnostic laboratory at Shush City. DNA was extracted from slid samples by phenol- chloroform- Isoemil alcohol method, and subjected to Nested-PCR as template. k DNA of the parasites were amplified by CSB1XR and CSB2XF in the first round of PCR and 13Z and Li R primers for the second round. After PCR, electrophoresis of products was performed and 750bp band from *L. tropica* and 560bp band from *L. major* were detected.

Results: A total of 100 cases comprising 47 females and 53 males were studied. The highest infected age group was under 10 years with a rate of 42% and the lowest rate was 4% at the age group of above 40 years. The results of PCR electrophoresis indicated that 90(90%) cases were *L. major* and 10 (10%) *L. tropica*. The predominant species in this area was *L. major*.

Conclusion: It is concluded that Nested PCR is a reliable test for diagnosis and identification of *Leishmania* species and can apply in epidemiological investigations.

Key words: *Cutaneous leishmaniasis*, PCR, Iran

Introduction

Cutaneous leishmaniasis (CL), a zoonotic disease and a major public health problem in the world, is endemic in the Middle East, Brazil and Peru (1). Annually 1, 500, 000 cases of leishmaniasis are reported from all around the world including 1, 000, 000 cases of cutaneous and 500, 000 visceral leishmaniasis (2). In south of Iran both forms of urban and rural disease are found (3).

A variety of diagnostic methods have been developed for *Leishmania* identification including

isoenzym analysis, monoclonal antibody, DNA hybridization, Schizodeme analysis, and other molecular techniques (4). In molecular methods both kinetoplast DNA and chromosomal DNA can be used as template for identification of *Leishmania* species by PCR.

The kinetoplast, an organelle unique to the kinetoplastids, contains approximately 10,000 small circular DNAs known as k DNA minicircle which are between 600 and 800bp in size in members of the genus *Leishmania*. A minicircle compromised conserved region (120bp) and a variable region (600bp). The high copy num-

bers of the *Leishmania* minicircles make an ideal target for diagnostic tests.

A Nested-PCR based method that permits both very sensitive detection and high-resolution identification of *Leishmania* parasites directly from clinical samples is presented here (5).

Materials and Methods

Sampling

In this descriptive study samples from lesions of 100 patients with cutaneous leishmaniasis at the age of 1-80 year referred to the health centre and a private medical diagnosis laboratory in Shush city, Khuzestan Province, Southwest Iran were collected using a sterile vaccinostyle to make an incision in the edge of the lesion, then fixed on a microscopic slide and stained with Giemsa and examined microscopically.

DNA extraction

DNA was extracted from samples scraped from the slides. Phenol- chloroform- Isoemil alcohol-extraction method was used to extracting DNAs described by Sambrook (6). The DNA samples dissolved in 50µl deionized distilled water and stored at 4 °C (7).

Nested-PCR

Special primers related to variable regions of k DNA were used. External primers CSB2XF and CSB1XR in the first-round PCR and internal primers 13Z and Li R in the second-round of PCR were applied (5). PCR products of the second-round of the PCR were loaded onto a 1.5% agarose gel.

Results

The results of second-round PCR showed that *L. tropica* generated a 750bp fragment whereas *L. major* generated a 560bp (Fig.1). These products are very specific for the species. A total of 100 cases aged 1-80 yr comprising 47 females (47%) and 53 males (53%) were studied. The highest infected age group was <10 yr with a rate of 42% and the lowest rate was 4% in the

age group of >40 yr. As to cases with active lesion, 38% had one lesion, 17% two lesions, 27% not location information lesions and the rest had ≥ 3 lesions. The prevalence of *L. major* was 90% (47.7% female) and 10% for *L. tropica* (40% female) among the cases.

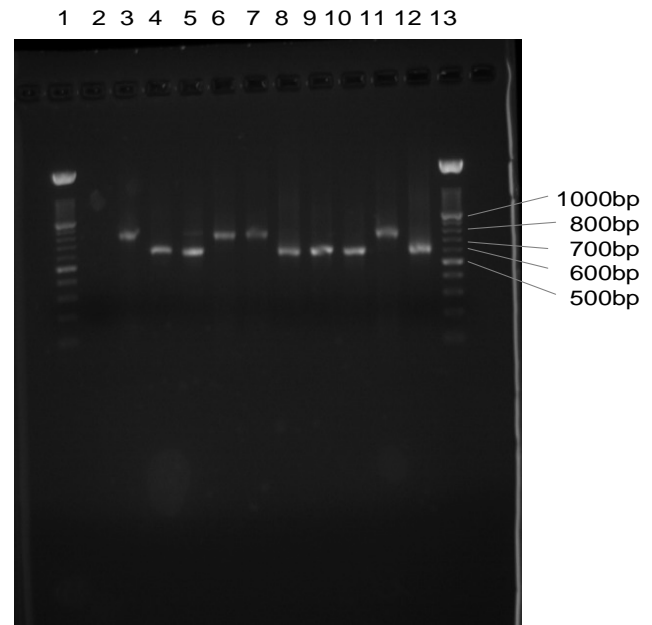


Fig.1: Lanes 1 and 13: ladder markers; lane 2: negative control; lanes 3, 6 and 7: *L. tropica*; lanes 4,5,8,9 and 10: *L. major*; lane 11: *L. tropica* (positive control); lane 12: *L. major* (positive control); A 750bp band and a 560bp represent *L. tropica* and *L. major*, respectively.

Discussion

So far, the etiology of CL has been unknown in Khuzestan Province (1, 3). It seemed that on the basis of clinical symptoms, presence of wild rodents (*Tatera indica*) as reservoir hosts (8), climate and geographical location, *L. major* should be the major species of CL. These issues were proved by the results of this study.

Tashakori *et al.* in their study of various location of Iran showed that *L. major* was the major cause of disease in Isfahan whereas *L. major* in Dehloran and Kashan and five cases of seven in Dezful, the two remaining in Dezful was *Crithidia*

lucilia (9). Study of Hajjaran *et al.* in Mashhad indicated that *L.tropica* was the predominant cause of cutaneous leishmaniasis (10). The results of Kolaczinski *et al.* study in Afghan refugee camps in Northwest Pakistan indicated that age, but not gender or residency, was correlated with the risk of active CL (11). After PCR product electrophoresis, a band of 750bp for *L. tropica* and a band of 560bp for *L. major* were observed that were similar to previous studies (5, 6).

It was concluded that Nested-PCR is a reliable method for diagnosis and identification of *Leishmania* species and can be applied in epidemiologic investigations.

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