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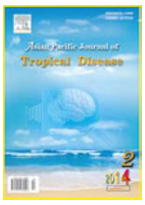

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Molecular epidemiological study of cutaneous leishmaniasis in the east north of Iran

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ABSTRACT

Objective: To identify and study the epidemiology of *Leishmania* species isolated from patients with leishmaniasis by PCR method in Sabzevar, Khorasan Razavi Province, Iran.

Methods: Aspirated samples from the lesions of 86 patients with confirmed leishmaniasis used for direct smear preparation. The samples are prepared and cultured in the Novy-MacNeal-Nicolle and Roswell Park Memorial Institute-1640 culture mediums. After extraction of DNA by using phenol-chloroform, parasite kintoplast DNA gene amplification was done by using PCR. The electrophoresis pattern of each species was compared with standard species of *Leishmania tropica* (*L. tropica*) and *Leishmania major* (*L. major*).

Results: Results of PCR patterns of kintoplast DNA gene suggested that two types of *L. tropica* and *L. major* isolated from leishmaniasis patients in Sabzevar and their findings indicated that both species of parasites, *L. tropica* and *L. major* are prevalent in Sabzevar.

Conclusions: According to this information, Sabzevar can be divided in two focal points of dry and dry-wet in terms of leishmaniasis, while it was previously known as the dry focal point. Likewise a significant relationship observed between *Leishmania* species to time distribution, type of lesion, amount of parasites and infection districts.

1. Introduction

Cutaneous leishmaniasis is a parasitic infection that is caused by various species of *Leishmania*. The disease occurs due to *Leishmania* inoculation to human skin via the bite of sand fly infected with promastigotes of the parasite. After infection, promastigotes enter into macrophages[1-3]. In macrophages, promastigotes convert to amastigotes which will induce nodules after proliferation and gradually the progressive lesion turns into papules and vesicles. After the lesion got well, it leaves a scar. Clinically, the lesions are divided into dry and wet forms[4-7]. *Leishmania major* (*L. major*) and *Leishmania tropica* (*L. tropica*) are responsible for wet and dry forms of leishmaniasis, respectively. There are different reservoirs for these parasites. Mice and wild-type rodents are reserviores of *L. major*, while human and dog (accidental hosts) are as reservoir hosts of *L. tropica*[8].

The disease is considered as a public health problem in some parts of Iran, particularly Sabzevar in Khorasan Province (Figure 1). Since reservoirs of the disease are

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Figure 1. Map of the city of Sabzevar in Khorasan Razavi Province, north eastern of Iran showing the geographical location and study sites.

different in the two species of parasites, in order to eradicate the disease, the species need to be identified. All forms of cutaneous leishmaniasis have the same morphology and it is not possible to differentiate species of *Leishmania* by clinical signs or microscopic methods. Among the existing approaches, molecular methods, such as PCR are very sensitive and rapid for identification of infectious agents. Since Sabzevar is one of the important focal points of the disease in Iran, we decided to study the epidemiology of cutaneous leishmaniasis using PCR method there[9-12].

2. Materials and methods

In this study 86 biopsies were collected from cutaneous leishmaniasis suspected persons in Sabzevar from May 2010 to May 2011. Biopsy was done with a scalpel under the lesion through picking up the macrophages. Two slides of each patient's lesions were prepared and stained with Giemsa. For amostigote cultivation, aspiration was performed from lesion using an insulin syringe containing 0.5 mL sterile saline buffer. The stained slides were examined by microscope with 40× and 100× magnification for observing amastigotes. If amastigotes were observed, the sample obtained from aspiration of the lesion would be entered into the Novy-MacNeal-Nicolle medium. To reduce microbial contamination potential, 250 to 500 IU of penicillin and 2 mg streptomycin per mililiter of culture was added to Novy-MacNeal-Nicolle medium. The culture mediums were incubated at 25 °C. After 3 to 4 d, the wet slides were prepared from the culture medium for observation of the promastigotes forms of parasites. Promastigotes with flagella were counted using a Neobar slide. If they reached one to two million per mililiter, they would be transferred to Roswell Park Memorial Institute-1640 culture medium containing fetal bovine serum to mass cultivation of promastigotes. After proliferation adjusted (1 000 promastigotes per mililiter) in culture medium, the promastigotes were centrifuged and washed with phosphate buffered saline for DNA extraction. DNA extraction was performed with phenol-chloroform method[13-15].

2.1. Kintoplast DNA (kDNA) amplification by PCR

A pair of primers for conserved sequences of *Leishmania*, kDNA including forward: (5′ TCG CAG AAC GCC CCT ACC 3′) and reverse: (5′AGG GGT TGG TGT AAA ATA GG3′ were