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Case Report

Molecular Evaluation of a Case of Visceral Leishmaniasis Due to *Leishmania tropica* in Southwestern Iran

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Abstract

We describe a case of visceral leishmaniasis (VL) due to *Leishmania tropica* in a 50-year-old Iranian man lived in a VL-endemic area in southwest of Iran. The patient presented with a 3-month history of fever and splenomegaly. Clinical signs and serological findings were suggestive of VL. Spleen biopsy was taken from the patient and intracellular forms of *Leishmania* amastigotes was seen in Giemsa stained smears. The patient was treated with pentavalent antimonial compound with complete resolution of his systemic signs and symptoms. DNA was extracted from the microscopic slides of the spleen biopsy and the nagt (N-Acetylglucosamine-1-Phosphate Transferase) gene of *Leishmania* was PCR-amplified. Sequence analysis of the PCR product demonstrated that the case has 99% identity with those of available sequences of *L. tropica*. Intra-species variation within isolate was 0-0.1%; whereas, inter-species differences of the isolate with those of *L. major* and *L. infantum* was significantly higher.

Introduction

Leishmaniases are protozoan vector-borne diseases caused by the genus *Leishmania*. Based on the clinical features, the disease is classified into main three forms of visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL). Visceral leishmaniasis (VL) is caused by *L. donovani* in the Indian

subcontinent and Eastern Africa, *L. infantum* in Mediterranean area and Middle East, and *L. chagasi* in Latin America (1). Iran is amongst the regions in the world where both cutaneous and visceral forms of the disease are present (2-3). Northwest and Southern parts of the country are the main foci of VL while zoonotic CL, caused by *L. major*, is common in more than 15 provinces and represents about 70% of cutaneous leishmaniasis cases in the country (4-5). Moreover, anthroponotic CL caused by *L. tropica*, is present in several urban foci in the country (5, 6).

The main causative agent of VL in Iran is *L. infantum* and carnivores (dogs, foxes and jackal) serve as the reservoirs (7). Both *L. tropica* and *L. major* which are the causative agents of cutaneous leishmaniasis have been reported from VL patients in Iran (8-10). In such cases *L. tropica* visceralize and cause systematic illness without obvious cutaneous symptoms. In the Persian Gulf War, several cases of VL caused by *L. tropica* were reported in American soldiers (11). Here we report a case of VL, caused by *L. tropica* without any cutaneous symptoms. The strain that caused the infection was characterized.

Case presentation

We describe a 50-year-old Iranian man who had lived in the VL-endemic area in Kohgiluyeh and Boyer-Ahmad Province, southwest of Iran, presented with a 3-month history of fever and splenomegaly. Results of serological studies were positive and suggestive of VL; though previous leishmaniasis infection was difficult to rule out. Since VL in Iran is mainly among children under 10 years old, the case was rather atypical. Spleen biopsy was taken from the patient and smear was stained with Giemsa staining. Spleen biopsy showed macrophages with intracellular forms of *Leishmania* amastigotes. The patient was treated with a 28-day course of intravenous pentavalent antimonial compound sodium stibogluconate with complete resolution of his systemic signs and symptoms. The case was not immunocompromised. Verbal informed consent was obtained from the patient for publication of this Case Report.

To determine the molecular characteristics of *Leishmania* species isolated from the patient, DNA was extracted from the microscopic slides of the spleen biopsy. Briefly, the slides were washed with absolute ethanol and covered with 250 µl of lysis buffer (50 mM Tris, 50 mM NaCl, 10 mM EDTA, pH 7.4, 1% v/v Triton X-100) for 1-2 minutes. Smears were removed from the slides and transferred to a 1.5 ml tube. Cell lysis was done by incubating the sample with 100 µg of proteinase K for 3 h at 56 °C. Phenol-chloroform was used to extract the lysate and ethanol was used to precipitate the extracted DNA. Precipitated DNA was re-suspended in double distilled water.

PCR-amplification of N-Acetylglucosamine-1-Phosphate Transferase (nagt) gene

The nagt (N-Acetylglucosamine-1-Phosphate Transferase) gene of *Leishmania* was PCR-amplified from the extracted genomic, using the primers L1 (Forward): (5' TCA TGA CTC TTG GCC TGG TAG) and L4 (Reverse): (5' CTC TAG CGC ACT TCA TCG TAG). PCR was carried out, as previously described (5). PCR products were separated by electrophoresis in 1.5% agarose gel and stained with safe stain. DNAs from Iranian reference strains, *L. tropica* (Acc. No. EF653267), *L. major* (Acc. No. JN860745) and *L. infantum* (Acc. No. EU810776) were included as positive controls in all PCR assays.

PCR amplification of the nagt gene of *Leishmania* produced fragments of about 1.4 kb, corresponding to *Leishmania* parasite. The PCR product was sequenced and the sequence was aligned and compared with those of existing sequences related to *Leishmania* in GenBank. The sequence analysis demonstrated that the case has 99% identity with those of available sequences of *L. tropica* in the GenBank. Alignment of sequence of the current isolate with those of *L. tropica* existing in the

GenBank showed just one DNA variable site in which nucleotide at position of 106 was single-base substituted.

A phylogenetic tree was constructed, using MEGA 5 software, and using Tamura 3-parameter option (Fig. 2). Intra-species variation within isolate of *L. tropica* in this study with another isolates of *L. tropica* amounted to 0-0.1%; while, inter-species sequence differences among *L. tropica* in this study with isolates of *L. major* and *L. infantum* was significantly higher, being 1%.

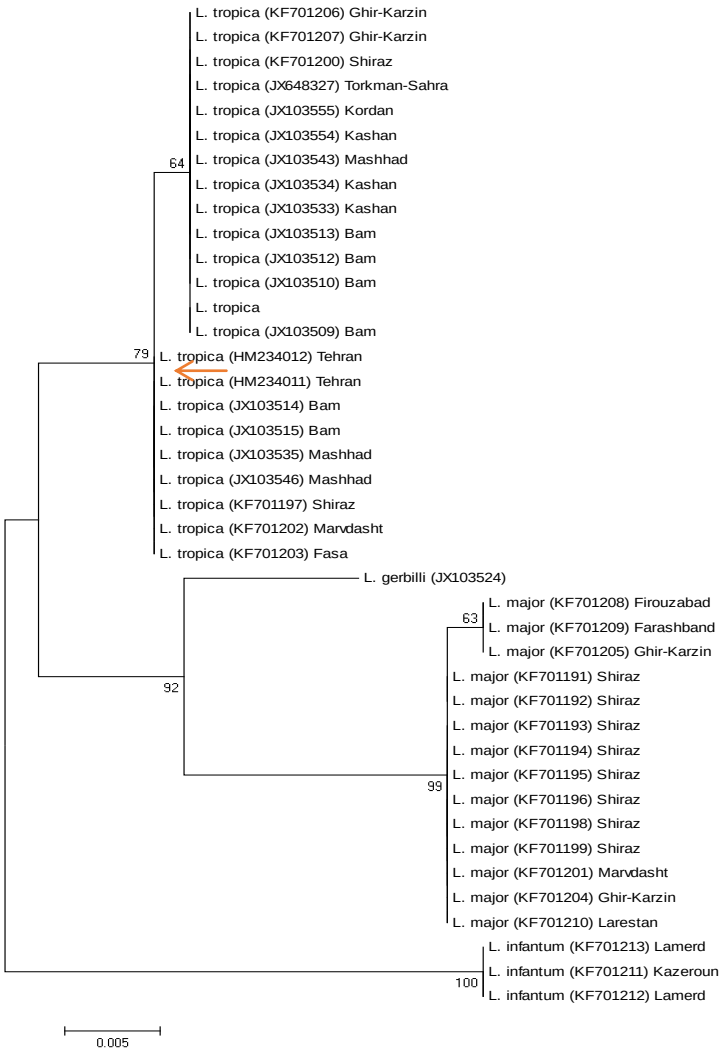

Fig. 1: Phylogenetic relationship of nagt sequence of *L. tropica* isolated from VL patient and *L. trpoica*, *L. major* and *L. infantum* from Iran, using Maximum Likelihood method

Discussion

The main causative agent of VL in south of Iran, as demonstrated by molecular methods, is *L. infantum* (6). This is the causative agent of VL in northwest of the country as well (2). However, VL due to *L. tropica* has been reported in few studies in south and northwest of the country (8-10).

Study of Alborzi et al., in southern Iran on 64 VL patients revealed that the dominant strain of *Leishmania* in these patients is *L. infan-*

tum (63 out of the 64 cases), but *L. tropica* was also isolated from one of VL patient (9). In another study, a case of diffuse cutaneous leishmaniasis (DCL) accompanied by visceral leishmaniasis caused by *L. tropica* was reported from Southern Iran (12). Moreover, two cases of disseminated leishmaniasis due to *L. tropica* in patients with HIV infection have been reported from Iran (13).

Concurrent mucosal and visceral leishmaniasis due to *L. tropica* was reported in a puppy from Iran (8). Moreover, VL due to *L. tropica* was reported in a domestic dog without any cutaneous involvement (7). Hajjaran et al. reported a case of canine VL in an 8-yr old dog infected with *L. tropica* (14).

Here we described a case of VL with *L. tropica* in a patients from a VL-endemic areas in southwest of Iran. The main reservoirs of VL in this area are dogs, although infection in cats is present, and the main causative agent of the disease is *L. infantum* (15-17). This is the first case of VL due to *L. tropica* which has been reported from this area. Molecular characterization of the isolate showed that the case has more than 99% homology with those of *L. tropica* isolated from the CL patients. Sequence of nagt gene was aligned with those of *L. tropica* available sequences in the GenBank which showed just one DNA variable site. Differences in the genotype of the parasite might lead to the type of cutaneous or visceralized forms of leishmaniasis caused by *L. tropica*. In a study, genetic differences of Indian strains of *L. tropica* isolated from human cases of CL with those of Indian strains of *L. tropica* isolated from human cases of VL were evaluated (18). Microsatellite analyses of dermatotropic and the viscerotropic strains of *L. tropica* consigned them to the same main genetic population. Furthermore, it was found that Indian strains isolated from human cases of VL fell into the same sub-population but were not genetically identical to the strains of *L. tropica* isolated from human cases of CL. In our study, intra-species variation within isolate of *L. tropica* with another isolates of *L. tropica* was very low while, inter-species sequence differences among the isolates of *L. major* and *L. infantum* was meaningfully high.

Conclusion

L. tropica might be a causative agent of VL in southwest of Iran. This should be considered in any measurements which target the control of the disease in the region.

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