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### Short Communication

## Causes of Pediatric Visceral Leishmaniasis in Southeastern Iran

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### **Abstract**

*Leishmania infantum* is the most frequent cause of visceral leishmaniasis and *L. tropica* has been rarely linked to the disease in Iran. In this study, bone marrow aspirates were collected from 10 child patients, suspected with visceral leishmaniasis referred to the Pediatric Wards of Kerman Medical Hospitals, Kerman, Iran during 2002–2011. *Leishmania* species were identified by using nested PCR in all slides. The PCR samples from nine patients indicated *L. infantum* as principal causative agent of visceral leishmaniasis and one *L. tropica* as a minor species.

### Introduction

Visceral leishmaniasis (VL) or kala-azar is an infectious parasitic disease with symptoms such as prolonged fever, hepatosplenomegaly, and reduction in various types of peripheral blood cells. If left untreated, the disease is fatal. Routine diagnosis of the disease is based on the presence of the causative parasite in clinical samples. Specific serology and polymerase chain reaction are useful tools for the diagnosis (1). Anti-K39

strip test is a highly sensitive test for the diagnosis of VL in symptomatic patients, but its specificity may vary in different geographic locations (2).

VL is endemic to southeastern regions of Iran (3). The following *Leishmania* species have been reported to cause VL in various geographies: *L. infantum*, *L. donovani*, and *L. chagasi*. Among those affected by visceral leishmaniasis, most of the patients are children under the

age of 5 years. In Iran, *L. infantum* seems to be the most common cause of VL. *L. infantum* also can cause cutaneous leishmaniasis (4). *L. tropica* is also known as an uncommon cause for visceral disease in the Middle East. Some studies have implicated *L. tropica* as another agent of VL in humans and animal reservoirs reported from north-west and south of Iran (5, 6).

This study was reviewed and approved by the Ethic Committee of Kerman University of Medical Sciences in Southeast Iran. The purpose of the study was to identify the specific causative *Leishmania* species for kala-azar in children.

## Materials and Methods

### Bone marrow smears

Ten children referred to the pediatric wards of Kerman Medical Hospitals, Kerman, Iran during 2002–2011 under the suspicion of VL were examined for amastigotes in bone marrow aspirates. Marrow slide preparations were fixed with methanol, stained by Giemsa, and observed for the presence of Leishman bodies (amastigotes) under light microscope with high magnification (1000x). Percent of parasitemia was not clear. A questionnaire containing questions on demographic characteristics, clinical symptoms, laboratory data, therapeutic measures, and treatment results was completed for each patient.

Informed consent was taken from each patient and the study was approved by local Ethics Committee.

### DNA extraction

DNA was extracted from the patients' samples. DNA samples were prepared from bone marrow smears. The smears were scraped using sterile blades, transferred to microtubes, and centrifuged thrice in a saline solution. Cell lysis was accomplished after incubation for at least 3 h or overnight at 56 °C. The lysate was extracted by phenol-chloroform followed by

ethanol precipitation. The DNA was resuspended in 50 µl double distilled water (DDW) and stored at 4 °C (7). DNA was extracted using Proteinase K Kit (Roche, Germany) according to the manufacturer's instructions.

### Nested PCR

Variable regions of minicircle fragments of kinetoplast DNA (kDNA) were amplified by nested PCR using the method described by Noyes and colleagues (8). Briefly the procedure was performed in 2 steps: first, 2 external primers were used: CSB1XR (CGAGTAG-CAGAAACTCCCGTTCA) and CSB2XF (ATT TTTCGCGATTTTCGCAGAACG). Next, 2 specific internal primers of LiR (TCG-CAGAACGCCCT) and 13Z (ACTGGGGG-TTGGTGTAATAAG) were used. The PCR products were analyzed on 1.5% agarose gel (Uvitech, UK) at a wavelength of 260 nm. *L. infantum* (MCAN/IR/07/Moheb-gh), *L. tropica* (MHOM/Sudan 158/OD strain), and *L. major* (MRHO/IR/75/ER) were used as positive controls, and distilled water was used as the negative control. *L. infantum*, *L. tropica*, and *L. major* yielded 680-bp, 750-bp, and 560-bp fragments, respectively.

## Results

Based on the bone marrow smear examination, we made a diagnosis of VL in all 10 patients (8 male and 2 female patients). Their mean age was 3.5 years. The PCR samples collected from 9 patients generated fragments of 680 bp, and the sample from the remaining 1 sample generated a fragment of 750 bp, indicating *L. infantum* to be the principal causative agent of VL and *L. tropica* to be the minor causative agent. From the 10 patients studied, 10 had fever and splenomegaly, eight had hepatomegaly, 7 had loss of appetite, and 9 showed pallor. Eight out of the nine patients with *L. infantum* infection had splenomegaly and seven had hepatomegaly. Intergroup differences in the clinical symptoms between the

patients with *L. infantum* infection and that with *L. tropica* infection were not significant. Eight out of the ten patients received sodium stibogluconate therapy. Of these eight patients, two died.

The lowest and the highest peripheral blood leukocyte levels were 1900/m<sup>3</sup> and 38,400/m<sup>3</sup>, respectively. The mean peripheral blood leukocyte count was 8400/m<sup>3</sup>. The peripheral blood leukocyte count for the patient with *L. tropica* infection was 6,200/m<sup>3</sup>. All 10 patients were anemic, with the average hemoglobin level being 7.16 g/dL. The lowest and the highest hemoglobin levels in the patients with *L. infantum* infection were 4.2 g/dL and 10.1 g/dL, respectively, while the hemoglobin level of the patient with *L. tropica* infection was 6.8 mg/dL.

All 10 patients had thrombocytopenia, with the average platelet count being 87,000/m<sup>3</sup>. The lowest and the highest platelet counts for the 9 patients with *L. infantum* infection were 14,000/m<sup>3</sup> and 134,000/m<sup>3</sup>, respectively. The platelet count of the patient with *L. tropica* infection was 86,000/mm<sup>3</sup>.

## Discussion

Visceral leishmaniasis is endemic in north-western and southern of Iran, and mainly affects children; *L. infantum* is the dominant species (3). The results of this study show that the main cause of kala-azar in Kerman province is *L. infantum*. An additional cause, the frequency of which has increased in recent years, is the viscerotropic strain, *L. tropica*, as demonstrated in the present study. Some studies have implicated *L. tropica* as another agent of VL in humans and animal reservoirs reported from north-west and south of Iran (5, 6). In another molecular study in Iran, of 28 patients, two VL patients were infected with *L. tropica* and 26 with *L. infantum*. Forty-seven Molecular samples revealed *L. infantum* in 43 and *L. tropica* in 4 dogs (9).

Another 2005 study conducted by Alborzi et al. on 64 patients with kala-azar from the southern provinces of Iran, examination of bone marrow slides revealed *L. infantum* to be the major causative agent (found in 63 patients); only 1 patient showed *L. tropica* infection (10).

On the other hand, a study conducted on 8 kala-azar patients from the American armed forces deployed in the Persian Gulf revealed *L. tropica* to be the causative species in 6 patients (11). *L. tropica* is also found as causative agent of human and canine VL in Turkey (12).

## Conclusion

*L. infantum* is the most common causative species of kala-azar in children in southeastern Iran; however, *L. tropica* can also cause visceral infection in individuals in this region. Nested PCR could be a valuable tool for species identification and selection of optimal therapy for VL.

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