LEISHMANIA TROPICA–ISOLATED PATIENT WITH VISCERAL LEISHMANIASIS IN SOUTHERN IRAN

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Abstract. Visceral leishmaniasis (VL) is caused by various strains of Leishmania donovani, Leishmania infantum, and Leishmania chagasi with different geographical distribution. The aim of this study was to identify the strains of Leishmania that can cause VL in southern Iran. DNA of Leishmania were extracted from the slides of bone marrow aspirates (#42) and spleen punctures (#22), which were positive for leishman body from the patients who were referred to the hospitals affiliated with Shiraz University of Medical Sciences. Differences in Leishmania strains were determined by size difference of the polymerase chain reaction (PCR) amplification as visualized on agarose gel. PCR results and smears had 100% correlation. The dominant strain of Leishmania was L. infantum (63 out of the 64 cases), but one case of L. tropica was also detected. VL mostly involves children below 2 years of age in Iran, therefore infection with L. infantum was expected, but this study is the first report of VL that is caused by L. tropica in Iran.

Visceral leishmaniasis (VL) is often caused by Leishmania donovani, Leishmania infantum, and Leishmania chagasi and is endemic in Middle East and Mediterranean regions. Northwestern and southern Iran are the primary foci for VL, which mainly affects children, so L. infantum is expected to be the dominant Leishmania strain in Iran.1–3 In the Persian Gulf War (1990–1991), L. tropica was the causative agent for leishmaniasis in American soldiers at bases in Bahrain and northern Saudi Arabia.4–6 Southern Iran is very similar to Bahrain and northern Saudi Arabia geographically, so L. tropica may be a causative agent for VL in southern Iran.

This study was done with molecular method to identify the dominant Leishmania strains causing VL in southern Iran and to evaluate if L. tropica can be one of the causative agents. Two groups of patients were selected.

• Group 1: Twenty-two patients who had proven positive spleen punctures for leishman bodies and were admitted to hospitals of Shiraz University of Medical Sciences during 1993–1994, and their spleen punctures had been archived.

• Group 2: Forty-four patients who had positive bone marrow aspirates for VL.

Leishmania DNA was extracted from the stained slides of spleen punctures (in Group 1) and bone marrow aspirates (in Group 2) using proteinase-K phenol chloroform method. At the first step, the slides were scratched and dissolved in 200 μL of extraction solution (EDTA 1%, Tris 15 mM, 10 mM Tween 20, pH = 8) then 160 μL of 800 μg/mL of proteinase-K solution was added, and the tubes were incubated at 56°C for 2 hours. In the next step, 200 μL phenol chloroform (pH = 8.5) was added to the prepared solution, mixed vigorously, and centrifuged at 6000 rpm (Eppendorf 5415 R, Eppendorf, Germany) for 1 minute, and the supernatant layer was transferred to another 1.5-mL micro tube, and 200 μL phenol chloroform was added again and centrifuged in the same manner as in the previous step, and cold glacial ethanol was added and centrifuged at 14,000 rpm (Eppendorf 5415 R) for 10 minutes to sediment the DNA. Then, 30 μL deionized distilled water was added to the dried sediment.

Polymerase chain reaction (PCR) was carried out using the couple primers 5’-CGA TCA GCA GAA ACT CCC GTT CA-3’ and 5’-ATT TTT CGC GAT TTT CGC AGA ACG-3’, which were introduced by Noyes and colleagues.7 In an 0.5-mL micro tube, PCR buffer (1x), 10 pm of each primer, 200 mM of each dATP, dGTP, dCTP, dTTP, and 2 mM MgCl₂ were added to 5 μL of DNA. The mixture was totally 20 μL and was covered with 15 μL mineral oil and put in the thermal cycler for the following program: 35 cycles consisting of 95°C for 1 minute, 57°C for 1 minute, and 72°C for another 1 minute. The mixture was finally put in 72°C for 10 minutes. The PCR product was studied by electrophoresis on 1.5% agarose gel, which was prepared in TAE buffer then was stained with ethidium bromide to be inspected with UV trans-illuminator for the bands.

Totally, 64 samples from 30 girls and 34 boys with mean age of 2 years were evaluated. Of them, 63 samples had 680-bp band suggesting L. infantum, and only one of the samples showed 750-bp band that is suggestive for L. tropica (Figure 1).

All slides, which were diagnosed microscopically as visceral leishmaniasis, were PCR positive, so our method for DNA extraction and PCR has had 100% correlation. Table 1 shows the causative agents for VL in children in our study.

VL is endemic in Iran, especially in the south of the country.1 There was not precise data about the Leishmania strains causing visceral leishmaniasis in humans in Iran before this study.

The results show that the major causative agent for VL in Iran is L. infantum, which was expected because most of the patients who have visceral leishmaniasis in Iran are children, but there was a sample of spleen puncture infected by L. tropica, which proves that L. tropica can be a causative agent for VL in Iran. Before the report about VL caused by L. tropica in American soldiers, which was the first report in our region, there were several reports from various parts of the world suggesting that L. tropica can cause VL. A study was done in Kenya in 1989 showed visceral leishmaniasis caused by L. tropica.8 Another study was done in India in 1995 that showed 4 out of the 15 cases of classic VL were caused by L. tropica, and in 1997 the first case from Morocco with visceral leishmaniasis caused by L. tropica was reported.9,10

Sixty-three of the 64 children with VL in our study had infection with L. infantum, but there was a single patient

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infected with *L. tropica*. The patient infected with *L. tropica* was not immunocompromised and had not any differences with the other patients based on primary workup and had been discharged from the hospital. As the American soldiers who were infected by *L. tropica* in the Persian Gulf War were all adults, we can hypothesize that *L. tropica* may occasionally be the causative agent for VL in adults. Thus, further studies are needed for documentation.

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