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Case Report

First Report of Kala-azar from Qeshm Island in Persian Gulf

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Abstract

Visceral Leishmaniasis (VL) is a sever disease that is prevalent in Iran. We report a case of VL in a 3.5 year-old boy. Prolonged fever, chill, abdominal distention, and weight loss were important symptoms. Blood count showed pancytopenia and hypohemoglobinemia. Specific anti-leishmanial antibodies were detected by serological test (IFAT, DAT) but no Leishman body was observed in bone marrow. However, a 145 bp band of KDNA belong to *L. infantum* was detected by PCR method. Glucantime was administered and treatment was well tolerated. This is the first report of VL from Qeshm Island in Persian Gulf.

Keywords: *Kala-azar, Visceral Leishmaniasis, Case report, Iran*

Introduction

Visceral leishmaniasis (VL) or Kala-azar is a systemic disease which is caused by *Leishmania donovani* complex (1). It is estimated that the annual occurrence of human visceral leishmaniasis cases worldwide to be 500,000 (2). The clinical signs in human include prolonged fever, hepatosplenomegaly, substantial weight loss, progressive anaemia and even may result to death (3).

Visceral leishmaniasis in the countries of Mediterranean basin and Middle East including Iran is caused by *L. d. infantum* (4). The most highly endemic areas of Iran are Fars and Bushehr Provinces in South, the districts of Meshkin-shahr and Moghan in Northwest and Qom Province in center of Iran (4-6). Other parts of Iran are considered as sporadic areas of VL, but so far, no cases were reported from Southern islands. In this paper we report a case of Kala-azar from Qeshm Island in Persian Gulf.

Case Report

The patient was, a 3.5 yr-old-boy from Qeshm Island in Persian Gulf, referred to Nemazee Hospital in Shiraz from Bandar-Abbas City with chief complaints of prolonged fever, chill, abdominal distention, weight loss, low appetite and icterus since three years ago. In physical examination, the patient had the following findings: axillary temperature= 39 °C, pulse rate= 170/ min, respiratory rate= 20/ min, splenomegaly (BCM= 4 cm) and hepatomegaly (BCM= 4 cm). However, abdominal sonography estimated the liver size as 14cm. Blood

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count showed pancytopenia with hemoglobin concentration of 4 g/dl, white blood cell count 2800/mm^3, platelet 60000/mm^3 and NRBC 3%. Liver function test showed protein 7.9 mg/dl, albumin 2.1 mg/dl, globulin 5.8 mg/dl, AST 66 IU/L, ALT 14 IU/L, ALK-Ph 174 IU/L, direct billirubin 0.1 mg/dl and total billirubin 0.4 mg/dl. Further laboratory data included: ESR 70cm, and CRP 48 mg/dl.

Specific anti-leishmanial antibodies were detected by Indirect Fluorescent Antibody Test (IFAT) and Direct Agglutination Test (DAT) at titters of 1: 128, 1: 3200, respectively. Bone marrow aspiration showed marked normocellular marrow but Leishman body was not seen.

Total DNA was extracted from blood buffy coat as described by Motazedian *et al*. (7). Briefly, 200 µl of buffy coat was homogenized with 200 µl lysis buffer [50 mM Tris-HCl (pH 7.6), 1 mM EDTA and 1% Tween 20] and 10 µl of a proteinase K solution (containing 19 mg of the enzyme/ml), in a 1.5 ml microcentrifuge tube. The homogenate was then incubated at 37 ºC overnight before 200 µl of a phenol: chloroform: isoamyl alcohol mixture (25:24:1, by volume) was added. After being shaken vigorously, the tube holding the mix was centrifuged (10,000g for 10 min) and then the DNA in the supernatant solution was precipitated with 400 µl cold, pure ethanol, resuspended in 50 µl double-distilled water and then stored at 4 ºC until it could be tested. Each 50 µl reaction mixture contained 200 µl of each deoxynucleoside triphosphate (dNTP), 3 mM MgCL2, 1.5 unit Taq polymerase (Sina gene, Tehran), 50 pM of each primer RV1 (5- CTTTTCTGGTCCCGCGGGTAGG-3) and RV2 (5-CCACCTGGCCTATTTTACACCA-3) and 5 µl of DNA extract, in PCR buffer (Boehringer Mannheim, Mannheim, Germany). Each reaction mixture was overlaid with mineral oil before being transferred to a CG1-96 thermocycler (Corbett Research, Sydney, Australia) set to give 5 min at 94 ºC followed by 40 cycles, each of 30 s at 94 ºC, 30 s at 59 ºC, and 30 s at 72 ºC, and then a final extension at 72 ºC for 10 min (8).

The PCR products were separated by electrophoresis in 1.5%-agarose gel, and 145 base pairs (bp) band of KDNA belong to *L. infantum* was detected by ultraviolet trans-illumination after staining with ethidium bromide.

Patient received 130 ml packed cell due to hypohemoglobonemia. Glucantime was intramuscularly used (starting 260mg and then increased to 520 mg and 780 mg) for twelve days. Granolocyte and platelete count increased by time. Treatment was well tolerated and and he was discharged with good condition.

Discussion

At the present time, VL is known as an endemic disease in some areas and also sporadic disease in all parts of Iran (9). So far, no cases reported from Iranian Island in South of Iran. Of course the patient had history of travelling to Bandar-Abbas City, center of Hormozgan Province where previously few cases reported from that area.

The early diagnosis and appropriate treatment of VL is important. The clinical signs of the patient were typical but no Leishman body was observed in bone marrow aspiration. Although observation of Leishman bodies in bone marrow aspiration is highly specific for diagnosis of the disease; this method is an invasive approach and low sensitive test (10). Serological methods such as DAT and IFAT are routinely carried out but in some patients, the titer of antibody may be not risen and have cross reaction with other diseases such as brucellosis, tuberculosis and malaria. On the other hand, these

methods are not appropriate and reliable at detecting the early stages of established infections and transient and self-limiting infections. However, the advantages of performance PCR on blood buffy coat are non-invasive, reliable, sensitive, specific, used in immunocompromised patients and also be able to identify species of parasite (11).

Many factors such as environmental conditions, density of dogs as a reservoir host and *Phlebotamus* as a vector are effective on acquisition of VL.

Qeshm is an island situated in the Strait of Hormuz off the South coast of Iran and east of the Persian Gulf (26°50′N 56°0′E). It is the largest island in Persian Gulf, and is almost 100 km long with an area of over 1295 km². The surface is mostly rocky and barren. The climate is quite dusty and hot and humid with warm summers, generally mild winters, and a great deal of sunshine throughout the year. The population of Qeshm Island is about 200,000 (12).

Since this is the first case report of Kala-azar in Qeshm Island, we suggest that seroepidemiological survey in human and animal reservoires (dogs) and identification of vectors should be performed in this area. It seems that VL diagnosis based on several methods particularly associated PCR as a molecular method is efficient and supplied in different parts of Iran especially in endemic areas.

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