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BRIEF COMMUNICATION

Co-infection of *Leishmania infantum* and *Brucella* spp in Iran

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Abstract In 2004, a 27-year-old man from Sirjan in Kerman Province (southeastern Iran) presented to Nemazee hospital with high-grade fever, chills, wet cough, abdominal pain, and mild splenomegaly. Anti-*Brucella* antibodies and anti-leishmanial antibodies were both shown to be present. *Leishmania* dipstick (rK39) was positive, but no amastigote was observed in the bone marrow. *Leishmania* kDNA corresponding to *L. infantum* was detected by nested-polymerase chain reaction (PCR) on liver biopsy and whole blood. These results confirmed co-infection of *Leishmania infantum* and *Brucella* spp.

Keywords Visceral leishmaniasis · Brucellosis · Co-infection · Zoonosis

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Introduction

Visceral leishmaniasis (VL) is a protozoan disease associated with *Leishmania donovani* intracellular parasites, which are transmitted by different species of sand flies (WHO 1990). It is estimated that the annual occurrence of human visceral leishmaniasis cases worldwide is 500,000 and that this condition accounts for 59,000 deaths per year as well as an expected disease burden of 2,357,000 disability-adjusted life years (WHO 2002). *L. infantum* is responsible for Mediterranean visceral leishmaniasis (MVL) in children and infants in the Mediterranean basin countries including Iran, and the dog is considered a major reservoir for the vector. The clinical signs in humans include prolonged fever, hepatosplenomegaly, substantial weight loss, progressive anemia, and even death (WHO 1990; Caldas et al. 2006). The diagnosis of VL is complex because commonly occurring diseases such as malaria, typhoid, and tuberculosis have clinical features similar to VL. Some VL cases have been misdiagnosed as autoimmune hepatitis, acute lymphoblastic leukemia, and malignant lymphoma (Kawakami et al. 1996; Jones et al. 2003; Dalgic et al. 2005).

Case report

In 2004, a 27-year-old-man from Kerman Province southeast of Iran presented to Nemazee hospital, which is affiliated to Shiraz University of Medical Sciences in Shiraz, southern Iran, with high-grade fever, chills, wet cough, and abdominal pain. Physical examination showed the patient to have mild splenomegaly. Blood count showed pancytopenia with Hb of 9 g/dl, WBC 3,200/mm^3, and platelet count of 91,000/mm^3. Liver function tests showed

AST 100 IU/L, ALT 90 IU/l and ALK-Ph 395 IU/l. Other lab findings were Alb/Glob ratio <1, CPK 55 U/C and LDH 636 U/C. No microorganisms were detected. Diagnostic tests such as tuberculosis (TB), VDRL, HBsAg, HIV, and Epstein–Barr virus were negative. However, Wright and Coomb's Wright tests were positive. These results confirmed infection with acute brucellosis. Chemotherapy with rifampicin and co-trimoxazole was started. After standard duration of treatment, no response was seen and the fever had not subsided. The patient was suspected to have visceral leishmaniasis, and the following analyses were performed: indirect fluorescent antibody test (IFAT) and direct agglutination test (DAT), the titers of anti-leishmanial antibody were 1/64 (cut off=1:128), 1/6,400 (cutoff= 1:3200), respectively. Moreover, *Leishmania* dipstick (rK39) as a rapid test was positive. No amastigote was observed in bone marrow aspiration but a markedly hypocellular marrow was observed. However, amastigotes of the parasite and associated granuloma reaction were detected in liver biopsy. *Leishmania* kDNA corresponding to *L. infantum* was detected on liver biopsy and whole blood using nested polymerase chain reaction (PCR), (Noyes et al. 1998). These results confirmed visceral leishmaniasis. The patient was from Sirjan city in Kerman Province (southeast of Iran) where VL is sporadic in the area but brucellosis is endemic (personal communication).

Discussion

The most highly endemic areas of VL in Iran are parts of Fars and Bushehr Provinces in the south, the districts of Meshkin-shahr and Moghan in northwest, and Qom Province in central Iran. Other parts of Iran are considered as sporadic areas for VL. Visceral leishmaniasis is common (over 98%) among children under 12 years old in different endemic foci in Iran and adult cases frequently present with subclinical and asymptomatic forms in endemic regions (Mohebali et al. 2005; Edrissian et al. 1999). Our report shows the importance of the awareness of physicians of adult cases of MVL in endemic regions.

In this case, using IFAT the result was negative and no amastigotes were seen in the bone marrow. Although the observation of amastigotes in bone marrow aspiration is highly specific for diagnosis of the disease, extensive examination of many smears may be required to demonstrate the parasite. Often the parasite may be observed only in tissues such as spleen, bone marrow, and liver. It is important to confirm any negative results of direct parasite visualization methods with additional serological and PCR assays (Sundar and Rai 2002).

We have not found any previous report of *L. infantum* / *Brucella* co-infection in Iran and possibly the world. el-Safi et al. (2004) did not find any association between the risk of infection with *Leishmania donovani* and *Mycobacterium tuberculosis* in hyper-endemic regions of both infections in Sudan. It seems that immunological interaction mechanisms between this organism and other intracellular pathogens exist and often inhibit the occurrence of co-infection. In rapid and early diagnosis of VL, especially in areas where diseases such as brucellosis, malaria, typhoid, and tuberculosis are endemic and clinical signs of other similar conditions are similar to VL, a differential diagnosis with appropriate laboratory analyses must be performed.

In conclusion, we suggest the application of PCR assay on whole blood as a fast, non-invasive, and reliable approach to accurate diagnosis and as an alternative to bone marrow aspiration organ biopsies.

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