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FIRST CASE OF VISCERAL LEISHMANIOSIS/HIV COINFECTION IN NIŠ – SOUTHEASTERN SERBIA

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Abstract - Visceral leishmaniosis (VL) has emerged as an important opportunistic parasitosis associated with human immunodeficiency virus (HIV) infection. The aim of this paper is to report the first case of *Leishmania*/HIV coinfection in a patient from Niš (Southeastern Serbia). Microscopical examination of Giemsa-stained bone marrow (BM) smears show the presence of *Leishmania* spp. *amastigotes* based on their morphological characteristics. In spite of the parasitological finding, the serological test applied gave negative results. Molecular analyses confirmed the infection and allowed us to identify the leishmania species as *Leishmania infantum* (100% identity). VL/HIV coinfection has important clinical, diagnostic and epidemiological implications. In fact, the failure of serological tests is expected in this condition, and the application of molecular diagnostics to the blood may offer, apart from an easy and non-invasive diagnostic opportunity, the possibility of warning about the risk of possible nosocomial infections.

Key words: Visceral leishmaniosis, HIV co-infection, molecular diagnosis

INTRODUCTION

The first report of HIV and VL coinfection appeared in 1985 and since then the number of cases has increased rapidly in southern Europe. Countries from other parts of Europe have also reported this coinfection (Alvar et al., 2008).

Visceral leishmaniosis has emerged as an important opportunistic parasitosis associated with HIV infection. In southern Europe, it was observed that up to 70% of cases of VL in adults were associated with HIV infection (Alvar et al., 1997, Lopez-Velez et al., 1998). Of the first 1700 cases of coinfection reported to the WHO by 1998, 1440 were from southwestern Europe (Guerin et al., 2002).

As for the other continents, most coinfections reported in the Americas are from Brazil. In Africa, the number of cases is expected to rise and it is further impaired by social adversities such as mass migration, displacement, civil unrest, and war. In Asia, coinfections are increasingly being reported from India, Bangladesh and Nepal (Sundar, 2001, Sundar et al., 2002).

A recent study (del Giudice et al., 2002) revealed that the incidence of VL in HIV-infected patients decreased from 11.6 ± 1.2 per 10 000 persons in the years before 1996 to 6.3 ± 0.7 per 10 000 persons after 1996, the year when highly active antiretroviral therapy (HAART) was initiated in France. Similar data have been reported from Spain (de La Rosa et al., 2002). However, at present the benefits of HAART are only available to 5% or less of HIV-infected patients in the world, so a decrease in this coinfection can be expected only in developing countries (UNAIDS: AIDS Epidemic Update 2002. www.unaids. org.).

In Serbia, from the beginning of this century to date, 22 cases of visceral leishmaniosis have been described, but no case of HIV/leishmania coinfection has been reported (Dakic et al., 2009). To report the first case of this opportunistic parasitosis in AIDS patient from Niš (Southeastern Serbia) is the aim of this paper.

MATERIALS AND METHODS

Case History

A 58 year old patient, permanently resident in the Nišava district (in a village near the Niš municipality) who had travelled as a construction worker in Iraq in 1992 and Ukraine in 2002, was hospitalized at the Clinic of Hematology in March of 2011 with severe malaise, weight loss (40 kg weight loss over a period of 12 months), anemia, hypergammaglobulinemia, pancytopenia, splenomegalia and asthenia.

After a bone marrow (BM) aspiration, intracellular and extracellular parasites (amastigotes of *Leishmania* spp.) were detected using a conventional microscopic examination of the BM smears. A sample of the BM and serum were sent to the Department of Parasitology, Faculty of Medicine, Niš.

Diagnosis of VL was confirmed by a double test of the microscopic examination of Giemsa-stained BM smears that was carried out by a clinical doctor and parasitologist to check for the repeatability of the morphological identification. The serologic test showed negative results. Leishmaniosis was confirmed by molecular methods and the identification of parasites was completed. Due to suspected HIV infection, a serological diagnosis was performed and HIV positivity was established by an enzyme-linked immunosorbent assay (ELISA) and confirmed by Western Blot (WB) analysis.

After being transferred to the Clinic for Infectious Diseases, the patient underwent treatment for leishmaniosis (liposomal amphotericin B, 4 mg/kg bodyweight/day (he lived until receiving first three doses, after days 1, 5 and 10). In addition to antileishmanial chemotherapy, the patient started to receive HAART and prophylactic therapy for other opportunistic infectious diseases, according to the official protocols for HIV&AIDS treatment, as the level of his CD4 and CD8 cells was 21 cells/μl and 118 cells/μl, respectively. During the course of therapy, adult respiratory distress syndrome (ARDS) occurred, which was the immediate cause of death.

For hematological investigations, BM and peripheral blood were taken. BM smears were stained by the Giemsa method and were microscopically examined (500-1000X magnification). Blood was used to prepare a serum sample; blood was also submitted to the Rapid Dipstick rK39 test (DiaSys Europe Ltd, Wokingham, UK). This is a qualitative membrane-based immunoassay using the recombinant antigen K39, which is part of the *Leishmania chagasi* kinesin-related protein and is specific for all members of the *Leishmania donovani* complex (Burns et al., 1993). DNA was extracted from the remaining blood (200 μL) and subjected to polymerase chain reaction with specific primers LEI 1-2 (Rogers et al., 1990), which amplify a fragment of 116 bp of the kinetoplast DNA, followed by sequencing of the amplicon and submission of the sequences obtained to a BLAST Identity Search to give the most likely identification of leishmania species involved in the infection.

RESULTS

Microscopical examination of Giemsa-stained BM smears proved the presence of *Leishmania* spp.

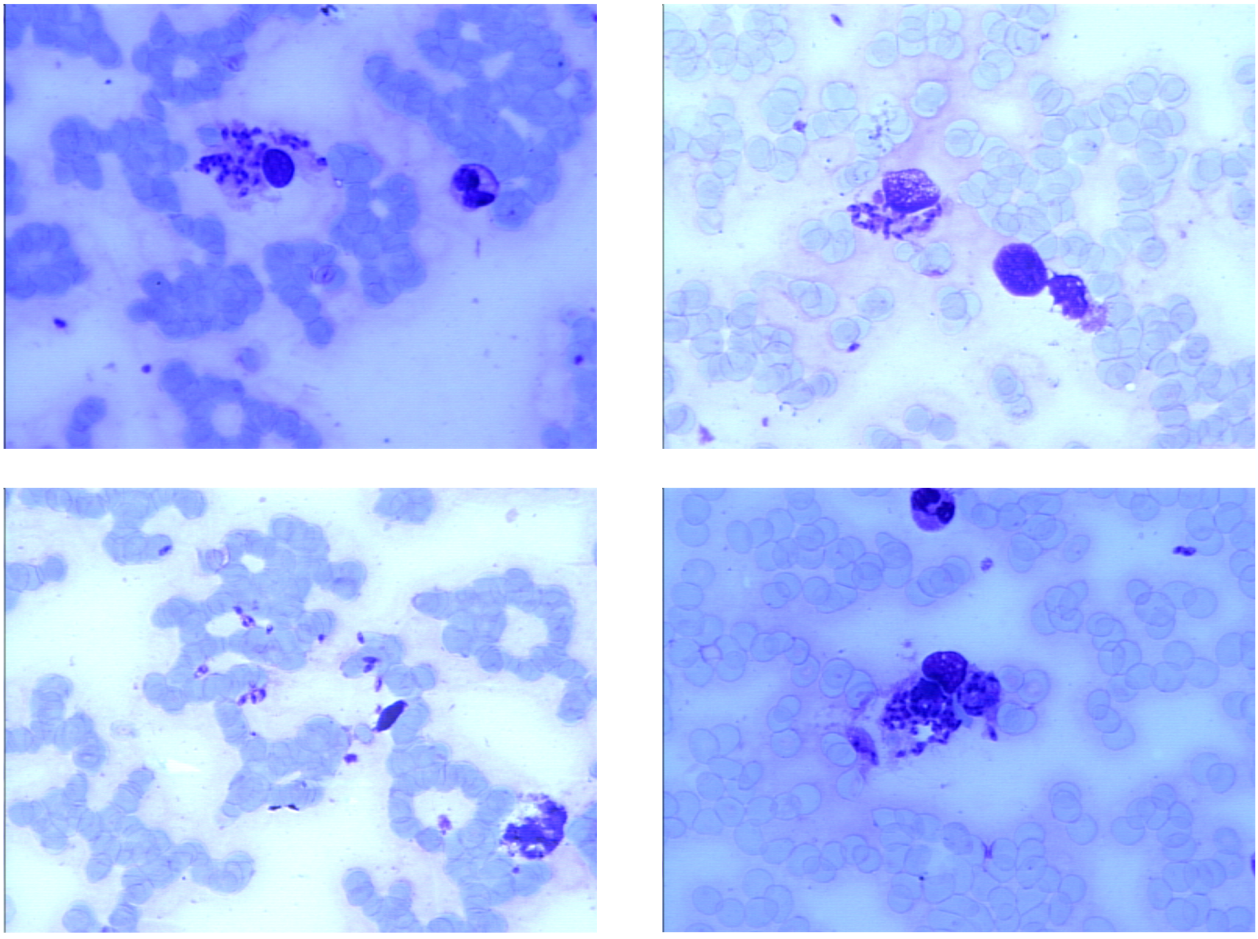

Fig. 1. Direct microscopic findings of amastigotes of *Leishmania* spp. in bone marrow (BM) smear Giemsa-stained (500-1000x)

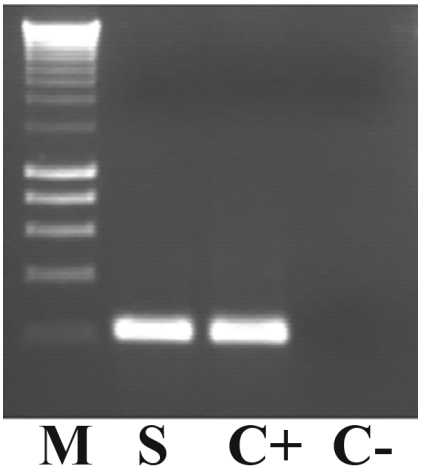

Fig. 2. PCR with specific primers LEI 1-2

amastigotes based on their morphological characteristics, and allowed us to diagnose the visceral form of leishmaniosis (Fig. 1).

In spite of the parasitological finding, the serological test applied gave a negative result. Molecular analyses confirmed the infection and allowed us to identify the leishmania species as *Leishmania infantum* (100% identity) (Fig. 2).

DISCUSSION

An increasing spread of *Leishmania infantum*, which causes zoonotic visceral and cutaneous leishmaniosis in humans and dogs (the reservoir host), and *L.*

tropica, which causes anthroponotic cutaneous leishmaniasis, has been evidenced in recent years in European countries. The high prevalence of asymptomatic human carriers of *L. infantum* in southern Europe (Moral et al., 2002, Martín-Sánchez et al., 2004, Pratlong et al., 2004, Marty et al., 2007) suggests that this latent parasitosis is a great public health problem, as demonstrated by the increase of HIV and *leishmania* coinfection prevalence (Alvar et al., 1997).

Leishmaniosis is becoming the third most frequent opportunistic parasitic disease in HIV patients after toxoplasmosis and cryptosporidiosis (Desjeux et al., 2003). The HIV/AIDS pandemic has modified the natural history of leishmaniosis (Alvar et al., 1997). HIV infection increases the risk of developing VL by 100 to 2,320 times in areas of endemicity: it reduces the likelihood of a therapeutic response, and greatly increases the probability of relapse (Lopez-Velez et al., 1998). It is thought that the parasitic infection found concurrently with HIV induces chronic immune activation and therefore an increased HIV load and accelerated progression of AIDS (Alvar et al., 2008), whereas immunological disturbances caused by HIV are particularly favorable for the uncontrolled multiplication of the parasite (Alvar et al., 2008).

The patient with HIV/leishmaniosis coinfection reported here is the first diagnosed case in Serbia. It is very difficult to say if this leishmaniosis was an imported or an autochthonous infection. The patient's travel history suggests he could have been infected in Iraq where he was working; in this country some endemic areas for *L. infantum* have been determined (WHO, 2010). However, people can be infected in southern Serbia as well.

The first autochthonous cases of VL in Serbia were recorded in 1945 in Niš and the Dobrič district, and this was where the patient was resident (Simić, 1957). Moreover, in the period from 1946 to 1948 in the territory of southern, eastern and western Serbia, more than 350 cases of kala-azar (VL) were recorded (Simić, 1957). The studies performed at that time established that the type of kala-azar was similar to that observed in the Mediterranean basin. As for the reservoirs of infection, the presence of *Leishmania spp.* was proven in dogs (most commonly in asymptomatic infection) in each region where kala-azar was identified in humans. The studies performed in Niš in 1955 showed that over 2% of dogs in the area had an asymptomatic infection (Simić, 1957). Rare autochthonic cases were reported in the Niš municipality in 1968 and 1969 when the presence of vectors, such as *Phlebotomus major*, *P. simici* and *P. perfiliewi*, was also reported (Petrović, 1980).

Epidemiologic data show that in the period from 1991 to 2000 there were 39 cases of VL reported in Serbia and Montenegro, with only one case of imported leishmaniosis (Dakic et al., 2009).

A recent retrospective epidemiologic and diagnostic study of VL in Serbia for the period 2001-2007 has demonstrated a visit to the Montenegrin coast to be a predominant risk factor in the 22 individuals diagnosed with VL, apart from one case of VL that occurred in southern Serbia and which probably represented a dormant focus of infection (Dakic et al., 2009).

The WHO concluded that more extensive and efficient surveillance is necessary in Europe to assess the emergence of leishmaniosis (WHO, 2009). Increased awareness of leishmaniosis is mandatory even in areas where it is not endemic.

In view of the growing epidemiologic problem of VL spread and especially VL/HIV coinfection, the surveillance of leishmaniosis is imperative, both in human and dog populations in Serbia, paying special attention to the southern regions of the country. Epidemiologic surveillance would certainly prevent an epidemic outburst of the infections.

As regards diagnostic tools, the negative serological findings in our patient were to be expected because HIV/*Leishmania*-coinfection induced a deficit in the host's humoral and cellular responses. This makes both serological and delayed type IV hypersensitivity-based tests of limited use in coinfected patients. Only about 40-50% of HIV/*Leishmania* coin-

fected patients have a positive *Leishmania* serology (Montalban et al., 1990, Gari-Toussaint et al., 1994). In fact, anti-*Leishmania* antibodies in AIDS patients are 50 times lower than in those with an intact immune system (Mary et al., 1992). Therefore, using serological methods many false-negative results should be expected in HIV-infected individuals. Ideally, at least two different serological tests should be used for each patient, and the leishmanial antigens employed should be freshly prepared, to increase their sensitivity (Desjeux et al., 2003).

Polymerase chain reaction (PCR) revolutionized the possibility of diagnosing the etiologic agents of infectious diseases. In the past decade, PCR-based techniques have been progressively more applied to diagnosis leishmaniosis, but its use is, to date, limited to tertiary health centers. To avoid invasive procedures, peripheral blood is often used, and the reported sensitivity of PCR on blood ranges from 70% to 96% (Takagi et al., 2009).

CONCLUSION

VL/HIV coinfection has important clinical, diagnostic and epidemiological implications. In this condition, the failure of serological tests is to be expected and, apart from being an easy and non-invasive diagnostic approach, the application of molecular diagnostics to the blood may give a warning about the risk of possible nosocomial infections.

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