Visceral leishmaniasis in an immunocompetent Hungarian adult patient

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ABSTRACT

Background: Visceral leishmaniasis caused by L. infantum Nicolle 1908 (Kinetoplastida, Trypanosomatidae) is very rare in immunocompetent adults. Clinical manifestation of the infection occurs in children and immune-compromised patients associated with AIDS, haemopoietic malignancies or after renal, liver, and heart transplantations in Europe. Hungary is regarded free of leishmaniasis. Except for a single infection in a small girl without a travel history, only a few imported cases have been recorded among Hungarians returning from endemic areas. Case presentation: Visceral leishmaniasis was diagnosed in a 32 years old immunocompetent Hungarian man who had spent his holidays during the previous 3 years in Dalmatian territories (Dubrovnik, Makarska and Trogir) of Croatia. He had two months history of fever, chills, and night sweating associated with weight loss. Physical examination showed mild hepatomegaly and extreme splenomegaly. The protozoon infection was confirmed by parasitological, serological and molecular biological methods. Clinical recovery of the patient was observed after treatment with amphotericin B. Conclusion: We want to highlight the effects of climate changes and to call attention of health care professionals in Central and Eastern European countries to consider the possibility of leishmaniasis as an emerging disease of tourists returned from endemic regions.

Keywords: Visceral Leishmaniasis; Immunocompetent; Human; Imported; Hungary; Amphotericin B

1. INTRODUCTION

Leishmaniasis is a group of the protozoan diseases, transmitted by the bite of sandfly infected with Leishmania parasites [1]. The infection of humans appears with multiple clinical manifestations including cutaneous (CL), mucocutaneous, diffuse and visceral (VL) leishmaniasis. The latter is responsible for approximately 59,000 deaths per year, a parasitic disease surpassed only by malaria [2]. There are two types of VL, anthroponotic and zoonotic. Zoonotic VL (ZVL) is wide spread and occurs in Latin America, Northern Africa, Southern Europe and in areas of the Middle East and Asia [2,3].

Two forms of leishmaniasis (VL and CL) present in Europe are caused by Leishmania infantum [4]. While cases of cutaneous leishmaniosis were reported in France, Italy and Spain, ZVL is endemic in all countries bordering the Mediterranean Sea [5].

The reservoirs of the pathogen can be several wild and domestic canid and rodent species but domestic dogs are considered the main reservoir of L. infantum playing a key role as the source of human infection. Indeed, there is a clear association between a high rate of infection in dogs and an increased risk of human disease [1,2].

Geographically, the distribution of leishmaniasis is limited by the distribution of its vector sandfly species. The sandfly vectors are mainly active during the night, and therefore the highest risk for contracting the infective stages of the parasite from sandfly bites is between dusk and dawn [6]. Five haematophagous sandfly species of the subgenus Larroussius belonging to Phlebotomus genus (Diptera, Psychodidae) are known in Europe as the vectors of L. infantum. Phlebotomus ariasi and P. perniciosus are present in the western Mediterranean from the Iberian to the Italian peninsula, while P. perlifelvi, P. neglectus and P. tobbi are distributed from Italy across
Balkan to Turkey [4,7,8]. The presence of these vector species is essential for the endemic transmission of the parasite among dogs and humans [9]. Another sandfly species, P. mascitti is mentioned as a suspected vector species of the parasite which is widely distributed in Europe with a northern limit in Belgium [6].

Our knowledge of the geographical distribution of phlebotomine sandflies in Hungary is incomplete due to the lack of systematic monitoring. According to historical records a few dozen specimens of P. macedonicus (recently known as P. perfiliewi) were caught at the southeastern part of the country in the early 1930s [10]. During the recent surveys in the EU FP6 EDEN project small numbers of two sandfly vectors (P. neglectus and P. perfiliewi) were trapped close to the Croatian and Serbian borders of the country (Farkas, personal communication).

In Europe, human VL is considered to be a rare disease, although its incidence increased significantly in the region during the 1990s [6], about 700 autochthonous cases have been reported each year from southern countries of Europe [3, 11]. Asymptomatic Leishmania infection of humans is more frequent than clinically apparent form. Once the clinical illness begins the disease rapidly progress and patients die due to VL unless treated. The factors that determine the progress of VL have not yet been completely identified, but a Leishmania specific cellular immune response seems to play a fundamental role in the control of infection [12]. After an incubation period of 2-8 months (range: from 10 days to longer than 2 years), the patient develops pyrexia, wasting, and hepatomegaly which may become excessive. Amastigotes of L. infantum disseminate throughout the reticuloendothelial system causing hyperplasia of phagocytic cells in spleen, liver, bone marrow and sometimes lymph nodes. In endemic European areas most patients are children [13].

A historical review on human leishmaniasis in Croatia documents the presence of stable disease foci in coastal and insular territories of central and southern Dalmatia since the beginning of the 20th century with a substantial increase in incidence from the 1990s [14]. In contrast to the endemic occurrence of the disease in the neighbor Balkanic countries Hungary is regarded to be free of leishmaniasis, only imported cases have been reported. Over 30 cases are known that were imported from the hyperendemic countries of the Middle East [15] and only a single human case of ZVL has been diagnosed by identification of amastigotes from the iliac crest biopsy in Hungary that originated from the neighboring Croatia that is regarded hypoendemic for leishmaniasis [16].

2. CASE PRESENTATION

On the 4th November, 2009, a 32 years old man was admitted to the Department of Internal Medicine, University of Pécs because of two months history of fever, chills, and night sweating associated with weight loss. The patient had no history of any kind of previous illnesses. Laboratory results revealed severe pancytopenia: white blood cell: 0.85 G/l with 24% monocytes, hemoglobin: 86 g/l and platelet: 50 G/l. These findings can be signs of hematological disorders (such as myelodysplastic diseases) or may indicate several infections (e.g., brucellosis, Q fever, leishmaniasis). The erythrocyte sedimentation rate and C-reactive protein (135 mg/l) were elevated but normal level of alanine aminotransferase and creatinine were observed. The gamma globulin level was beyond the measurable range (>40 g/l) with decreased albumin level and albumin-globulin ratio of 0.4.

Physical examination showed mild hepatomegaly and extreme splenomegaly (the spleen reached the upper rim of the pelvis). The patient’s general condition was relatively good. The reason for referral was a suspicion of malignant hematological disease but the bone marrow trephine biopsy excluded malignancy. The patient had never received any transfusions or injections of blood or blood products before the onset of his illness.

Detailed medical history revealed that the patient spent his holidays during the previous 3 years in Dalmatian territories (Dubrovnik, Makarska and Trogir) of Croatia. This raised the possibility of an imported “exotic” disease. Such imported disease which does not occur in Hungary can be leishmaniasis although immunocompetent adults are affected very rarely. Examination of the immune status of our recent patient excluded immunosuppression or HIV-1 co-infection. HIV-1 test were negative as well as the blood cultures, the Brucella agglutination and Coxiella burnetti indirect immunofluorescent assays.

The clinical diagnosis of visceral leishmaniasis was confirmed by detection of amastigotes in bone-marrow aspirate followed by serological and molecular assays. Acute-phase serum sample was tested by Leishmania Western-blot IgG qualitative Immunoblot Assay (Ldbio Diagnostics, France), that showed well-defined bands which are indicative for the presence of specific anti-Leishmania IgG in the sample. The patient’s bone marrow aspirate and blood sample were collected before and after two week therapy, respectively. These samples were studied with a L. donovani sl. kDNA minicircle specific PCR assay [17]. DNA extracted with QIAamp DNA Mini Kit (QIAGen, Hilden, Germany) was processed in 25 µl final reaction-volume with RV1 (5’-CTTTTCTGTCCCGGGTGGG-3’) and RV2 (5’-CCACCCGTGTTTTTATACCCA-3’) primers (25 pmole both) and the PCR Core System I. (Promega, Madison, WI, USA) to amplify an approximately 145 bp long region of the parasites DNA. Final concentrations
Leishmania kDNA in the assay proved the presence of ethidium bromide stained 1.5% agarose gel. The PCR step at 4 minutes at 94 ℃ anellation and 30 s 72 ℃ elongation. A final elongation at 72 ℃ for 10 minutes was followed by the hold step at 4 ℃. Products of amplification were visualized in ethidium bromide stained 1.5% agarose gel. The PCR assay proved the presence of Leishmania kDNA in the patient’s bone marrow, but blood sample was negative probably due to the efficacy of the treatment (Figure 1).

Amphotericin B of total dose of 20 mg/body weight was used conform to international recommendations. After second day of treatment the patient become free of fever, the leukocyte and platelet count continuously increased reaching 3.63 G/l and 130 G/l respectively. During treatment only temporary mild creatinine elevation (156 μmol/l) were observed, which normalized after hydration. The patient’s spleen size decreased gradually; on the second week of treatment declining half of the original size whilst normalize at the end of treatment. In the follow-up period no relapses were observed.

3. DISCUSSION

Classic ZVL in immunocompetent individuals in Mediterranean countries is found mainly among children, although since 1989 an increase in the number of cases among adults has been observed in south-western European countries mostly (20-70%) due to Leishmania and HIV-1 co-infections. After a peak in 1990s, the incidence of co-infections decreased notably due to introduction of highly active antiretroviral therapy for AIDS. Even in those areas as well where leishmaniasis is widespread the incidences of ZVL among immunocompetent adults were extremely rare [1,11,13,18,19].

Human VL has known to occur in Croatia since 1911, although the first well-documented, microscopically confirmed case was reported only in 1930 in the Dubrovnik area [14]. From 1931 through 1957 an average of 15 cases were recorded yearly in several Dalmatian territories. Starting from the 1960s, only sporadic cases of both VL and CL were identified in central and southern Dalmatia [14]. However, a substantial increase was recorded in VL cases during the recent (1991-1995) war in Croatia and in the post-war period in that region the most of those cases (64.3%) were found in children below 10 years [14].

Except for a single infection in a small girl without a travel history [20], only imported human cases have been recorded in Hungary. Várnai et al. [10] reported 31 cases of cutaneous or visceral leishmaniasis (CL, VL) among Hungarians returning from endemic areas, and Fried et al. [16] diagnosed VL in a Hungarian woman who had spent her holidays in Dalmatia.

In the past two decades VL incidence rates without HIV-1 co-infection have increased in Italy and France (including a four-fold increase in Alpes-Maritimes), and new endemic areas have been detected where no previous autochthonous cases had been reported (e.g. in northern Italy, North Croatia, Switzerland and Germany) [11,21]. The distribution of VL in Europe is significantly more restricted than the distribution of the sandfly vectors. The transmission of the disease agent within the range of the vectors depends on vector abundance, vector survival, vector biting rate (i.e. gonotrophic cycle), the extrinsic incubation period, and the length of the transmission season. Each of these parameters is climate dependent [6]. Climatic changes are likely to extend northwards the range reached by the sandfly vectors. In currently endemic areas, higher seasonal temperatures would lead to prolonged activity periods and shorter diapauses periods. This could result in an increased number of sandfly generations per year. In addition, higher temperatures may accelerate the maturation of the protozoal parasite, thereby increasing the risk of infection [6,7,21]. Thus, climate-induced changes may increase the risk of the emergence of new diseases including leishmaniasis in Central and Western Europe [22,23]. Imported infected dogs can also contribute to the emergence of leishmaniasis in new locations.

Whereas Hungary is regarded as free of leishmaniasis the tourist traffic towards to the leishmania endemic regions of Croatia is increasing year by year. So the chance of appearance of new imported cases can be taken into account. To prevent this greater emphasis should be placed in education of travellers with a focus on the available prevention methods.
4. CONCLUSIONS

Zoonotic VL is a re-emerging disease in the Mediterranean area primarily due to climate induced changes. Classic clinical pictures of the disease in immunocompetent individuals are found mainly among children. Adults are affected very rare. Nevertheless as our case suggests the occurrence of ZVL should be also considered in patients presenting with fever of unknown origin, hepatosplenomegaly, and pancytopenia who returned from endemic areas.

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