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A Case of Visceral Leishmaniasis in the Northern Adriatic Region

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A B S T R A C T

A 33-year-old male patient with fever, splenomegaly, pancytopenia and lymphocytosis was admitted to the Department of Hematology in Rijeka. Laboratory findings, bone marrow aspiration and biopsy excluded hemoblastosis and aplastic anemia. To exclude primary splenic lymphoma we performed splenic aspiration where Leishmania amastigotes were found. No cases of visceral leishmaniasis have been previously described in the Northern Adriatic region. Considering epidemiology, a contraction of the disease in the Velebit mountain range could be possible despite the current non-endemic status of the region.

Introduction

The leishmaniases are a group of diseases caused by the genus Leishmania and transmitted by female sandflies of the genus Phlebotomus in the Old World and Lutzomyia in the New World. Human leishmanial infection can result in three main forms of disease: visceral form (Kala-azar), cutaneous disease and mucocutaneous leishmaniasis[1,2]. Visceral leishmaniasis is the most serious, potentially fatal form of the disease which has a worldwide distribution. Certain regions are endemic areas of the disease including the countries of Europe, North Africa and the Middle East surrounding the Mediterranean basin[3,4]. Endemic regions in Croatia include middle and southern Dalmatia, but the disease remains sporadic. The Northern Adriatic has been considered a non-endemic region. The organisms causing visceral leishmaniasis in the Mediterranean region (L. infantum) and those in Americas (L. chagasi) are very similar and the disease is mainly characterized by fever, weight loss, hepatosplenomegaly, lymphadenopathy and pancytopenia. Routine diagnostic procedures include several serological methods

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and microscopic examination of Giemsa--stained material obtained from the bone marrow, lymph nodes or spleen. Most commonly used drugs in the treatment of visceral leishmaniasis are pentavalent antimony compounds.

Case Report

A previously healthy, 33-year-old male patient was admitted to the Department of Hematology, University Hospital Center »Rijeka«, presenting with remittent fever which lasted for two months, weakness, hepatosplenomegaly and pancytopenia. On admission he was febrile (38.5 °C), the liver was palpable 1 cm and the spleen 5 cm below the costal margin.

Laboratory tests revealed elevated ESR, pancytopenia and mild lymphocytosis with atypical cells in the peripheral blood (Table 1). Total serum proteins were raised (93 g/L), albumins 39.1 g/L, gamma globulins 36.4 g/L. Serum prothrombine time was prolonged to 19 seconds. Lactate dehydrogenase (LDH) was elevated (505 IU/L).

The patient remained febrile for 15 days with his body temperature showing a remittent pattern. Repeated blood cultures were negative as well as Vidal's reaction. Malaria was excluded by examination of thick and thin blood films. Broad spectrum antibiotic therapy administered during the diagnosing process included Cefuroxime 3 750 mg/day i.v. and Netilmycin 2 150 mg/day i.v. for 5 days followed by Imipenem 3 1 g/day i.v. for 7 days, but remained *sine effectu*.

Clinically non-progressive splenic enlargement (200 mm) was visualized and measured by abdominal ultrasound which also showed an enlarged liver. Bone marrow aspiration, ordered to exclude aleukemic leukemia and malignant non-Hodgkin's lymphoma, revealed only mild plasmocytosis (7.5%) (Figure 1), while Leishmania amastigotes could be found neither in the bone marrow aspirate nor in the bone marrow biopsy specimen.

The patient's condition worsened with persisting fever accompanied by sweats and discomfort in the left hypochondrium. In order to exclude primary splenic

TABLE 1
LABORATORY TEST RESULTS ON ADMISSION, AFTER THERAPY AND ONE YEAR LATER

Laboratory tests	On admission	After 21 days of therapy	One year later
ESR (mm/h)	98	35	9
E (10^{12}/l)	3.7	4	4.8
Hb (g/L)	98	118	141
WBC (10^9/L)	2.2	4.2	6.6
Lymphocytes (%)	44	40	39
Platelets (10^9/L)	62	205	297
dctlparPlasma cells (%)	4	0	0
Atypical cells (%)	13	2	1
Blast cells (%)	2	0	0
Total serum proteins (g/L)	93	–	80
Albumins (g/L)	39.1	–	44
Gamma globulins (g/L)	36.4	–	14
LDH (IU/L)	505	382	195

lymphoma we decided to do splenic aspiration biopsy. Unexpectedly, Leishmania amastigotes were found in the splenic aspirate (Figure 2), but not in the bone marrow aspirate and biopsy specimens. Immunofluorescent assay (IFA) for Leishmania

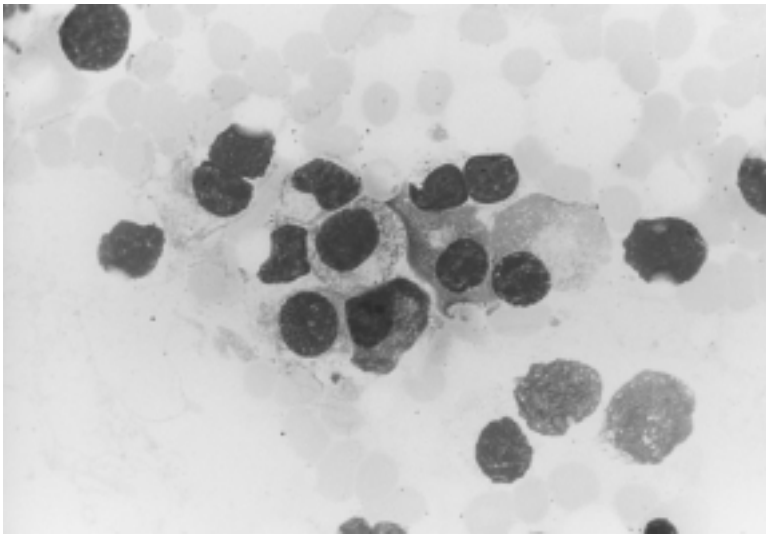

Fig. 1. Bone marrow plasmocytosis – aspiration biopsy smear (1000, May-Grünwald-Giemsa stain).

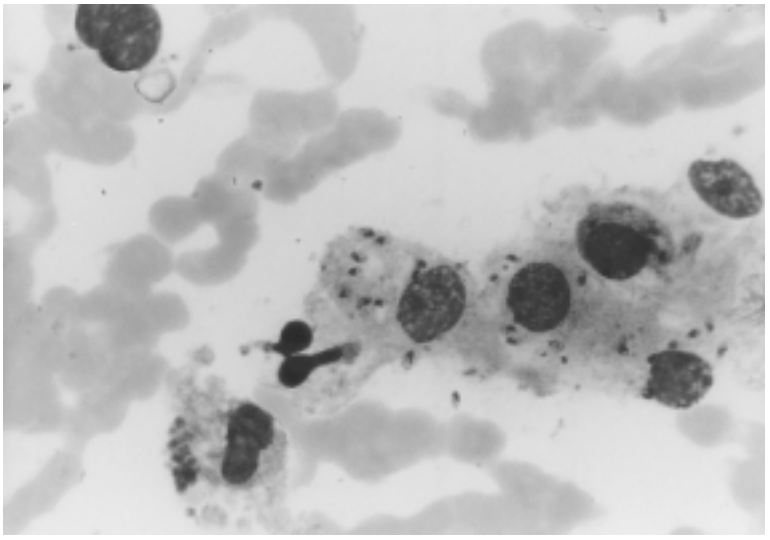

Fig. 2. Leishmania amastigotes in the splenic aspirate (1000, May-Grünwald-Giemsa stain).

showed an IgG titre of 1:2560 and the Formol gel reaction was also positive.

A repeated analysis of the epidemiological history indicated that the patient resided in a military setting located in the non-endemic region of the Velebit mountain range (Northern Adriatic) for two years and 4 months.

Treatment was initiated with Pentostam (pentavalent antimony derivate) 850 mg/day for 21 days. On the third day of Pentostam therapy the body temperature fell to the normal value and the patient's condition continued to improve. On the 12th day of Pentostam therapy the patient got Staphylococcus aureus pneumonia. Ceftriaxon therapy was administered and the body temperature normalized after three days. Our patient left the hospital after 50 days of hospitalization, feeling very well and being afebrile. The spleen was palpable 1 cm below the costal margin. We registered an improvement in the laboratory findings: ESR 35 mm/h, WBC 4 10^9/L, lymphocytes 40%, LDH 382 U/L, atypical cells 2%, blast cells disappeared. IFA IgG antibody titre to Leishmania was 1:256 six months after the diagnosis was made. However, after one year the spleen was not palpable and the laboratory tests were normal (Table 1).

Discussion

A certain protraction of this patient's diagnostic process has been partly caused by previous diagnostics which ruled out most of the epidemiological problems and infectious diseases in the mountainous region of Velebit and contemporary military setting where the patient spent two years and four months prior to the admission to our Department of Hematology. Other non-hematological disorders were also considered, shifting our attention to the possible hematological causes of the leading symptoms. Therefore, our diagnostic algorithm was targeted to exclude hemoblastosis, lymphoma or aplastic anemia primarily, despite the fact that fever, splenomegaly and pancytopenia were also attributable to visceral leishmaniasis. In addition to a number of more frequent disorders, especially within the chronically stressed populations in the post-war years, an atypical epidemiological history and non-endemic status of the Velebit mountain range, certainly affected our diagnostic algorithm in this unexpected case.

Conventional approaches to the diagnosis of visceral leishmaniasis include several serological methods and microscopic examinations of the material obtained from the bone marrow, lymph nodes and spleen. The bone marrow may be infiltrated with parasitized macrophages which usually rupture on smearing and free parasites must be searched. In this case, laboratory examination, as well as bone marrow aspiration and biopsy, excluded hemoblastosis but no Leishmania amastigotes were found. To rule out primary splenic lymphoma we considered spleen biopsy which is also a diagnostic procedure in visceral leishmaniasis. Although the examination of the material obtained from the spleen detects parasites in 95–99% of cases[1,2], splenic aspiration is an invasive procedure with hemorrhage as its possible complication. Follo - wing our criteria for the spleen aspiration biopsy (prothrombin time preferably within normal values or less than 5 seconds longer than in the normal control (10–14s), and platelet count not less than 80 10^9/L), in order to optimize the safety of the procedure, K vitamin and platelet transfusions were administered prior to the splenic aspiration. We were surprised to find Leishmania amastigotes in the spleen macrophages since there were no parasitized cells found in the bone marrow specimens which might have also been diagnostic for the disease. In this case, splenic aspiration biopsy was the

key diagnostic procedure, confirmed later by serological tests.

In the past two decades there has been a resurgence of visceral leishmaniasis in the regions where it was eradicated after the widespread use of chlorophenothane (DDT) in malaria prevention. Phlebotomine populations were greatly reduced around houses but zoonotic transmission was not affected. When the use of residual insecticides was discontinued in many Mediterranean countries, transmission to people was reestablished with an increase in the number of leishmania cases[4]. Rare autochthonous cases of visceral leishmaniasis in the middle and southern Dalmatia have recently been described in literature[5,6], as well as cases of imported visceral leishmaniasis from southern Croatia into the neighboring countries. However, these sporadic cases hardly represented a significant public health problem[7], even in the endemic regions. There have been no leishmania cases reported in the northwestern, littoral part of the Adriatic coast where the infection is considered extremely rare and the region non-endemic. However, outbreaks of visceral leishmaniasis have often followed famine, wars, civil and political disturbances resulting in malnutrition and migration of populations. It is also necessary to underline the existing differences in the clinical course of the disease: some infected individuals develop subclinical infection and others clinical disease. Reasons responsible for differences in the outcome of infection are not known, but possible factors include: variations in the species of parasite and the intensity of transmission, nutritional status of the patient (malnourished infected children develop clinical disease, while equally exposed well-nourished children develop subclinical infection), or combinations of these or other factors.

According to the epidemiological data in the medical history of our patient and the usual length of the incubation period, there is strong ground to suspect an autochthonous infection in this region, although the possibility of a previously acquired infection with a long incubation period and clinical manifestation of the disease after more than three years spent in a chronically stressful, non-endemic environment, cannot be completely ruled out. A combination of factors including stress, malnutrition and a modified immune response might have played an important role in any of these scenarios.

Considering the changes in climate, higher temperatures are likely to accelerate maturation of the protozoal parasite, thereby increasing the risk of infection. The spread of vectors from the adjacent southern regions seems logical, although multidisciplinary studies onsite should backup these speculations in the future[7–11]. In addition to the changes in microclimate, it would be interesting to investigate possible changes in the distribution and relations between reservoirs and vectors responsible for the transmission of the disease. Future studies of the medically relevant entomofauna, not adequately investigated in Croatia[7], could concentrate on the possible spread of sandflies along the northern coast and adjacent mountains, which would confirm the possibility of the autochthonous acquisition of the disease in the Velebit region.

Considering the increased number of autochthonous and imported cases of visceral leishmaniasis[8–14], its chronic clinical course and a high mortality rate if not recognized, pancytopenia, splenomegaly and fever should be timely associated and serologically tested for visceral leishmaniasis even in non-endemic regions, and during diagnostic efforts reasonably aimed at non-infective diseases of similar presentation. The importance of the epidemiological history and continuous evaluations is crucial[15], therefore, this case

and the emerging reports raise a considerable amount of questions for both, epidemiologists and clinicians, to be answered by the future analyses that might help reconsider the leishmania endemic regions in Croatia.

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PRIKAZ SLUČAJA VISCERALNE LIŠMENIOZE NA PODRUČJU SJEVERNOG JADRANA

SAŽETAK

Na Hematološkom odjelu u Rijeci hospitaliziran je 33-godišnji muškarac zbog povišene temperature, splenomegalije, pancitopenije i limfocitoze. Laboratorijski nalazi, punkcija i biopsija koštane srži isključili su hemoblastozu i aplastičnu anemiju. Zbog sumnje na primarni limfom slezene, učinjena je aspiracijska biopsija slezene te su u makrofagima pronađeni amastigoti lišmenije. Do sada nije opisan slučaj pojave visceralne lišmenioze na području sjevernog Jadrana, iako se ista javlja endemski u srednjoj i južnoj Dalmaciji. Prema epidemiološkoj anamnezi, moguća je infestacija na području Velebita.