International Journal of Infectious Diseases 34 (2015) 46–50

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Current clinical, laboratory, and treatment outcome characteristics of visceral leishmaniasis: results from a seven-year retrospective study in Greece

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ARTICLE INFO

Article history:
Received 30 December 2014
Received in revised form 24 February 2015
Accepted 25 February 2015

Keywords:
Visceral leishmaniasis
Parasite
Liposomal amphotericin
Haemophagocytic syndrome
PCR

SUMMARY

Objectives: Visceral leishmaniasis (VL) is re-emerging in endemic areas. The epidemiological, clinical, laboratory, and treatment outcome characteristics in a large cohort of VL patients is described herein.
Methods: The cases of 67 VL patients (57% male, mean age 56 years) treated in two Greek hospitals over the last 7 years were identified and evaluated retrospectively.
Results: Forty-six percent of patients reported contact with animals. Seventeen patients (25%) were immunocompromised, and 22% were co-infected with another pathogen. Sixty-four percent of patients had fever, 57% had weakness, 37% had sweats, 21% had weight loss, and 13% had a dry cough, while 6% developed haemophagocytic syndrome. The median duration of symptoms was 28 days. Fifty-eight percent of patients had splenomegaly, 49% had hepatomegaly, and 36% had lymphadenopathy. The diagnosis was established by positive PCR in peripheral blood (73%) and/or bone marrow specimens (34%). Sixty-one patients (91%) received liposomal amphotericin (L-AMB). Six patients (10%) did not respond or relapsed but were eventually cured after a second cycle of L-AMB. During a 6-month follow-up, the overall mortality was 9%, although none of these deaths was attributed to VL.
Conclusions: VL is still a common disease in endemic areas, affecting immunocompetent and immunocompromised patients. Its diagnosis is challenging, and molecular techniques are valuable and helpful tools to achieve this. Treatment with L-AMB is safe and very effective.

1. Introduction

Visceral leishmaniasis (VL) is an endemic and potentially life-threatening disease in the tropics, subtropics, and Mediterranean basin, including Greece. It is characterized by a broad range of clinical and laboratory findings, such as fever, cachexia, hepatosplenomegaly, pancytopenia, hypergammaglobulinemia, and hypoalbuminemia.[1]

Leishmaniasis is a mandatory notifiable disease in Greece.[2] *Leishmania infantum* is the species responsible, while the most common vectors are *Phlebotomus neglectus*, *Phlebotomus tobbi*, and *Phlebotomus perfiliewi*.[3,4] Nevertheless, the true prevalence of VL is probably underestimated, as infectious diseases are generally under-reported in many countries, including Greece.[5,6]

Data on VL in Greece are scarce. Therefore, the aim of this study was to report the epidemiological and clinical characteristics, risk factors, diagnostic tools, treatment, and outcome of VL among patients treated in two tertiary care Greek hospitals during the last 7 years.[2,3,7–9]

2. Patients and methods

All adult patients (age >14 years) with well-established VL diagnosed at the Department of Medicine of the University General Hospital of Larissa, and the Infectious Diseases Unit of the Pathophysiology Department of the General Hospital of Athens "Laikon" from January 1, 2007 to December 31, 2013, were evaluated. The patients' electronic records/written charts were

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http://dx.doi.org/10.1016/j.ijid.2015.02.021

reviewed for demographic characteristics, underlying diseases, laboratory parameters, treatment outcome, and the mortality rate at 24 weeks after diagnosis. The clinical manifestations and potentially concurrent infections were also recorded.

An index patient was defined according to international criteria as a confirmed VL case in the presence of clinical and laboratory manifestations compatible with VL (i.e., two or more of persistent fever >38 °C, hepatosplenomegaly, substantial weight loss, anaemia, leukopenia, polyclonal hypergammaglobulinemia, or lymph node enlargement), along with a positive result for two of the following three tests: serology (immunofluorescence antibody test), demonstration of parasite by smear in tissue samples (i.e., bone marrow smear, lymph node), or molecular techniques.[1,10]

Regarding molecular techniques, DNA extraction was performed from 2 ml of blood or 1 ml of bone marrow, which was suspended in 1 ml phosphate buffered saline (PBS) and layered over Biocoll Separating Solution (Biochrom, Germany). After centrifugation at 1800 rpm for 30 min, the interface was collected and suspended in 8 ml of PBS. The suspension was centrifuged for 10 min at 2000 rpm and the supernatant was discarded. The pellet was re-suspended in 0.2 ml of PBS. DNA extraction was then performed using the QIAamp DNA Mini Kit (Qiagen, Germany), following the manufacturer's instructions. The DNA was eluted in 0.1 ml of buffer AE. A simple PCR reaction amplifying a fragment of the SSU rRNA gene of Leishmania was used for detection, as described previously.[11] For *Leishmania* species identification, another previously described PCR method was also available.[12]

The patient was considered as immunosuppressed when there was a history of malignant disease (haematological malignancy or solid tumour) or a history of inherited or acquired immunodeficiency (e.g., splenectomy, HIV infection) or was under treatment with immunosuppressive agents (e.g., corticosteroids, azathioprine, methotrexate, and chemotherapeutic or biological agents). With regard to corticosteroids, immunosuppression was considered if the patient had received a dose of ≥5 mg of prednisone or equivalent, every day, for at least the last 30 days. Anaemia was defined when haemoglobin was <12 g/dl in females and <13 g/dl in males. Leukopenia and neutropenia were defined when leukocyte and neutrophil counts were less than 4×10^9/l and 1×10^9/l, respectively. Thrombocytopenia was defined when the platelet count was less than 140×10^9/l. Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), C-reactive protein, albumin, and gamma-globulins, and the erythrocyte sedimentation rate (ESR), were determined using standard techniques.

The treatment schedule for liposomal amphotericin (L-AMB) was administered according to the US Food and Drug Administration (FDA)-approved regimen for immunocompetent (3.0 mg/kg on days 1 to 5, 14, and 21) and immunosuppressed patients (3–5 mg/kg daily or intermittently for 10 doses (days 1–5, 10, 17, 24, 31, and 38) up to a total dose of 40–60 mg/kg).

Clinical response was assessed at the completion of treatment and was defined as cure (defervescence, restoration of laboratory parameters, or significant reduction in spleen size) or failure (persistent or worsening of clinical and laboratory findings). Relapse was defined as the reappearance of signs and symptoms of the disease within 6 months, in association with the identification of the parasite in a bone marrow smear, after initial successful treatment.

The study was approved by the institutional review boards of the two tertiary medical centres. All participants gave their informed consent.

3. Results

Over the 7-year study period, 67 patients were identified with well-established VL (56 in Larissa and 11 in the Athens medical centre); their mean ± SD age was 56.1 ± 19.5 years and 38 were males. The age distribution of the patients is shown in Figure 1. The main clinical, physical, and laboratory findings of the patients are shown in Table 1. Forty-four patients (66%) were living in rural areas and 31 (46%) had been in frequent contact with animals (strays or domestic dogs). At diagnosis, 17 patients (25%) were considered immunocompromised; seven of them were under immunosuppression with corticosteroids, azathioprine, methotrexate, or anti-tumour necrosis factor regimens due to autoimmune rheumatic diseases, seven had an active underlying malignancy, and one patient each had a history of splenectomy, hypogammaglobulinemia, and HIV infection. Moreover, nine patients (13%) suffered from diabetes mellitus.

The median duration of symptoms at diagnosis was 28 days (range 1–240 days). Overall, 56 patients (84%) reported low-grade fever, whereas 43 (64%) had a fever >38 °C. Thirty-eight patients (57%) reported weakness, 14 (21%) loss of weight, and nine (13%) a dry cough. Of interest, one patient presented only with fever and a single intranasal bleeding lesion and two patients presented with prolonged fever and neurological symptoms (acute polyradiculitis and sensory-motor axonal neuropathy, respectively). Two patients were recorded as asymptomatic, as the first patient had only splenomegaly and the second had enlarged inguinal lymph nodes.

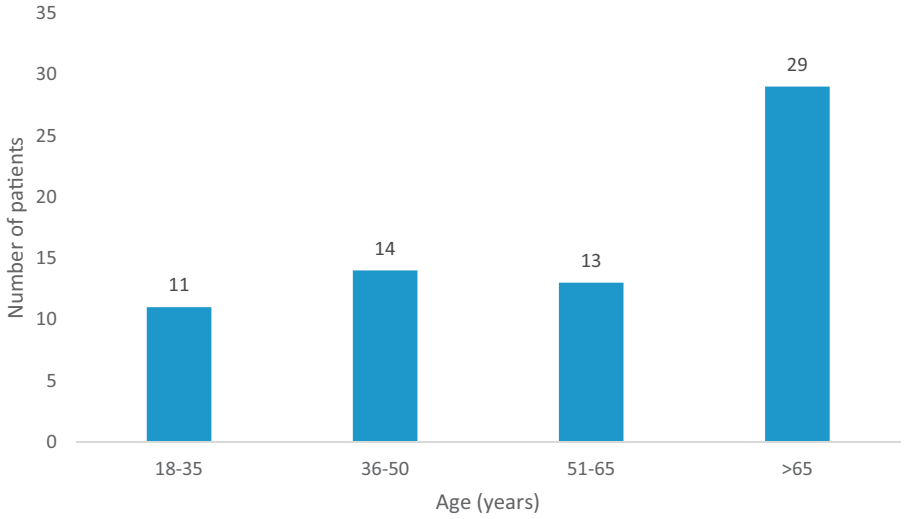

Figure 1. Distribution of patients with visceral leishmaniasis according to age.