Mediterranean visceral leishmaniasis in HIV-negative adults: a retrospective analysis of 64 consecutive cases (1995–2001)

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Aim: To evaluate in a retrospective analysis cases of Mediterranean visceral leishmaniasis (VL) diagnosed in HIV-negative adults during a 7-year period.

Materials and methods: Demographic data, previous or underlying diseases, clinical and laboratory features and therapeutic findings were considered.

Results: A total of 64 patients were included, of whom 10 (16%) had underlying diseases and two were pregnant. Fever and hepatosplenomegaly were the main presenting symptoms, whereas pancytopenia and an elevated erythrocyte sedimentation rate were observed in all cases. Smears from bone marrow aspirate were positive at microscopy in 62 cases (97%). Twenty-four patients received meglumine antimoniate (MA) given during 21 consecutive days (20 mg/kg per day), and 40 patients liposomal amphotericin B (l-AmB) given at days 1–5 and 10 (3 mg/kg per day). Both groups' clinical and laboratory findings improved, but patients on l-AmB therapy had a faster recovery (85% on l-AmB therapy and 50% on MA therapy showed defervescence at day 5, \( P < 0.01 \)). Treatment failures were observed in five cases, three (12%) on MA and two (5%) on l-AmB therapy. No significant toxicity was observed in patients treated with l-AmB, whereas three (12%) patients treated with MA showed electrocardiographic abnormalities.

Conclusions: l-AmB therapy may be considered the treatment of choice for any adult patients with Mediterranean VL, since it permits a faster recovery, has a lower incidence of side effects and is useful also in immunosuppressed patients.

Keywords: visceral leishmaniasis, liposomal amphotericin B, meglumine antimoniate, Leishmania infantum, treatment

Introduction

Visceral leishmaniasis (VL) is a disseminated protozoan infection transmitted by phlebotomine sandflies, caused by Leishmania donovani in the Indian subcontinent and East Africa and by Leishmania infantum (syn.: Leishmania chagasi) in both the Old and New Worlds.1,2 In Mediterranean countries, about 1000 people are affected annually, with high prevalence in children and an increasing rate in immunocompromised and immunosuppressed subjects, such as HIV-positive and transplant patients.3,5

Meglumine antimoniate (MA) and sodium stibogluconate have been the traditional antimonial drugs for VL treatment. However, the increasing rate of resistance reported in some areas with high prevalence of VL,5 and the incidence of serious adverse events7,8 have limited the use of antimonial drugs and promoted alternative treatments, such as amphotericin B (AmB).9,10 Because renal toxicity of the conventional drug formulation limits daily dosage, lipid formulations of AmB, i.e. liposomal (l-AmB), colloidal dispersion and lipid complex, have been developed and found effective and safe since they result in higher concentrations in liver and spleen tissue macrophages and are poorly recovered in the kidney.11–13

Currently, l-AmB is approved by the U.S. Food and Drug Administration for the treatment of VL,14 whereas in Italy and other Mediterranean countries, where resistance to antimonials has been rarely reported,15 it is still under evaluation in cost-effectiveness studies. Because no studies have compared the efficacy of l-AmB versus MA
Mediterranean visceral leishmaniasis in HIV-negative adults

in HIV-negative adult patients with Mediterranean VL, we present here our experience of cases observed during a 7-year period, with particular reference to efficacy, tolerability and long-term response to treatments with MA or l-AmB.

Materials and methods

A retrospective analysis was carried out on HIV-negative adult patients with diagnosis of VL referred to our Division of Infectious Disease at the ‘D. Cotugno’ Hospital, Naples, and admitted between January 1995 and December 2001.

Inclusion criteria for the study were: (i) no history of previous VL; (ii) presence of clinical signs of VL, mainly fever (defined as a body temperature >37.5°C) and/or hepatosplenomegaly; (iii) demonstration of Leishmania from Giemsa-stained smears of bone marrow or spleen aspirates, or from cultures carried out with this material; (iv) treatment with MA at a dosage recommended by the World Health Organization16 for Leishmania infantum infection (Sb 20 mg/kg per day × 21 days) or l-AmB at a dosage which had proved highly effective in adult Italian patients in a multicentre trial11 (3 mg/kg at days 1–5 and 3 mg/kg at day 10); (v) post-therapy follow-up of at least 12 months. Exclusion criteria were: (i) age < 14 years; (ii) use of other lipidic formulations of AmB; (iii) co-infection with HIV.

At admission, we considered demographic data (age, sex and residence), previous or underlying disease(s), presenting signs and symptoms (fever, hepatic and spleen size), non-specific laboratory data such as full blood count, gammaglobulin concentration, erythrocyte sedimentation rate (ESR), blood urea and creatinine, and specific laboratory data such as antileishmanial antibody titre determined by the immunofluorescent antibody test (IFAT), culture results from bone-marrow and splenic aspirates, and zymodeme analysis of Leishmania strains obtained.

Laboratory assessments including full blood count, ESR and IFAT titre were considered at the end of therapy, and monthly during the subsequent follow-up period.

A second marrow aspirate was scheduled 2–6 weeks after the end of therapy.

Patients were considered cured when symptoms disappeared, no Leishmania was demonstrated at a second bone-marrow aspirate (when available) and IFAT titre decreased at each determination. A VL relapse was defined as recurrence of symptoms associated with demonstration of parasites on smears or cultures of bone marrow or splenic aspirate after apparently successful treatment.

Efficacy of therapy was evaluated in respect to the drug received (MA group versus l-AmB group). Student’s paired t-test, Fisher’s exact test, Kaplan–Meier method and log-rank test were employed as appropriate for significance in the data analysis.

Results

A total of 67 consecutive patients were diagnosed as having VL during the study period. Three of them (4%) were not included in the study because they had already been treated with a lipid-complex formulation of AmB (two patients) or were lost to follow-up (one patient). Hence, we included in the study 64 patients (59% males) aged 14–82 years (median 40 years).

Underlying conditions were reported in 12 patients (19%). Three patients were affected by chronic hepatitis, two by diabetes mellitus, two by chronic renal failure (one of them had end-stage renal failure and underwent haemodialysis three times weekly), one patient by hereditary spherocytosis, one by pulmonary tuberculosis, one patient was malnourished and two patients were pregnant at the time of the diagnosis.

The onset of clinical symptoms varied between 2 and 26 weeks (median 5 weeks) before diagnosis. Fever was present in 60 cases (94%), asthenia in 44 (69%), weight loss in 33 (52%) and abdominal pain in 25 (39%).

At admission, all patients had hepatosplenomegaly, two (3%) jaundice, one (2%) concomitant lobar pneumonia.

Twenty-four (37%) patients received MA and 40 (62%) l-AmB at the dosages currently recommended.11,16

Underlying conditions were recorded among three (12%) patients on MA and nine (22%) on l-AmB therapy. Both pregnant patients were treated with l-AmB. Figure 1 shows the relationship between patients’ admission and treatment during the study period. Patients were treated mainly with MA until 1998; subsequently, l-AmB was the only drug administered.

Table 1 reports the main demographic, clinical and laboratory findings at admission. Patients, divided here by treatment group, were comparable in respect to all variables considered. Haemoglobin concentration below 7 g/dL was observed in five patients (8%) (three from MA group and two from l-AmB group). Three cases (5%) had signs of renal failure and two (3%) had alanine-aminotransferase levels over three times greater than normal values. Gammaglobulin expressed as % of total blood proteins ranged between 15.2 and 64.1 (median 35.5), without any statistical difference between the groups of patients.

Table 2 reports the main parasitology data at admission. Anti-Leishmania antibodies were present in all reported cases, with a range between 1/160 and 1/20 480 (median 1/640). In 62 patients (97%), amastigotes were observed from Giemsa-stained smears of bone marrow aspirate. In the remaining two (3%) patients, parasite demonstration was obtained by culture of bone marrow aspirate and by Giemsa-stained smear of spleen aspirate, respectively. Cultures of Leishmania were attempted for 33 patients and found positive in 13 cases. Zymodeme analysis was carried out on seven established isolates; six were characterized as L. infantum zymodeme Montpellier (MON) 72 (i.e. a typical genotype of the Naples area17), and one as L. infantum MON 1 (the commonest genotype in the Mediterranean area).

Table 3 reports changes from baseline data of laboratory results obtained at the end of treatment (day 21 for patients treated with MA, day 10 for patients treated with l-AmB). Both groups’ clinical and
laboratory findings improved, without any significant difference in respect to treatment. Defervescence occurred between day 1 and 22 (median 5 days) from the first drug administration in patients of the MA group, and between day 1 and 15 (median 3 days) in patients of the l-AmB group. Furthermore, after the first 5 days of therapy, defervescence occurred in 12 patients (50%) of the MA group and in 34 (85%) of the l-AmB group ($P < 0.01$). Figure 2 reports Kaplan–Meier curves of defervescence in respect to treatment, which show a significantly faster recovery in patients treated with l-AmB (log-rank test analysis, $\chi^2 = 4.34, P = 0.037$).

A post-therapy bone-marrow aspirate was carried out in 38 patients (16 in the MA group and 22 in the l-AmB group) and found negative in all cases.

Treatment failures were observed in five cases, three (12%) on MA therapy and two (5%) on l-AmB therapy. Four of these patients had a relapse 6–24 months after the end of treatment and one died after 10 days of MA therapy. This patient had been diagnosed as having VL 11 months before our observation and refused treatment until he was admitted to our division. An increase in IFAT titre was observed in all relapsing patients at least 2 months before the clinical evidence of VL recurrence. All relapsing patients were treated with a 10-day course of l-AmB at the dosage of 3 mg/kg per day and recovered fully. Among the 12 patients with underlying conditions, only one (8%) relapsed, a patient who had chronic renal failure and received a complete course of l-AmB. Both pregnant patients showed a complete and long-term response. No abnormality was observed in newborns, who were disease-free after 2 years of follow-up.

Treatment regimens were well tolerated and patients were treated with a full dosage of both drugs; no significant biochemical toxicity was observed. Three (12%) patients treated with MA had electrocardiographic abnormalities that did not require withdrawal from treatment but closer monitoring. No patient on l-AmB therapy had phlebitis at the infusion site.

Discussion

Mediterranean VL has a high prevalence in areas of southern Europe.\(^1\) During the study period, both incidence and age/sex distribution of the disease did not change in our region, from which most of our

| Table 1. Main demographic, clinical and laboratory findings at admission |
|-----------------------------|-----------------------------|-----------------------------|
| Parameter                  | Group MA                    | Group l-AmB                 | $P$ value |
| Age (years)                | 35.1 ± 13                   | 39.6 ± 17                   | N.S.     |
| % Males                    | 58                          | 65                          | N.S.     |
| % Immunosuppressed         | 12                          | 22                          | N.S.     |
| Spleen size (cm)\(^a\)     | 8.1 ± 3.6                   | 7.4 ± 2.0                   | N.S.     |
| Haemoglobin (g/dL)         | 8.37 ± 1.0                  | 9.31 ± 1.4                  | N.S.     |
| ESR (mm/h)                 | 81.4 ± 27                   | 77.8 ± 24                   | N.S.     |
| White blood cells (×10\(^3\) cells/mm\(^3\)) | 2.54 ± 0.74                 | 2.88 ± 0.8                  | N.S.     |
| Platelets (×10\(^3\) cells/mm\(^3\)) | 110 ± 36                    | 116 ± 52                    | N.S.     |

Data are expressed as mean ± s.d.; MA, meglumine antimoniate; l-AmB, liposomal amphotericin B. \(^a\)Spleen size was measured from left costal margin.

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<tr>
<th>Table 2. Parasitological findings at admission</th>
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<tr>
<td>Anti-Leishmania antibodies</td>
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<tr>
<td>BM aspirate smear</td>
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<td>BM aspirate culture</td>
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<td>Spleen aspirate smear</td>
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BM, bone marrow.

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<th>Table 3. Improvement of clinical and laboratory findings at the end of treatment</th>
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<td>Parameter</td>
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<tr>
<td>Haemoglobin (g/dL)</td>
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<td>ESR (mm/h)</td>
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<td>Spleen size (cm)</td>
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Mediterranean visceral leishmaniasis in HIV-negative adults

patients originate. About 20% of our patients had underlying conditions associated with some degree of immunodepression (chronic hepatitis, chronic renal failure or diabetes mellitus) that may have predisposed them to clinical disease. These findings indicate an early evaluation of VL in differential diagnosis of febrile patients with chronic diseases living in an endemic area.

We also observed two patients who were pregnant at the time of diagnosis. Alterations of immune response that may favour VL and other infections sustained by intracellular pathogens have been reported in pregnancy.18,19 A careful evaluation of the diagnosis of leishmaniasis in pregnant women and newborns with fever of unknown origin is recommended if a history of exposure in endemic areas has been reported.20

The gold standard of VL diagnosis is parasite demonstration in bone marrow aspirates. In our study, we have reported a smear positivity rate exceeding 95%. On the other hand, bone marrow cultures are ineffective for a rapid VL diagnosis since they may become positive after several weeks, and can have low sensitivity (39% in our study) if not properly carried out; however, a bone-marrow aspirate culture was essential to diagnose VL in one case with negative smear. Furthermore, having identified L. infantum MON 72 in cultured organisms from six out of seven patients investigated, we confirmed that most of our cases contracted the infection in our area.17

We also investigated the value of IFAT serology in diagnosing VL. The efficacy of this method in the screening of potentially infected patients and in the post-treatment follow-up has been confirmed in our cases (100% sensitivity), but variability of titre and the occurrence of cross-reactions may result in some degree of non-specificity. However, the value of serology may be relevant during the post-therapy follow-up, since all relapsing patients showed an increase in IFAT titre before the recurrence of VL symptoms.

Efficacy of treatment with AmB, either in conventional or in lipid formulations, has been thoroughly investigated, but no controlled clinical trials have compared efficacy and safety of l-AmB versus MA in the treatment of HIV-negative patients with Mediterranean VL.21 In our study, we reported a cure rate exceeding 80% in both groups of treatment, but time to defervescence was shorter in patients treated with l-AmB and over 80% of these patients had no fever by day 5 of treatment. Furthermore, haematological parameters reported at the end of treatment showed equal improvement in both groups, unless l-AmB therapy was conducted for a shorter period in respect to MA.

No significant side effects were reported in patients on l-AmB therapy, whereas electrocardiographic abnormalities were seen in three cases treated with MA. This observation has to be considered in treating patients with renal failure and abnormalities in serum electrolyte balance, who may be considered at high risk for antimonal related cardiac toxicity.22

The advantages of l-AmB over MA in the treatment of Mediterranean VL are poorly sustained by the response rate since the drugs show equal efficacy and resistance to antimonia is rarely reported in the Mediterranean area.23 The main advantage of l-AmB in developed countries is the reduction of the long hospitalization period. Patients on l-AmB therapy have faster resolution of symptoms and may be discharged even after 5 days of therapy, receiving the sixth dose in a day care centre. Instead, life-threatening side effects of MA therapy, mainly cardiac abnormalities such as arrhythmias, and a slower resolution of symptoms warrant a close evaluation of patients on MA therapy.24

At present, the high cost makes l-AmB use impracticable in less developed countries. In developed countries, reduction of hospitalization period may balance the high cost of the treatment. In our department, VL therapy accounts for 4100 Euros for patients treated with l-AmB and for 4200 Euros for patients on MA treatment (assuming a patient weight of 70 kg and a 5-day hospitalization period for patients on l-AmB and a 21-day period for patients on MA).

In conclusion, l-AmB therapy should be considered the treatment of choice for any adult patients with Mediterranean VL for reduction of treatment period and lower rate of side effects.

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References