Liposomal amphotericin B versus pentavalent antimony salts for visceral Leishmania in children

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The aim of this study was to investigate the efficacy of a 21-day schedule of liposomal amphotericin B compared to pentavalent antimony salts in the treatment of patients during a first episode of visceral leishmaniasis.

In this study, 17 cases of visceral leishmaniasis admitted to Behçet Uz Children's Hospital between January 2005 and April 2012 were reviewed retrospectively. The study group was composed of 11 males (64.7%) and 6 females (35.3%). One group included 11 patients who were treated with pentavalent antimony salts, sodium stibogluconate or meglumine antimoniate, intramuscularly for 28 days. The second group was treated with amphotericin B intravenously at a dosage of 3 mg/kg on days 1-5, 10 and 21 (a cumulative dose of 21 mg/kg/day). While pentavalent antimony salts were found to increase biochemical and hematological findings, liposomal amphotericin B was responsible for rapid recovery in fever and shorter hospital stay. As a result, our study shows the advantages of both medications independent of their costs.

Key words: childhood, visceral leishmaniasis, liposomal amphotericin B, pentavalent antimony salts.

Visceral leishmaniasis (VL) is a life-threatening systemic infection caused by protozoa of the genus Leishmania and is transmitted by phlebotomine sandflies. Leishmania is widespread in most countries in the Mediterranean basin, including Turkey. Leishmania infantum is responsible for most VL cases in Turkey[1]. The disease is characterized by prolonged fever, weight loss, splenomegaly, and pancytopenia, and carries a high risk of mortality in the absence of treatment[2,3]. Diagnosis of VL has depended on detection of anti-leishmanial antibodies by serologic tests or demonstration of Leishmania amastigotes in tissue specimens[1]. The conventional treatment of kala-azar consists of pentavalent antimony salts (PAS) - sodium stibogluconate and meglumine antimoniate. However, within the last decade, due to the development of resistance to previous drugs and side effects, amphotericin B deoxycholate and liposomal amphotericin B (L-AMB) have been recommended for treatment of VL despite the cost, which is highly important in under-developed and developing countries[4-12].

The present retrospective study was designed to investigate the efficacy and tolerability of a 21-day schedule of L-AMB, compared to a reference treatment, PAS, in patients during a first episode of VL.

Material and Methods

Dr. Behçet Uz Children's Hospital is one of the tertiary health care hospitals in Turkey. In this study, 17 cases of VL admitted to our hospital between January 2005 and April 2012 were reviewed retrospectively.

The diagnosis of VL was based on the following criteria: clinical picture, indirect immunofluorescence antibody test (IFAT) at a titer of ≥1/64 and demonstration of Leishmania amastigotes in Giemsa-stained bone marrow aspirates. One group included 11 patients who were treated with PAS, sodium stibogluconate or meglumine antimoniate, intramuscularly for 28 days at a dosage 20 mg/kg per day.

The second group was treated with L-AMB (AmBisome®) intravenously at a dosage of 3 mg/kg on days 1-5, 10 and 21 (for a cumulative dose of 21 mg/kg).

Patients whose symptoms and clinical and laboratory findings diminished (disappearance of fever, decrease in spleen size, normalization of laboratory findings) were accepted as full response. Therapy failure was defined as parasite persistence in any sample after a complete course of therapy. Relapse was defined as the reappearance of clinical symptoms of disease plus the presence of amastigote forms of Leishmania in bone marrow smears after initial successful treatment[1]. The hospital stay and the time required for the recovery of fever were calculated starting with the administration of one of the two drugs.

The statistical analysis was performed using the Statistical Package for the Social Sciences version 15 (SPSS Inc, Chicago, IL, USA). The proportions of patients with treatment success were compared among treatment groups by means of the Fisher's exact test. Numerical variables such as the patient's age, hospital stay and duration required for recovery were expressed as means ± standard deviation, and comparisons of the two groups were performed by t-tests. The changes over time in hemoglobin (Hb), white blood cell (WBC) count and platelet (PLT) count were analyzed with paired t-tests. A p value of <0.05 was considered to be significant.

Results

Clinical Features

Totally, 17 patients were included in this retrospective study. The study group was composed of 11 males (64.7%) and 6 females (35.3%). The PAS group included 11 patients, while 6 patients were in the L-AMB group. The median age in the PAS group was 36 months and in the L-AMB group was 33 months, and no statistically significant difference was present between the two groups ($p>0.05$). The symptoms, IFAT titers and bone marrow aspiration results are reviewed in Table I.

The median treatment duration in the PAS group was 28 days and the regimen for L-AMB treatment was alternatively days 1, 2, 3, 4, 5, 10, and 21. The mean duration of the hospital stay was 16±2.7 (minimum 12 - maximum 20) days in the L-AMB group, while it was 30.18±0.98 (minimum 29-maximum 32) days in the PAS group, and hospital stay was significantly longer in the PAS group ($p=0.0003$). The mean time required for recovery of fever in the L-AMB group was 2.17 ± 0.753 days (minimum 1-maximum 3) days and in the PAS group was 4.45 ± 1.50 days (minimum 2 - maximum 7) days, with recovery significantly longer in the PAS group ($p=0.0009$).

A one-way repeated measures ANOVA was conducted to compare WBC, PLT, Hb, and albumin levels (Table II). The mean WBC levels significantly increased in time (in the PAS

Table I. The Clinical and Specific Laboratory Feature Symptoms of the Patients with Visceral Leishmaniasis

Symptoms	Number of cases	%
Fever	17	100
Loss of appetite	10	58.82
Abdominal distension	14	82.35
Abdominal pain	3	17.64
Weight loss	8	47.05
Fatigue	13	76.47
Night sweating	3	17.64
Cough	4	23.52
Headache	1	5.88
Jaundice	1	5.88
IFAT(+)	13	76.47
Amastigotes in bone marrow	17	100

IFAT: Immunofluorescent antibody test.

Table II. Laboratory Changes in the First 15 Days According to the Drug Group

	PAS group (mean ± SD)	L-AMB group (mean ± SD)
PLT count (/mm^3)		
PLT before treatment	78363-11106	133166-25917
3rd day	109363-14280	116500- 31804
7th day	175272-15616	152166-33475
15th day	250727-23302	246666-36395
WBC (/mm^3)		
WBC before treatment	3011-1458	5680-1889
3rd day	3717-1316	3918-1746
7th day	4718-1590	5180-1615
15th day	5790-2091	5480-2502
Hb (g/dl)		
Hb before treatment	5.83-1.40	6.417-3.5997
3rd day	7.39-1.90	8.333-1.7648
7th day	7.9.-2.18	9.216-2.00840
15th day	9.58-1.17	9.300-1.56844
Albumin level (g/dl)		
Before treatment	2.2-0.3	2.8-0.5
15th day	2.8-0.3	2.850-0.6
Total protein level (g/dl)		
Before treatment	7.2-0.7	7.2-1.0
15th day	7.7-0.9	7.3-1.1

PLT: Platelet. WBC: White blood cell. Hb: Hemoglobin. PAS: Pentavalent antimony salts. L-AMB: Liposomal amphotericin B.

group, Fig. 1) ($p<0.05$); however, there was no significant increase in WBC over time in the L-AMB group ($p=0.147$). The mean Hb levels across the 15 days during the treatment had increased in both the PAS group ($p=0.0007$) and L-AMB group ($p=0.043$) (Fig. 2). The mean PLT levels significantly increased in time (in the PAS group, Fig. 1) ($p=0.002$); however, there was no significant increase of PLT over time in the L-AMB group ($p=0.223$) (Fig. 3).

The albumin levels measured on the 7th day ($p=0.002$) and 14th day ($p=0.001$) were found to be significantly higher compared to the initial albumin level before treatment in the PAS group, but no significant difference was present in albumin in the L-AMB group ($p>0.05$). There was no significant change over time in total protein levels in either of the groups ($p>0.05$).

No relapses or unresponsiveness to L-AMB was observed, but there was one non-responder in the PAS group (9%), who later required treatment with L-AMB. The patient experiencing relapse was a two-year-old male child who had been admitted to our hospital with the complaints of fever, pallor, weight loss, and loss of appetite. His fever recovered on the fifth day of admission, but he was diagnosed as relapse 1.5 months after the initiation of PAS, and was retreated with L-AMB.

Discussion

Visceral leishmaniasis (VL) is prevalent in more than 80 countries in Asia, Africa, Southern Europe, and South America[4,13]. It is considered as sporadic in western and central regions of Turkey. According to the Turkish Ministry of Health, 40 cases are reported annually on average in Turkey[14].

The definitive diagnosis of VL is based on the positive culture of the organism and/or demonstration of the amastigotes in Giemsa-stained tissue samples. Bone marrow aspiration is a reliable diagnostic method, and Giemsa-

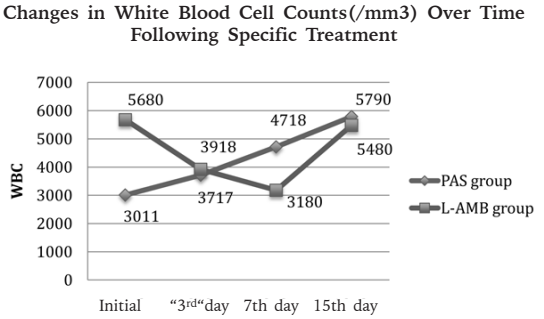

Fig.1. The changes in WBC counts over time: The mean WBC levels significantly increased over time in the PAS group ($p<0.05$); however, no significant increase in WBC over time was observed in the L-AMB group ($p=0.147$).

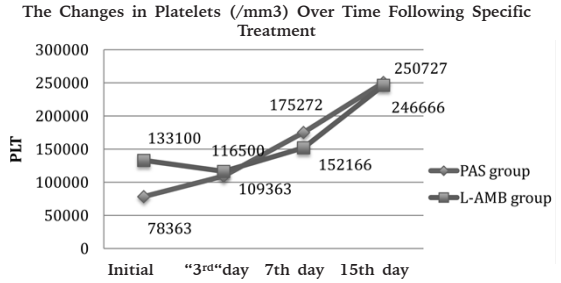

Fig. 3. The changes in PLT count over time: The mean PLT levels significantly increased over time in the PAS group ($p=0.002$); however, there was no significant increase in PLT levels over time in the L-AMB group ($p=0.223$).

stained amastigotes have been reported to be present in 54-86% of VL patients[15-17]. Bone marrow aspirates may be cultured in Novy-MacNeal-Nicolle (NNN) or Schneider insect medium. In Turkey, culture positivity in NNN medium was determined as 16.6% and 14.2%[10,18]. In our study, no culture positivity was present. The methods such as polymerase chain reaction (PCR), IFAT, complement fixation, and hemagglutination are also used for the diagnosis of VL. The sensitivity and specificity of the ELISA test with recombinant K39 antigen are reported as approximately 100%[19,20]. In our cases, elevated IFAT titers were detected in 13 patients (76.5%), and presence of the Giemsa- stained amastigotes in bone marrow aspirates was the mainstay for diagnosis.

Recently, PAS had been widely used as the first-choice of VL treatment for 28 days at a dosage of 20 mg/kg per day[4,5]. Cure rates were reported as more than 90%, but occasionally recurrence or resistance to the treatment

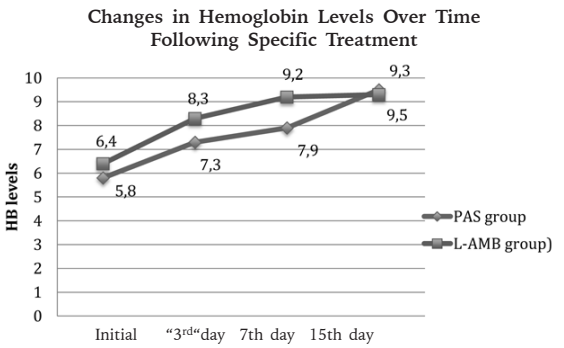

Fig. 2. The changes in Hb over time following treatment: The mean Hb levels had increased in both the PAS ($p=0.0007$) and L-AMB ($p=0.043$) groups during treatment.

regimen was reported to develop. Nearly 10% of the patients were reported to fail to respond to the initial therapy[4,21]. In our study, only one patient did not respond to PAS therapy and required L-AMB treatment. Even though the PAS regimens are effective in the treatment of VL, some disadvantages were reported including prolonged hospitalizations and adverse effects. Although some adverse effects of PAS, such as malaise, myalgia, arthralgia, persistent cough, abdominal pain, increased levels of liver transaminases, amylase and lipase, vomiting, renal effects, and T-wave changes in ECG, have been reported[22], none of these adverse effects was observed in the current study. However, a study conducted in France reported adverse effects in 46.4% of the patients under meglumine treatment[17].

The mean duration of hospital stay was also longer for the PAS group (16±2.7 days for L-AMB and 30.18±0.98 days for the PAS group, $p=0.0003$). In a similar study conducted in Turkey, the mean duration of hospital stay was 22 days in patients treated with amphotericin B; however, all the patients who were treated with meglumine antimoniate were reported to have a hospital stay of at least 31 days[23]. As a result, especially in developed countries, evaluation of hospital costs versus the cost of L-AMB itself should be done, since it provides a shorter hospital stay[24].

In recent years, L-AMB therapy was used in patients with VL who were resistant to PAS treatment[25]. In animal models, L-AMB therapy was found to be 200-400 times more effective than stibogluconate sodium treatment[26]. The aim of therapy is to increase the phagocytosis by macrophages in infected tissues[4]. Amphotericin deoxycholate may be

used intravenously at a dosage of 0.5-1 mg/kg/day for eight weeks for VL treatment. For the cases unresponsive to previous PAS therapy, 1 mg/kg on alternate days for a total of 15 infusions (for 30 days) was reported to succeed, with cure rates of 98%. L-AMB and cholesterol dispersion of amphotericin B were reported to be more effective and less toxic compared to conventional amphotericin B. Plus, L-AMB was found to be five times more effective and 25 times less toxic than conventional amphotericin B in animal models[17].

In a multi-center study of Davidson et al.[24], earlier recovery in symptoms and laboratory findings in patients with VL treated with L-AMB was reported. The same study reported that the patients' fever recovered within a short period of time after starting the treatment. They also reported that short-course treatment regimens of L-AMB were also associated with a shorter hospital stay[24]. In our study, treatment with L-AMB was found to be associated with faster recovery of fever compared to the PAS group, suggesting its clinical effectiveness (p=0.0009).

Unfortunately, there is no specific laboratory analysis to evaluate the response to VL treatment. Thus, resolution of fever, gain in weight, and recovery of splenomegaly and of the hematologic parameters (such as the levels of Hb, PLT, and leukocytes) may help as the appropriate treatment indicators[27,28]. In our study, a significant increase in PLT counts, leukocyte counts, Hb, and serum albumin levels were statistically higher in the PAS group on days 3, 7, and 15 of the treatment compared to the initial values; however, Hb levels were found to increase in time only in the L-AMB group. In contrast to our findings, Davidson et al.[24] reported that a short-course treatment regimen of L-AMB led to a significant rise in the same parameters stated above. In that study, they had not compared results with PAS treatment. However, the small number of patients in our study precludes our giving general suggestions on the efficacy of the treatment options, while a number of unexplained points in VL treatment remain.

In conclusion, despite the limited number of patients in this study, while the use of PAS increases biochemical and hematological findings more rapidly than L-AMB, a rapid recovery in fever and shorter hospital stay were achieved with L-AMB treatment. Thus, the evaluation for selecting one of these drugs should be made taking into account the differences between countries. Our study shows the advantages of both medications were independent of costs.

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