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Visceral Leishmaniasis in 13 Pediatric Patients in Turkey: Treatment Experience

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SUMMARY: Visceral leishmaniasis (VL) is a life-threatening systemic infection caused by protozoa of the genus *Leishmania* and transmitted by phlebotomine sandflies. Leishmaniases are widespread in most countries in the Mediterranean basin, including Turkey. *Leishmania infantum* is responsible for VL in Turkey. We previously reported 19 children with VL who were diagnosed during the period of January 2000 to December 2003. In this study, 13 consecutive cases of VL admitted to our hospital between December 2003 and January 2008 were analysed retrospectively. Fever, splenomegaly and hepatomegaly were most common findings in physical examination while anemia, elevated erythrocyte sedimentation rate and C-reactive protein were the most common laboratory findings. Bone marrow aspirate was obtained in all cases and *Leishmania* amastigotes were detected in 9 of them (69.2%). *Leishmania* antibodies by the immunofluorescent antibody test were positive in all cases. All of the patients were treated initally with meglumine antimonate. Treatment failure occurred in two children, who were subsequently cured with liposomal amphotericin B. One additional child was treated with liposomal amphotericin B because of the side effects of meglumine antimonate. All the children were finally cured. Meglumine antimonate still seems to be the first choice in the treatment of pediatric VL in Turkey.

Key Words: Visceral leishmaniasis, children, Turkey

Türkiye'den Visseral Leishmaniasisli 13 Çocuk Vaka: Tedavi Deneyimi

ÖZET: Visseral leishmaniasis (VL), *Phlebotomus*'larla taşınan *Leishmania* protozoonunun neden olduğu hayatı tehdit eden sistemik bir enfeksiyonudur. *Leishmania*'lar Türkiye'nin de içinde bulunduğu Akdeniz ülkelerinde yaygın olarak görülürler. Türkiye'deki VL'den *Leishmania infantum* sorumludur. Daha önce Ocak 2000-Aralık 2003 tarihleri arasında VL tanısı almış 19 olgu yayınlamıştık. Bu çalışmada Aralık 2003-Ocak 2008 tarihleri arasında hastanemize kabul edilen 13 visseral leishmaniasisli olgu retrospektif olarak incelendi. Hastaların başlıca fizik muayene bulguları ateş, splenomegali ve hepatomegali iken başlıca laboratuar bulguları anemi, artmış eritrosit sedimentasyon hızı ve C-reaktif protein idi. Tüm hastalara kemik iliği aspirasyonu yapıldı ve 9 tanesinde (%69,2) *Leishmania* amastigotları görüldü. Tüm hastaların immun floresan antikor testi ile *Leishmania* antikorları pozitifti. Tüm hastalar başlangıçta meglumin antimonat ile tedavi edildi. Tedaviye cevap vermeyen 2 hasta Amfoterisin B ile tedavi edildi. Meglumin antimonatın yan etkisinden dolayı 1 hasta da liposomal amfoterisin B ile tedavi edildi. Hastaların hepsinde kür sağlandı. Türkiye'deki çocukluk çağı visseral leishmaniasis tedavisinde meglumin antimonat hala ilk seçenek gibi görünmektedir.

Anahtar Sözcükler: Visseral leishmaniasis, çocuk, Türkiye

INTRODUCTION

Visceral leishmaniasis (VL) is a life-threatening systemic infection caused by protozoa of the genus *Leishmania* and transmitted by phlebotomine sandflies (1). VL usually affects young children and adults. *Leishmania* is widespread in most countries in the Mediterranean basin, including Turkey. *Leishmania infantum* is responsible from VL in Turkey (2). Typical VL is associated with prolonged intermittent fever, pallor, massive hepatosplenomegaly, pancytopenia and hypergammaglobulinemia (3). Diagnosis of VL relies on demonstration of *Leishmania* amastigotes in tissue specimens or detection of antileishmanial antibodies by serologic tests (4). Almost all untreated patients die. The pentavalent antimony compounds are the first choice treatment except some regions of the World (5). Lipid formulation of amphotericin B useful in cases of treatment failure with antimonials (5). We reported previously 19 children with VL who were diagnosed in the period of January 2000 to December 2003.

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MATERIALS AND METHODS

Dr Sami Ulus Children's Hospital in Ankara is one of the largest children's hospitals in Turkey. In this study, 13 consecutive cases of VL admitted to our hospital between December 2003 and December 2007 were analysed retrospectively. None of the patients was coinfected with human immunodeficiency virus or known to be immunocompromised. Diagnosis of VL was based on the following criteria; clinical picture, IFAT at a titer of ≥1/64 and demonstration of *Leishmania* amastigotes in Giemsa stained bone marrow aspirates. Meglumine antimonate (Glucantim®) was adminis-tered im for 28 Days at a dosage 20 mg/kg per day. Liposomal amphotericin B (AmBisome®) was administered iv at a dosage of 3 mg/kg on days 1-5,10 and 21. Patients whose symptoms and clinical and laboratory findings diminished (dissappearance of fever, decrease in spleen size, normalization laboratory findings) accepted that they had been cured. 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.

RESULTS

The median age of the 13 patients [8 male (61.5%) and 5 female (38.5%)] was 76 months (range 23- 168 months, mean ± SD, 76 ± 42.9). Most of the patients were between 2 - 10 years old (77%). The symptoms began 7 days to 6 months before admission. The symptoms, epidemiological, clinical features, the presence of amastigotes on bone marrow, IFAT titers and treatment, hematological and biochemical features of the pa-tients with VL are shown in Table 1, Table 2, Table 3 respecti-vely. None of the patients had neither hyperpigmented skin lesion nor lymphadenopathy. One patient had haemophagocytic lymphohistiocytosis in his bone marrow aspiration.

Table 1. The symptoms of the patients with visceral leismaniasis

Symptoms	Number of cases	%
Fever	11	84.6
Loss of appetite	5	38.4
Abdominal distension	5	38.4
Abdominal pain	4	30.7
Weight loss	4	30.7
Fatigue	3	23
Night sweating	2	15.3
Cough	2	15.3
Headache	1	7.7
Jaundice	1	7.7

Eleven of thirteen patients were treated initally with meglumine antimonate, one of them was treated with liposomal amphotericin B later, because of the side effects of meglumine antimonate. The patient with haemophagocytic lymphohistiocytosis was treated initally with liposomal amphotericin B because of the clinical severity and IVIG was given as an adjunct therapy. VL relapse occurred in two patients. At the time of relapses, the patients were febrile and suffered from fever, severe anemia, leucopenia, thrombo-cytopenia and splenomegaly. One of them treated with liposomal amphotericin B initially because he had been treated with meglumine antimonate previously in another center. He relapsed again 12 months after treatment course was completed and then he was treated with a second course of meglumine antimonate. The other patient who relapsed after 5 months of treatment with meglumine antimonate was treated with liposomal amphotericin B succesfully. All the children were finally cured and no death was observed.

DISCUSSION

In Turkey, notification of VL and CL are compulsory. VL + CL incidence was reported as 1.6–8.53 cases in 100.000, number of the imported cases is unknown. The mean annual number of VL and CL cases reported to the Ministry of Health was 37 and 2.300, respectively. Canine leishmaniasis sero-prevalance is average 15.7% (6). The clinical features of VL patients in the pediatric age group in Turkey suggest that it is the Mediterranean type which is mostly seen in children younger than 10 years (2, 7, 8). Most of our patients were ranged in age from 2-10 years old (77%), similar to Mediterranean VL who presented predominantly by fever, splenomegaly and hepatomegaly. Anemia, hypergammaglo-bulinemia, elevated ESR and CRP are most common laboratory findings. Lymphadenopaty and skin hyperpigmen-tation are uncommon in the Mediterranen basin in contrast to kala-azar (2, 7). All of our patients had splenomegaly and most of them had fever, elevated ESR and hypergammaglobu-linemia, none of them had neither lymphadenopaty and none skin hyperpigmentation. We have diagnosed of haemophagocytic lymphohistiocytosis in a 23 month old patient with VL. VL associated haemophagocytic lympho-histiocytosis is rare, with 56 cases reported in the English literature. It has been reported that VL related HLH is often under-recognized because of overlapping clinical features and negative marrow evaluation at onset, leading to high mortality rates (9). Examination of bone marrow smears is an easy method for diagnosing VL and was positive in 55– 80% of cases (2). Diagnosis of VL can be difficult when the amastigote burden is low (10). Bone marrow aspirates could not obtained from two patients with thrombocytopenia and the diagnosis of VL was based on IFAT. Antimony remains the therapeutic cornerstone in all regions except Bihar State India and southern Europe. In northenn Bihar where man is the reservoir, there has been an epidemic of primary resistance (5). Another study from Turkey reported that out of 40 patients treated with meglumine antimonate, 38 responded well without significant side-effects (2). In our previous study, all of 19 patients were treated initially with meglumine antimonate or sodium stibogluconate (7). One child, whose condition did not improve with meglumine antimonate, recovered when treated with liposomal amphotericin-B which is the drug of choice in the cases with VL associated with haemophagocytic lymphohistiocytosis (9).

Table 2. Epidemiological, clinical features, the presence of amastigotes on bone marrow, IFAT titers and treatment of patients with visceral leishmaniasis.

Cases	Age (months)	Sex	Local origin	SM (cm)	HM (cm)	BM	IFAT	Treatment
1	49	M	North Anatolia	4	1	-	1/1024	Glucantime
2	74	M	East Anatolia	3	5	+	1/512	Glucantime
3	148	F	North Anatolia	10	3	+	1/1024	Glucantime
4	112	M	East Anatolia	14	9	+	1/64	Glucantime
5	23	M	Central Anatolia	6,5	6	+	1/256	Glucantime
6	70	F	East Anatolia	7	12	+	1/128	Glucantime
7	40	M	North Anatolia	8	4	+	1/64	Glucantime
8	168	F	South Anatolia	15,5	13,5	+	1/64	Glucantime
9	43	F	Central Anatolia	4	1	-	1/64	Glucantime
10	146	M	Central Anatolia	12,5	4,5	+	1/512	Glucantime
11	84	M	South Anatolia	12	4	+	1/512	Glucantime
12	60	F	North Anatolia	10	3	+	1/512	Glucantime
13	72	M	West Anatolia	10	8	+	1/1024	Glucantime

BM, bone marrow; **F**, female; **HM**, hepatomegaly (the distance below the right costal margin); **IFAT**, immunofluorescent antibody test; **M**, male; **SM**, splenomegaly (the distance below the left costal magrin); **+**, amastigot present; **-**, amastigot absent.

Table 3. Hematological and biochemical features of the patients with visceral leishmaniasis

Cases	Hgb (g/dL)	WBC (x10³/µL)	Neutrophil (x10³/µL)	Platelet (x10³/µL)	PT (sec)	PTT (sec)	CRP (mg/dl)	ESR (mm/h)	AST (U/L)	ALT (U/L)	IgG (mg/dl)	Protein (g/dL)	Albumin (g/dL)
1	6,2	2,4	0,37	157	NT	NT	41,4	85	45	22	2212	7,48	3,09
2	8,5	4,2	2,26	153	12,3	39,1	9,5	55	49	75	2350	7,78	3,73
3	7,9	3,1	0,74	110	13,5	31,1	70,1	110	26	6	7880	11,2	2,86
4	8,5	4,1	2,05	144	14,9	33,1	8,45	144	134	86	3390	9,09	3,09
5	8,9	4,8	1,08	76	16	35	11,4	25	229	120	2220	6,8	2,9
6	8,6	5,4	1,31	227	14	27,1	38,7	115	44	17	4020	8,5	2,5
7	6,1	3,6	0,96	84	15	39,1	107	60	63	25	NT	6,4	1,9
8	10,3	2,5	0,87	154	15,2	41	9,32	100	225	93	5830	9,9	1,1
9	11,9	11,5	2,65	105	NT	NT	5,9	30	209	204	1820	6,97	4,22
10	8,2	5,5	1,65	84	13,6	43,8	2,94	60	27	12	3630	7,3	2,35
11	9,2	5,5	1,1	150	13,6	48,5	76,6	93	27	16	3380	8,83	3,42
12	6,3	3	1,2	59	13	29	136	105	30	14	NT	8	2,8
13	6,7	2,1	0,54	66	NT	NT	71,5	30	73	21	1640	6,4	2,95

Hgb, hemoglobin; **WBC**, White blood cell, **PT**, prothrombin time; **PTT**, partial prothrombin time; **CRP**, C-reactive protein; **ESR**, Erythrocyte sedimentiation rate; **AST**, aspartat aminotranspherase; **ALT**, alanine aminotranspherase; **IgG**, immunoglobulin G; **NT**, Not tested

In conclusion VL should be suspected in patients with fever, hepatosplenomegaly and cytopenia. Our present findings also show that treatment of VL with antimonials in Turkish pediatric patients is a relatively inexpensive, safe and effective treatment with low relapse rates. Interestingly meglumine antimonate was useful in a patient with twice relapse who treated with liposomal amphotericin-B at the time of first relapse. However we thought that liposomal amphotericin-B may be useful in cases of treatment failure or relapse with antimonials or significant adverse effects of the drug or VL associated with haemophagocytic lymphohistiocytosis.

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