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Treatment of resistant visceral leishmaniasis with interferon gamma in combination with liposomal amphotericin B and allopurinol

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ABSTRACT

This report describes the first case of visceral leishmaniasis (VL) resistant to pentavalent antimonials and also the first use of combinational therapy in Iran. The patient was a two-year old boy, from a non-endemic area for leishmaniasis in northern Iran, presenting with pentavalent antimonial resistant VL. Additional treatment with conventional and liposomal amphotericin B was not effective. A complete cure was achieved following a three week treatment with liposomal amphotericin B (5 mg/kg/day for 5 days, then on the 14th and 21st days), allopurinol (25 mg/day for 5 days, then on the 14th and 21st days) and interferon gamma (50 μg/m^2 subcutaneously three times weekly). Our results suggest a need for further studies to identify resistant *Leishmania* species and their susceptibility to different treatment regimens.

1. Introduction

Leishmaniasis, including cutaneous (CL), mucocutaneous, and visceral (VL) forms, is a vector-borne infection caused by at least 20 species of the protozoan parasite of the genus *Leishmania* [1,2]. These neglected infectious diseases are transmitted to humans through the bite of blood-sucking sand flies (female *Phlebotomus* species). *Leishmania* spp. are endemic in more than 90 countries and affect several million individuals worldwide. The World Health Organization (WHO) estimates that VL is endemic in more than 70 countries and is responsible for approximately 0.2–0.4 million new cases and 40,000 deaths each year [3].

Pentavalent antimonials, including sodium stibogluconate (Pentostam) and meglumine antimoniate (glucantime) are the most common treatment for leishmaniasis worldwide, and have been used for the past 70 years. Following the loss of effectiveness of antimonial drugs in several cases in recent years, other drugs including miltefosine, paromomycin, pentamidine and amphotericin B (conventional or liposomal) have been used for the treatment of VL [4].

Iran is endemic for both cutaneous and visceral leishmaniasis [5,6], and pentavalent antimonials are generally the drugs of choice for the treatment of both leishmanial forms. Although there has been some evidence of glucantime resistance in *Leishmania tropica* (the causative agent of CL) infections [7,8], there has not been any report showing drug resistance by *Leishmania infantoum* (the causative agent of VL) in Iran. Here we present a case of drug resistant VL in a child and an effective treatment using combinational drug therapy.

2. Case report

The patient was a two-year old boy admitted to Children's Medical Center with fever, chills, weight loss, abdominal distension and pallor. The patient's village, in Rudsar County, located in the Guilan province of northern Iran, is a non-endemic area for leishmaniasis. Based on medical records from the local health center, the patient had fever and pallor for approximately three weeks and suffered from severe weight loss. There was no history of night sweats or cough, or travel or contact with someone with active tuberculosis. The patient was treated with broad-spectrum antibiotics in the local health center. He had a gastrointestinal bleeding episode, which was controlled by supportive care.

Abbreviations: VL, visceral leishmaniasis; CBC, complete blood count; PMN, Polymorphonuclear leukocytes; Hb, hemoglobin; PLT, platelet; AST, Aspartate aminotransferase; ALT, alanine aminotransferase; ALP, Alkaline phosphatase; ESR, erythrocyte sedimentation rate; CRP, C-Reactive Protein; PT, prothrombin time; PTT, Partial thromboplastin time; INR, international normalized ratio; WBC, white blood cells; MCV, mean cell volume; MCH, mean cell hemoglobin.

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He had no diarrhea or vomiting, but abdominal pain was mentioned. His mother reported that the child had been pale, excessively tired and anorexic during the four previous weeks. His abdomen was distended and the spleen was palpable approximately 13 cm below the costal margin. The patient had been referred with a primary diagnosis of malignancy.

The primary results of laboratory tests were as follows: WBC: 2000/μL, PMN: 60%, lymphocytes: 38%, Hb: 7 mg/dL, PLT: 300 k/μL, AST: 96 U/L, ALT: 135 U/L, ALP: 345 U/L, ESR: 88 mm/h, CRP: 66 mg/L, PT: 15 s, PTT: 36 s, and INR: 1.3 IU. There was no evidence of malaria or malignant cells in peripheral blood samples. Blood and urine cultures were negative for bacteria and the urine analysis was negative for WBC and RBC. There were no issues identified in a chest x-ray.

Abdominal ultrasound demonstrated an enlarged spleen (splenomegaly), and multiple lymphadenopathy. The patient was admitted to the hematologic department with a primary diagnosis of malignancy. Subsequent bone-marrow aspiration and splenic biopsy identified *Leishmania* bodies, confirming VL. The bone marrow sample was negative for malignancy. The patient's parents were consulted for approval of treatment and glucantime (meglumine antimoniate) therapy was initiated (20 mg/kg/day intravenously for 20 days).

Based on a suspicion of secondary infection and neutropenia, ceftazidime (150 mg/kg/day) was also added to the treatment regimen. The patient's general condition deteriorated and petechiae was seen. A second laboratory examination revealed the following values: ESR: 90 mm/h, CRP: 67 mg/dL, WBC: 1500/μL, PMN: 15%, Hb: 6.5 g/dL, MCV: 72 fL, MCH: 22 fL, and PLT: 220 k/μL.

Bone marrow aspiration was repeated and the presence of *Leishmania* parasites was observed again. Therapy with amphotericin deoxycolate (conventional Ambisome or amphotericin B) was started (0.5 mg/kg/day) and platelet transfusions (10 cc/kg) were done simultaneously. After a week, the patient's general condition and fever did not improve. Intravenous liposomal Ambisome (5 mg/kg/day) was replaced with conventional Ambisome, but the patient's clinical condition deteriorated further. The patient was subsequently treated for three weeks using a combinational drug therapy as follows; liposomal amphotericin B 5 mg/kg/day (for 5 days, then on the 14th and 21st days), allopurinol (25 mg/day for 5 days, then on the 14th and 21st days) and interferon γ (IFN-γ) (50 μg/m^2 subcutaneously three times weekly). The patient responded rapidly to this combinational therapy. Within three days of this therapeutic regime, the patient became afebrile, and after five days, the general condition of patient improved significantly. His spleen size was reduced to 8 cm (span of size) after two weeks. By the 21st day, the patient was fully recovered and a bone marrow aspirate was negative for *Leishmania* bodies. The patient was asymptomatic at 3 and 6 months post-treatment and his spleen was not palpable. Blood hemoglobin had increased to 9.5 g/dL with no transfusion, and his weight had increased from 10 kg to 12 kg. We did not observe any side effects or notable complications resulting from the combinational therapy during treatment and follow up.

3. Discussion

Chemotherapy is the most effective measure for control and management of VL. The failure of treatment with antimonial drugs may be the result of several factors including immune or nutritional status of patient, species of parasite, and location of the parasite within the patient's body (i.e., in tissues not readily accessible by drugs). Although resistance to antimonial drugs in northeast India first emerged in the 1990s, antimonials are still the drug of choice in several parts of the world, including Latin America, East Africa and the Middle East [9]. To our knowledge this report is the first regarding unresponsiveness of VL to antimonials and conventional/liposomal Ambisome in Iran and is also the first report of the use of combinational therapy.

In a previous report, Hadighi et al. reported resistance to glucantime resulting in treatment failure among patients with cutaneous leishmaniasis in an endemic area in the northeast region of Iran [7]. The treatment regimen used in the present report (liposomal Ambisome, allopurinol, and interferon gamma) was used here for first time in combination, although each of these drugs has been used in other regimens and in combination with other drugs. It should be noted that drugs used in combinational therapy should have synergistic effects without drug interactions.

Conventional or liposomal amphotericin B have been the most commonly used drugs in cases of drug-resistant VL [10–12]. These compounds bind to ergosterol-related sterols in cell membranes and change membrane permeability that can lead to leakage of intracellular components. A single dose of liposomal Ambisome (5 mg/kg for 7–14 days) followed by miltefosine (100 mg/day for 7 days) was highly efficacious (98% of patients cured) and was well tolerated in a randomized trial in India [13]. Kumar et al. used a combination of liposomal amphotericin B and miltefosine to treat a patient with multiple relapsing and multi-drug resistant VL [12]. In the present study, amphotericin was not effective. Resistance to amphotericin B has previously been reported in a non-endemic region for leishmanisis in India [10], which is consistent with our case from Guilan province in northern Iran, where VL is also not endemic.

Allopurinol has been used successfully in combination with pentavalent antimonials, pentamidine and ketoconazole in patients with resistant VL and with high relapse rates [14–16]. Based on our experience in this case report, allopurinol appears to be an appropriate drug for combinational therapy based on minimal toxicity as evidenced by our patient not showing signs of clinical complications.

Immunotherapy, the use of biological immune-stimulating molecules or compounds combined with drugs, is one of the most interesting approaches for improving the efficacy of drug treatment regimens for VL. It is based on stimulating Th1 responses (IL-12, IFN-γ, NK cells and etc.) which play an important role in host immunity against *Leishmania* parasites. Several immuno-stimulatory cytokines, such as IFN- γ, IL-12 and granulocyte macrophage colony-stimulating factor (GM-CSF), have been investigated for individual therapy or in combinational therapies with pentavalent antimonials or other drugs [17]. IFN-γ was one of the first immunological compounds used for this propose and has shown strong anti-parasitic effects with good clinical outcomes [18–20]. Badaro et al. reported that a combination of recombinant IFN-γ and pentavalent antimony was highly efficacious against resistant VL. In their study, six of eight patients who were resistant to multiple courses of pentavalent antimonial drugs were successfully treated with this therapeutic regimen [20]. In two subsequent studies by Squires et al. [18] and Sunder et al. [19] it was found that combinational therapy using IFN-γ was safe and well tolerated, and accelerated the recovery of patients with previously untreated VL.

In conclusion, for treatment of VL, combinational therapies may have substantial benefits including increasing the efficacy of treatment, reducing the risk of toxicity, overcoming drug resistance, and reducing treatment duration and cost. This report suggests the emergence of *Leishmania* spp. resistant to conventional drugs used in Iran. Further studies are necessary to identify the resistant *Leishmania* species in different parts of Iran and their susceptibility to different treatment regimens.

Conflict of interests

No reported conflicts.

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