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Short Report: Relapse of Visceral Leishmaniasis after Miltefosine Treatment in a Nepalese Patient

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Abstract. We report the first case of visceral leishmaniasis (VL) relapse in a healthy individual after complete miltefosine treatment. The patient attended hospital with a history of fever for 2 months, splenomegaly, hepatomegaly, and weight loss. The case was confirmed as VL by microscopical detection of *Leishmania* parasites in a bone marrow specimen and by a positive result for the immunochromatography-based test targeting the *Leishmania donovani* rK39 antibody. A polymerase chain reaction (PCR) specific for the *Leishmania* kinetoplast minicircle gene was positive, and subsequent sequencing of the PCR-amplified product confirmed that this case was a *L. donovani* infection. The patient was treated with miltefosine for 28 days, during which time the response was good, and the *Leishman-Donovan* body (LD body) was negative on discharge. Ten months later, however, this patient again developed high fever and splenomegaly, and LD bodies and rK39 antibody were positive, thus indicating a relapse of VL. The patient was subsequently treated with 1 mg/kg of amphotericin B for a total of 14 days and recovered completely.

Visceral leishmaniasis (VL), also known as Kala azar, is a parasitic disease caused by *Leishmania donovani* and is responsible for about 59,000 deaths per year and 2.4 million disability-adjusted life years lost.[1] India, Nepal, and Bangladesh account for 300,000 cases annually and thus suffer 60% of the global burden of the disease.[2] The disease displays a wide epidemiologic diversity, which can mainly be attributed to co-infection with HIV, malnutrition, and host genetic factors.[3] There are an estimated 6.5 million people at risk from the disease in Nepal, where it caused a total of 28,424 cases with 582 deaths between 1980 (when cases were first recorded) and 2006. The highest case fatality rate was recorded in 1982 (13.16%), and the highest case incidence was in 2003.[4]

The first line treatment of VL is a pentavalent antimonial (sodium stibogluconate [SSG]) as recommended by the World Health Organization (WHO).[5] On the Indian subcontinent, the efficacy of SSG has gradually declined despite the regular increasing of both dose and duration of treatment. Current chemotherapy for leishmaniasis has been bolstered by the introduction of new drugs and formulations such as miltefosine (MLF) and liposomal amphotericin B. Although MLF is an effective oral drug, its teratogenic potential (observed in rats at a no-effect dose level of 0.6 mg/kg) and long half-life *in vivo* remain drawbacks.[6] One of the major dangers is that its long half-life (150–200 hours) might encourage the emergence of resistant parasites in the field.[7]

We describe here a patient with VL infection from midwestern Nepal whose disease completely resolved clinically and parasitologically after 28 days of oral MLF (2.5 mg/kg/day) administration but relapsed 10 months later. To the best of our knowledge and available literature, such a relapse of VL in a healthy individual after complete MLF treatment has not previously been reported in Nepal.

A 19-year-old male patient from the Bardiya district (Figure 1) in midwestern Nepal was transferred to Sukraraj Tropical and Infectious Disease Hospital, Kathmandu, Nepal, from Bheri Zonal Hospital, Nepalgung, Banke, in July 2007. The patient had a high-grade fever, associated with rigor, and had experienced a loss of appetite and weight for 2 months. The patient had a history of travel to the Indian State of Uttar Paradesh, which borders the Bardiya district of Nepal. During the first month of fever, the patient was locally treated with antipyretics and taken to Bheri Zonal Hospital, the biggest government hospital in the Midwestern region, 40 km from Bardiya district. He was treated with antibiotics and antimalarial drugs at Bheri Zonal Hospital, but after 2 weeks of continuous fever, he was transferred to Sukraraj Tropical and Infectious Disease Hospital for definite diagnosis and treatment. The patient was found to be anemic, and the spleen size was 4 cm at the first time of admission. Initial basic hematologic examination showed a white blood cell count of 6700/mm^3, hemoglobin level of 8.7 g/dL, and total neutrophil count of 59%, with a lymphocyte fraction of 40%. The liver enzyme levels were moderately low but within normal range. Total serum protein level was 7%, and globulin level was increased to 5%. Random blood glucose level was 128 mg/dL, and the electrocardiogram was normal. Renal function was within normal range with blood urea of 28 mg/dL and creatinine of 0.6 mg/dL. The provisional diagnosis of leishmaniasis using rK39 (Insure; Inbios, Seattle, WA) was positive, and bone marrow aspiration was also positive for *Leishman-Donovan* body (LD body).

Polymerase chain reaction (PCR) analysis was performed for species identification. DNA was extracted from bone marrow smears using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) and was subjected to a PCR specific for a *Leishmania* minicircle kinetoplast gene. The detailed PCR process was given previously.[8] Positive PCR product with the size similar to those of confirmed *L. donovani* samples (T4 and D10 Nepalese isolates) suggested that the case was *Leishmania* infection (Figure 2). The DNA fragment was recovered from agarose gel and purified using QIAquick Gel Extraction kit (Qiagen). The purified DNA fragment was directly sequenced using an ABI Prism BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) with the LIN 19 primer according to the manufacturer's instructions.[8] The obtained sequence (486 bp) showed strong homology with the *L. donovani* Dd8 Indian strain (MHOM/IN/DD8; accession no. Y11401), T4 isolate, and D10 isolate (98.7%, 100%, and

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FIGURE 1. District map of Nepal. Districts endemic for leishmaniasis are indicated in gray. The VL patient reported resides in Bardiya district (black), where Bheri Zonal Hospital is located. STIDH, Sukraraj Tropical and Infectious Disease Hospital.

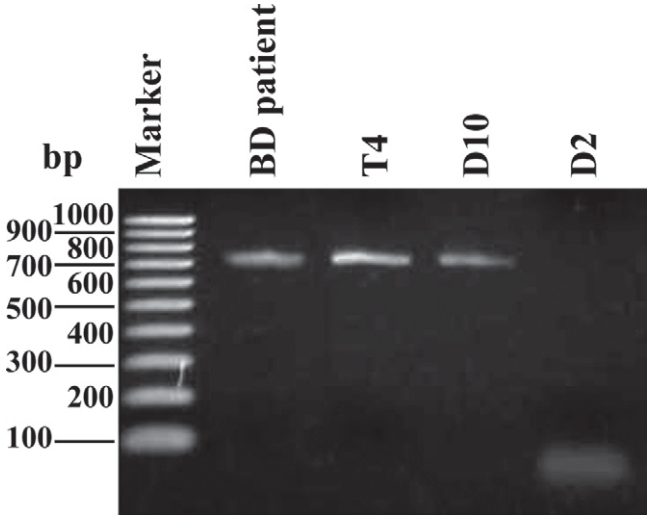

FIGURE 2. Agarose gel electrophoresis of the PCR-amplified DNA fragments. BD, reported patient bone marrow sample; T4 and D10, Nepalese samples infected with *L. donovani*; D2, negative sample for *Leishmania*. Marker indicates 100-bp DNA ladder.

99.2%, respectively), supporting *L. donovani* infection diagnosis at the molecular level. Sequence data have been deposited in the GenBank/EMBL/DDBJ databases under accession numbers AB458388, AB458399, and AB458390.

The patient was treated with 2.5 mg/kg of MLF orally for a period of 28 days in the hospital as a directly observed therapy (DOT) and evaluated every week for the period of 28 days during the course of treatment to ensure compliance, detect any MLF-related adverse reactions, and evaluate the drug response. The patient was cured completely after 28 days of treatment as indicated by the absence of fever, impalpable spleen, and negative result for LD body.

The patient was completely free from disease for 9 months after treatment, until the development of a fever at the end of May 2008. During July 2008, the patient visited Sukraraj Tropical and Infectious Disease Hospital to investigate the cause of fever and for treatment. Physical examination showed that the patient had fever, mild anemia, and splenomegaly measuring ~4 cm in size. Routine examination including white blood cell count, erythrocyte sedimentation rate, stool, and urine was normal beside decreased hemoglobin level (7.1 g/dL). Positive rK39 test and positive LD body in the bone marrow aspiration confirmed *Leishmania* infection. Thus, this case was diagnosed as a relapse of leishmaniasis. After treatment with amphotericin B (1 mg/kg body weight) for a total of 14 days, the patient's fever subsided and the spleen became impalpable. The patient has been free from disease until October 2008 when this manuscript was prepared. The patient will be observed for relapse for 1 year by the Sukraraj Tropical and Infectious Disease Hospital.

In Nepal, some data on the efficacy of SSG have been documented. Karki and others[9] reported that the definite cure rate after treatment with 20 mg/kg/day SSG was 78% for 20-day treatment and 93% for 30-day treatment. In another study in southeastern Nepal from July 1999 to January 2001, treatment failure with SSG was observed in 10% of patients.[10] Treatment failure with SSG may have multiple origins—related to the drug regimen, the host immune status, and/or the parasite's inherent tolerance itself.[11] One of the important contributing factors to the drug resistance in Bihar has been attributed to the use of infra-therapeutic doses and/or insufficient duration of SSG therapy.[11] This phenomenon also exists in Nepal. Moreover, the socio-cultural similarity and the open border between southern Nepal and northern Bihar facilitate cross-border population movements, which may also play an important role in the spread of SSG-resistant strains of *L. donovani.*

It has been previously shown that *in vitro* generation of *L. donovani* promastigotes resistant to MLF is possible,[12] and relapse cases of MLF-treated parasites have been observed in HIV-positive patients.[13,14] Relapse cases in HIV-positive patients suggest that host immunity plays a role in the elimination of parasites after MLF treatment. The first hints of the emergence of MLF resistance were associated with an increase in treatment failure caused by relapses, which subsequently became more common. Therefore, this report of a VL relapse after complete MLF treatment in a healthy individual may suggest an increase in MLF resistance of parasites in Nepal.

MLF is an alkylphospholipid, an analog of phosphocholine, and its chemical similarity to the natural phospholipids of cellular membranes suggests that it probably inhibits transmembrane signals and the synthesis of the cellular membrane itself. The basic mechanism for MLF resistance in *Leishmania* parasites consists of inactivation of the MLF transporter, LdMT.[15] Certainly, in India, the same factors as those that favor the selection of SSG resistance could also select for MLF resistance, because the drug is too expensive for impoverished patients to buy a full course of treatment and, additionally, has a comparatively long half-life. A simple mechanism for the emergence of MLF resistance is the selection of inactivating point mutations in any of the genes essential for MLF uptake. All these intrinsic features would suggest that the emergence of MLF resistance is highly likely in such areas. It should be noted that relapse cases after MLF treatment have already been reported for cutaneous leishmaniasis caused by other *Leishmania* species.[16,17]

The visceral form of leishmaniasis is endemic in the Terai region of Nepal, particularly in 13 districts of the southeast. Although this is the first reported VL case from the midwestern part of Nepal, it is difficult to confirm directly as an indigenous case because the patient was from the border district of Bardiya and has previously visited India. The patient's family

was evaluated for leishmaniasis with the rK39 test and were all shown to be negative. Thus, it remains unclear whether VL is endemic in the midwestern part of Nepal.

In conclusion, although MLF treatment induced an early clinical response in the patient, the risk of relapse indicates a need for studying new drug combinations or maintenance regimens. Because cell-mediated immunity against *Leishmania* parasites is poor or absent in patients with VL, combination treatments of MLF with agents that increase host immune responses, such as immunotherapy, should be considered. Policies concerning the proper use of this drug should be followed and supervised by health authorities in endemic areas of Nepal to minimize the risk of the appearance of drug failures and to ensure a long life span for this drug.

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