

A Case Report of Visceral Leishmaniasis in Singapore

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Abstract

Visceral leishmaniasis (Indian kala-azar) caused by infection due to the protozoan *Leishmania donovani* is endemic in the Indian subcontinent and adjoining regions. Prolonged fever, hepatosplenomegaly, anaemia and pancytopenia, in the appropriate setting, are important clinical markers towards the diagnosis. Diagnosis is established by blood film or bone marrow examination for Leishman Donovan (LD) bodies and/or culture. Treatment with sodium stibogluconate, pentamidine isethionate or even amphotericin B is usually successful.

We report the first case of culture-proven visceral leishmaniasis in Singapore, in a 30-year-old Bangladeshi worker who presented with pyrexia of unknown origin (PUO). He had the classical constellation of symptoms and signs as mentioned above. Diagnosis was confirmed by culture on the Novy, NeNeal and Nicolle (NNN) medium. He was successfully treated with 20 days of pentamidine isethionate daily infusions at a dose of 2 mg/kg/day.

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Introduction

Leishmaniasis, caused by infection by the protozoan *Leishmania donovani*, was almost simultaneously identified by Leishman in May 1903 in London and Donovan in July 1903 in Madras.¹ The genus *Leishmania* has several species which can result in cutaneous, mucocutaneous or visceral disease. Visceral leishmaniasis may be caused by *L. donovani*, *L. infantum*, *L. chagasi*, *L. tropica* and other *Leishmania* spp.² Of these, *L. donovani* causes human disease in the Indian subcontinent, *L. infantum* in northwestern China, *L. chagasi* in South America, *L. tropica* in the Mediterranean regions and other *Leishmania* spp in the African countries particularly Sudan.²

The cardinal clinical features are fever of several weeks to months in duration, anaemia with a hepatosplenomegaly and pancytopenia on laboratory examination. Occasionally, there may be no organomegaly in children.³

The diagnosis may be established by direct visualisation of the amastigote form on smear or the promastigote from culture.⁴ Treatment is with antimonials, pentamidine or amphotericin B.²

We report the first case of culture-proven Indian kala-azar (visceral leishmaniasis) in Singapore.

Case Report

A thirty-year-old worker in the construction industry had arrived in Singapore from Bangladesh in October 1995. He presented in April 1996 with complaints of unrelenting fever of one month's duration. His fever had been of insidious onset with daily febrile episodes often reaching a maximum of 40°C. The fever was not associated with rigors, vomiting, diarrhoea or other systemic symptoms. There was no dysuria, cough, headache or other symptoms of note. He had received anti-pyretics periodically with no significant relief. Physical examination revealed an asthenic male with moderate pallor, hepatomegaly of about 4 cm below the right costal margin and splenomegaly of about 6 cm below the left costal margin, both of which were firm and non-tender. Abdominal examination revealed no other significant findings. There was no significant lymphadenopathy and respiratory and cardiovascular examinations were

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clinically unremarkable. He was mentally alert and coherent, there was no neck stiffness and neurological, ocular and oropharyngeal examination was normal.

Laboratory investigations on admission revealed a haemoglobin of 9.9 gm/dl, a total white count of 3100/cu.mm, a differential count with 36% polymorphs, 47% lymphocytes and 13% monocytes and a platelet count of 76 000/cu.mm. Blood film examination for *Plasmodium* species was negative on three different occasions. Serial bacterial blood cultures on three occasions were also negative. His reticulocyte count was 3% and erythrocyte sedimentation rate was (ESR) 100 mm in the first hour.

Serology for Widal, Weil-Felix reaction, amoebiasis and dengue fever were negative. Liver function tests revealed normal bilirubin levels with a marginal rise of transaminase levels. Urinalysis was normal and stool microscopy revealed ova of hookworm which were treated with mebendazole 100 mg twice daily for 3 days. A repeat stool microscopy was negative for parasites. Serum B12 and folate levels were normal and haemoglobin electrophoresis for thalassaemia was negative. Serology for Epstein-Barr virus and cytomegalovirus were negative. His indirect and direct Coomb's tests as well as anti-double stranded DNA and Rheumatoid factor were also negative. The chest radiograph was normal. Mantoux test was negative and sputum for acid-fast bacilli on three occasions were negative. A computed tomographic (CT) scan of the abdomen revealed hepatosplenomegaly without significant lymphadenopathy or intra-abdominal abscesses. Bone marrow trephine and aspiration were performed. The aspirate revealed erythroid hyperplasia and specific examination to look for Leishman Donovan (LD) bodies was negative. The trephine was similarly unrevealing.

The patient continued to have fever ranging between 37°C and 40°C. Serial blood counts after admission were no different from the first. In view of his clinical presentation and the fact that he had recently come from Bangladesh, a country known to be endemic for kala-azar, a diagnosis of visceral leishmaniasis was strongly considered. A second bone marrow aspiration was performed 5 days after the first and a specimen was inoculated on the Novy, McNeal and Nicolle (NNN) medium for culture at the Department of Microbiology, Faculty of Medicine, National University of Singapore. After 17 days, promastigotes were seen in the culture (Fig. 1).

The patient was treated for 20 days with intravenous pentamidine isethionate in a dose of 2 mg/kg/day as a daily infusion. He tolerated therapy well and had no hypoglycaemia. After a week of therapy his fever showed a gradual decline. Over the last 4 to 5 days of therapy, he was afebrile throughout. The patient was discharged from hospital and on follow-up at the end of May 1996, one month after his initial presentation, haematological parameters showed a haemoglobin of 11 gm/dl, total

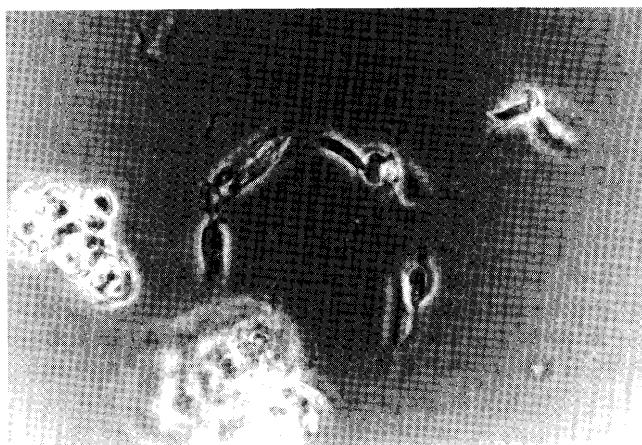


Fig. 1. Promastigotes cultured on the Novy, McNeal and Nicolle medium

white count of 8700/cu.mm, a differential count with 52% polymorphs, 31% lymphocytes and 16% monocytes, 1% eosinophils and a platelet count of 380 000/cu.mm.

Discussion

Visceral leishmaniasis of the variety called Indian kala-azar is caused by the protozoan *Leishmania donovani*. The vector is the sand-fly, *Phlebotomus argentipes*, found in the Indian sub-continent and Bangladesh. Indian kala-azar is endemic in eastern and north-eastern India in the states of Bihar, Orissa and eastern Uttar Pradesh, as well as some parts of Tamil Nadu in south India. It is also endemic in Bangladesh and surrounding regions. The parasite exists in two forms, the amastigote which is the aflagellate form and seen in the human body and the promastigote, the flagellate form which exists in the vector and culture. The parasite resides in the gut of the sand-fly and multiplies to form promastigotes which migrate to the proboscis of the fly. When a person is bitten by the sand-fly, the parasite is regurgitated into the human tissues in order for the fly to suck blood leading to transmission of the disease. Subsequently, the parasite invades the reticulo-endothelial tissues causing hypertrophy over time. This accounts for the organomegaly as well as the pancytopenia.

The organism may be visualised by examining bone marrow smears for Leishman Donovan (LD) bodies, the amastigotes, using Leishman's or Giemsa stain after fixing with 100% methanol.⁴ In cases of heavy parasitemia, a thick blood film using Leishman's stain may also demonstrate the parasite. A recent report⁵ mentioned a method called leukocytoconcentration in which blood parasites are concentrated on a small surface of a microscope slide while the red blood cells are removed by lysis. This method compares well in efficiency of isolation of the organism with other conventional methods. A number of serological tests have been developed and compared for their efficacy in the diagnosis of visceral leishmaniasis. A comparison of

various serological tests show that the indirect fluorescent antibody test (IFAT) is 100% sensitive and specific while the direct agglutination test (DAT), which involves detection of agglutinating antibodies, has a comparative efficiency of 97.2%.⁶ The enzyme-linked immunosorbent assay (ELISA), counter immunoelectrophoresis (CIEP), aldehyde and Sia water tests have an efficacy in the range of 84.3% to 97.2% only.⁶ Other authors have also evaluated the efficacy of DAT as a diagnostic tool and found it to be simple and reliable.⁷ Recent advances include the development of a polymerase chain reaction (PCR) method⁸ for diagnosis, possibly helping to avoid invasive procedures such as bone marrow aspiration.

Although bone marrow aspiration has been favoured as a diagnostic method, a marrow smear alone may not always reveal LD bodies. Sinha et al⁹ reported that the smear may be positive only 60% of the time while a culture is positive 97.8% of the time. Culture of blood or marrow may be achieved on the NNN medium and would be positive even if smears are negative as occurred in our patient (Fig. 1).

Splenic aspirate has been attempted as a means of acquiring a sample for diagnosis and has been reported to yield a high rate of positivity of over 90%.¹⁰ However, the procedure is not without inherent risks, chiefly a haemoperitoneum. Since we did not have sufficient experience with this procedure, this was not attempted in our patient.

Sodium stibogluconate is considered the first-line drug in the treatment of kala-azar and is recommended in a dose of 20 mg/kg/day for 28 days for primary visceral leishmaniasis.¹¹ A number of studies from India have demonstrated the superiority of pentamidine isethionate and amphotericin B over antimonials and are now recommended for primary as well as relapsed cases.¹²⁻¹⁴ Amphotericin B is administered in a dose of 1 mg/kg/day for 14 days and is considered safe even in pregnancy where other drugs cannot be used;¹⁵ while pentamidine, though not recommended during pregnancy, is extremely effective in a dose of 2 mg/kg/day for 15 to 20 days,¹³ as in our patient. A combination of sulphadiazine, trimethoprim and metronidazole has also been tried, with success, in pregnancy.¹⁶ The pancytopenia and marrow suppression associated with visceral leishmaniasis has been treated with granulocyte-macrophage colony-stimulating factor (GM-CSF) in severe cases, with success.^{17,18} Adverse effects of treatment include abdominal pain, anorexia, vomiting, nausea, myalgia, headache, arthralgias, pancreatitis and acute tubular necrosis with antimonials,^{19,20} headache, flushing, nausea, vomiting, vascular collapse if infused too quickly and hypoglycaemia with pentamidine and chills, rigors, fever and hypokalaemia with amphotericin B.² In our patient, we decided to use pentamidine because of the easy

availability of the drug in our centre and our greater experience with it over antimonials.

This case serves to highlight the importance of considering Indian kala-azar (visceral leishmaniasis) as a differential diagnosis in unrelenting fever among persons who hail from endemic areas such as the Indian subcontinent. This is particularly relevant since a significant proportion of the workforce in Singapore is from the Indian sub-continent. When a diagnosis of kala-azar is strongly suspected and the blood film and bone marrow smears are unrevealing, it is important to consider culturing the blood or bone marrow aspirate to confirm the diagnosis.

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