CASE SERIES

CLINICO-HEMATOLOGICAL PROFILE OF VISCERAL LEISHMANIASIS AMONG IMMUNOCOMPETENT PATIENTS

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Abstract. We studied cases of visceral leishmaniasis (VL) over a 2-year period among immunocompetent patients who presented to a rural medical college in West Bengal, India to determine a clinical and hematological profile among these patients. We studied a total of 36 cases of VL; the male to female ratio of the cases was 1.6:1 and the mean age was 20.1±11.1 years. A detailed history, physical examination, hemogram, bone marrow or splenic aspiration and chest x-ray were conducted on all cases. A CT-scan of the thorax and fiberoptic bronchoscopy were performed in selected cases. Fever and splenomegaly were present in all cases. Weakness, abdominal pain, bleeding, and hepatomegaly were seen in 63.9, 27.8, 8.3 and 58.3% of cases, respectively. Pancytopenia, bicytopenia, leukopenia and thrombocytopenia were seen in 58.3, 41.7, 61.1 and 83.3% of cases, respectively. Five patients (13.9%) had cough, 2 (5.6%) had hemoptysis, 6 (16.7%) had an abnormal chest x-ray and 3 (8.3%) had localized reticulo-nodular opacities on a CT-scan of the thorax. Bronchoalveolar lavage showed gram-positive cocci in 2 cases (5.6%). One patient died of acute respiratory distress syndrome. Cytopenia was common among the series of VL patients. Pulmonary complications, usually secondary infection, were less frequent (found in 13.9% cases) but was fatal in one patient.

Keywords: visceral leishmaniasis (VL), immunocompetent patient, hematological changes, pulmonary involvement

INTRODUCTION

Leishmaniasis, transmitted by female phlebotomus sandflies, occurs in three forms: visceral, cutaneous and mucocutaneous. The distribution of visceral leishmaniasis (VL) or kala-azar, includes southern Europe, the Mediterranean region, East Africa, China, the Indian subcontinent, and endemic foci are found in South and Central America, particularly in Brazil (Pearson *et al*, 2000). After con-

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traction, the subject may be completely asymptomatic, or have an oligosymptomatic illness that either resolves spontaneously or evolves into active VL. The major complication leading to death is bacterial superinfection (Pearson and Sousa, 1996). The name kala-azar (meaning "black fever" in Hindi) is derived from hyperpigmentation which often develops among patients in India (Pearson and Sousa, 1996). Kala-azar is re-emerging as a major health problem in India (Bora, 1999). The Indian subcontinent and Sudan accounted for over 90% of the estimated 2.5 million cases in the world (Bhattacharya *et al*, 2006). The disease is a serious problem in the eastern region of India. Ninety percent of all leishmaniasis cases reported in India occur in the state of Bihar. The disease is also being detected in several districts bordering Bihar, in the States of West Bengal and Uttar Pradesh (Bhattacharya *et al*, 2006). One survey conducted in Africa over a period of 10 years found mortality rates in VL of 38-57% (Seaman *et al*, 1996).

There have been reports of secondary bacterial pneumonia occurring in VL (Garcés *et al*, 1990; Mehmet *et al*, 2003). Few cases of pulmonary involvement with VL have been reported among human immunodeficiency virus (HIV) infected patients (Peters *et al*, 1990; Romeu *et al*, 1991; Matheron *et al*, 1992; Casado *et al*, 1998).

In this case series we analyzed the clinical and hematological profiles of immunocompetent patients with VL attending a rural medical college serving six northern districts of West Bengal, India located near the state of Bihar. We have also determined pulmonary involvement in some of these patients.

MATERIALS AND METHODS

We conducted this cross sectional observation study at North Bengal Medical College (NBMC), West Bengal, India over a two year period. Only cases of visceral leishmaniasis were included in the study but patients with diabetes, HIV infection, malignancy, a BMI<25, immunosuppressive conditions and those taking corticosteroids or other immunosuppressive therapy were excluded from the study.

A detailed clinical history and physical examination were conducted on all patients included in the study. We also conducted the following studies on all subjects: hemoglobin, total and differential leukocyte count, platelet count, peripheral smear study for abnormal cells, liver function tests, urea, creatinine and uric acid. All patients were tested for diabetes and HIV infection. Other immunosuppressive conditions were excluded by history and appropriate investigations. The diagnosis of VL was suspected on the basis of clinical manifestations, and was established by direct agglutination test, aldehyde test, demonstration of amastigote forms from bone marrow or splenic aspirates. Aspirated bone marrow smears were stained with Leishman's stain and Prussian blue. Splenic aspirates were air dried and stained with Leishman-Giemsa (LG) stain.

Pulmonary involvement was assessed by pulmonary symptoms (cough, chest pain, shortness of breath or hemoptysis) and pulmonary signs (tachypnea, dullness on percussion, abnormal breath sounds or other abnormal sounds). A posterior-anterior view chest x-ray (CXR) and spirometry was done on all cases. A high resolution computed tomography (HRCT) of the thorax and fiberoptic bronchoscopy (FOB) with broncho-alveolar lavage (BAL) were conducted in selected cases (patients with chest symptoms or abnormal chest radiography). Due to lack of facilities

Table 1
Age and sex distribution of patients.

Sex	Age group in years Number (%)				Total
	0-9	10-19	20-29	≥30	
Male	5 (22.7)	4 (18.2)	8 (36.3)	5 (22.7)	22
Female	5 (35.7)	5 (35.7)	2 (14.3)	2 (14.3)	14
Total	10 (27.8)	9 (25.0)	10 (27.8)	7 (19.4)	36

Table 2
Observed hematological parameters in the study population.

Parameter	Range	Average (Standard deviation)
Hemoglobin (g/dl)	3.6-10.2	7.1 (±1.9)
Total leukocyte count (/mm^3)	1,200-9,400	3,700 (±1,800)
Platelets (/mm^3)	20,000-212,000	108,500 (±40,500)
Neutrophils (%)	5-65	42 (±17)
Lymphocytes (%)	30-95	55 (±17)

transbronchial lung biopsy could not be performed. BAL fluids were tested for cytology, Gram stain, bacterial culture and acid-fast bacilli (AFB).

RESULTS

Twenty-two of the 36 cases were male (male:female ratio of 1.6:1). The mean age was 20.1 (SD)±11.1 years (range 6-42 years). Sixteen subjects (44.4%) were aged <15 years (Table 1). More than 80% of the cases were aged less than 30 years. The duration of symptoms were: <2 months in 7 cases (19.4%), 2 to 6 months in 16 cases (44.4%) and >6 months in 13 cases (36.1%). Fever was present in all cases, intermittent in 29 cases (80.6%) and associated with chills in 4 cases (11.1%). Weakness, abdominal pain and bleeding were seen in 23 cases (63.9%), 10 cases (27.8%) and 3 cases (8.3%), respectively. Pallor was present in 34 cases (94.4%), skin hyperpigmentation was present in 2 cases (5.6%) and jaundice was seen in 1 case (2.8%). The spleen was palpable in all cases and was between 2-10 cm below the left costal margin in 28 cases (77.8%) and >10 cm in 8 cases (22.2%). The liver was palpable in 21 cases (58.3%); in these cases it was <5 cm below the costal margin.

Cytopenia in the peipheral blood was common (Table 2). Pancytopenia was seen in 21 cases (58.3%); 15 cases (41.67%) had bicytopenia (decreased RBC and WBC or platelets). Leukopenia was seen in 22 cases (61.1%). The platelet count was <150,000 in 30 cases (83.3%), <100,000 in 16 cases (44.4%) and <50,000 in 2 cases (5.6%). The peripheral smear showed a dimorphic differential count in 22 cases (61.1%) and macrocytosis in 3 cases (8.3%). A direct agglutination test was positive in 29 cases (80.6%) and an aldehyde test was positive in 13 cases (36.1%). Amastigotes were detected in fair numbers in all cases. The marrow was hypercellular in 23 cases (63.9%) and normocellular in 12 cases (33.3%).

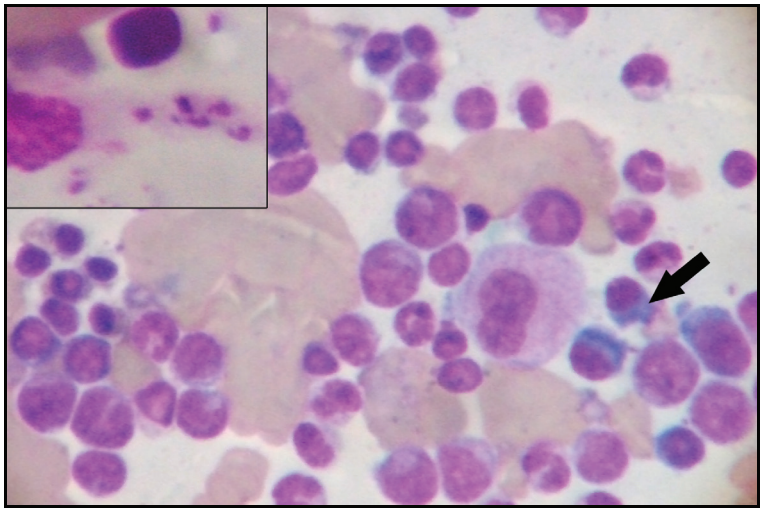

Fig 1–Bone marrow aspirate showing hypercellular marrow, mild dyserythropoiesis and increased plasma cells (arrow) (Leishman's stain, x400). Inset showing leishmania amastigote (Leishman's stain, x1,000).

Megaloblastic changes with occasional dyserythropoiesis was noted in 10 cases (27.8%). Megakaryocytes were adequate in all cases. The plasma cells ranged from 3 to 10% (Fig 1). The stainable iron in marrow ranged from low to normal.

Seven patients in our series had pulmonary abnormalities; 2 (5.6%) had old tuberculosis (TB), 5 (13.9%) had chest symptoms and 2 (5.6%) had chest signs (Table 3). One subject had a normal CXR but the HRCT-thorax showed an interstitial pattern. One subject developed acute respiratory distress syndrome (ARDS) with hemoptysis, severe dyspnea. His partial pressure of oxygen with inspiratory oxygen fraction ratio (PaO_2/FiO_2) was 150. His CXR showed bilateral perihilar infiltrative lesions. A HRCT-thorax could not be done due to his severe dyspnea. The patient was treated with parenteral antibiotics but he expired on the third day of hospitalization. Two patients had radiological findings suggestive of old TB, one of them had a past history of TB. Sputum stains and BAL fluids were negative for AFB in both these patients. A HRCT demonstrated a localized reticulo-nodular pattern in 3 cases (8.3%). Two asymptomatic patients had an abnormal CXR and HRCT-thorax (one had a history of TB and another had an interstitial pattern and the BAL showed a high neutrophil count. One patient had radiological findings suggestive of lingular consolidation and BAL fluid showed gram-positive cocci. One case had interstitial and alveolar opacities on CXR and HRCT-thorax with gram-positive cocci in BAL fluid. Bacterial culture was negative in all cases. Hilar lymphadenopathy and pleural involvement were not detected in any of our patient. Five patients (13.9%) (excluding 2 cases with old TB) had pulmonary manifestations attributable to VL; all of them, except the ARDS case, responded to antibiotics and antileishmania treatment.

DISCUSSION

Leishmania spp are obligate intracellular parasites of macrophages in mammalian hosts. The clinical manifestations of VL include prolonged fever, weight loss, cachexia, splenomegaly, hepatomegaly, lymphadenopathy and pancytopenia. Petechiae, ecchymoses and mild edema may be present; jaundice and ascites are rare. The clinical picture may be similar to that of malaria, typhoid fever, miliary tuberculosis, schistosomiasis, brucellosis, amebic liver abscess, infectious mononucleosis,

Table 3
Pulmonary manifestations in the study population.

Abnormalities		Sings/Symptoms (No.)
Pulmonary abnormalities in the study population (*n*=36)		
Pulmonary symptoms	5 (13.9%)	Cough (5) Hemoptysis (2) Dyspnea (1)
Pulmonary signs	2 (5.6%)	Localized crepitations (2) Tracheal shift (1)
X-ray abnormalities	6 (16.7%)	Symptomatic with normal CXR (1) Abnormal CXR but no symptoms (2)
ARDS	1 (2.8%)	Patient expired
Abnormalities detected (*n*=6)		
High resolution computed tomography (HRCT) of thorax	6 (16.7%)	Findings suggestive of old tuberculosis (2) Lingular consolidation (1) Interstitial pattern (3)
Fiberoptic bronchoscopy (FOB)	0	Intra-luminal pathology (none)
Broncho-alveolar lavage (BAL)	3 (8.3%)	High neutrophil count (1) Gram-positive cocci (2)

lymphoma, and leukemia (Wilson and Streit, 1996). Fever may be intermittent with twice daily fever spikes, or less commonly, continuous. Fine-needle aspiration of the spleen is the most sensitive method of diagnosing VL in 96-98% of cases (Chulay and Bryceson, 1983). Organisms can also be identified in the bone marrow (positive in 55-88% of cases), liver (positive in 70% of cases) and lymph nodes (Mehmet *et al*, 2003). Patients gradually develop compromized immunity and bacterial superinfection; pneumonia, bronchitis, pyoderma, or otitis occurs in 60% of infected patients, and are often caused by *Pseudomonas aeruginosa* or *Staphylococcus aureus* (Pearson *et al*, 2000).

An increasing incidence of VL has been observed as a co-infection in HIV positive subjects in countries bordering the Mediterranean Sea (Russo *et al*, 2003). In these subjects, the length of the incubation period of VL is presumably very short, particularly in those who have severe immunodepression (Russo *et al*, 2003). At diagnosis, almost all cases of VL/HIV co-infection have been found to have fewer than 200 CD4+ cells/microliter of blood, and about 50% meet the AIDS-defining criteria during their first episode of VL (Russo *et al*, 2003). The clinical manifestations of VL in HIV-infected individuals may be similar to those seen in HIV-negative cases (Russo *et al*, 2003). Almost all cases of co-infection are prone to VL relapses, even after carefully managed anti-leishmanial treatment (Russo *et al*, 2003). However, HIV-positive cases may develop unusual multi-organ pathology and atypical presentations, as an impaired immune system allows spread and atypical localization of leishmania amastigotes more easily than in immunocompetent individuals (Yaduvanshi *et al*, 1999; Kumar *et al*, 2007). Oral ulcers and lymphadenopathy mimicking tuberculosis as a presentation of VL in HIV positive patients have been reported for India

(Yaduvanshi *et al*, 1999; Kumar *et al*, 2007).

Rare pulmonary involvement occurring in VL in patients with HIV co-infection have been reported from Spain (Nigro *et al*, 2003). Pleural and peritoneal involvement in VL has been reported in an HIV positive individual (Muñoz-Rodríguez *et al*, 1997). Bilateral pleural effusions with pulmonary symptoms have been reported in VL-HIV coinfection, where histologic evaluation of pleural fluid and bone marrow revealed histiocytes with intracellular leishmania amastigotes (Chenoweth *et al*, 1993).

Respiratory tract involvement in immunocompetent subjects is rare. Mucosal involvement confined to the upper respiratory tract (nose, upper lip, tongue, and mouth) due to *Leishmania donovani* and *Leishmania infantum* have been reported from the Sudan (Abdalla *et al* 1975). Lower respiratory tract involvement in immunocompetent individuals is rare; interstitial pneumonitis with fibrosis was reported in only one series involving HIV seronegative persons with VL without mediastinal lymphadenopathy (Duarte *et al*, 1989). Leishmaniasis involving the lung may mimic interstitial pneumonitis with radiographic evidence of multiple nodules consistent with bronchiolitis obliterans (Herrejón *et al*, 2005). In one such situation, the diagnosis of pulmonary leishmaniasis was established by the discovery of amastigotes in the transbronchial biopsy (Herrejón *et al*, 2005). Interstitial pneumonitis related to visceral leishmaniasis was found to enhance Th2 cell mediated inflammation with increased macrophages and CD8 cells, increased expression of IL-4, TNF-alpha and low IFN-gamma expression and the microenvironment have been reported to be responsible for increasing the chance of bacterial infection (Tuon *et al*, 2009). In one case report, progressively enlarging granulomatous mediastinal lymphadenopathy, worsening hemoptysis, and intense mucosal granulomatous inflammatory response in the large bronchi in an immunocompetent patient improved with a course of treatment with amphotericin B (Ben *et al*, 2000). In a retrospective study of 50 children with VL, fever, nonproductive cough and splenomegaly were found in all patients whereas anemia, leukopenia, neutropenia, thrombocytopenia and pancytopenia were found in 100, 80, 60, 60, and 60% of cases, respectively (Mehmet *et al*, 2003). Five children had pneumonia, there was bronchopneumonic infiltration in three patients, lobar pneumonia in two patients; the predominant pathogens isolated were *Staphylococcus aureus* and *Streptococcus pneumoniae* (Mehmet *et al*, 2003).

In our cases series of immunocompetent patients with VL, we found cytopenias (pancytopenia, bicytopenia and monocytopenia) were common hematologic complications. Pulmonary complications related to VL, probably due to secondary infection, were observed in 5 cases (13.9%) and 2 patients had old TB not related to VL. One patient in our series developed ARDS and expired despite all our efforts and four other patients responded to antibiotics along with anti-leishmania treatment.

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