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CASE REPORT

ETHIOPIAN PATIENT WITH VISCERAL LEISHMANIASIS-ASSOCIATED HEMOPHAGOCYTICLYMPHOHISTIOCYTOSIS

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ABSTRACT

A 29-year-old male patient was diagnosed to have visceral leishmaniasis at initial presentation, and later developed visceral leishmaniasis-associated hemophagocyticlymphohistiocytosis, as evidenced by persistent fever, worsening organomegaly and cytopenia, hyperferritinemia, hyperlipidemia, elevated transaminases, and hemophagocytosis in bone marrow aspirate. The case is presented and discussed with available literature review.
***Key words**: Visceral leishmaniasis, hemophagocyticlymphohistiocytosis.*

INTRODUCTION

Hemophagocyticlymphohistiocytosis (HLH) was initially described as "histiocytic medullary reticulosis" by Scott and Robb-Smith in 1939 (1). HLH is a rare and often fatal clinical syndrome characterized by an uncontrolled hyper inflammatory state due to excessive immune activation (2,3). Infections are known to be one of the major triggering factors for acquired HLH. Viral, bacterial, fungal, protozoan and parasitic-associated HLH have been reported. Other associated underlying clinical conditions include rheumatologic/autoimmune diseases, malignancies and metabolic disorders (3-6). Diagnosis of HLH requires molecular genetic testing, or 5 of 8 clinical parameters fulfilled. Clinical parameters of diagnostic criteria include fever, splenomegaly, bi- or pancytopenia, hyperferritinemia, hypertriglyceridemia and/or hypofibrinogenemia, low or absent NK-cell activity, high levels of soluble IL-2 receptor (sIL-2r), and hemophagocytosis in aspirates of bone marrow, spleen or lymph nodes (3,5,6). Standard treatment strategies for HLH include etoposide and dexamethasone as remission-induction, followed by cyclosporine as continuation therapy. Diagnosis of HLH in patients with visceral leishmaniasis is extremely challenging due to overlapping clinical features. High index of clinical suspicion is required for early diagnosis. Prompt treatment of the underlying cause is necessary for better outcome (5,6,9,10).Here we discuss the clinical presentation, diagnosis, and treatment associated with this rare and potentially lethal clinical disorder.

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CASE PRESENTATION

A 29-year-old male patient from Abderafi, North Gondar zone, North West Ethiopia, admitted to the Leishmaniasis Research and Treatment Centre, University of Gondar hospital in November, 2015 with fever of 15 days duration associated with drenching night sweats, loss of appetite and significant weight loss. He had blurred vision, light headedness, tinnitus and fatigue. He complained of nose bleeds but no gum bleeding or petechial rash. He repeatedly visited a kala-azar endemic area as a daily laborer. The patient did not experience swelling in the neck, axillae or groin area. He had no history of cough, sputum production, abdominal swelling or change in bowel habits. He had been admitted four months previously in the treatment centre with the diagnosis of visceral leishmaniasis, treated with sodium stibogluconate and had improved upon discharge. Test-of-cure (TOC) was not done due to logistic reasons. Serologic testing for HIV infection was negative during the previous admission.

On physical examination, he appeared chronically ill. His blood pressure (BP) was 100/70 mmHg, pulse rate (PR) 90 beats per minute (bpm), respiratory rate (RR) 20 breaths per minute and temperature (T) 39.7^0C. The patient was underweight with a body mass index (BMI) of 16.3 kg/m^2. He had conjunctival and buccal mucosal pallor. No lymphadenopathy was observed in accessible sites. The lungs were resonant to percussion and with good air exchange bilaterally. Abdomen was scaphoid with splenomegaly apparent 4 cm below the left costal

margin, and a total liver span of 14 cm. Multiple centrally umiblicated papules were observed over the face. The remainder of the examination was unremarkable.

Investigations on admission showedwhite blood count (WBC) of1600/µl, hemoglobin (Hgb) of 7gm/dl, platelet count of 109, 000/µl. Splenic aspirate was positive for Leishman-Donovan (LD) bodies (Grade 2+). Serologic testing for HIV infection was negative, and his CD4 count was 234/mm^3. Chest X-ray was normal, ultrasound examination of the abdomen revealed hepatosplenomegaly. A diagnosis of visceral leishmaniasis (first relapse) and Molluscumcontagiosum was made, and he was started on ambisome, 5 mg/kg day 1-5, 10, 17, 24 and miltefosine 50 mg po twice daily for 1 month. Cryotherapy was scheduled for skin lesions in a dermatology clinic. Complete blood count and biochemical tests were determined weekly and other investigations as required.His fever subsided and over all sense of well-being improved after 10 days of anti-leishmanial therapy. After three weeks of admission, he became acutely ill with persistent high grade fever, drenching sweats, fatigue, shortness of breath and palpitations. On examination, BP was 90/50 mmHg, PR was120 bpm, RR was23bpm, T was 39^0C.

He had paper-white conjunctivae and icteric sclerae. He had an ejection systolic murmur and S3gallop on cardiovascular examination. Abdominal examination revealed worsening organomegaly. Laboratory findings showed WBC of 600/µl, Hgb of5gm/dl, platelets of 3,000/µl. Serum creatinine, total bilirubin and transaminases were elevated. Presumptive diagnoses of suboptimal response to anti-leishmanial therapy, sepsis syndrome and drug toxicity were made. Intravenousceftriaxone and ciprofloxacin were started. Blood cultures were collected, but were negative for growth up to day 7.

Repeat bone marrow examination was done and revealed cellular marrow with maturation of all cell lines, and macrophagesengulfingerythroid and myeloid cells, suggestive of hemophagocytosis (Fig 1).Serum ferritin and fasting triglycerides were determined, and both were elevated. In sum, the clinical presentation met criteria for visceral leishmaniasis-associated hemophagocyticlymphohistiocytosis, and hence he was immediately started on dexamethasone, 4mg intravenously (IV)3 times daily for 2 weeks with taper over a month, and cotrimoxazoleas bacterial infection and pneumocystis pneumonia (PCP) prophylaxis. He required frequent transfusions of compatible packed blood cells. After completion of anti-leishmanial therapy, Test-of-Cure was done and revealed positive LD-bodies in a bone marrow aspirate (Grade 1+). The course of ambisome was extended with dose adjustments after development of hypokalemia and elevated creatinine. The patient's clinical condition deteriorated after 7 weeks of admission and died (Table1).

Fig 1. Photomicrograph shows marrow microphages with engulfed erythroid and myeloid cells. Wright stain, Oil immersion

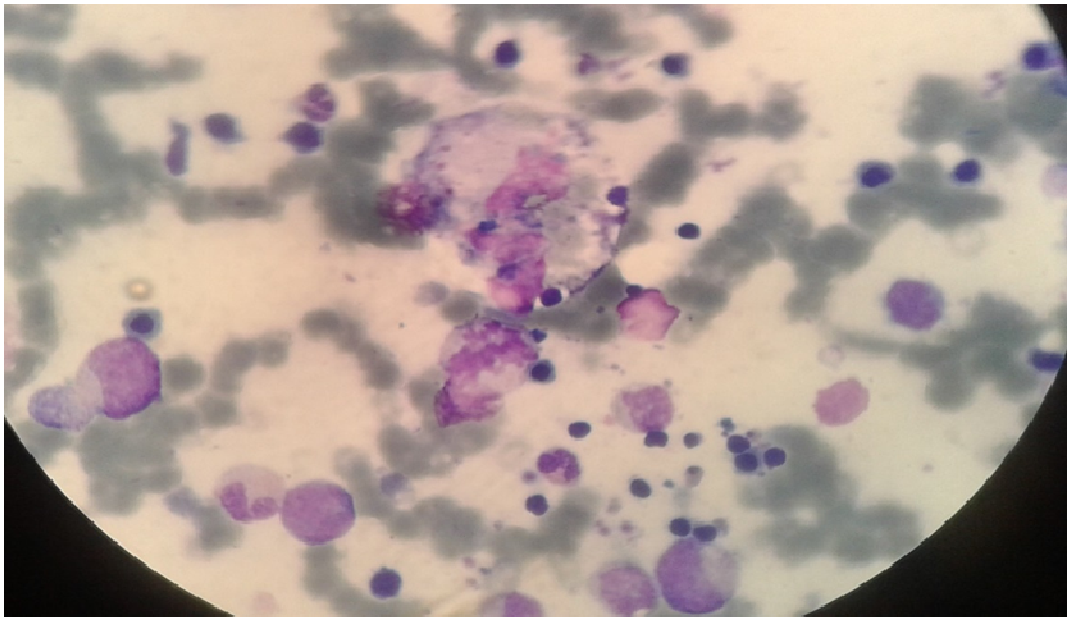

Table1. Laboratory results during initial admission, time of diagnosis of hemophagocyticlymphohistiocytosis, and one week before death.

Variables	On admission Nov. 2015	Time of HLH diagnosis Dec. 2015	A week before death Feb. 2015	Reference value
Complete blood count				
Hemoglobin (gm/dl)	7.0	5.0	4.6	12-18
WBC ($x10^3$/µl)	1.5	0.6	0.3	4-11
Platelets ($x10^3$/µl)	109	32	24	150-450
ESR (mm/hr)	94	58	-	0-20
Renal function tests				
BUN (mg/dl)	20	34	65	7-20
Cr (mg/dl)	1.2	1.6	1.8	0.6-1.2
Liver biochemical tests				
ALT (IU/dl)	42	125	74	5-40
AST (IU/dl)	36	102	23	5-40
Bilirubin (mg/dl)	0.9	2.0	-	0.3-1.5
Total protein (mg/dl)	4.8	4.2	-	6-8
Serum Albumin (gm/dl)	2.4	-	-	3.5-5.0
Electrolytes				
Potassium (meq/L)	3.8	3.5	3.2	3.5-5.0
Sodium (meq/L)	135	135	142	135-145
Chloride (meq/L)	110	106	119	102-109
Others				
Serum amylase (U/L)	90	215	188	20-96
Serum ferritin (ng/ml)	-	6909	-	30-250
Triglyceride (mg/dl)	-	300	-	30-200
Anti-rK 39 ELISA	positive	-	-	
Anti-HIV antibody	negative	-	-	
Anti-HCV antibody	negative	-	-	
HBs AG	negative	-	-	
ANA	negative	-	-	
BM for LD-bodies	positive	positive	-	
CD4 count	234	131	-	500-1300

HLH: Hemophagocyticlymphohistiocytosis

DISCUSSION

Hemophagocyticlymphohistiocytosis (HLH) is a hyper-inflammatory disorder resulting in hyperactivation of lymphocytes and histiocytes leading to aggressive and uncontrolled hemophagocytosis in the reticuloendothelial system. It comprises two clinical forms, primary or genetic affecting cytolytic secretory vesicles of immune cells, and secondary or acquired as a result of infectious diseases, autoimmune/rheumatologic diseases, malignancies and metabolic disorders(2-4).Acquired forms of HLH are more common than genetic forms, and most acquired forms have an infectious etiology. The possible role of infectious diseases in causing HLH was first elucidated in 1979 by Risdall *et al* in a case series describ-ing patients with a viral-associated HLH. After the viral-associated HLH cases were identi-fied by the group, associations with bacterial, protozoan, parasitic, and fungal infections were soon reported. (3-5). Although the exact pathogenesis is not well understood, HLH is thought to be a hyper-inflammatory statedue to cytokine storm, as the hyper stimulated lymphocytes and histiocytes infiltrate and damage host tissues (3-5).

Clinical features of HLH include prolonged fever, organomegaly, and manifestations of cytopenia of 2 or 3 of the major hematopoietic lineages, while hyperlipidemia, hyperferritinemia and hypofibrinogenemia are observed laboratory findings. Presence of hemophagocytosis in tissue is a parameter for diagnosis of HLH. The diagnosis can be challenging, as the early symptoms and signs are non specific and no specific laboratory tests exist (5,6). The Histiocyte Society formed a standard definition of HLH as part of the HLH-94 clinical trial in 1994. It has been revised as part of the HLH-2004 trial and is the definition most commonly employed for diagnostic purposes. Five out of 8 criteria are necessary to make a diagnosis of acquired HLH (Table 2) (5,6,9). Visceral leishmaniasis is parasitic infection caused by the genus *Leishmania* and transmitted by a bite from the infected sand fly, *Phlebotomus*. It is characterized by fever, organomegaly and cytopenia. Demonstration of Leishman-Donovan bodies in tissue aspirate remains the standard to diagnose visceral leishmaniasis (6-8). It is known that Leishmania spp. establish chronic intracellular infection in macrophages of the reticuloendothelial system, which could prompt uncontrolled macrophage activation with excessive release of cytokines and development of HLH (6-8). The case developed recurrent fever, worsening of organomegaly and cytopenia, and histo-pathologic features of hemophagocytosis in bone marrow aspirate during the second week of therapy for relapsed visceral leishmaniasis. Biochemical tests revealed hypertriglyceridemia, hyperferritinemia, elevated transaminases and hyperbilirubinemia, supportive of a diagnosis of visceral leishmaniasis-associated hemophagocyticlymphohistiocytosis.

The HLH-94 protocol described treatment strategies of immunosuppression and immune modulation with 8 weeks of remission-induction using etoposide and high-dose dexamethasone with or without intrathecal methotrexate, followed by continuation therapy with cyclosporine A. The HLH-2004 protocol intensifies treatment with cyclosporine A at the beginning of induction and adds hydrocortisone to intrathecal methotrexate, followed by hematopoietic stem-cell transplant with a suitable allogeneic donor. Most cases of infection-related HLH should be treated with standard HLH protocol in addition to addressing underlying infection (9,10). In conclusion, HLH is a catastrophic and fulminant clinical syndrome of immune activation. A high index of suspicion for early clinical recognition is required, since no specific diagnostic parameters exists and the disease is often fatal (9,10).

Table 2. Diagnostic criteria for HLH according to the HLH-2004 protocol

A diagnosis of HLH can be made if either criteria 1 or 2 is met:
1. Molecular diagnosis consistent with HLH
2. Clinical and laboratory criteria
(at least 5/8 criteria should be fulfilled)
- Fever
- Splenomegaly
- Cytopenia ≥2–3 cell lines in peripheral blood (hemoglobin <9 g/dl, platelets $<100 \times 10^3$//µl neutrophils $<1.0 \times 10^3$//µl)
- Hypertriglyceridemia and/or hypofibrinogenemia (fasting triglycerides ≥ 265 mg/dl, fibrinogen ≤1.5 g/L)
- Hemophagocytosis in bone marrow, spleen, CSF, or lymph nodes.
- Decreased or absent NK-cell activity (according to local laboratory reference)
- Ferritin ≥500 µg/L
- sCD25 (soluble IL-2–receptor) ≥2,400 U/mL
Supportive evidence includes:
Cerebral symptoms with moderate pleocytosis and/or elevated protein
Elevated transaminases
Elevated bilirubin

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