Original Article

Visceral Leishmaniasis-associated Hemophagocytosis: A Single Center Experience

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Abstract

Background: Kala-azar is a multisystem infection of the reticuloendothelial system. Various hematologic abnormalities have been described in kala-azar including hemophagocytic syndrome (HPS).

Methods: We reviewed bone marrow aspirate smears from 18 documented cases of kala-azar complicated by HPS.

Results: The bone marrow smears were hypercellular with erythroid hyperplasia. Megaloblastic changes, foamy macrophages, activated macrophages with cytoplasmic vacuoles and elongated cytoplasmic process, intra- and extracellular amastigotes, cytoplasmic fragments (blue bodies), plasma cells with inclusions and hemophagocytic cells were seen. Leishman bodies (amastigotes) were also found in some hemophagocytic cells.

Conclusion: Kala-azar should be considered as an etiology of HPS, particularly in endemic areas.

Keywords: Bone marrow aspiration findings, hemophagocytic syndrome, kala-azar

Cite the article as: Mokhtari M, Kumar PV. Visceral Leishmaniasis-associated Hemophagocytosis: A Single Center Experience. *Arch Iran Med*. 2013; **16(8):** 471–473.

Introduction

Kala-azar is a generalized protozoan infection of the reticuloendothelial system caused by the genus leishmania. The disease is endemic in the Middle East, East Africa and Southern Europe.[1] Kala-azar is characterized by fever, hepatosplenomegaly, lymphadenopathy and various hematological abnormalities.[2–4] The most common hematologic manifestation of kala-azar is anemia however the patient may develop leukopenia, thrombocytopenia, pancytopenia, and coagulation abnormalities. Rarely, hemophagocytic syndrome (HPS) – a disorder caused by activation and proliferation of T-cells and macrophages with subsequent cytokine production, leading to fever of unknown origin, hepatosplenomegaly, pancytopenia, hypofibrinogenemia and hypertriglyceridemia may develop.[5,6] The disease may be primary and familial or secondary to various etiologies that include viral, bacterial, fungal and parasitic infections as well as malignancies. It may occur in the setting of some immunodeficiency syndromes (Chediak-Higashi, Griscelli and X-linked lymphoproliferative syndrome) and as a complication of autoimmune disorders, particularly rheumatoid arthritis.[6–9]

Clinical presentations of HPS mimic visceral leishmaniasis. In addition, visceral leishmaniasis may be complicated by hemophagocytosis, thus differentiating between the primary and secondary forms of HPS is an important task. Infection-associated HPS resolves with treatment of the underlying infection whereas in the primary form, cytotoxic drugs are the mainstay of therapy.

In this study we described the bone marrow findings of visceral leishmaniasis complicated by HPS.

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Accepted for Publication: 19 June 2013

Materials and Methods

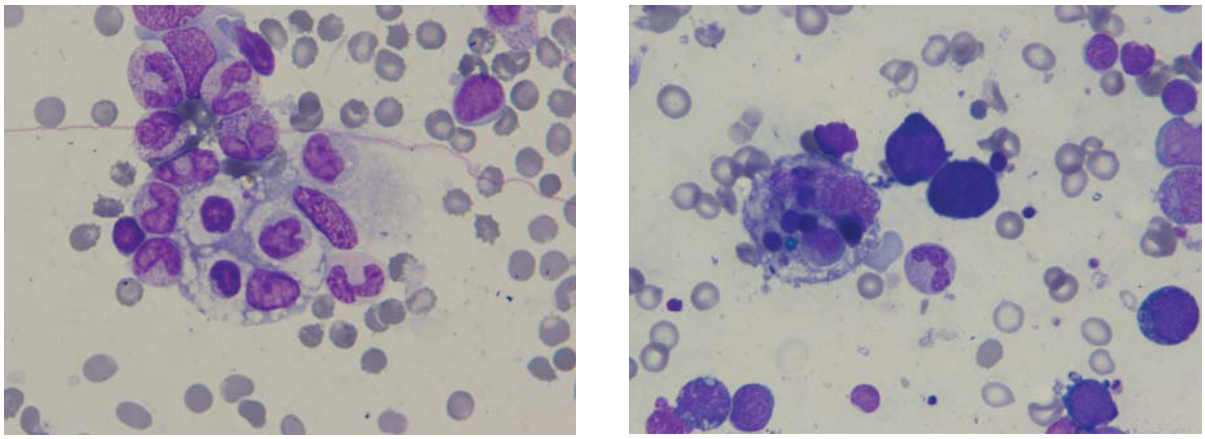
Bone marrow aspirates and peripheral blood samples from 18 (11 females and 7 males) documented cases of kala-azar complicated by HPS were reviewed in order to determine clues for the diagnosis of leishmaniasis-associated HPS. Patients ages ranged from 9 months to 10 years (mean: 4.5 years).

Results

The bone marrow smears were hypercellular with decreased myeloid/erythroid ratio (erythroid hyperplasia). Megaloblastic changes, foamy macrophages, activated macrophages with cytoplasmic vacuoles and elongated cytoplasmic process, intra- and extracellular amastigotes, cytoplasmic fragments (blue bodies), as well as plasma cells with inclusions and hemophagocytic cells were observed. Leishman bodies (amastigotes) were also found in some hemophagocytic cells (Figures 1–4). Based on bone marrow search, the parasite load varied between cases of kala-azar and ranged from heavy to sparse. In one patient who had a negative serologic test and bone marrow smears, we diagnosed visceral Leishmaniasis based on *Leishmania*-specific polymerase chain reaction (PCR). In this patient reexamination of the bone marrow aspirate after three weeks revealed intracellular leishman bodies. The most common hematological abnormality among patients was anemia in 16 (88.8%) patients. Other hematological changes were thrombocytopenia in 9 (50%) and pancytopenia in 10 (55.5%) patients.

Discussion

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, hepatospenomegaly and various hematological abnormalities.[3,4,10] The disease affects



A B

Figure 1 (A and B). Bone marrow aspiration smear shows macrophages with engulfed erythroid, lymphocytes and polymorphonuclear cells (hemophagocytosis) in a polymorphic background. Wright stain, oil immersion.

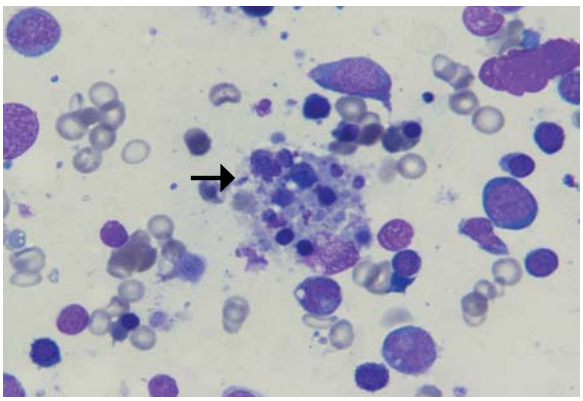


Figure 2. Bone marrow aspiration of visceral leishmaniasis complicated by hemophagocytosis. Leishman bodies are evident in the cytoplasm of hemophagocytic cells. Wright stain, oil immersion.

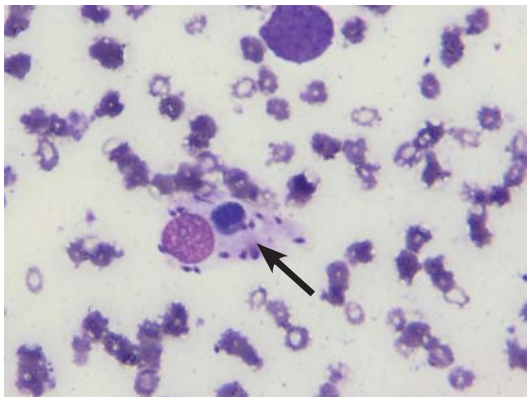


Figure 3. Hemophagocytic cells loaded with leishman bodies. Wright stain, oil immersion.

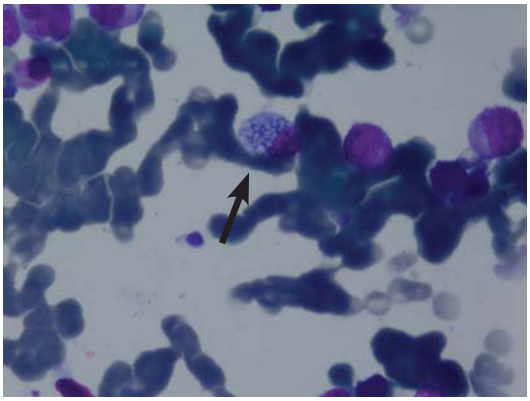


Figure 4. Plasma cells with cytoplasmic inclusions in bone marrow aspirate of visceral leishmaniasis complicated by hemophagocytosis. Wright stain, oil immersion.

approximately 500,000 individuals each year.[10,11]

Diagnosis of visceral Leishmaniasis is based on the presence of amastigotes (Leishman bodies) in bone marrow or tissue sections, serologic assays, cultivation of the organism and PCR.[11,12] Demonstration of Leishman bodies remains the gold standard in the diagnosis of kala-azar.[12]

The most common hematological abnormality in our patients was anemia followed by thrombocytopenia and pancytopenia.

The development of anemia in kala-azar may be due to malnutrition, sequestration, an immune mechanism, hemolysis, bone marrow depression and hemophagocytosis. Thrombocytopenia and leukopenia may occur as a consequence of hypersplenism and hemophagocytosis.[4]

Some authors previously described bone marrow smear findings of visceral Leishmaniasis such as intra- and extracytoplasmic amastigotes, plasma cells, free cytoplasmic bodies, hemophagocytic cells and myelodysplasia.[13–15] In keeping with their results we found intra- and extracellular amastigotes, megaloblastic changes, plasma cells, free cytoplasmic bodies, and hemophagocytic cells in bone marrow aspirates of our patients. We also observed the

presence of foamy macrophages, activated macrophages with cytoplasmic vacuoles and elongated cytoplasmic processes in addition to Leishman bodies in the hemophagocytic cells.

HPS is a life threatening condition characterized by uncontrolled activation of the immune system secondary to various inherited and acquired disorders.[5,6]

It was first thought that the viruses were the main etiology of infection-associated HPS, however, numerous other agents such as gram-negative bacteria, tuberculosis, leishmaniasis, and fungi have been reported to cause HPS.[16] Epstein-Barr virus is the most common virus in inducing HPS. This virus is proposed to be a triggering agent in both the familial and malignancy-associated forms of HPS.[6,8,9,17,18]

HPS is characterized by an increased inflammatory response due to hypersecretion of pro-inflammatory cytokines that lead to cytopenia, coagulopathy and high serum triglyceride levels.[6,16,19] Despite the pro-inflammatory state, there is impaired function of cytotoxic T lymphocytes and natural killer cells. In infection-associated HPS, high levels of cytokines and interference by infective agents may be the cause for cytotoxic T cell dysfunction.[6,8]

The cardinal signs of HPS of fever, hepatosplenomegaly and cytopenia may occur in response to various infections such as visceral Leishmaniasis but increasing severity of these signs should raise the possibility of a more serious problem such as HPS.[6]

Conclusion

The presentation of HPS and visceral Leishmaniasis are very similar. Therefore in the presence of hemophagocytic cells, plasma cells, free cytoplasmic bodies, and activated macrophages an extensive search for Leishmania amastigotes should be performed, particularly in endemic areas. Performing serological tests and PCR on bone marrow or peripheral blood specimens will increase the sensitivity of diagnosing Leishmaniasis-associated HPS.

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