Visceral Leishmaniasis: Bone Marrow Biopsy Findings

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Summary: Visceral leishmaniasis (VL) or Kala-azar is a common parasitic infection among children in Iran. The records of 249 children with VL were evaluated retrospectively. The clinical, hematologic, and bone marrow biopsy findings were studied. In particular, we assessed whether there was an association between bone marrow biopsy findings and prognosis. Five major groups were identified: (1) hypercellular marrow with many Leishman Donovan (LD) bodies, (2) multiple noncaseating granulomas with a few LD bodies, (3) diffuse fibrosis with rare LD bodies, (4) benign lymphoid nodules with many LD bodies, and (5) marrow necrosis with many LD bodies. The patients with hypercellular marrow and benign lymphoid nodules were alive and responded well to glucantime therapy. The patients with marrow fibrosis and marrow necrosis died and were resistant to any type of therapy. Patients with granulomas did not respond to glucantime therapy but responded to amphotericin B. However, less than half of the patients died owing to malnutrition and misdiagnosis. We correlated the bone marrow biopsy findings with the treatment outcomes and prognosis. The outcome was excellent in cases of hypercellular marrow, very poor in cases of fibrosis and necrosis, and intermediate in cases of granulomas. As a result, we believe that bone marrow biopsy findings can be helpful for assessing the prognosis of VL patients.

Key Words: visceral leishmaniasis, Kala-azar, splenomegaly, bone marrow biopsy, marrow lymphoid nodules, marrow necrosis

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Visceral leishmaniasis (VL) is a chronic inflammatory disease produced by several species of *Leishmaniae*, *L. donovani*, *L. infantum*, and *L. chagasi*. This infection is common among the poor in the central parts of India, Latin America, Middle East, China, Africa, and Southwestern Asia. Sandflies transmit this disease to humans via bite. Children are mainly affected by this infection and the clinical infection is characterized by chronic fever, anorexia, weight loss, and splenomegaly.[1–7]

Microscopically, the infection is characterized by a massive parasitic (amastigote) infiltration of macrophages. In bone marrow smears, the parasites are oval-round bodies with large nuclei, 2 to 4 μm in diameter and display the characteristic kinetoplast. In biopsy specimens, the parasites are 1 to 2 μm round bodies inside macrophages visualized under the oil immersion objective. VL produces a variety of hematologic abnormalities, such as anemia, leukopenia, thrombocytopenia, and pancytopenia. The bone marrow, spleen, and liver are common sites of infection, but other organs such as the lung, kidney, and gastrointestinal tract can also be involved.

VL is a common childhood parasitic infection in Iran, is primarily caused by *L. infantum*. This disease is endemic to the southern regions of Iran. Here, we present a retrospective study of 249 children with VL who were treated with glucantime and also amphotericin B. This report focuses on the significance of marrow biopsy findings and its relation to patient prognosis.

PATIENTS AND METHODS

The records of 249 children who were admitted to the Shiraz university Hospitals (Namazi and Faghihi) in the Shiraz and Fars provinces of Iran over a 25-year period were studied retrospectively. The hospitals in Shiraz are referral centers for the southern provinces of Iran (Khuzestan, Busher, Yasuj, and Fars). Patients' ages ranged between 5 and 9 years. One hundred eighty-three males and 66 females were included in the study.

In addition to the history and physical examination, all children had a complete blood count (coulter electronics, Ltd, UK), VL serology (hemagglutination titer-Behring werke Ag, Germany), and bone marrow study. Serologic tests and cultures were performed in the microbiology department of Shiraz medical school. Bone marrow aspirations and trephine biopsies were performed on the posterior iliac crest. The smears were Wright-Giemsa stained. Histologic sections were stained with hematoxylin-eosin, Ziehl-Neelson (acid-fast bacilli), and Masson-trichrome (fibrosis). Four pathologists reviewed the biopsies and marrow smears. The parasite load was calculated arbitrarily by counting the number of parasites

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per 10 consecutive oil immersion fields (OIL). Patients with AIDS or tuberculosis (TB) were excluded.

The preferred treatment regimen of VL consisted of antimony (glucantime) 20 mg/kg/d for 3 weeks parenterally. We administered amphotericin B, 0.5 to 1 mg/kg/d for 4 weeks intravenously, to patients with granulomas unresponsive to glucantime therapy. However, 9 cases of granulomas were initially misdiagnosed as TB and treated with anti-TB drugs at outside hospitals. Another 11 patients were under-nourished and suffered from severe anemia.

RESULTS

This disease occurred most often in residents of rural and mountainous areas. The duration of illness varied from 1 month to 1 year. Four of the 45 granulomatous cases were readmitted for relapse within a 5 to 6 months period. A diagnosis of VL was made on the basis of a clinical picture compatible with disease, together with high indirect hemagglutination titers for leishmania (at least 1:128) or identification of Leishman Donovan (LD) bodies in marrow smears or both.

The most common symptoms and signs were fever (100%), malaise (100%), anorexia (100%), splenomegaly (100%), hepatomegaly (80%), arthritis (30%), and weight loss (4%). The results of the initial peripheral blood cell counts revealed mild anemia (75%), mild thrombocytopenia (39%), mild elevation of the erythrocyte sedimentation rate (25%), pancytopenia (10%), and mild leukopenia (7%).

Examination of bone marrow aspirations and biopsies were performed upon admission. The marrow smears revealed adequate megakaryocytes, reverse myeloid-erythroid ratio, plasmacytosis (ranges 8% to 15%), and mast cells. Hemophagocytosis was noticed rarely in 7 cases. LD bodies were observed in all cases; however, the load of parasites varied from case to case. We counted the total number of LD bodies in 10 consecutive OIF. If the parasitic count was between 50 and 100 in 10 OIF or if there were 2 to 3 fully loaded macrophages in 10 OIF, we placed that sample into the "many" category. If the parasite count was 10 to 50, we placed that sample into the "frequent" category. A parasitic count between 5 and 10 per 10 OIF was categorized as "few." If 1 to 2 parasites were found after an extensive search in 10 OIF, we categorized that sample as "rare." The morphology of LD bodies is easily detected in marrow smears (Figs. 1A, B).

Marrow biopsies (Figs. 2A–E) revealed 5 major findings: hypercellularity, granulomas, fibrosis, benign lymphoid nodules, and necrosis. The details of 5 groups, treatment response, and prognosis are presented in Table 1 and the statistical data comparing the outcome of the 2 groups (hypercellular vs. granulomas) are presented in Figure 3. The LD bodies were small round bodies under oil immersion but the morphology was not as clear as detected in the smears. The special stains, such as acid-fast and Masson-trichrome were performed to identify acid-fast bacilli and fibrosis, respectively. Post-treatment bone marrow examination after 6 months did not reveal leishmanial parasites and showed normocellular marrow.

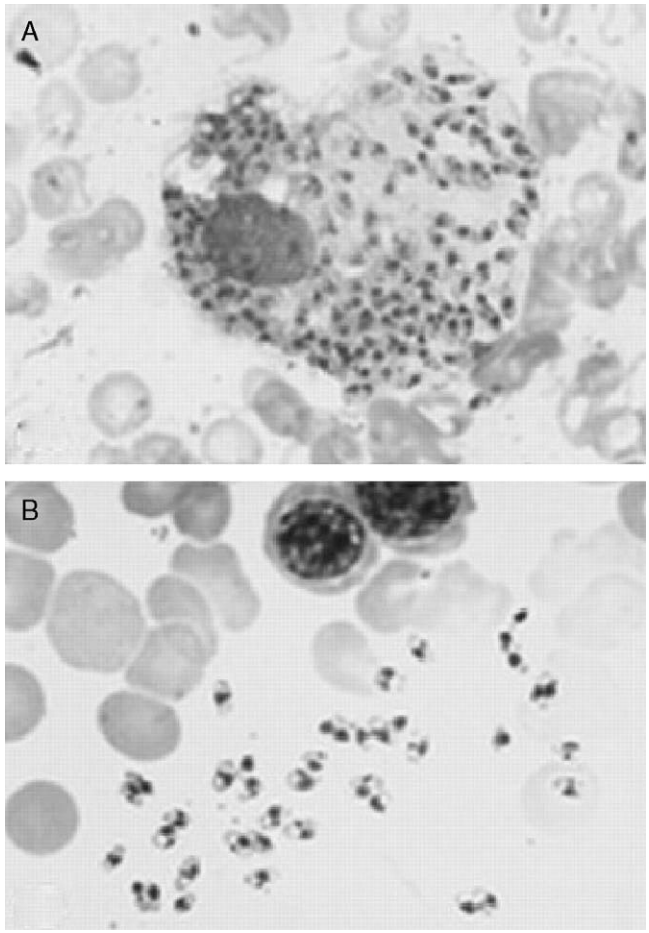

FIGURE 1. A, Bone marrow smear shows one macrophage loaded with nearly 100 oval to round-shaped LD bodies. The nucleus and kinetoplast are seen clearly inside the LD body. Wright-Giemsa: 1200. B, The background of a bone marrow smear shows isolated LD bodies outside the macrophage. Wright-Giemsa: 1200.

DISCUSSION

Bone marrow biopsy findings in VL have rarely been reported. In this report, we describe marrow biopsy findings and discuss the differential diagnoses of VL. We also tried to correlate marrow biopsy findings with the treatment response and prognosis. Our study revealed 5 major groups: hypercellular, noncaseating granulomas, fibrosis, benign lymphoid nodules, and necrosis.

Patients in the hypercellular marrow group had numerous LD bodies identified by smear. These patients responded well to glucantime therapy and continued to do well. Relapse was only observed in 1 patient within a period of 6 months. These hypercellular marrow biopsies

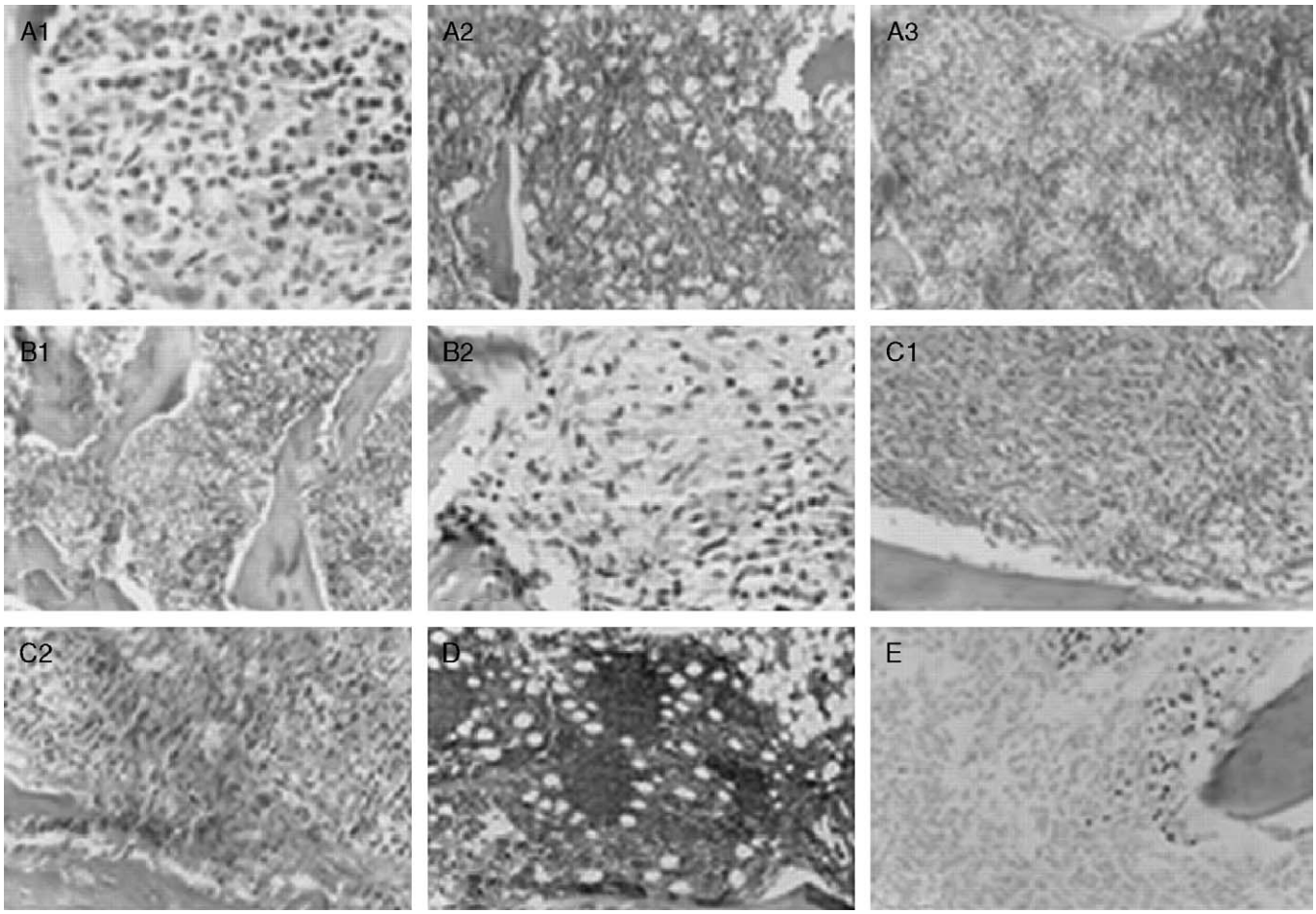

FIGURE 2. Bone biopsy shows: A1, hypercellular marrow infiltrated by numerous macrophages loaded with LD bodies. No fat or other marrow cells were observed. Hematoxylin and eosin (H&E): 900. A2, Hypercellular marrow infiltrated with large macrophages with clear cytoplasm resembling the ''starry-sky appearance.'' No fat or other marrow cells were observed. H&E: 400. A3, Hypercellular marrow infiltrated with small groups of pale-looking macrophages resembling storage disease. H&E: 400. B1 and B2, Hypercellular marrow with noncaseating granulomas composed of epitheloid histiocytes, plasma cells, and fibroblasts. No giant cells were observed. H&E: 400, 900. C1 and C2, Hypercellular marrow shows focal and diffuse infiltration with spindle-shaped fibroblasts with hyperchromatic nuclei. H&E: 400, 900. D, Hypercellular marrow shows benign lymphoid nodules of varying sizes without germinal centers, resembling lymphoma. H&E: 400. E, Severe marrow necrosis shows pink granular material and a few viable cells. H&E: 400.

can be confused with many diseases such as lymphoma-leukemia, hemoloytic anemias, megaloblastic anemias, refractory anemias, myeloproliferative disorders, and hemoglobinopathies. However, the above diseases were excluded because of the presence of numerous LD bodies. A few interesting findings were also noticed in this group such as starry-sky appearance (Fig. 2A2) resembling Burkitt lymphoma and focal collection of macrophages (Fig. 2A3) resembling storage diseases. However, these macrophages were loaded with LD bodies.

In the noncaseating granuloma group, LD bodies were rarely identified and generally only after an extensive

TABLE 1. Marrow Biopsy Findings in 249 Patients With VL and Outcome

Marrow Findings	No. Cases	Load of LD Bodies	Response to Glucantime	Prognosis
(1) Hypercellular marrow	187	Many	Good	Alive
(2) Noncaseating granulomas	45	Few	Not good	Dead: 20, alive: 25
(3) Fibrosis	9	Rare	Not good	Dead
(4) Benign lymphoid nodules	5	Many	Good	Alive
(5) Marrow necrosis	3	Many	Not good	Dead

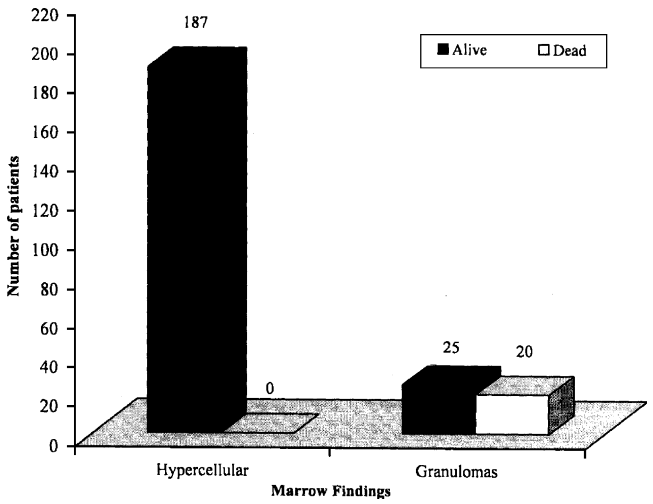

FIGURE 3. Statistical data comparing the outcome of 2 groups (VL, hypercellular, vs. granulomas).

search. Polymerase chain reaction was more useful for diagnosis of these cases. Response to glucantime therapy was not poor. Relapses were seen in 4 cases within a period of 5 to 6 months. Some patients responded to amphotericin B. The overall prognosis was not good in this group. Less than half of the patients (20) died owing to misdiagnosis and bleeding problems. Nine of these 20 cases were initially misdiagnosed as TB and were treated with anti-TB drugs. The other 11 patients were under-nourished and anemic. If the LD bodies had not been identified in the screening, one could easily mistake these cases for TB, brucellosis, viral or fungal infections. We believe VL should be considered in the differential diagnosis of marrow granulomas, especially in endemic countries.[8]

Diffuse or focal fibrosis in association with VL has rarely been reported.[9] Patients in the fibrotic marrow group were commonly misdiagnosed with myelofibosis, radiation fibrosis, and repair process. In such situations, identification of LD bodies and a Polymerase chain reaction study were helpful for the diagnosis. These patients did not respond to any type of treatment and died during hospitalization owing to severe hemorrhage and infection.

The lymphoid nodule marrow group comprised a small number of cases in our study. The marrow biopsy findings of these patients can be easily mistaken for lymphomatous involvement.[10] Marrow lymphoid nodules in association with VL has not been reported so far. These nodules might have formed secondary to the parasitic reaction. These patients responded well to glucantime therapy.

Necrotic marrow was observed in 3 cases in this study. This has never been reported in association with VL. However, marrow necrosis has been reported in association with many conditions, such as infections, sickle cell anemia, leukemia-lymphoma, metastatic tumors, and radiation. Our patients did not respond well to glucantime therapy and died because of disseminated intravascular coagulation. The exact cause of necrosis was unclear. It was likely due to thrombosis of the capillary lumen by parasites resulting in the necrosis.

In this study, the majority of cases belonged to 2 groups, hypercellular (187 cases) and granulomatous (45 cases). The patients with hypercellular marrow responded well to glucantime therapy, whereas granulomatous cases responded to amphotericin B. The prognosis was excellent in the hypercellular group; however, in the granulomatous group patients fared poorly (20 deaths). The exact cause of death was unclear. It is likely that many factors were involved such as misdiagnosis, malnutrition, anemia, and secondary infection. Statistical analysis in Figure 3 shows striking differences in the treatment outcomes between these 2 groups.

In conclusion, our study revealed that VL could produce various histologic changes in bone biopsy specimens, such as hypercellularity, granulomas, benign lymphoid nodules, fibrosis, and necrosis. Patients with hypercellular marrow responded well to glucantime therapy and continued to do well. The patients with fibrosis and necrosis died despite treatment with glucantime. Cases with granulomas responded to amphotericin B well. Overall, their prognosis was not good. We also noticed a few interesting histologic findings, such as marrow necrosis, benign lymphoid nodules, and starry-sky appearance, which had not been reported before in association with VL.

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