BONE MARROW HISTOPATHOLOGICAL CHANGES IN VISCERAL LEISHMANIASIS

Mohammed O. Al-Sohaibani, MD, FCAP

Over a period of two years (June 1989-May 1991), bone marrow trephine biopsies from 16 patients with visceral leishmaniasis were examined histologically to assess the correlation between the peripheral blood counts and bone marrow status. Only 25% of patients had normal peripheral blood counts. Of the remainder, 25% had anemia alone, 25% had anemia with thrombocytopenia, and 25% had pancytopenia. The anemia was microcytic/hypochromic in 88.7% of cases and normocytic in 12.3% of cases. Thrombocytopenia was a notable feature, with mean platelet count of $115 \pm 72.47 \times 10^9$/L, and eight patients having thrombocytopenia below 120×10^9/L. There was diffuse bone marrow hypercellularity in 13 cases and focal hypocellularity in three. The increased cellularity was attributable to trilineage hyperplasia, with a predominance of erythroid activity in most cases. Increase in the histiocytic population was a prominent feature in all the cases. In 10 cases, most of the histiocytes were found to be full of LD bodies, while in the other six, the parasite load was much less. Megakaryocytes were abundant in number, forming aggregates in most cases. Other notable features included erythrophagocytosis (nine cases) and moderate perivascular plasmacytosis (13 cases). *Ann Saudi Med 1996;16(3):304-307.*

Visceral leishmaniasis (VL), also known as kala-azar, is endemic in the southern (Gizan Province) and southwestern regions (Asir Province) of Saudi Arabia.[1-3] On the other hand, the cutaneous form of leishmaniasis is prevalent in the eastern and central regions, particularly in Al-Hassa and Al-Qassim.[2] Previous reports from the Kingdom[4-6] have dealt with the clinical features of VL, which are found to be similar to those described in patients from elsewhere.

To the best of our knowledge, a detailed study demonstrating bone marrow findings in patients with VL has no routine histologic techniques. Four micron sections were cut and stained with hematoxylin and eosin, Giemsa, and reticulin stain by silver impregnation.

Material and Methods

The study was conducted on 18 patients of pediatric age group, admitted to King Fahad Hospital at Al Baha with clinical features suggestive of VL. The duration of their illness ranged from two weeks to one year. Each patient underwent a complete clinical workup, including a detailed history and physical examination, followed by diagnostic bone marrow or splenic puncture in which Leishman-Donovan bodies (LDB) or amastigotes were demonstrated.

From the Department of Pathology, College of Medicine, King Saud University, Riyadh.

Address reprint requests and correspondence to Dr. Al Sohaibani: Department of Pathology, College of Medicine, King Saud University, P.O. Box 2925, Riyadh 11461, Saudi Arabia.

Accepted for publication 12 December 1995. Received 10 October 1995.

Adequate trephine bone marrow biopsies were obtained from the posterior iliac crest with a Jamshidi needle in 16 patients, who are the subjects of this report. Biopsies were fixed in 10% neutral buffered formalin, then decalcified for one hour and processed by routine histologic techniques. Four micron sections were cut and stained with hematoxylin and eosin, Giemsa and reticulin stain by silver impregnation.

Microscopically, the marrow cellularity was assessed according to the criteria described before.[5] Four hundred cell differential counts were performed on Giemsa-stained sections. The myeloid:erythroid ratio (M:E) was calculated. Sections were also examined for the presence and the extent of parasitemia. The reticulin content of each silver-impregnated section was evaluated. The plasma cell and histiocyte population contents were also assessed.

The peripheral blood counts were performed on a Coulter S-Plus multi-analyzer (Coulter Electronics, Hialeah FL).

Results

There were 16 patients in the present study, with a mean age of 1.4 years (range four months to 12 years), with the majority being under the age of four years. There were 10 males and six females. The most common presenting clinical features were fever (90.6%), pallor (89%), abdominal distention (60%), splenomegaly (100%), hepatomegaly (95%), and weight loss (50%). Intercurrent infections, mainly of the respiratory and gastrointestinal tracts, were detected in 50%.

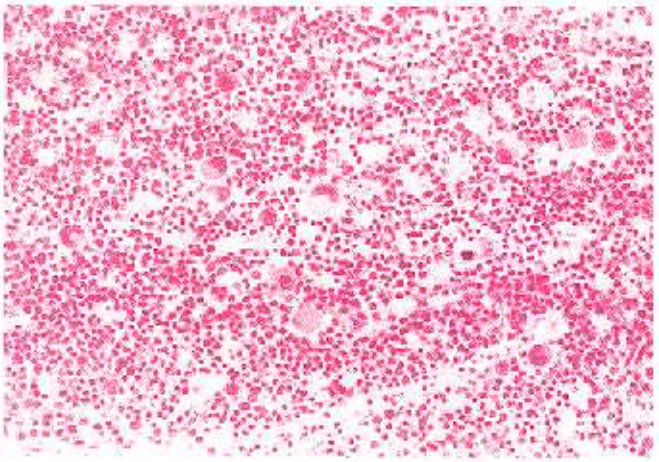

FIGURE 1. Diffuse increased cellularity with increased number of megakaryocytes (250x).

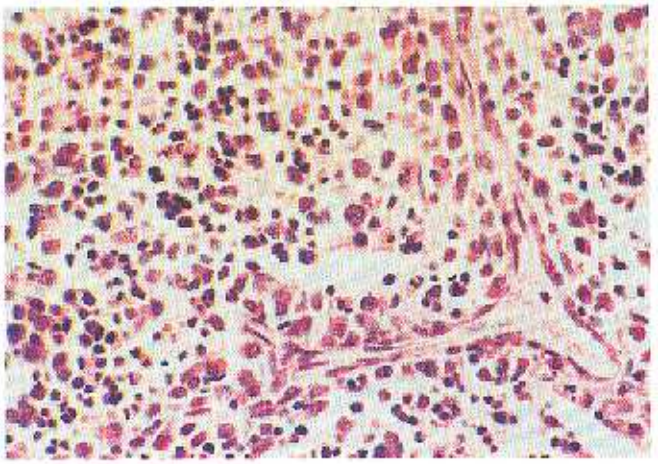

FIGURE 2. Marrow section with blood vessel surrounded by slightly increased numbers of plasma cells (250x).

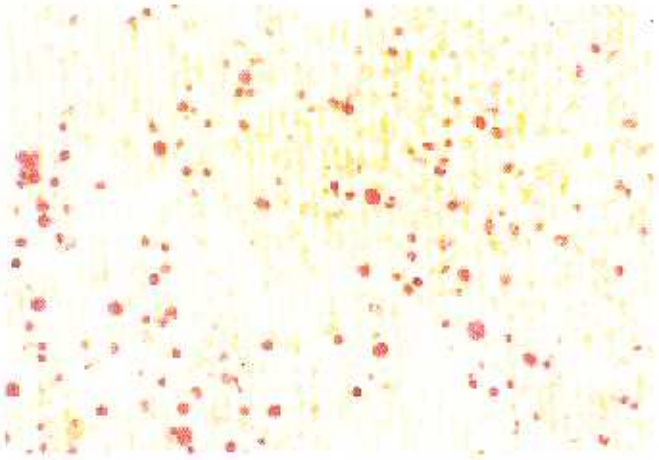

FIGURE 3. Hyperplastic cells with erythrophagocytosis (250x).

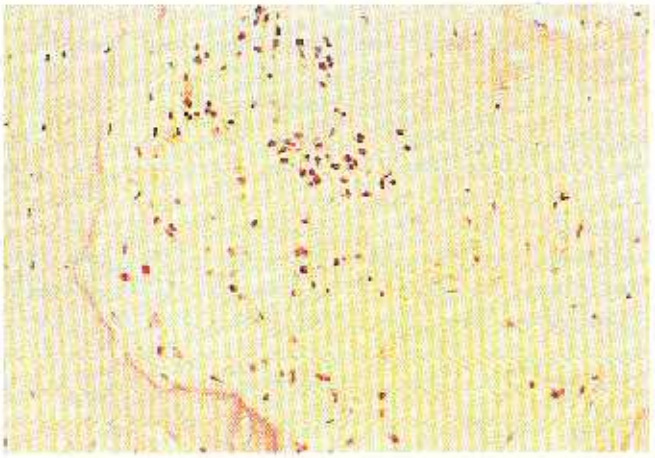

FIGURE 4. Marrow section showing lysis of marrow fat cells (100x).

Peripheral Blood Counts

Peripheral blood counts on admission were normal in 25% of cases. Of the remaining cases, 25% had anemia alone, 25% had anemia and thrombocytopenia, and 25% had pancytopenia. Thus, anemia was a common finding in most patients. The mean hemoglobin concentration was 70.2 ± 13.3 g/L. The anemia was microcytic and hypochromic in 88.7% of the cases and normocytic in the rest. Iron deficiency contributed to the hypochromic-microcytic anemia, as was evident from the bone marrow iron depletion in 12 cases. The mean platelet count was 115 ± 73 x 10^9/L, with eight patients (50%) presenting with a platelet count below 120 x 10^9/L (Table 1).

Bone Marrow Findings

Evaluation of hemopoiesis in the trephine biopsy sections revealed diffuse hypercellularity in 13 cases (Figure 1) and focal hypocellularity in the rest. There was trilineage hyperplasia, predominantly affecting the erythroid cells with an M:E ratio of 1:1 or less. Megakaryocytes were abundant, forming aggregates in 14 cases (Figure 1). Plasmacytosis, ranging between 4% and 11%, was evident in 13 cases, with plasma cells mainly clustering around blood vessels (Figure 2). Histiocytes were increased in 10 cases; in six of these, the histiocytes contained large numbers of LDB. In some sections, these histiocytes were present in aggregates with apparent necrosis, resulting in release of free LDB (Figure 3). Erythrophagocytosis was seen in 14 cases. In two cases, clusters of histiocytic cells showing large hyperchromatic nuclei were seen. Lysis of the marrow fat cells was seen in two sections (Figure 4). Vascular changes in the form of endothelial swelling and subendothelial deposition of hyaline-like material in small arteries (Figure 5) were seen in three cases. There were no granulomata in any of the sections.

Reticulin stain revealed mild diffuse increase in reticulin in 10 cases (Figure 6) and focal increase in six cases. The latter was seen in the areas of reticulum cell aggregates.

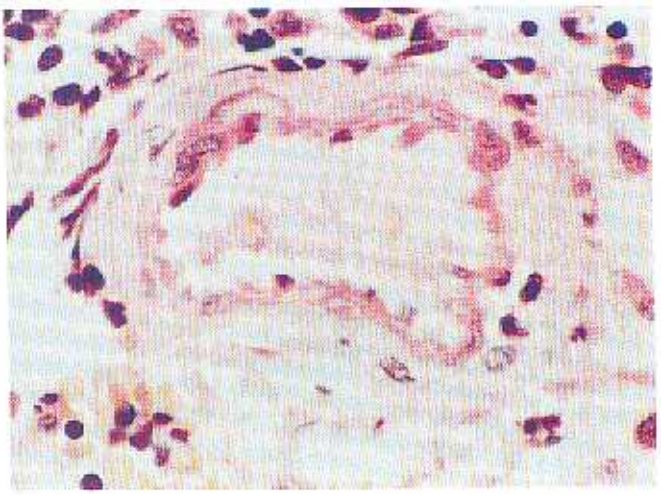

FIGURE 5. Evidence of endothelial cells swelling and subendothelial deposition of hyaline-like material (400x).

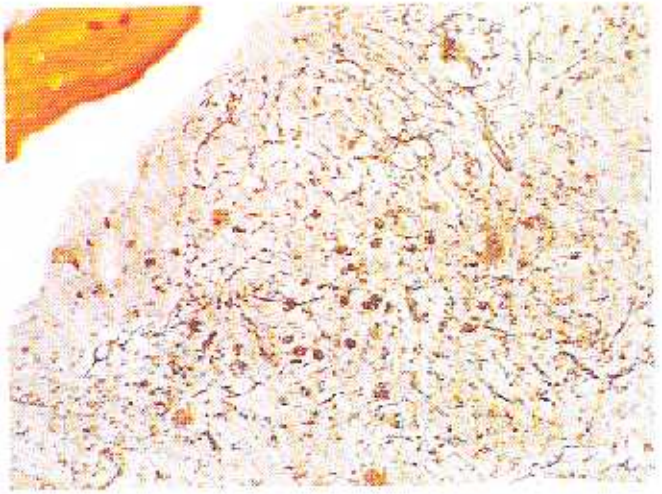

FIGURE 6. Marrow with mild diffuse increased fibrosis (250x).

TABLE 1. *Peripheral blood findings in 16 patients with visceral leishmaniasis.*

	Mean + SD	Range
Hemoglobin (g/L)	70.2 ± 1.33	45 – 94 (12)*
PCV	0.213 ± 0.39	0.133 – 0.264 (12)*
MCV (fL)	67.2 ± 9.5	51.8 – 84
MCH (pg)	22.2 ± 3.8	17.3 – 29.2
MCHC (g/L)	330.7 ± 23.5	276 – 361
WBC ($x10^9$/L)	5.0 ± 2.9	1.5 – 11.1 (4)*
Neutrophils (%)	23.5 ± 15.8	43 – 91 (5)*
Platelets ($x10^9$/L)	115 ± 72.5	4.0 – 268 (8)*

*Number of cases with low values; PCV=packed cell volume; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration.

Discussion

The hematological changes noted in a group of children with VL include anemia and thrombocytopenia, as well as pancytopenia. Anemia was found to be the most common finding in these patients, affecting more than half. This could be due to hemophagocytosis, which was a prominent feature in our cases and which appears to be the principal contributing factor to the development of hemolytic anemia seen in VL, analogous to that observed in the virus-associated hemophagocytic syndromes.[8,12,13]

Thrombocytopenia in association with anemia was noted in 25% of patients. In VL it is likely to be due to increased peripheral platelet destruction, which may be caused by an immune-mediated mechanism, or may merely be a reflection of the increased hemophagocytosis. The marrow megakaryocytic hyperplasia is in keeping with compensatory thrombopoietic response.

Cytopenias associated with VL have been the subject of many previous studies.[1,3-8] Although in one report reduced activity of the bone marrow was considered to be responsible for the cytopenias,[9] subsequent studies have failed to confirm this and instead, increased marrow activity has been demonstrated. Several mechanisms have been suggested to account for pancytopenia in VL, the most acceptable of which is histiocytic hemophagocytosis. Besides its occurrence in the bone marrow, hemophagocytosis is found to be even more pronounced in extramedullary reticuloendothelial tissues such as the spleen and the liver.[8,10,11] Similarly, in the present study, pancytopenia was associated with hyperplasia of the hemopoietic tissue in the majority of cases with greater increase in erythropoiesis than in granulopoiesis, as reflected by the reduced M:E ratio. This could indicate that red blood cells are more prone to increased destruction than the granulocytes in VL, and that the marrow response favored erythropoiesis.

The increase of marrow plasma cells seen in most of our cases is yet another salient feature of VL. Bone marrow plasmacytosis could be a reflection of increased polyclonal B-cell activation in VL, which is usually accompanied by hypergammaglobulinemia.

The bone marrow vascular changes observed in the present study have been previously reported[11] and could be attributed to gamma globulin deposition by the surrounding plasma cells, or alternatively, the result of immune complex deposition.

The focal bone marrow hypocellularity noted in some cases is interesting and could be the result of necrosis and its aftereffect. Increased reticulin is known to occur secondary to inflammatory lesions, as in granulomata. In our patients, such increase could be explained by the increased proliferation and/or necrosis of histiocytes.

In conclusion, we consider that the hematological abnormalities in VL can be adequately explained on the bases of histological changes in the marrow, namely the increased hemophagocytosis and necrosis of histiocytes, and the perivascular increase of marrow plasma cells, perhaps reflecting the increased polyclonal B-cell activation.

Acknowledgment

I would like to thank Dr. M.N. Al-Nasser, Director General of King Fahad Hospital in Al-Baha, for permitting publication of this material.

References

1. Sabbah RS, Al-Sahban E, Akhtar M. Kala-Azar: Approaches to diagnosis and treatment. King Faisal Specialist Hospital. Saudi Med J 1983;93-7.
2. Peters W, Al-Zahrani MA. The leishmaniasis as a public health problem in Saudi Arabia. Saudi Med J 1987;8:333-43.
3. Patil SB, Rodrigue OP. Visceral leishmaniasis in children. Saudi Med J 1990;11:99-104.
4. Al-Jurayyan N, Al-Ayed IH, Al-Nasser MNS, Al-Mugeiren NMA, et al. Visceral leishmaniasis in infancy and childhood. Epidemiology and clinicopathological study of 63 cases in Al-Baha province, Saudi Arabia. J Trop Pediat 1992;38:12-6.
5. Hartsock RJ, Smith EB, Petty CS. Normal variation with aging of the amount of hematopoietic tissue in bone marrow from the anterior iliac crest. Am J Clin Pathol 1965;43:326-31.
6. Van Peenen PFD, Peid TP. Leishmaniasis in the Sudan Republic., IV. Clinical and laboratory aspects of kala-azar in hospitalized patients from the upper Nile Province. Ann J Trop Med Hyg 1962;11:723-30.
7. Maru, M. Clinical and laboratory features and treatment of visceral leishmaniasis in hospitalized patients in northwestern Ethiopia. Am J Trop Med Hyg 1979;28:15-8.
8. Livolti S, Fischer A, Musumeci S. Haematological and serological aspects of Mediterranean Kalaazar in infancy and childhood. Acta Trop 1980;30:551-65.
9. El-Hassan AM, Ahmed MAM, Rahim AA, et al. Visceral leishmaniasis in the Sudan: clinical and hematological features. Ann Saudi Med 1990;10:51-6.
10. Edington GM, Gilles HM. Pathology in the tropics (1st ed). London: Arnold Ltd., 1969:45.
11. Verss B, Malik MOA, Satir AA, El-Hassan AM. Morphological observations on visceral leishmaniasis in the Sudan. Trop Geogr Med 1974;26:198-203.
12. Woodruff AW, Topley E, Knight R, Downie CGB. The anemia in kala-azar. Brit Med J 1972;22:319-29.
13. Musumeci SD, Agata A, Fischner A. Antiglobulin consumption test in kala-azar. Trans R Soc Trop Med Hyg 1974;68:261-7.