The Haematological Manifestations of Visceral Leishmaniasis in Infancy and Childhood

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Summary

The haematological manifestations were reviewed in 94 patients (55 males and 39 females) with visceral leishmaniasis. Their ages ranged from 4 months to 12 years (mean [redacted] 1.8 years). All patients had splenomegaly and were anaemic, while (73 per cent) were neutropenic and (56 per cent) thrombocytopenic. Coagulation abnormalities were encountered in 10 (11 per cent) patients; in four patients this was associated with disseminated intravascular coagulopathy. Bone marrow was hypercellular in (90 per cent), normocellular in (5 per cent), and hypocellular in (4 per cent). Also variable degrees of erythrophagocytosis and leukophagocytosis were noted with preponderance of histiocytes (46 per cent) and granulomatous formation (25 per cent). Low haemosiderin content in the bone marrow was noted, which together with the finding of high serum ferritin is consistent with anaemia of chronic inflammation.

Hypersplenism, haemophagocytosis and granulomatous lesions of the bone marrow, chronic inflammation, and dietary factors appear to be the most important factors in the causation of the haematological changes in visceral leishmaniasis.

Introduction

Visceral leishmaniasis (Kala-Azar) is a systemic protozoal parasitic infection caused by *Leishmania donovani* (LD) and constitutes a major health problem in many parts of the world.[1] The disease is being increasingly reported from Saudi Arabia, in particular the south-western region.[2–5]

The parasite multiplies primarily in the reticuloendothelial cells of the liver, spleen, bone marrow, and lymph nodes, and the disease manifests with fever, weight loss, hepatosplenomegaly, and pallor. Various haematological manifestations, ranging from a life-threatening disseminated intravascular coagulation (DIC) to mild anaemia, have been reported.[5–13] majority were from adult population with only few detailed reports on infants and children.[10]

Prompted by this, we report here the haematological manifestations of 94 cases of visceral leishmaniasis in infants and children who were admitted to King Fahad Hospital (KFH) at Al-Baha over a 10-year period.

Patients and Methods

The haematological findings in 94 infants and children, who were admitted to King Fahad Hospital (KFH) at Al-Baha over a 10-year period from June 1982 to July 1992, were reviewed retrospectively. The diagnosis was made on the basis of:

1. identification of *Leishmania donovani* (LD) bodies on bone marrow, liver biopsy or splenic puncture; or
2. clinical features and laboratory findings compatible with the diagnosis in patients in whom LD bodies were not seen, but who had a significantly high indirect haemagglutination titre (IHAT) for *Leishmania*, i.e. $\geq$1:64 (haemagglutination titre—Behring Werke AG, West Germany), and who responded favourably to sodium stibogluconate (Pentostan).

Fifty-five patients were males and 39 females. Their ages ranged from 4 months to 12 years (mean 1.8 years). Eighty-three (88 per cent) patients were under the age of 4 years. The duration of illness ranged from 2 weeks to 1 year (mean 10 weeks).

A complete history and physical examination were obtained. Initial laboratory investigations included complete blood count (Coulter Electronics, Ltd, UK), peripheral smear, white cell differential count, reticulocyte count, platelets count, peripheral blood film for malaria, erythrocyte sedimentation rate (Wintrobe method), renal and liver function tests, and urinalysis. Specific haematological values at various age groups were considered to be abnormal according to the established reference values in infancy and

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TABLE 1
Haematological findings in visceral leishmaniasis on admission and discharge

Test	On admission			On discharge		
	Mean ± SD	Range	No. of cases	Mean ± SD	Range	No. of cases
Haemoglobin (g/l)	69 ± 15	27–102	94	112 ± 15	80–153	83
White blood cells (10^9/l)	4.8 ± 2.7	1.4–11.3	94	7.4 ± 3.8	2.7–17	83
Platelets (10^9/l)	109 ± 82.3	4–633	94	279 ± 100	66–469	83
Reticulocyte (10^9/l)	3.3 ± 2.1	0.4–8.1	94	ND*	—	—
Erythrocyte sedimentation rate (mm/h)	55.5 ± 15	25–78	94	ND*	—	—

*ND = Not done.

childhood.[14] Bone marrow aspiration and biopsy were performed in all the patients following the standard procedures.

Coagulation tests; prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen level (Diagnostica Stago, France), fibrin/fibrinogen degradation products (Thrombo-Wellcotest, Wellcome Diagnostics, UK), Coomb's test, haemoglobin electrophoresis, glucose-6-phosphate-dehydrogenase (G-6-PD) assay, and serum ferritin were done when appropriate. Vitamin B12 and folic acid levels were not done.

Patients were treated with intravenous sodium stibogluconate (Pentostan). The duration of therapy ranged from 14 to 30 days (mean 21). Haematinics were prescribed if indicated. Blood transfusion, fresh frozen plasma and platelets were given, for severe anaemia and or bleeding, when indicated.

Patient's response to therapy was monitored, and complete blood count, peripheral smear, and liver function tests were regularly checked during and at the end of therapy.

Chi-square test (χ^2) and Fisher's Exact probability test were used. $P < 0.05$ was considered significant.

Results

The most common presenting clinical features in this series were; splenomegaly (100 per cent), fever (94 per cent), hepatomegaly (94 per cent) pallor (91 per cent), abdominal distension (60 per cent), and weight loss (43 per cent). Intercurrent infections were detected in fifty (53 per cent) patients. Respiratory and gastrointestinal infections were the commonest infections detected and presented in 70 per cent of cases.

The haematological findings are summarized in Tables 1 and 2. Patients were divided into three groups according to the age, but without distinction of sex. All patients were anaemic. The mean haemoglobin was 69 ± 15 g/l (range 27–102). Twenty three (25 per cent) patients had severe anaemia with haemoglobin of ≤ 60 g/l. When the patients were classified into three groups according to their ages as shown in Table 2, there was no significant difference between the first and second groups; while the differences between first and third, and second and third groups were significant ($P < 0.05$ and 0.01, respectively). Red cell morphology was characterized by anisopoikilocytosis in all, with preponderance of macrocytes in 21 (22 per cent) patients. Slight to moderate polychromasia was found in 68 (72 per cent) patients. The mean white blood cell count was (4.8 ± 2.7) × 10^9/l (range 1.4–11.3). Sixty-nine (73 per cent) patients were neutropenic. The mean platelets count was (109 ± 82.3 × 10^9/l (range 4–633) and 53 (56 per cent) patients were thrombocytopenic. No significant differences in RBC, MCV, MCH, reticulocyte, white blood cells, and platelets counts were observed between the three different age groups. Hepatic dysfunction was present in 65 (69 per cent) patients, which improved in all cases after therapy. Coagulation abnormalities, mainly prolonged PT and APTT, were anoted in 10 (11 per cent) patients, and in four patients this was associated with disseminated intravascular coagulopathy (DIC). Forty-eight patients required blood products transfusion, no problems were encountered at cross-match in all except one patient who had a positive reaction both to direct and indirect Coomb's test.

Bone marrow aspiration and biopsy were performed in all patients for diagnostic purpose. Sixty-eight (72 per cent) were positive for LD bodies on microscopy. Increased cellularity of the bone marrow was found in 85 (90 per cent) patients. Variable degrees of erythrophagocytosis and leukophagocytosis were noted together with abundance of histiocytes in 43 (46 per cent) patients, and granulomatous formation in 23 (25 per cent) (Fig. 1). In five (5 per cent) patients the bone marrow was normocellular, while hypocellularity was noted in four (4 per cent). Erythropoiesis was normoblastic in 79 (84 per cent) patients and megaloblastic in 15 (16 per cent) patients. Bone marrow staining for haemosiderin content was performed in 76 patients, 59 of which demonstrated low or absent iron stores. Among this group, serum ferritin was measured in 15 patients, 12 of them demonstrated abnormally high levels, while the other three was within normal.

The Hb electrophoresis was performed in 30

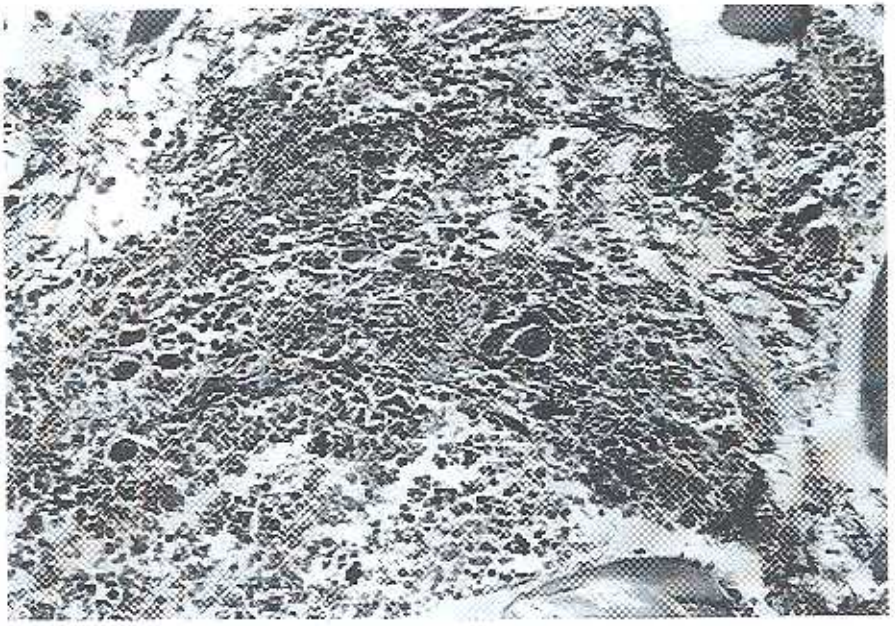
TABLE 2

Initial haematological changes (mean ± SD) in 94 patients with visceral leishmaniasis according to age group

Age (years)	No. of cases	Haemoglobin (g/l)	RBC × 10^9/l	MCV (fl)	MCH (Pg)	Reticulocytes (10^9/l)	White blood cells × 10^9/l			Platelets × 10^9/l
							Total	Neutrophils	Lymphocytes	
0–2	54	71 ± 16	3.22 ± 0.67	65.6 ± 8.8	21.3 ± 3.0	3.0 ± 1.7	5.6 ± 2.9	1.8 ± 1.6*	3.7 ± 2.2	121.8 ± 105
> 2–4	29	75 ± 12	3.22 ± 0.69	67.9 ± 7.1	21.8 ± 2.9	4.0 ± 2.2	4.4 ± 2.1	0.9 ± 0.4*	3.2 ± 1.7	113.7 ± 75
> 4–12	11	61 ± 13	2.97 ± 0.73	68.6 ± 11.5	21.9 ± 3.6	2.9 ± 2.2	4.5 ± 2.9	1.3 ± 0.9	3.0 ± 2.5	92.7 ± 67

RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin.
* $P = 0.003$.

(A)



(B)

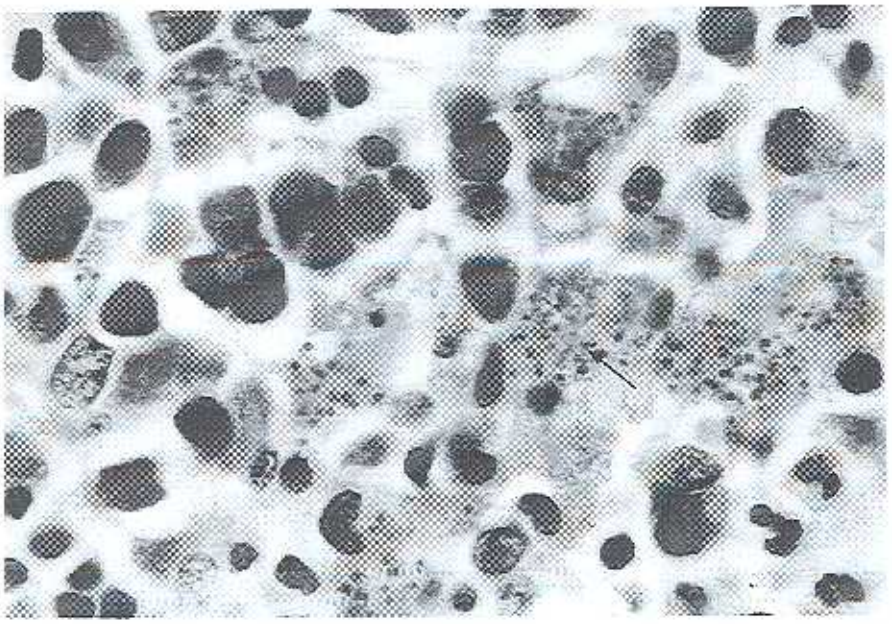


FIG. 1. Bone marrow smear showing increased cellularity with preponderance of histiocytes and early granulomatous formation (A), and amastigotes (B) (Haematoxylin and eosin stain; magnification ×400).

patients and showed normal pattern in all, except four who demonstrated HbAS pattern. G6PD activity was assayed in 26 patients and demonstrated normal activity.

During the period of hospitalization, the fever subsided, the patients gained weight, and the spleen and liver size started to decrease with the most significant reduction occurring in the spleen.

The haematological findings in 83 patients on discharge are shown in Table 1. The rise in haemoglobin concentrations was statistically significant ($P < 0.005$) with a mean haemoglobin concentration of 112 ± 15 g/l (range 80–153). Furthermore, although there was a significant ($P < 0.005$) rise in the mean white blood cell count $(7.4 \pm 2.8) \times 10^9$/l (range, 2.7–17). Twelve patients were still neutropenic. Also, the mean platelets count rose to $(279 \pm 100) \times 10^9$/l (range 66–469), which was statistically significant ($P < 0.001$), but three patients were still thrombocytopenic at discharge from the hospital.

Discussion

Visceral leishmaniasis is characterized by fever, hepatosplenomegaly, and severe haematological alterations. Anaemia and neutropenia are always present. Usually anaemia is of hypochromic microcytic type, but hyperchromic macrocytic anaemia have been reported.[5,7] Reduction of platelets count completes the picture of the progressive pancytopenia which is typical of visceral leishmaniasis.[10] The pathogenesis of the pancytopenia is probably complex, and still far from defined. Henderson[15] first described this in 1937 and Cole[6] has reported one of the largest series of the disease at that time and postulated 'haematopoietic depression' as a factor. The view that the 'crowding out' effect on the erythrocyte precursors in the marrow as suggested by Charterjee,[16] in 1946, has not been supported by the finding of unimpaired erythropoiesis in two patients with visceral leishmaniasis and by the observation of erythroblastic hyperplasia, with normal or increased cell maturations.[5,10–12,17–19] Dyserythropoiesis and ineffective erythropoiesis of the bone marrow have also been reported.[18]

While increased plasma volume due to splenomegaly could play a role, sequestration and destruction of blood cells in the enlarged spleen proved to be a major factor.[8,10–12,19,20] Immune mechanisms (auto-antibodies and immune complexes) have been incriminated as a contributory factor for the anaemia.[8,10,12,21,22] Concomitant infections and malnutrition with deficiency of folic acid, vitamin B12, and iron are additional factors.[7,10–12]

In the present study, a diversity of haematological abnormalities were noted. We found anaemia of varying degrees in all patients, comparable to what has been reported in adults[11–15] and pediatric series with visceral leishmaniasis.[4,7,9,10,12] The pathogenesis of anaemia in our patients is multifactorial. Nutritional factors have an important bearing. Red cell morphology in the majority of patients is microcytic and hypochromic, and suggestive of iron deficiency. This is supported by low or absent iron stores of the bone marrow, associated with low serum ferritin in few patients and marked improvement of the anaemia with iron therapy. Although data on the iron status of the population are not available from the region, Babiker *et al.*[23] and Al-Fawaz[24] have shown, in two different studies on children from Riyadh, a high prevalence of iron deficiency anaemia. Although, vitamin B12 and folic acid levels were not done, in our patients, the finding of macrocytes in 21 (22 per cent) patients and presence of megaloblastic changes in their bone marrow are consistent with dietary deficiency of these vitamins. The finding on the other hand, of high serum ferritin in some other patients with low amounts of haemosiderin in their bone marrows is consistent with anaemia of chronic inflammation.[10,11,25]

Although, studies with labelled blood elements to confirm splenic sequestration were not performed,[8,10–12] hypersplenism seems to play a major role in the causation of pancytopenia in this series. This is suggested by increased bone marrow activity, progressive pancytopenia and constant splenomegaly. Leukopenia and thrombocytopenia are features of hypersplenism; as much as 50–90 per cent of the total platelets mass may be incarcerated in the spleen.[11] Other additional factors in our series might include bone marrow hypoplasia or depression, haemophagocytosis, and granulomatous formation.

The constant presence of leukopenia associated with neturopenia in the majority of our patients is comparable to what has been reported before,[10] but agranulocytosis observed in Indian Kala Azar[7] was not observed. The reduced number of platelets, as in our series, is seldom associated with haemorrhagic manifestations, as well as with alterations of coagulation factors.[10] The bleeding episodes among our patients were less frequent (4 per cent), and it was mainly associated with disseminated intravascular coagulopathy (DIC) and thrombocytopenia.[10–12]

In conclusion, the haematological abnormalities in visceral leishmaniasis of infancy and childhood are common. The pathogenesis is complex and multifactorial. Hypersplenism, haemophagocytosis, and granulomatous lesions of the bone marrow, chronic inflammation, and dietary factors appear to be the most important factors. Visceral leishmaniasis should be included in the differential diagnosis of patients presenting with anaemia, leukopenia, thrombocytopenia, pancytopenia, or DIC in particular in geographical areas where the disease is still endemic.

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