ORIGINAL ARTICLE

MORPHOLOGY OF BONE MARROW IN VISCERAL LEISHMANIASIS

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Background: Visceral Leishmaniasis (Kala Azar), a vector borne parasitic disease is endemic in many parts of the world including South East Asia. It is a chronic febrile ailment caused by *Leishmania Donovani* (LD). More than three hundred million people living in the endemic areas are at high risk and fourteen million are living with the disease. **Methods**: This prospective study was conducted on seventy-five patients over a period of twelve years at the department of Pathology Ayub Medical College Abbottabad, focusing on the morphology of bone marrow aspirate obtained from patients. The aspirate was smeared on glass slides, fixed with alcohol and stained with Giemsa stain. The microscopic examination of stained slides was carried out by the single microscopist to avoid the difference of opinion. The initial diagnosis was further confirmed by tow experienced microscopists. **Results:** A minor difference was observed in the number of male and female participants, 38 versus 37, with male to female ratio of 1.02:1. The majority of the patients were 1–15 years old. As much as 45.2% patients were 1–5 years old. In this age group, males were affected more than the females with male to female ratio of 1.4:1. Most of the patients belonged to district Battagram and Tor Ghar. Bone marrow aspiration was easy and the marrow aspirate was found having marrow fragments on naked eye examination in majority of the patients. Extrahistiocytic LD bodies alone were seen in 100%, extra and intrahistiocytic in 80% and intrahistiocytic alone in 33.3% patients. The parasite index was 1–5 to 1–10 in 53.4% and 26.6% patients respectively. **Conclusion**: Visceral Leishmaniasis is endemic in the study area. Bone marrow examination, although an invasive procedure, gives direct microscopic diagnosis of visceral Leishmaniasis and may be considered where indicated.

Keywords: Visceral Leishmaniasis; Bone marrow aspirate; Kala Azar; Sandfly

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INTRODUCTION

Visceral Leishmaniasis (also known as Kala Azar), is a vector borne chronic febrile ailment caused by *Leishmania Donovani* (abbreviated as LD bodies) after the bite of sand fly having this parasite.[1] According to an estimate, more than 300 hundred million people living in the endemic areas are at high risk, while another 14 million are living with the disease.[2] The disease is endemic in many parts of the world including South East Asia.[3]

In Pakistan, the disease is prevalent in hilly areas of Khyber Pakhtunkhwa, upper Punjab, Northern areas and the adjoining areas of Kashmir. Cases have also been reported from Sindh and some areas of Baluchistan.[4–6] It mostly affects children less than twelve years age[7] but cases have been reported in adults as well[8,9]. Different studies are available on visceral Leishmaniasis form Pakistan, and other countries which were carried out in the past twenty years on different aspects of this disease including diagnosis, clinic-haematological features, treatment and resistance to the commonly used drugs.[10–17]

MATERIAL AND METHODS

The present study aimed at knowing the different haematological responses of bone marrow to infection by Leishmania, which may not be uniform in all the patients. It may vary from patient to patient depending upon the immune status of patient, any coexisting nutrient deficiency and even from region to region. The studies conducted in other countries have revealed varying results. But no study until now has been conducted on this aspect of the disease in our country. This was the rationale of our study. This may be helpful in the initial diagnosis and follow-up of patients with visceral leishmaniasis after treatment. This was a prospective study, conducted at the Department of Pathology, Ayub Medical College Abbottabad, from January 2009 to January 2017 over a period of eight years on 55 patients of either gender, all ages, races and ethnic groups. Informed written consent was taken from the patient for bone marrow study. In case of a minor, the consent was taken from parents or guardian. The patients participating in this study were mostly referred from Hospitals, including Ayub Teaching Hospital, District Headquarter Hospitals Mansehra, Abbottabad and Haripur. Patients were also referred from private clinics throughout Hazara Division and the adjoining areas of Azad Jammu and Kashmir. Bone marrow was aspirated from posterior iliac spine under 2% lignocain with bone marrow aspiration needle. The marrow aspirate was smeared on glass slides, fixed with alcohol and

stained with giemsa stain. The microscopic examination of stained marrow slides was carried out by single microscopist to avoid the difference of opinion.

RESULTS

The study results are shown in tables 1–4. There was minor difference in the number of male and female participants, 38 versus 37, with male to female ratio of 1.02:1. Majority of the patients (86.6%) was 1–15 years old, 34 (45.2%) patients were 1–5 years old. In the age group, males were affected more than the females with male to female ratio of 1.4:1 (Table-1)

Most of the patients belonged to district Battagram (29.3), while Abbottabad was the second most commonly affected (22.6) district (Table-2). Bone marrow was aspirated from the medial aspect of upper end of tibia in 58(77%) patients. Bone marrow aspiration was easy and the marrow aspirate was found having marrow fragments on naked eye examination in 53.3% patients. The amount of marrow aspirate was ≤0.5 ml in as much as 80% patients (Table-3).

Regarding microscopic examination of bone marrow, extracellular LD bodies alone were seen in 100%, extra and intra-histiocytic in 80% and intra-histiocytic alone in 33.3% patients. The parasite index was 1–5 to 1–10 in 53.4% and 26.6% patients respectively. Those with higher parasite index were more symptomatic than the others. Increased lymphocytes, plasmacytosis, haemophagocytosis and eosinophilia were the next common abnormalities (33.3%, 40%, 40% & 33% respectively) seen on microscopic examination of bone marrow of the patients. Initially, the aspirate was dry in five patients, not fit for reporting and excluded from the study with suggestion to repeat the test. On repeating, scanty aspirate was obtained, showing LD bodies on microscopic examination (Table-4)

Table-1: Demographic profile of patients (n=75)

Age in years	n (%)	Male n (%)	Female n (%)
<1	07 (9.3)	03 (4)	04 (5.3)
1 to 5	34 (45.3)	20 (26.6)	14 (18.6)
5 to 10	18 (24)	10 (13.4)	08 (10.6)
10 to 15	13 (17.3)	05 (6.6)	08 (10.6)
>15	03 (4)	00	03 (4)
Total	75 (100)	38 (50.6)	37 (49.4)

Table-2: Residence of patients (n=75)

Address	n (%)
District Battagram &Tor Ghar	22 (29.3)
District Abbottabad	17 (22.6)
District Mansehra	10 (13.3)
Northern areas	07 (9.3)
Kohistan	05 (6.6)
Afghan refugees	05 (6.6)
Azad Jammu and Kashmir	04 (5.3)
Murree hills	03 (4)
District Haripur	02 (2.6)

Table-3: Macroscopic parameters of bone marrow aspiration (n=75)

Parameters		n (%)
Site of aspiration	Tibia	58 (77.3)
	Iliac bone	17 (22.7)
	Others	00 (00)
Ease of aspiration	Easy	40 (53.3)
	Difficult	20 (26.7)
	Very difficult	15 (20.0)
Amount of aspirate	<0.5 ml	35 (46.7)
	0.5 ml	25 (33.3)
	≥1 ml	15 (20.0)
Quality of aspirate	Cellular	40 (53.3)
	Diluted	30 (40.0)
	Dry	05 (06.7)

Table-4: Microscopic parameters of bone marrow aspirate (n=75)

Parameter		n (%
Background	Clean	35 (46.6)
	Dirty	25 (33.4)
	Pinkish	15 (20.0)
Cellularity	Normal	55 (73.3)
	Increased	05 (06.7)
	Decreased	15 (20.0)
Erythropoiesis	Normal	55 (73.6)
	Hyperplastic	05 (06.4)
	Depressed	15 (20.0)
Myelopoiesis	Normal	50 (66.7)
	Hyperplastic	10 (13.3)
	Depressed	15 (20.0)
Megakaryocytes	Normal	50 (66.7)
	Increased	10 (13.3)
	Decreased	15 (20.0)
Lymphocytes	Normal	40 (53.4)
	Increased	25 (33.3)
	Decreased	10 (13.3)
Plasma cells	Normal	35 (46.7)
	Increased	30 (40.0)
	Decreased	10 (13.3)
Parasite index	1–5/100X	40 (53.4)
	5–10/100X	20 (26.6)
	>10/100X	15 (20.0)
Stainable iron	Absent	37 (49.4)
	Normal	23 (30.6)
	Increased	15 (20.0)
Intrahistiocytic LD bodies	Present	25 (33.3)
	Absent	50 (66.7)
Extrahistiocytic LD bodies	Present	75 (100)
	Absent	00 (00)
Extra & Intrahistiocytic LD bodies	Present	60 (80.0)
	Absent	15 (20.0)
Bare nuclei of parasite	Present	10 (13.3)
	Absent	65 (86.7)
Haemophagocytosis	Present	30 (40.0)
	Absent	45 (60.0)
Eosinophilia	Present	25 (33.3)
	Absent	50 (66.7)
LD bodies in other cells	Seen	05 (06.7)
	Not seen	70 (93.3)

DISCUSSION

Bone marrow examination is an authentic method of diagnosis of visceral Leishmaniasis. Studies have reported different morphological pictures of bone marrow from patients with visceral Leishmaniasis. Haemo-phagocytosis and increased histiocytes were reported the common microscopic findings in a recent study.[18] Both Intra-histiocytic and Extra-histiocytic LD bodies were seen. In another study normocellular marrow was seen in majority of the patients. Megaloblastic change, erythroid

hyperplasia and dyserythropoiesis were other significant findings.[19] Hypercellular marrow, increased plasma cells, granulomas, haemo-phagocytosis and gelatinous transformation of marrow were reported in an earlier study.[20] Daneshbod and colleagues reported some other interesting findings including leukemic blasts, Reedsternberg –like cells, tart cells and foamy cells in addition to the other findings mentioned above.[21] In the present study, macroscopic features of bone marrow aspiration were also studied, including site and ease of aspiration, amount and quality of aspirate on naked eye examination for marrow particles. Aspiration was done from tibia, in 77%, patients and was easy in 53% patients. As much as 0.5 ml marrow aspirate was obtained in 77% and the aspirate was cellular on naked examination in 70% patients. These features have not been studied in the other studies cited above. Microscopic examination of marrow aspirate revealed more or less the same microscopic features as have been reported by the earlier researchers with some minor differences. Background of stained slide was clean in 30% cases and was dirty or pinkish in 55%. This was not due to staining artefacts. It is thought that this finding may be due to increased immunoglobulin giving a pinkish background, as in multiple myeloma. The cause of dirty background of stained slides cannot be explained on the basis of increased immunoglobulin. It might have been due to necrotic material aspirated during the procedure. Necrosis could not be confirmed on giemsa staining of aspirate. Secondly, neither a clot biopsy nor a trephine biopsy was taken from any patient. The parasite index was 1–5, 5–10 and >10 per oil immersion field in 45%, 25% and 20% patients respectively. Those with higher parasite index were more symptomatic than the others. The sensitivity of bone marrow for visceral Leishmaniasis has been reported 70.2%, compared to 96.4% for splenetic puncture[22]; it has been proposed as a technique of choice for establishing the parasitologic diagnosis of visceral Leishmaniasis[23].

AUTHORS' CONTRIBUTION

MI: Performing tests, data collection and final study compilation. JF: Literature review. NG: study Compilation

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