

## Morphological Study of Bone Marrow in Visceral Leishmaniasis at SKMCH, Muzaffarpur, Bihar

Shweta Kumari<sup>1</sup>, Anita Kumari<sup>2</sup>, Mahesh Prasad<sup>3</sup>

<sup>1</sup>Tutor, Department of Pathology, Sri Krishna Medical College (SKMC), Muzaffarpur, Bihar.

<sup>2</sup>Tutor, Department of Pathology, Govt. Medical College and Hospital (GMCH), Bettiah, West Champaran, Bihar.

<sup>3</sup>Associate Professor, Department of Pathology, Sri Krishna Medical College (SKMC), Muzaffarpur, Bihar.

---

Received: 08-02-2023 / Revised: 10-03-2023 / Accepted: 02-04-2023

Corresponding author: Dr. Anita Kumari

Conflict of interest: Nil

---

### Abstract

**Background:** In Muzaffarpur district of Bihar, Visceral Leishmaniasis (Kala Azar), a parasitic illness spread by vectors, is widespread. global persistent febrile illness brought on with Leishmania Donovanii (LD). More than 300 million residents of endemic regions are at high risk, and 14 million are afflicted with the illness.

**Methods:** This prospective study, which examined the morphology of bone marrow aspirates taken from patients, was carried out on 75 patients over the course of nine months, from November 2020 to July 2021, at the pathology department of Sri Krishna Medical College and Hospital, Muzaffarpur, Bihar. On glass slides, the aspirate was spread, fixed with alcohol, and stained with Giemsa.

**Results:** The ratio of male to female participants was 1.02:1, with 38 male participants to 37 female individuals being the only significant difference. Most of the patients were between the ages of 1 and 15. Up to 45.2% of patients were between 1 and 5 years old. With a male to female ratio of 1.4:1, men in this age range were more affected than women. The majority of the patients came from the Muzaffarpur district. Bone marrow aspiration was simple, and the majority of patients had marrow fragments in the aspirate upon visual inspection. 100% of patients had extrahistiocytic LD bodies alone, 80% had extra and intrahistiocytic LD bodies, and 33.3% had intrahistiocytic LD bodies alone. In 53.4% and 26.6% of patients, the parasite index ranged from 1 to 10, respectively.

**Conclusion:** The research area has an endemic case of visceral leishmaniasis. Despite being invasive, bone marrow analysis provides a direct microscopic diagnosis of visceral leishmaniasis and may be used as necessary.

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

---

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

---

### Introduction

Leishmania Donovanii, commonly referred to as LD bodies, is the parasite that causes the chronic fever illness known as visceral

leishmaniasis (sometimes referred to as Kala Azar), which is transmitted by sand flies.[1] An estimated 14 million individuals are

infected with the disease, and over 300 million people who live in endemic areas are at high risk.[2] Numerous regions of India, particularly the district Muzaffarpur (Bihar), are plagued by the illness.[3]

People contract the illness from the bites of sandflies, which acquired a parasite while consuming the blood of another person who had the parasite. Leishmania parasites, which are more than 20 different species in total, are the disease's primary cause and are carried by 90 different species of sandflies.[4] However, there is only one typical kind of parasite, *Leishmania donovani*, and only one species of sandfly, *Phlebotomus argentipes*, that spreads the disease on the Indian subcontinent.[5] There are recommendations in place for India, and there are variations in the disease's form, the medication used to treat the parasite, and the insecticide used to avoid bug bites.[4]

In addition to the personal costs, the disease has a significant negative economic impact on the affected areas and India as a whole. [6]

The goal of the current investigation was to understand the various, potentially variable haematological responses of bone marrow following *Leishmania* infection. Depending on the patient's immunological health, any coinciding vitamin deficiencies, and even the

geography, it may differ from patient to patient.

### Material and Methods

On 75 patients of either gender, all ages, races, and ethnicities, this prospective study was carried out in the Department of Pathology, Sri Krishna Medical College, Muzaffarpur, Bihar, between November 2020 and July 2021. The patient's signed informed consent was obtained for the bone marrow testing. Parental or guardian consent was required in the case of a minor. The majority of the patients who took part in this study were referred by Muzaffarpur district private nursing home and its surroundings. Additionally, private clinics made referrals of patients. Under 2% lignocain, bone marrow was extracted with a bone marrow aspiration needle from the posterior iliac spine. On glass slides, the marrow aspirate was applied, fixed with alcohol, and dyed with giemsa.

### Results

Tables 1-3 display the study findings. 38 against 37 individuals were male, with a male to female ratio of 1.02:1. There was a little difference in the number of participants. 34 patients (45.2%) were 1 to 5 years old, making up the majority of patients (86.6%) who were 1 to 15 years old. With a male to female ratio of 1.4:1, men in this age range were more affected than women (Table-1).

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

Age in years	n (%)	Malen (%)	Femalen (%)
<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)

In 58 (77%) of the patients, bone marrow was aspirated from the medial aspect of the upper end of the tibia. Bone marrow aspiration was simple, and 53.3% of patients had marrow fragments in the aspirate upon visual inspection. Up to 80% of patients had marrow aspirates that were less than 0.5 ml (Table-2).

**Table 2: 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)

Extracellular LD bodies alone were observed in 100% of cases, extra and intra-histiocytic in 80%, and intra-histiocytic alone in 33.3% of patients during microscopic examination of the bone marrow. In 53.4% and 26.6% of patients, the parasite index ranged from 1 to 10, respectively. The symptoms were worse in those with higher parasite indices than in the other groups. On microscopic analysis of the patients' bone marrow, elevated lymphocytes, plasmacytosis, haemophagocytosis, and eosinophilia were the next frequently observed abnormalities (33.3%, 40%, 40%, and 33%, respectively). Five patients initially had aspirates that were dry, unfit for reporting, and they were asked to repeat the test because they were being excluded from the research. Repeating the procedure yielded a sparse aspirate that, upon microscopic inspection, revealed LD bodies (Table-3).

**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)

<b>Plasma cells</b>	Normal	35 (46.7)
	Increased	30 (40.0)
	Decreased	10 (13.3)
<b>Parasite index</b>	1–5/100X	40 (53.4)
	5–10/100X	20 (26.6)
	>10/100X	15 (20.0)
<b>Stainable iron</b>	Absent	37 (49.4)
	Normal	23 (30.6)
	Increased	15 (20.0)
<b>Intrahistiocytic LD bodies</b>	Present	25 (33.3)
	Absent	50 (66.7)
<b>Extrahistiocytic LD bodies</b>	Present	75 (100)
	Absent	00 (00)
<b>Extra &amp; Intrahistiocytic LD bodies</b>	Present	60 (80.0)
	Absent	15 (20.0)
<b>Bare nuclei of parasite</b>	Present	10 (13.3)
	Absent	65 (86.7)
<b>Haemophagocytosis</b>	Present	30 (40.0)
	Absent	45 (60.0)
<b>Eosinophilia</b>	Present	25 (33.3)
	Absent	50 (66.7)
<b>LD bodies in other cells</b>	Seen	05 (06.7)
	Not seen	70 (93.3)

## Discussion

Visceral Leishmaniasis can only be accurately diagnosed with a bone marrow test. Studies have revealed several morphological images of patients with visceral leishmaniasis bone marrow. In a recent investigation, hemophagocytosis and a rise in histiocytes were described as the typical microscopic results.[7] LD bodies were observed in both extra- and intra-histiocytic settings. In a different study, the majority of patients had normocellular marrow. Other noteworthy observations were megaloblastic change, erythroid hyperplasia, and dyserythropoiesis.[8] An earlier investigation revealed hypercellular marrow, enlarged plasma cells, granulomas, hemophagocytosis, and gelatinous marrow transformation.[9] In addition to the observations already discussed, Daneshbod and colleagues also revealed several additional intriguing findings, such as

leukemic blasts, Reedsternberg-like cells, tart cells, and foamy cells.[10] The site and ease of aspiration, the quantity, and the quality of the aspirate were all examined in the current study along with macroscopic characteristics of bone marrow aspiration. In 77% of cases, aspiration was performed from the tibia, and in 53% of cases, it was simple. In 77% of patients, a marrow aspirate larger than 0.5 ml was collected, and 70% of those aspirates were identifiable as cells upon naked inspection. The other research mentioned above do not address these characteristics. The marrow aspirate underwent microscopic analysis, which, with a few minor variations, showed essentially the same microscopic features as those described by the earlier studies. In 30% of cases, the stained slide's background was clean; in 55% of cases, it was muddy or pinkish. This has nothing to do with stained artefacts. This result is

hypothesised to be caused by an increase in immunoglobulin, which creates a pinkish background, similar to multiple myeloma. Increased immunoglobulin cannot be used to explain why stained slides have a dirty background. Necrotic material that was aspirated during the surgery could have been the cause. Giesa staining of the aspirate did not reveal any evidence of necrosis.

Second, no patient had either a clot biopsy or a trephine biopsy performed on them. In 45%, 25%, and 20% of patients, the parasite index was 1–5, 5–10, and >10 per oil immersion field. The symptoms were worse in those with higher parasite indices than in the other groups. As opposed to splenic puncture, [11] which has a sensitivity of 96.4%, bone marrow has a reported sensitivity of 70.2% for visceral Leishmaniasis, and has been suggested as the preferred method for making the parasitologic diagnosis of visceral Leishmaniasis [12].

### Conclusion

The research area has an endemic case of visceral leishmaniasis. Despite being invasive, bone marrow analysis provides a direct microscopic diagnosis of visceral leishmaniasis and may be used as necessary.

### References

1. Rab MA, Frame IA, Evans DA. The role of dogs in the epidemiology of human visceral leishmaniasis. *Trans R Soc Trop Med Hyg.* 1995;89(6):612–5.
2. Pace D. Leishmaniasis. *J Infect.* 2014; 69(Suppl 1): S10–8.
3. Meheus F, Boelaert M. The burden of Visceral Leishmaniasis. *Trop Med Int Health.* 2010;15 Suppl 2:1–3.
4. World Health Organization (14 March 2019). Leishmaniasis Fact Sheet. [www.who.int](http://www.who.int). World Health Organization.
5. Joshi AB, Das ML, Akhter S, Chowdhury R, Mondal D, Kumar V, Das P, Kroeger A, Boelaert M, Petzold M. Chemical and environmental vector control as a contribution to the elimination of visceral leishmaniasis on the Indian subcontinent: cluster randomized controlled trials in Bangladesh, India and Nepal. *BMC Medicine.* 5 October 2009;7: 54.
6. Regional Office for South-East Asia (2012), Regional strategic framework for elimination of kala-azar from the South-East Asia Region (2011-2015), New Delhi: World Health Organization, hdl:10665/205826
7. Chandra H, Chandra S, Kaushik RM. Visceral Leishmaniasis with Associated Common, Uncommon and Atypical Morphological Features on Bone Marrow Aspirate Cytology in Non-endemic Region. *J Trop Med.* 2013; 2013:861082.
8. Raina S, Raina RK. Hematological Profile of Newly Diagnosed Patients with Visceral Leishmaniasis from a Non-Endemic Hilly Region of India. *Ann Clin Pathol.* 2017;5(1):1100.
9. Dhingra KK, Gupta P, Saroha V, Setia N, Khurana N, Singh T. Morphological findings in bone marrow biopsy and aspirate smears of visceral Kala Azar: a review. *Indian J Pathol Microbiol.* 2010;53(1):96–100.
10. Daneshbod Y, Dehghani SJ, Daneshbod K. Bone marrow aspiration findings in kala-azar. *Acta Cytol.* 2010;54(1):12–24.
11. Zijlstra EE, Ali MS, el-Hassan AM, el-Toum IA, Satti M, Ghalib HW, *et al.* Kala-azar: a comparative study of parasitological methods and the direct agglutination test in diagnosis *Trans R Soc Soc Trop Med Hyg.* 1992;86(5):505–7.
12. da Silva Mr, Stewart JM, Costa CH. Sensitivity of bone marrow aspirates in the diagnosis of visceral leishmaniasis. *Am J Trop Med Hyg.* 2005;72(6):811–4.

