

Leishmaniasis from a Laboratory Perspective

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Abstract

Visceral leishmaniasis is a severe infectious disease caused by Leishmania spp., characterized by a strong host immune response and significant hematological abnormalities. Clinically, the disease presents with prolonged fever, splenomegaly, severe anemia, pancytopenia, and hypergammaglobulinemia, and delayed diagnosis may worsen patient outcomes. This retrospective descriptive study included 23 patients with confirmed visceral leishmaniasis admitted between January 2022 and March 2023 to the Infectious Diseases Service and the Pediatric Infectious Diseases Service at the Mother Teresa University Hospital Center in Tirana. Demographic characteristics, clinical manifestations, and laboratory parameters—including hematological indices, inflammatory markers, biochemical tests, and bone marrow examination—were collected and analyzed. The disease was more frequent in the pediatric population, with most affected children under 5 years of age, while the mean age among adults was 48 years. HIV–Leishmania coinfection was identified in three patients. Anemia was present in all cases and was frequently associated with neutropenia and thrombocytopenia. Bone marrow examination confirmed the presence of the parasite in all evaluated samples, supporting its role as the diagnostic gold standard. Additional laboratory abnormalities included changes in inflammatory markers, liver enzyme levels, and the albumin–globulin ratio. The correlation of laboratory findings with clinical manifestations may facilitate earlier suspicion and diagnosis of visceral leishmaniasis, particularly in non-specialized centers, enabling timely confirmation and management of the disease.

Keywords: Visceral leishmaniasis; laboratory findings; anemia; splenomegaly; bone marrow examination.

Introduction

Leishmaniasis is a vector-borne zoonotic disease caused by protozoan parasites of the genus *Leishmania*. Once considered primarily a disease of parasitological and veterinary importance, it has become a major global public health concern due to climate change, increasing human mobility, urbanization, and the growing number of immunocompromised individuals. Laboratory investigations play a central role in diagnosis, monitoring, and therapeutic management, particularly through parasitological, serological, and molecular methods, together with the evaluation of characteristic hematological abnormalities and their correlation with clinical findings. (1–4)

The disease has been recognized since antiquity, but the causative parasite was identified independently by Sir William Boog Leishman and Charles Donovan in 1903. Subsequent studies established the role of *Leishmania* species, domestic dogs as the principal reservoir in zoonotic visceral leishmaniasis, and sandflies of the genus *Phlebotomus* as the main vectors responsible for transmission in the Mediterranean region. (4,7–10)

Globally, approximately one billion people live in endemic areas, with transmission reported in nearly 100 countries across five continents. An estimated 700,000–1,000,000 new cases occur annually, including approximately 50,000–90,000 cases of visceral leishmaniasis, the most severe clinical form, which remains associated with substantial morbidity and mortality if left untreated. The highest burden is observed in the Americas, East Africa, the Mediterranean basin, the Middle East, and South Asia. (2,7–10)

Albania is an endemic country for zoonotic visceral leishmaniasis caused by *Leishmania infantum*. Since the first reported cases in 1937, several epidemiological studies have confirmed the persistent circulation of the disease. Between 1998 and 2004, 815 cases of visceral leishmaniasis were reported, predominantly in the districts of Shkodër, Lezhë, Kukës, Krujë, Tirana, Librazhd, and Gjirokastrë. More recent surveillance data indicate approximately 20–30 new cases annually, confirming the continued endemicity of the disease in Albania. (2,5,6)

Leishmaniasis is classified as a metazoonosis because transmission occurs through the bite of infected female sandflies. The parasite exists in two morphological forms: the extracellular promastigote within the sandfly vector and the intracellular amastigote, which survives and multiplies inside macrophages of the vertebrate host. Following inoculation, promastigotes are rapidly phagocytosed by macrophages, transform into amastigotes, and proliferate within phagolysosomes, resulting in persistent intracellular infection. In the Mediterranean region, *Phlebotomus* species are the principal vectors, whereas dogs constitute the main reservoir of infection. (4,7,9,15)

Three major clinical forms are recognized: cutaneous, mucocutaneous, and visceral leishmaniasis. Visceral leishmaniasis typically presents with prolonged fever, weight loss, hepatosplenomegaly, pancytopenia, and hypergammaglobulinemia. In children, the disease may initially mimic hematological malignancies, making laboratory confirmation essential for establishing the diagnosis. (7,10–12,21)

The host immune response plays a fundamental role in disease progression. Protective immunity is predominantly mediated by Th1-associated cytokines, including IFN- γ , IL-2, TNF- α , and IL-12, whereas Th2-mediated responses characterized by IL-4, IL-5, IL-6, and IL-10 promote parasite persistence and chronic infection. Persistent intracellular survival of *Leishmania* contributes to immune dysregulation, hypergammaglobulinemia, immune complex formation, and varying degrees of immunosuppression. (22,26,27)

Laboratory diagnosis of visceral leishmaniasis relies on both direct and indirect methods. Direct diagnosis includes microscopic demonstration of amastigotes in bone marrow, splenic, or other tissue aspirates stained with May–Grünwald–Giemsa, as well as molecular techniques such as polymerase chain reaction (PCR), which provide high sensitivity and permit species identification and parasite load assessment. Indirect diagnosis is based on serological methods, including ELISA, indirect immunofluorescence, direct agglutination tests, and rapid rK39 immunochromatographic assays. (12–20)

Routine laboratory investigations commonly reveal normocytic normochromic anemia, leukopenia, thrombocytopenia, hypoalbuminemia, hypergammaglobulinemia, and markedly elevated erythrocyte sedimentation rate. Although these findings are nonspecific, they frequently raise clinical suspicion and prompt definitive parasitological or molecular confirmation. (17–22)

Aim of the Study

The aim of this study was to identify the principal laboratory findings in patients with visceral leishmaniasis and to evaluate their correlation with the clinical presentation, including the main signs and symptoms of the disease.

Study Objectives

1. To evaluate hematological, biochemical, and inflammatory laboratory parameters in patients diagnosed with visceral leishmaniasis.
2. To analyze the relationship between laboratory abnormalities and clinical manifestations of the disease.
3. To assess the role of laboratory findings in supporting early diagnosis of visceral leishmaniasis.
4. To examine the evolution of the disease during the acute phase and following therapy, based on clinical and laboratory indicators.

Materials and Methods

This retrospective descriptive study included 23 patients with a confirmed diagnosis of visceral leishmaniasis who were admitted between January 2022 and March 2023 to the Infectious Diseases Service and the Pediatric Infectious Diseases Service at the Mother Teresa University Hospital Center, Tirana.

Clinical records were reviewed, and demographic, clinical, and laboratory data were systematically collected and analyzed. The following variables were evaluated:

- **Demographic characteristics:** age, sex, place of residence, and confirmed diagnosis.
- **Laboratory parameters:** hematological indices including red blood cell count (RBC), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC); leukocyte differential count; platelet count; and additional laboratory tests including ferritin, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen, creatinine, and bone marrow examination (myelogram).
- **Clinical findings:** presence of high fever, splenomegaly, hepatomegaly, skin changes, asthenia, and cough.

Information regarding HIV status was also collected to identify possible HIV–Leishmania coinfection among the study participants.

The study was conducted in accordance with the principles of the Declaration of Helsinki. Patient data were anonymized prior to analysis, and confidentiality was maintained throughout the study. Approval was obtained from the institutional ethics committee according to local regulations.

Results

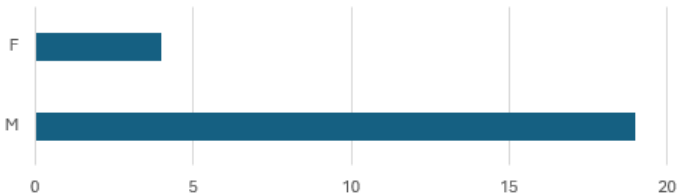
A total of 23 patients with confirmed visceral leishmaniasis were included in this study, of whom three were HIV-positive. Four patients were female and nineteen were male. Fourteen patients were younger than 10 years of age, while nine were between 11 and 55 years old. All patients were hospitalized at the Mother Teresa University Hospital Center, Tirana, during the period January 2022 to March 2023. Patients were recruited from multiple regions of Albania, and no specific geographic area showed a higher incidence. Specifically, 3 patients were residents of Krujë, 2 from Shkodër, 2 from Fier, and 1 patient each from Gjirokastër, Burrel, Lezhë, Vlorë, and Durrës, while the remaining patients resided in Tirana.

TABLE 1. Age

Age	Number of Patients
0-4 years old	12
5-10 years old	3
11-55 years old	8

Children younger than 4 years represented the most affected age group. The study identified a particularly high incidence among children under 2 years of age, with 7 recorded cases.

FIGURE 1. Distribution of Cases by Sex



Among our patients, males showed a higher prevalence of disease, accounting for approximately 70% of the cases.

FIGURE 2. Confirmed Diagnosis

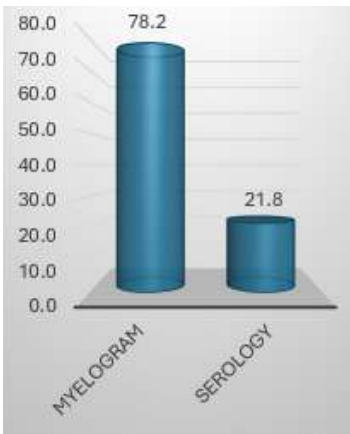
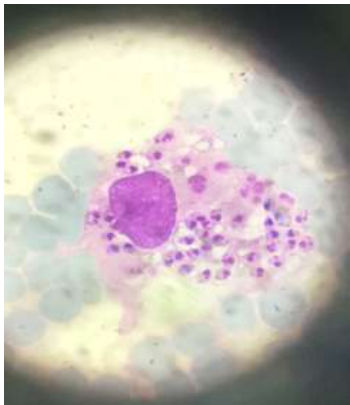


FIGURE 3. *Leishmania* in Bone Marrow



Of the 23 patients, 78.2% had their diagnosis confirmed through identification of *Leishmania* parasites on bone marrow examination (myelogram), where the organisms were observed in both intracellular and extracellular forms. The remaining patients had the diagnosis confirmed by *Leishmania* IgG serology.

Among the 23 patients with confirmed parasitic infection, clinical signs and symptoms consistent with this pathology were observed. The onset and evolution of clinical manifestations were assessed from the time of initial hospital admission. All patients presented with high fever, which in most cases persisted for more than 10 days and did not respond to antipyretic or antibiotic therapy. The majority reported weakness, asthenia, and myoarthralgia, while some experienced weight loss. Pallor was commonly observed, affecting both the skin and mucous membranes. No cases of other cutaneous lesions, such as erosions or nodules, were recorded. Less frequent and atypical symptoms included dry cough, although it was not possible to determine whether it was attributable to other underlying conditions. Vomiting and diarrhea were present in a small number of patients, particularly in the pediatric group. On physical examination, splenomegaly was detected in 100% of cases and confirmed by abdominal ultrasound, while hepatomegaly was present in 95% of patients. No hemorrhagic manifestations or edema were observed in our cohort.

It is noteworthy that approximately 59% of patients had experienced these symptoms within the previous 30 days, while a considerable proportion reported symptom onset within the last three months.

All laboratory investigations were performed at the Department of Laboratories of the Mother Teresa University Hospital Center. In the 23 patients with confirmed leishmaniasis, laboratory findings revealed alterations related to the inflammatory state as well as evidence of organ and tissue involvement. The presence of the parasite was demonstrated in patients' myelograms. Significant hematological abnormalities were observed, including anemia, deviations in the leukocyte differential count, and thrombocytopenia.

The biochemical parameters evaluated in this study, which showed significant alterations and correlation with the clinical presentation of these patients, include:

- **Transaminases:** In our study, most patients showed mildly elevated values, generally up to 100 IU/L, which is common in patients with visceral leishmaniasis. Seventeen patients had increased ALT and AST levels, with mean values of approximately 67.7 IU/L for ALT and 56.7 IU/L for AST. Transaminase levels exceeded 100 IU/L in only four cases, while two patients had normal values.
- **Urea and Creatinine:** Seven patients demonstrated reduced levels of urea and creatinine, with mean values of approximately 7.9 mg/dL and 0.3 mg/dL, respectively. These findings appeared closely related to the severity

of anemia, as well as to the patients' general condition, weight loss, and malnutrition.

- Lactate Dehydrogenase (LDH): Elevated LDH levels were observed in 13 patients. The mean value was 439.5 U/L, with a maximum recorded value of 747 U/L. The remaining patients had normal or minimally elevated values.
- Inflammatory Markers: 1. **C-reactive protein (CRP)**: CRP levels were elevated in all patients, ranging from 1.50 mg/dL to 20.72 mg/dL. 2. **White blood cell count (WBC)**: WBC levels were reduced in almost all cases, except for one patient with normal values and one HIV-positive patient who showed leukocytosis (17.9 K/ μ L).
- Total Proteins: Normal reference values range from 6.0 to 8.04 g/dL. Results showed that total protein levels were generally within the normal range. However, all patients exhibited decreased albumin levels, accompanied by hyperglobulinemia. Protein electrophoresis performed in eight patients demonstrated reversal of the albumin/globulin ratio, with a mean A/G ratio of 0.82. Gamma-globulin levels increased to 19–29% of total proteins in five cases and to 30–35% in three cases. In several patients, elevations in α 1- and α 2-globulins were also observed.
- Complete Blood Count Analysis

The principal laboratory abnormalities observed in the complete blood count of our 23 patients were:

- Anemia
- Microcytic, hypochromic erythrocytes
- Thrombocytopenia
- Leukopenia (not present in all cases)
- Neutropenia
- Lymphocytosis
- Monocytosis
- Left shift of the leukocyte differential count

Marked alterations in these parameters were observed in all patients.

In the six patients in whom reticulocyte counts were measured, values ranged from 11% to 44%.

TABLE 2. Characteristics of Red Blood Cells

	N	Minimum	Maximum	Mean	Normal Reference Range
MCV	23	56.0	97.3	74.81	80-100 fL
MCH	23	18.4	28.5	23.5	26-34 pg

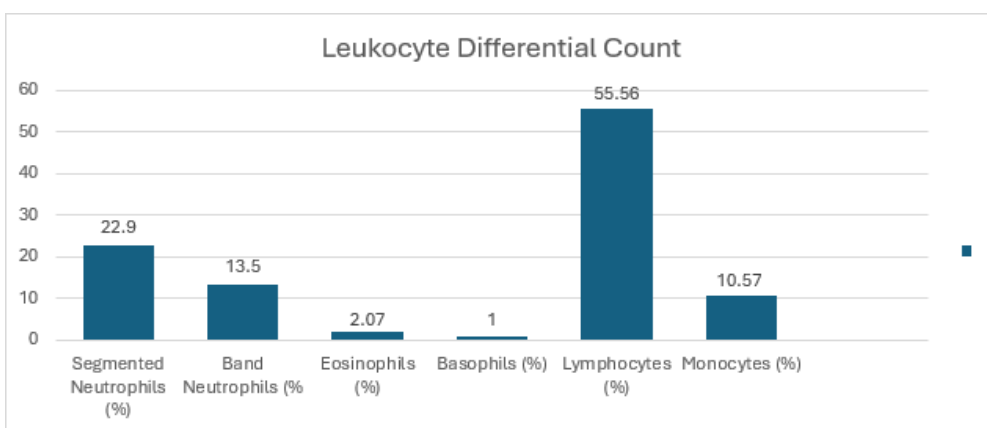
MCHC	23	28	35.50	31.00	31-35.5 g/dl
HCT	23	20.3	36.8	26.6	35-55 %
RBC	23	2.54x10 ⁶	4.6x10 ⁶	3.03x10 ⁶	4-5*10 ⁶ µL

The table above summarizes the data regarding both the quantity and morphological characteristics of erythrocytes in these patients. As observed, the mean red blood cell count was reduced, reaching markedly low minimum values in some cases. On average, the cells did not consistently show reduced MCV, although microcytosis was noted in certain cases. Overall, most cases demonstrated hypochromic erythrocytes, with mean MCH and MCHC values below the normal range.

TABLE 3. Hemoglobin

Severity of Anemia Hemoglobina (g/dl)	Number of Patients
I (12.8 - 9.6)	7
II (9.5 – 6.4)	12
III (6.3 – 3.2)	4
IV (<3.2)	0

Anemia was consistently present and was evidenced by reduced mean hemoglobin values. As shown in the table above, 12 patients had hemoglobin levels below 9.5 g/dL, and 4 of them were classified as having severe (grade III) anemia, with hemoglobin values below 6.3 g/dL.



Another peripheral blood alteration characterizing these patients was leukopenia. The mean white blood cell (WBC) count was below the reference range in 17 patients. In one HIV-positive patient, the WBC count was elevated, while in five patients leukocyte counts remained within normal limits. In addition

to leukopenia, changes were observed in the leukocyte differential count. Overall, a reduction in the percentage of neutrophils was noted, with a mean value of 22.9%, along with an increased proportion of immature neutrophils, with a mean value of approximately 13.5%. No significant changes were observed in basophils or eosinophils. Lymphocyte levels were elevated, with a mean value of 55.56%. Platelet counts were below the normal range in 100% of the cases included in the study. The majority of patients (approximately 73%) had platelet counts between 100,000 and 150,000/mm³, followed by 20.3% of cases with counts ranging from 30,000 to 100,000/mm³. A small proportion of patients presented with platelet counts below 30,000/mm³.

Bone Marrow Examination (Myelogram)

TABLE 4. Interpretation of the Myelogram

Leishmania Present	17
Leishmania Absent	0
Myeloid to Erythroid Ratio (M/E)	1/1 to 3,5/1
Plasma Cells > 5 %	17
5-10 %	0
> 10 %	0
Megakaryocytic Series Present	13
Rare	2
Absent	2

As observed from the bone marrow examinations of our patients, the presence of the parasite was identified in all smears, both extracellularly and intracellularly. The granulocytic-to-erythroid (M:E) ratio was preserved in nearly all patients. Plasma cells were present at levels below 5% in all cases. The megakaryocytic lineage was absent in two patients, while in the majority it was present at all stages of maturation. No abnormal cells were detected.

As highlighted above, the clinical presentation was characterized by prolonged high fever. Fever reflects an underlying pathological state, which in this case is caused by the presence of the parasite. By correlating the detection of *Leishmania* during the early stages of hospitalization—when persistent high fever was observed—with the markedly reduced blood cell counts indicating a longstanding infection, it can be inferred that fever correlates with the parasitic burden in these patients. Monocytosis, immune system hyperactivity, and hyperglobulinemia—present in nearly 100% of cases—further support the conclusion that the elevated temperature is also a consequence of these immunological changes.



The pronounced anemia observed in all patients correlated with their general condition and clinical manifestations, including pallor, weakness, fatigue, and overall poor health status.

As indicated by the epidemiology of the disease, leishmaniasis remains endemic and represents an ongoing health concern in our country. Delays in diagnosis and treatment worsen patients' conditions, leading to more severe clinical manifestations and significant laboratory abnormalities.

In our setting, all cases are confirmed at the Mother Teresa University Hospital Center, which may create logistical challenges and delays for patients living in regions distant from Tirana. The implementation of a standardized clinicopathological protocol could help minimize diagnostic delays, thereby reducing the morbidity and mortality associated with the disease.

Discussion

Visceral leishmaniasis remains an important public health concern in endemic Mediterranean countries, including Albania. The findings of the present study are consistent with the classical clinical and laboratory profile of visceral leishmaniasis reported in previous studies [13,14]. Anemia, leukopenia, thrombocytopenia, and splenomegaly were the most common findings observed in our cohort, reflecting the combined effects of bone marrow involvement, hypersplenism, and chronic inflammation [6,7,13].

The predominance of pediatric cases, particularly among children younger than five years of age, is in accordance with the epidemiological pattern reported in Mediterranean countries, where *Leishmania infantum* is the principal causative species [8,20]. Previous Albanian studies have similarly demonstrated a higher incidence among young children, confirming the continued importance of visceral leishmaniasis in this vulnerable population [4,8].

The hematological abnormalities observed in our study are consistent with the known pathophysiology of the disease. Anemia, leukopenia, and thrombocytopenia are primarily related to bone marrow involvement, splenic sequestration, and chronic inflammatory processes [6,13,14]. The universal presence of anemia in our cohort highlights its importance as a key laboratory finding and supports consideration of visceral leishmaniasis in patients presenting with prolonged fever and unexplained cytopenias in endemic areas [7,21].

Hypoalbuminemia, hypergammaglobulinemia, and elevated inflammatory markers were common findings among our patients and reflect chronic immune activation induced by persistent parasitic infection [14,25]. These laboratory abnormalities are well-recognized features of visceral leishmaniasis and may provide important diagnostic clues, particularly when associated with splenomegaly and hematological abnormalities [13,25].

Bone marrow examination demonstrated high diagnostic value and confirmed infection in the majority of patients. Although serological and molecular methods have become increasingly available, direct visualization of amastigotes remains a reliable diagnostic approach, particularly in patients presenting with prolonged fever, splenomegaly, and cytopenias [18,19,22].

Several limitations should be acknowledged. First, the retrospective design restricted data collection to information available in medical records. Second, the relatively small sample size may limit the generalizability of the findings. Nevertheless, this study provides valuable data regarding the clinical and laboratory characteristics of visceral leishmaniasis in Albania and highlights the importance of laboratory findings in facilitating early diagnosis and timely treatment.

Conclusions

Visceral leishmaniasis continues to represent a significant diagnostic challenge in endemic regions. In our cohort, anemia, thrombocytopenia, leukopenia, splenomegaly, hypoalbuminemia, and hypergammaglobulinemia were the most consistent clinical and laboratory findings. Bone marrow examination remained a reliable method for confirming the diagnosis. Recognition of these characteristic laboratory abnormalities may facilitate earlier clinical suspicion, prompt diagnostic investigation, and timely treatment, thereby reducing disease-related morbidity and improving patient outcomes. Strengthening awareness of the laboratory profile of visceral leishmaniasis is particularly important in non-specialized healthcare settings.

Declarations

Ethics approval and consent: This retrospective study was conducted in accordance with the Declaration of Helsinki. Data were obtained from routine clinical records and analysed anonymously.

Conflicts of interest: The authors declare no competing interests.

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Author contributions: All authors contributed to the study conception and design, data collection and interpretation, and drafting and critical revision of the manuscript, and approved the final version.

Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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