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Original Article

Visceral Leishmaniasis (VL) Clinical Presentation, Laboratory Findings, Treatment Options and Outcome

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Abstract

Background: Black disease, also known as visceral leishmaniasis (VL), is a parasitic illness caused by various *Leishmania* species. The risk of morbidity and mortality increases with delayed diagnosis and treatment. Early VL diagnosis and fast appropriate treatment are critical issues in endemic areas.

Methods: This study was a retrospective cross-sectional study to investigate the diagnostic and therapeutic course of patients admitted with the diagnosis of VL in the Children's Medical Center (CMC) Hospital, Tehran, Iran. All cases of VL in patients under the age of 18 hospitalized between the years 2012 to 2022 were enrolled.

Results: Twenty-seven patients were enrolled with an average age of 28.13 months with the majority of females (51.8%). Common clinical signs were fever (96.2%) and splenomegaly (92.59%). However, lymphadenopathy was rare. The largest number of patients was from Tehran Province, followed by Ardabil, Khuzestan, Gilan, and Alborz provinces. The most common hematological abnormalities were anemia (85.1%) and thrombocytopenia (44.4%). In accordance with the treatment strategy, liposomal amphotericin B and amphotericin B deoxycholate were given to 11 and 5 patients, respectively. Eleven of them received glucantime. The average length of hospitalization for liposomal amphotericin B was 15.36 ± 12.49 days. In comparison with glucantime (18.38 ±10.26 days) and amphotericin B deoxycholate (20.20± 6.18 days), liposomal amphotericin B group hospitalization was shorter than others were.

Conclusion: VL should be included in the differential diagnosis of any child who presents with fever, splenomegaly, and anemia. Concerning the treatment strategy in this study, liposomal amphotericin B had more efficiency and shorter hospitalization duration.

Introduction

Visceral Leishmaniasis (VL), also known as kala-azar is a tropical and subtropical parasitic disease, which is caused by various species of "*Leishmania*", transmitted through female phlebotomine sand flies (1). The parasite and its carrier are various, depending on the geographical area. Leishmania infantum, also known as Chagasi in South America, is the species that causes VL in the Middle East and Iran. (2). Canines are considered the main reservoir in Iran (3). VL is reported in over 70 countries from 5 continents, as well as it has become an endemic disease in more than 60 countries (4). The global incidence of VL decreased substantially in the past decade: from between 200000 and 400000 new cases in 2012, and to between 50000 and 90000 in 2017 (5). The average annual number of diagnosed cases of VL in Iran was 0.449 cases/100,000 inhabitants during the last decade (6-8).

In addition to overt clinical episodes of VL, most infected patients present with asymptomatic/ subclinical infections (9). Children typically suffer more from the VL in endemic places since adults may have developed immune protection from prolonged exposures (10). Common clinical manifestations include prolonged fever, loss of appetite, weakness, fatigue, and progressive abdominal enlargement due to hepatosplenomegaly (11). VL can also occur in immunocompromised hosts infrequently; therefore, patients with congenital or acquired immune deficiency should be assessed for VL if they develop Fever of Unknown Origin (FUO), cytopenia, or hepatosplenomegaly (12).

The traditional confirmatory diagnostic tests involve finding parasites in the patients' lymph node, bone marrow, or splenic tissue biopsies (13). The direct agglutination test (DAT) and rK39 antigen-based test are two of the serological diagnostics that are commonly used in clinical practice (14).

The conventional therapy for VL includes anti-leishmanial drugs for instance liposomal amphotericin B (L-AmB), amphotericin B deoxycholate (DAmB), miltefosine, paromomycin, pentamidine, and pentavalent antimonials (15). There are two forms of pentavalent antimony readily available: sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime) (16). Among the treatment options, each drug has its own pros, cons. Paromomycin is an inexpensive and effective drug, easily administered intramuscularly, and however it needs a 21-days treatment duration and has nephrotoxicity and ototoxicity as main side effects (17). Miltefosine is the only oral medication for VL treatment but complications including, diarrhea, vomiting, and dehydration limit its application (18). As another treatment, glucantime has numerous side effects including painful injection, diarrhea, emesis, abdominal discomfort and hepatic, renal and particularly cardiac toxicity. (19). Amphotericin B is an antifungal drug that can bind to ergosterol (the main component of the leishmanial cell membrane) with high affinity and can cause cell death. Furthermore, it can be a very effective treatment (20). This drug has two types, liposomal amphotericin B (L-AmB) and amphotericin B deoxycholate (DAmB) forms (21). Although serious side effects such as hypokalemia, nephrotoxicity, hypokalemia, infusion reactions, and myocarditis has limited the use of DAmB form. L-AmB is produced by combining Amphotericin B with liposomes, resulted in strong tropism for the macrophages and also offers improved stability in plasma and reduced renal and cardiac toxicity (22).L-AmB can be a more appropriate treatment compared to DAmB due to fewer side effects, and better pharmaco-kinetics and bioavailability, also it is the most common drug to used treat Leishmania-resistant species cases due to its efficiency and well tolerance. In addition to these advantages, its high costs and good cold

chain requirement considered as L-AmB disadvantages (18, 23).

Considering the prevalence of VL in Iran, this study was designed to investigate the demographic features, clinical presentation and laboratory findings in these patients. Three drug options mostly used to treat VL in Iran (L-AmB, DAmB and glucantime), therefor the aim of our study was to determine the effectiveness, length of hospital admission and relapse rate of each drug regimen.

Material and Methods

This cross-sectional retrospective study was carried out at the Children's Medical Center (CMC), a tertiary pediatric hospital in Tehran, Iran to look into the diagnostic and therapeutic trajectory of patients diagnosed with VL between 2012 and 2022. First of all, this study covered all children of both gender including male and female under the age of 18 years who were admitted and treated with a confirmed diagnosis of VL. The final diagnosis was made through clinicopathological approaches in patients with a suspicious clinical presentation of the VL, such as of FUO, cytopenia and hepatosplenomegaly. Related information of patients during the aforementioned period with a confirmed diagnosis of VL and who had previously given consent to the use of their medical records for research purposes extracted. The patients' file used to gather data such as demographics, clinical signs, laboratory tests results, type of treatment and outcome.

Because the study was conducted over ten years, the patients were followed up until 2022 in terms of recurrence by visiting in the clinic on an outpatient basis. Patients hospitalized in the early years of the study were followed for a longer time, and cases who were hospitalized in 2022 were followed up for at least one year after discharge.

This study received ethical approval from the Ethical Committee at Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran (code: IR.TUMS.CHMC.REC.1400.112).

All of the patients under the age of 18 years with final diagnosis of VL disease were included in this study. The patients with simultaneous presence of other internal diseases, which affect the results of our study and fail to receive treatment for any reason are excluded from our study.

SPSS version 22 (IBM Corp., Armonk, NY, USA)) was used to analyze the data. Continuous and categorical variables are expressed as mean (SD) and frequencies (percent), respectively. An Independent t-test was used for the comparison of continuous variables between study groups. P-value of <0.05 was considered statistically significant.

Results

The total numbers of VL patients from 2012 to 2022 were 27 cases, including 14 females (51.8%) and 13 males (48.1%), with the average age of 28.13± 23.45 months (Table 1). The most of these patients have resided in Tehran (30%) followed by Ardabil (20%) Province (Table 2).

Table 1: The mean and standard deviation of the age of patients diagnosed with VL who were admitted to CMC Hospital between 2012 and 2022

	Mean (standard deviation)	*Median (interquartile range)*	*Minimum*	*Maximum*
Age (month)	28.13± 23.45	20 (16-29)	4	98

Table 2: Frequency distribution of living locations of patients diagnosed with VL who were admitted to CMC Hospital between 2012 and 2022

Province name	***Frequency*** *(%)*
Tehran	8 (29.62)
Ardabil	5 (18.51)
khuzestan	3 (11.11)
Gilan	3 (11.11)
Alborz	2 (7.40)
Isfahan	1 (3.70)
Golestan	1 (3.70)
Kordestan	1 (3.70)
Ilam	1 (3.70)
Chaharmahal and Bakhtiari	1 (3.70)
Lorestan	1 (3.70)

The main and common clinical findings of patients were fever (96.2%) and splenomegaly (92.5%). Lymphadenopathy was seen only in 3 patients (11.1%). The most common hematologic abnormality was anemia followed by thrombocytopenia (Table 3). Hematologic values of ESR, WBC, CRP, PLT, and Hb demonstrated in Table 4. Notably, these hematological parameters did not have a normal distribution. Considerably, only 9 of (33.3%) VL considered as their primary diagnosis although VL is diagnosed in other patients during further investigations (Table 5).

Table 3: incidence of hematologic abnormalities of patients diagnosed with VL who were admitted to CMC

Hematologic abnormality	***Frequency (%)***
Anemia	23 (85.18)
Thrombocytopenia	12 (44.44)
Leukopenia	7 (25.92)
Leukocytosis	3 (11.11)
Thrombosis	1 (3.70)

Table 4: hematologic values of patients diagnosed with VL who were admitted to CMC Hospital between 2012 and 2022

Name	***Mean (SD)***	***(IQR) Median***	***Minimum***	***Maximum***
ESR	60.56±32.48	(41-84) 59	3	140
CRP	48.72 ±53.97	(14-58) 33.5	3	187
WBC	6939 ± 4690	(4100-7670) 5940	1800	22500
HB	8.8 ±1.92	(8-10) 8.85	1.8	12.1
PLT	185769 ±145658	(81000-256000) 150000	17000	672000

The most common differential diagnoses were malignancy and hemophagocytic lymphohistiocytosis (HLH)

Table 5: Initial diagnosis of patients diagnosed with VL who were admitted to CMC Hospital between 2012 and 2022

Initial diagnosis	*Frequency (%)*
VL	9 (33.3)
Malignancy	6 (22.2)
HLH	2 (7.4)
Immunodeficiency	2 (7.4)
Hepatitis	1 (3.7)
Anemia	1(3.7)
Fever without local sign	1(3.7)
Storage Disease	1(3.7)

VL diagnosis is confirmed by three tests. 12 cases through bone marrow aspiration (Figs. 1, 2), 12 patients by DAT, and 7 individuals through rK39 Antigen detection test detected (Table 6). L-AmB was prescribed for 11 patients, five cases received DAmB, and 11 received glucantime. The average length of hospitalization was 16.07±11.17 days. The patients were treated with L-AmB had shorter admission time (Table 7). L-AmB had a better therapeutic efficiency and a shorter hospitalization period. As a result, it brings less financial burden to the treatment system. None of the patients had a failure to treatment and no death occurred.

Table 6: diagnostic tests results of patients diagnosed with VL who were admitted to CMC Hospital between 2012 and 2022

Test name	*Positive Frequency (%)*	*Negative Frequency (%)*	*Unknown Frequency (%)*
Bone marrow aspiration	12 (44.44)	14 (51.86)	1 (3.70)
DAT	12 (44.44)	0	15 (55.56)
rK39 Antigen	7 (25.93)	0	20 (74.07)

Table 7: treatment of patients diagnosed with VL who were admitted to CMC Hospital between 2012 and 2022

Drug	*liposomal amphotericin B (L-AmB),*	*Glucantime*	*Amphotericin B deoxycholate (D-AmB)*
Frequency (%)	11 (40.74)	11 (40.74)	5 (18.51)
Hospitalization duration mean	15.36 ± 12.49	18.38 ±10.26	20.20± 6.18

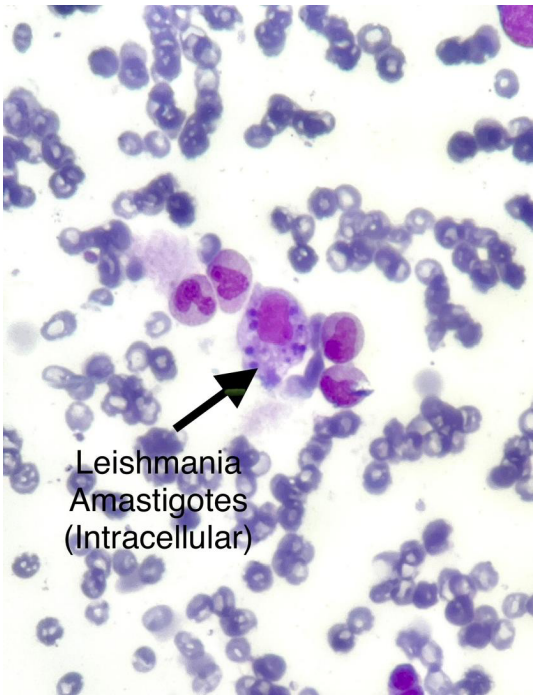

Fig. 1: Leishman body In Leishmaniasis, Leishmania Amastigotes (intracellular) that seen in bone marrow biopsy of one of the VL patients (Figure after Dr Maryam Sotudeh, pathologist)

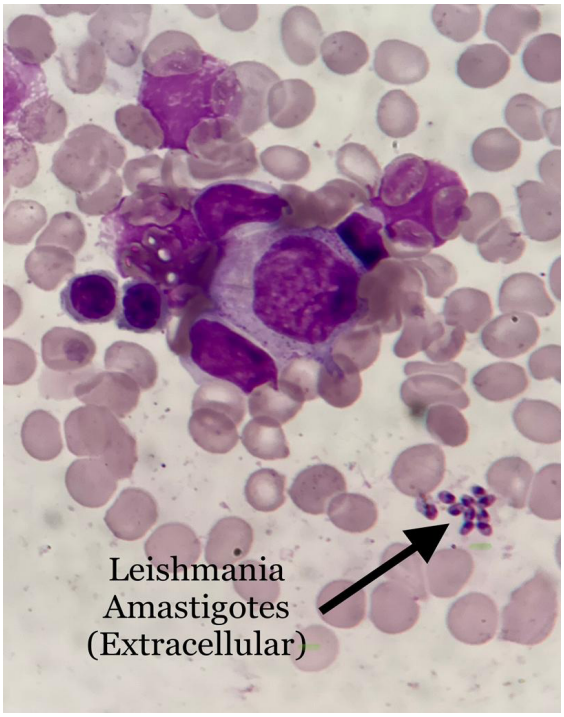

Fig. 2: Leishman body in leishmaniasis, *Leishmania* Amastigotes (extracellular) that seen in bone marrow biopsy of one of the VL patients (Original)

Discussion

In this study, the female gender was relatively (with an 8% difference) more frequent than male gender. This finding is consistent with the results seen in a study done in northeast of Iran, which represented that female to male ratio was 2.3:1 (24). However, a few studies have revealed that VL is more prevalent in men. For instance, 57.1% and 42.9% of patients were men and women, respectively (25). This idea is supported by another study conducted in Fars Province (26). There was not a significant difference in the genders, according to a report from the Booyerahmad district (27). Additionally, a systematic review found no significant gender difference in prevalence (28).

In the current study, the mean age is about 28 months (median age=20 months). Similar studies in other places have shown occurrence of this disease among children was more than other ages. According to a study, 90% of the studied patients were under 5 years old (29). In addition, study conducted in the Fars Province, reported VL cases are frequent among 2 years old children (26).

Currently, the VL in Iran is endemic in five provinces: Ardabil, East Azerbaijan, Fars, Bushehr, and Qom. In addition, 44.6% of the VL infection is reported from northwestern region of Iran. Although sporadic occurrences of VL have been reported in other regions. The highest incidence rate of VL was 57 cases/100,000 inhabitants from Ardabil province (7, 9). While in this study, the largest number of patients was from Tehran provinces, followed by Ardabil, Khuzestan, Gilan, and Alborz. Noteworthy, our center is an academic referral pediatric center in Tehran Province.

The common clinical features of VL in our study were fever, splenomegaly and anemia. Also, in similar studies done in southeast of Iran, northwest of Iran, and also in Alborz Province reported the most frequent signs and symptoms were fever, splenomegaly and anemia (30-32). Additionally, the main clinical presentations of the patients were fever (96.2%), hepatosplenomegaly (68.8%) and abdominal protrusion (71.9%) (26). Likewise, fever, splenomegaly, hepatomegaly, and anemia were reported to be the most common signs among hospitalized patients (33). An Ethiopian investigation accounted splenomegaly, fever, and anemia frequencies as follow: 98.6%, 96.5%, 95%, respectively in their region (34). Therefore, VL should be included in the differential diagnosis of any child who presents with fever, splenomegaly, and anemia. Moreover, in the current study prevalence of lymphadenopathy was 11%. A similar study in Ethiopia reported the prevalence of lymphadenopathy was 9.9% (34). Although a study in Meshkin-Shahr only a 4% of patients reported lymphadenopathy (29). Hence, lymphadenopathy is considered as one of the symptoms in VL patients; however, it is not the major symptom.

Another study in a pediatric hospital in Tehran mean hemoglobin concentration, mean platelet count and mean WBC count were 9.2 ± 2 g/dL, 152 ± 7.7/mL and 4.84 ± 1.6/mL, respectively. However, in comparison with our results (Hb: 8/8 ± 1.92g/dl, platelet: 185.769 ± 145.658 /mL, WBC: 6.939± 4.69/mL), the mean plt and WBC count was higher in our findings (35). According to Tofighi et al. laboratory results, anemia (97.1%), thrombocytopenia (91.2%), and leukopenia (67.6%) were the most common laboratory hematological abnormalities in VL. Since their results were similar to our study in terms of order, although their incidence was lower in our findings (36). The result of the Shiraz study has shown the average ESR was 65.43±22.36 mm/h (37). Likewise, in our study the average ESR was 60.56 ±32.48 mm/h, which was roughly similar to each other.

Bone marrow aspiration was positive in 58.8% of patients in Kerman, though in com-

parison to our study, only 44.44% of our patients had positive aspiration (38).

In our study, three treatment options (Glucantim, L-AmB, DAmB) were considered. The average hospitalization time in the treatment with L-AmB was less than other drugs with better efficacy and fewer side effects (15.3 days) in our study. Glucantime and DAmB were used in the treatment of VL in another pediatric hospital in Tehran, D-AmB for 15 days and glucantime for 4 weeks (35). In Sakari study, a 28-day course of glucantime has been used in most cases (25). L-AmB reduces the hospital stay, hospitalization costs, and risk of nosocomial infections (39). The average duration of the hospitalization in the L-AMB group was 16±2.7 days, while in the glucantime group was 30.18±0.98 days (40). A study conducted in Italy reported a mean duration of hospitalization with L-AmB 8±3 days, with a majority of 10 days (41).

In general, according to the available evidence, it seems that L-AmB is associated with fewer side effects, shorter hospitalization time and better efficiency. However, L-AmB is recently considered to treat VL, which is relatively more expensive than other therapeutics, which requires further investigations.

Conclusion

VL should be included in the differential diagnosis of any child who presents with fever, splenomegaly, and anemia. The disease may occur in different regions of Iran, but the prevalence of the disease is significantly higher in some regions. Using the appropriate diagnostic tests, starting the right treatment and choosing the best option based on the conditions of each patient in each community will lead to better management and outcome. Given that this study was conducted at a single-center, it is advised to develop a multi-center study, gather, and analyze the data from a large population in order to have a deeper knowledge of the disease.

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Competing interest

The authors of this study declare no conflicts of interest.

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