Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8648
https://doi.org/10.4025/actascihealthsci.v48i1.68269

Clinical and laboratory evaluation of patients with visceral leishmaniasis undergoing different therapeutic regimens of liposomal amphotericin B

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ABSTRACT. Visceral Leishmaniasis (VL) is an infectious disease caused by protozoa of the *Leishmania* genus and is transmitted to humans by the bite of infected female *Lutzomyia* sandflies. Several treatment protocols for VL are available, including the utilization of liposomal amphotericin B. Nevertheless, the optimal protocol with the minimal adverse effects remains uncertain. In this context, the present study aims to assess the clinical and laboratory outcomes of patients with VL undergoing two distinct therapeutic regimens of liposomal amphotericin B. A cross-sectional study was conducted to analyze data from the medical records of patients diagnosed with VL between 2015 and 2019 in an endemic area in the northern region of the State of Minas Gerais, Brazil. All patients in the study were predominantly under the age of 10 years, with 70.4% being male (19 cases). In terms of clinical conditions, fever was present in 92.3% of cases, while splenomegaly and hepatomegaly occurred in 100% of patients. No significant differences were observed between the treatment regimens of liposomal amphotericin B at 4 mg (kg day^{-1})$^{-1}$ for 5 days and 3 mg (kg day^{-1})$^{-1}$ for 7 days. The data suggest that patients who received liposomal amphotericin B for both 5 and 7 days exhibited no clinically or laboratory differences compared to those who utilized liposomal amphotericin B for a five-day duration.

Keywords: Visceral leishmaniasis; liposomal amphotericin B; therapeutic.

Received on December17, 2024.
Accepted on September 17, 2025.

Introduction

Visceral leishmaniasis (VL) is a systemic infectious disease endemic to tropical regions, caused by protozoa of the genus *Leishmania*. In the Americas, the etiological agent responsible for VL is *Leishmania infantum*, which is transmitted to humans through the bite of phlebotomine sandflies (Gutiérrez-Ocampo et al., 2021). In Brazil, two *Lutzomyia* species have been identified as vectors of the disease: *Lutzomyia longipalpis* and *Lutzomyia cruzi*, with the former being of greater epidemiological significance due to its wider distribution and higher transmission potential (Rangel et al., 2018).

In Brazil, from 2000 to 2013, 48,358 confirmed cases of VL were reported, corresponding to an average incidence rate of 1.88 cases per 100,000 inhabitants (Alvar et al., 2012). Despite the implementation of more specific guidelines for managing severe VL cases, the case fatality rate remains significantly high (Gama et al., 2013). In 1994, the lethality rate was 3.4%, increasing to 8.5% in 2003, 5.8% in 2009, and 7.2% in 2017 (Ministério da Saúde, 2018).

According to the Ministry of Health (MS) (2018), therapeutic failures have highlighted the need for alternative treatments with lower toxicity profiles. This led to the introduction of Amphotericin B formulations in the 1990s, which have since demonstrated notable clinical advantages. Amphotericin B liposomal formulations offer reduced toxicity and cure rates comparable to those of amphotericin B deoxycholate. Additionally, this liposomal form is characterized by better retention and activity in the reticuloendothelial system, along with shorter treatment regimens, which contribute to reduced hospital stays and overall treatment costs (Berman et al., 1998; Kumari et al., 2022).

Liposomal amphotericin B exhibits broad tissue distribution and penetration, resulting in higher plasma concentrations compared to amphotericin B deoxycholate. This formulation demonstrates particularly high

penetration in the spleen and liver, where therapeutic levels persist for several weeks post-administration (World Health Organization, 2010; Hamill, 2013; Wasan et al., 2022; Noor & Preuss, 2024). Reports of adverse reactions with liposomal amphotericin B are significantly lower than those associated with amphotericin B deoxycholate. Amphotericin B toxicity in humans arises from its interaction with cholesterol, leading to the oxidation of cell membranes and the generation of reactive oxygen species. Amphotericin B aggregates can also cause toxicity in human organisms due to their size and the differential distribution of various sized molecules throughout the system, as well as their elimination. Consequently, nephrotoxicity is the most prominent adverse effect associated with amphotericin B (Frézard et al., 2022).

In this context, the present study evaluated the clinical and laboratory data of patients with VL at the start and conclusion of treatment at the University Hospital Clemente Faria, an institution located in an endemic area. The study emphasizes the use of liposomal amphotericin B under two different treatment protocols.

Materials and methods

The study was conducted in accordance with ethical guidelines, adhering to the principles outlined in Resolution 466/12, and was submitted to the Research Ethics Committee (CEP) of the State University of Montes Claros (Unimontes) for approval.

This study is a descriptive, retrospective cross-sectional analysis with a quantitative approach, based on data collected from the medical records of patients diagnosed with VL between 2015 and 2019 at Clemente de Faria University Hospital (HUCF) of the State University of Montes Claros, MG, Brazil.

The study included 27 patients with VL who were treated with liposomal amphotericin B. Participants were divided into two groups: Group I consisted of individuals treated with liposomal amphotericin B for 5 days at a dose of 4 mg $(kg\ day^{-1})^{-1}$, while Group II included individuals treated for 7 days with 3 mg $(kg\ day^{-1})^{-1}$. The therapeutic regimen for each patient was selected randomly by the healthcare service.

Demographic variables collected included age, sex, and place of origin (rural or urban). Clinical variables such as fever, diarrhea, jaundice, edema, vomiting, and bleeding, as well as laboratory variables measured at the beginning and end of treatment, including blood count, platelet count, bilirubin levels, transaminases, amylase, lipase, urea, and creatinine, were also recorded. The study population comprised patients of all age groups with a parasitologically confirmed diagnosis of VL who were treated with liposomal amphotericin B for 5 or 7 days. Patients who received liposomal amphotericin B but also underwent other leishmanicidal therapies were excluded from the study.

All data collected regarding demographic and clinical-laboratory variables were digitized and subsequently analyzed using the SPSS software for Windows, version 22.0 (SPSS Inc., Chicago, IL). Initially, a descriptive analysis of the data was performed, including frequency distribution between the groups. The normality of the data was assessed using the Shapiro-Wilk test. Statistical significance was determined using either the unpaired T-test or the Mann-Whitney test, depending on the appropriateness for comparing differences between groups before and after treatment. To evaluate changes within each group before and after treatment, the paired T-test or Wilcoxon test was applied.

Results

During the study period (2015 to 2019), 27 patients treated with liposomal Amphotericin B at the Clemente de Faria University Hospital of the State University of Montes Claros (MG) were included. The mean age of the study population was 9.4 ± 13.6 years, ranging from six months to 52 years. In the evaluated groups, the mean age was 3.6 ± 3.3 years (ranging from 0.6 to 12 years) in Group I, and 15.6 ± 17.5 years (ranging from 0.6 to 52 years) in Group II. Among the participants, 19 (70.4%) were male and 8 (29.6%) females. All patients resided in the northern region of Minas Gerais, with the majority coming from urban areas.

All patients in both groups achieved clinical cure by the end of treatment, with no cases of relapse. Additionally, none of the individuals in the study sample, from either group, required discontinuation of therapy due to adverse effects or the use of alternative leishmanicidal therapy.

At the beginning of treatment, the most common clinical signs and symptoms were fever (92.3%), splenomegaly (100%), hepatomegaly (100%), edema (29.6%), vomiting (25.9%), diarrhea (11.1%), and bleeding (7.4%). None of the patients presented with jaundice. Furthermore, no significant differences were observed in the comparison of these clinical signs and symptoms between the two groups.

Upon evaluating peripheral hematological data, no significant differences were observed between the two treatment protocols at the beginning and end of treatment regarding hemoglobin, leukocyte, and platelet counts (Figure 1). However, notable differences were evident when analyzing the changes within each protocol.

For hemoglobin levels, both groups demonstrated an increase: the group treated for 5 days showed an increase from 5.76 ± 2.12 g dL^{-1} to 8.11 ± 1.81 g dL^{-1}($p<0.030$), while the group treated for 7 days exhibited an increase from 7.15 ± 1.31 g dL^{-1} to 7.96 ± 1.43 g dL^{-1} ($p = 0.14$) (Figure 1A).

Similar trends were observed for leukocyte counts, with significant increases noted after both treatment durations: in the 5-day group, leukocytes rose from 4013 ± 2067 mm^3 to 5739 ± 2103 mm^3 ($p = 0.006$), and in the 7-day group, they increased from 3946 ± 3247 mm^3 to 5088 ± 3646 mm^3 ($p = 0.041$) (Figure 1B).

A comparable pattern was also observed for platelet counts, which significantly increased at the end of treatment in both the 5-day protocol (from 99,400 ± 69,689 mm^3 to 181,067 ± 104,320 mm^3; $p = 0.016$) and the 7-day protocol (from 82,600 ± 80,841 mm^3 to 207,667 ± 186,367 mm^3; $p = 0.019$) (Figure 1C).

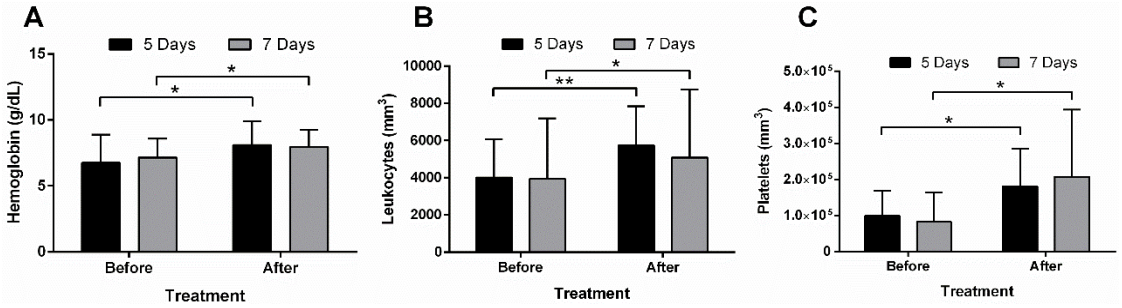

Figure 1. Hematological marks evolution of two groups of patients with visceral leishmaniasis in the period before and after treatment with liposomal amphotericin B.

Laboratory data regarding liver function markers were assessed (Figure 2). Among the evaluated parameters, no significant differences were observed between the two treatment protocols. However, for the transaminases assessed, a notable reduction in aspartate aminotransferase (TGO) levels was observed in the group treated with the 5-day protocol, decreasing from 234.2 ± 345.5 U $mL^{-1}$to 76.4 ± 89.3 U mL^{-1}($p = 0.019$). In contrast, no significant change was detected in the 7-day protocol, where TGO levels were recorded as 176.8 ± 205.3 U mL^{-1}before treatment and 82.5 ± 73.2 U mL^{-1}after treatment ($p = 0.953$) (Figure 2A).

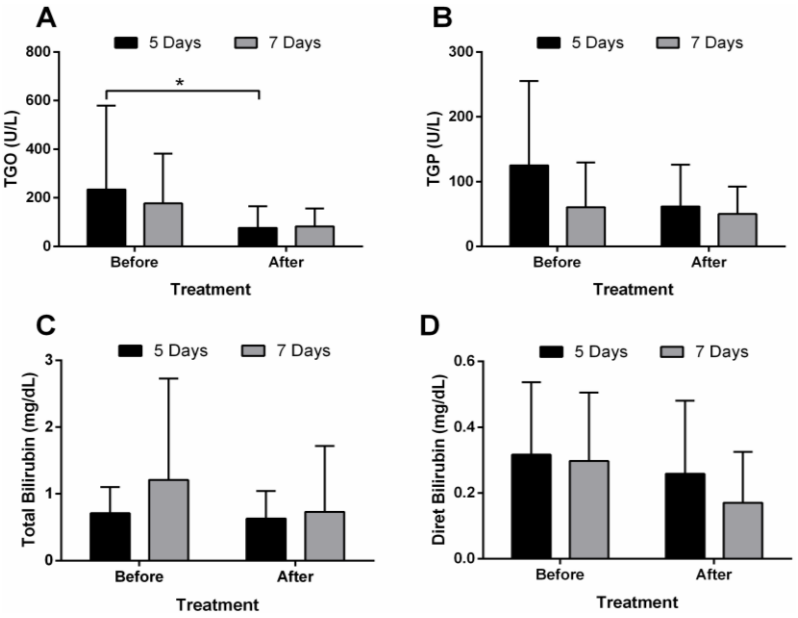

Figure 2. Liver marks evolution of two groups of patients with visceral leishmaniasis in the period before and after treatment with liposomal amphotericin B.

A reduction was noted for TGP in group 5 days of treatment, with levels decreasing from 125.0 ± 130.2 U mL^{-1} to 61.5 ± 64.6 U mL^{-1} ($p = 0.055$). No statistical significance was reached in the 7-day group with a decrease from 60.5 ± 68.9 U mL^{-1} to 50.2 ± 42.2 U mL^{-1} ($p = 0.110$) (Figure 2B).

The total bilirubin levels in the 5-day treatment group showed no statistical differences (before: 0.71 ± 0.39 mg dL^{-1}; after: 0.63 ± 0.41 mg dL^{-1} (p = 0.760)), just as the 7-day group (before: 1.21 ± 1.52 mg dL^{-1}; after: 0.73 ± 0.99 mg dL^{-1} (p = 0.139). Similarly, for direct bilirubin, the 5-day treatment group showed values of 0.32 ± 0.22 mg dL^{-1} before treatment and 0.26 ± 0.22 mg dL^{-1} after treatment (p = 0.308), whereas the 7-day treatment group recorded values of 0.30 ± 0.21 mg dL^{-1} before and 0.17 ± 0.15 mg dL^{-1} after treatment (p = 0.092). No statistically significant differences were found for these parameters in either treatment protocol (Figure 2C and Figure 2D).

In our study, renal function markers were also evaluated, as depicted in Figure 3. No significant differences were observed between the two treatment protocols for the investigated parameters. However, when analyzing each protocol individually at the beginning and end of treatment, distinct trends emerged for certain markers.

For creatinine levels, no significant changes were detected in either group: the 5-day treatment group showed values of 0.49 ± 0.16 mg dL^{-1} before treatment and 0.50 ± 0.21 mg dL^{-1} after treatment (p = 0.755), while the 7-day group had values of 0.67 ± 0.37 mg dL^{-1} before and 0.73 ± 0.42 mg dL^{-1} after treatment (p = 0.476). Similarly, urea levels showed no significant changes, with the 5-day treatment group recording values of 24.4 ± 8.6 mg dL^{-1} before and 25.3 ± 11.8 mg dL^{-1} after treatment (p = 0.534), and the 7-day group showing levels of 28.1 ± 16.7 mg dL^{-1} before and 31.1 ± 10.4 mg dL^{-1} after treatment (p = 0.462) (Figure 3A and Figure3B).

However, a substantial reduction in potassium levels was observed in the 7-day treatment group, decreasing from 5.56 ± 5.51 mEq L^{-1} to 3.56 ± 0.64 mEq L^{-1} (p = 0.012), while no significant change was noted in the 5-day treatment group (from 4.11 ± 0.43 mEq L^{-1} to 4.06 ± 0.75 mEq L^{-1}; p = 0.556) (Figure 3C).

Additionally, a significant increase in sodium levels was detected in the 5-day treatment group, rising from 133.83 ± 3.21 mEq L^{-1} to 139.22 ± 3.99 mEq L^{-1} (p = 0.011), whereas the 7-day protocol showed no significant change, with values of 137.64 ± 2.11 mEq L^{-1} before and 138.67 ± 4.09 mEq L^{-1} after treatment (p = 0.752) (Figure 3D).

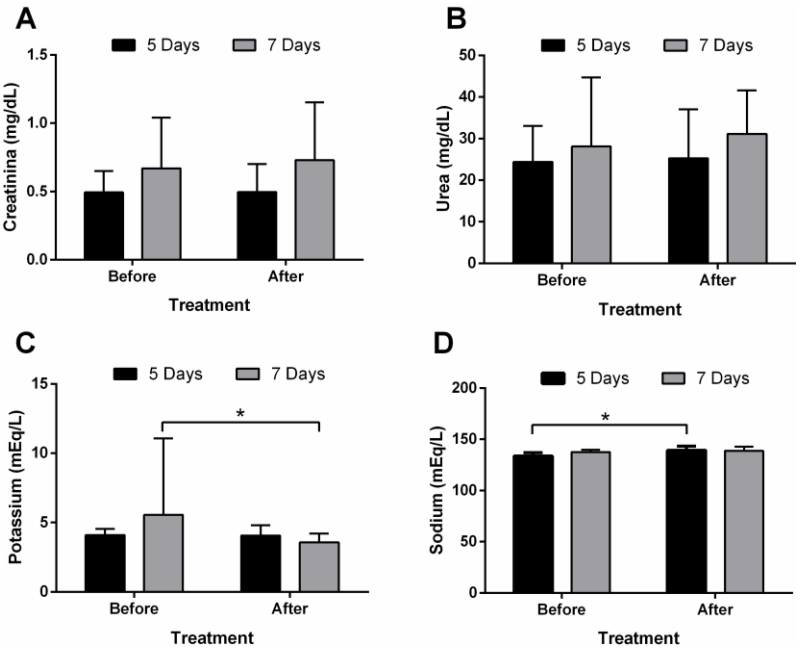

Figure 3. Renal marks evolution of two groups of patients with visceral leishmaniasis in the period before and after treatment with liposomal amphotericin B.

The digestive enzymes lipase and amylase were also assessed, as shown in Figure 4. No significant differences were observed between the two treatment protocols for either enzyme. However, when comparing each protocol individually before and after treatment, a significant increase in amylase levels was noted in both the 5-day treatment group (from 47.0 ± 32.0 IU mL^{-1} to 71.0 ± 37.0 IU mL^{-1}; p = 0.041) and the 7-day treatment group (from 40.0 ± 26.0 IU mL^{-1} to 68.0 ± 35.0 IU mL^{-1}; p = 0.05) (Figure 4A and Figure 4B).

Regarding lipase, no significant changes were detected before and after treatment in either group (5-day group before: 29.4 ± 36.5 IU mL^{-1}, after: 22.0 ± 13.7 IU mL^{-1} (p = 0.395); 7-day group before: 20.2 ± 12.4 IU mL^{-1}, after: 29.3 ± 15.1 IU mL^{-1} (p = 0.125)).

Finally, clinical monitoring of spleen and liver size was conducted, and the results are summarized in Figure 5. No significant differences were observed between the two treatment protocols regarding the size of these organs. A similar pattern was noted when each protocol was analyzed separately, before and after treatment.

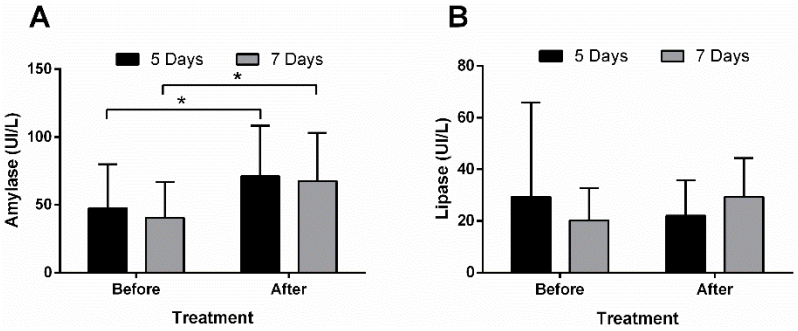

Figure 4. Pancreatic marks evolution of two groups of patients with visceral leishmaniasis in the period before and after treatment with liposomal amphotericin B.

For the liver size for either group. In the 5-day treatment group, liver size remained consistent (before: 4.30 ± 1.93 cm, after: 4.25 ± 1.90 cm; p = 0.374), and in the 7-day treatment group, there was a non-significant reduction in size (before: 4.33 ± 2.12 cm, after: 3.30 ± 2.11 cm; p = 0.257).

Similarly, no significant changes were seen in spleen, no significant reduction in size was detected for either group. The spleen sized remained stable in the 5-day treatment group (before: 6.13 ± 3.71 cm, after: 6.13 ± 3.09 cm; p = 0.395) and in the 7-day treatment group (before: 7.21 ± 2.91 cm, after: 6.00 ± 6.68 cm; p = 0.102).

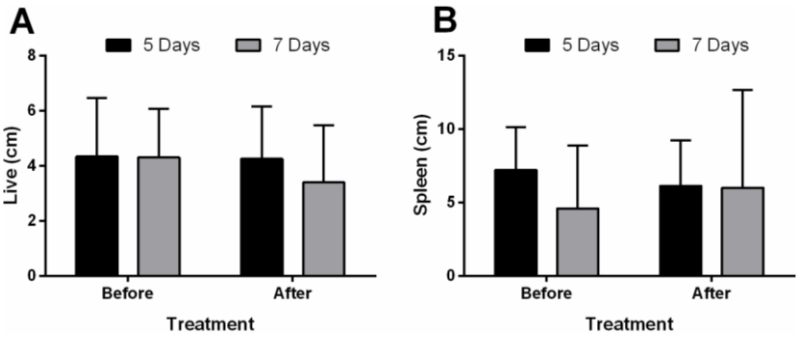

Figure 5. Spleen and liver evolution of two groups of patients with visceral leishmaniasis in the period before and after treatment with liposomal amphotericin B.

Since opened vials of liposomal amphotericin B cannot be stored for subsequent use, the daily surplus generated by the two treatment regimens (5 days and 7 days) was analyzed and is presented in Table 1. Given the similar efficacy observed between both regimens, the amount of daily leftover medication could serve as a relevant factor in deciding which regimen to adopt. Reducing waste would not only enhance cost-effectiveness but also optimize resource utilization, potentially benefiting healthcare systems, particularly in resource-limited settings.

Table 1. Daily excess of liposomal amphotericin B in both therapeutic regimens.

Patient	treatment time (days)	Weight (kg)	Dosage mg (kg day^{-1})$^{-1}$	Total mg day^{-1}	remnant *AMBL mg day^{-1}	Dosage mg (kg day^{-1})$^{-1}$	Total mg day^{-1}	remnant *AMBL mg day^{-1}
1	5	10,6	3	31,8	18,2	4	42,4	7,6
2	5	10	3	30	20	4	40	10
3	5	8,4	3	25,2	24,8	4	33,6	16,4
4	5	10,4	3	31,5	18,5	4	41,6	8,4
5	5	9	3	27	23	4	36	14
6	5	20,6	3	1fr + 11	39	4	1 fr + 32,4	17,6
7	5	10,9	3	32,7	17,3	4	43,6	6,4
8	5	9,6	3	28,8	21,2	4	38,4	11,6
9	5	10,9	3	32,7	17,3	4	43,6	6,4
10	5	9,5	3	28,5	21,5	4	38	12
11	5	20	3	1fr + 10	40	4	1fr + 30	20
12	5	10,3	3	30,9	19,1	4	41,2	8,8
13	5	13	3	39	11	4	1fr + 2	48
14	5	16	3	48	2	4	64	36
15	7	10	3	30	20	4	40	10

16	7	28	3	1fr + 34	16	4	2fr + 12	38
17	7	22	3	1 fr + 16	34	4	1fr + 38	12
18	7	72	3	4 fr + 16	34	4	5 fr + 38	12
19	7	7,1	3	21,3	28,7	4	28,4	21,6
20	7	63,5	3	4 fr	40,5	4	5 fr + 4	46
21	7	14	3	42	8	4	56/06	44
22	7	8	3	25	25	4	32	18
23	7	56	3	3fr + 18	32	4	4fr + 24	26
24	7	51,7	3	3fr + 5,1	44,9	4	4fr + 6,8	43,2
25	7	21,5	3	1fr + 14,5	35,5	4	1fr + 36	14
26	7	10,4	3	31,2	18,8	4	41,6	8,4
27	7	21	3	1fr + 13,0	37	4	1fr + 34	16

Discussion

The study assessed the clinical and laboratory evolution of patients with VL treated with liposomal amphotericin B, administered at 4mg (kg $day^{-1})^{-1}$ for 5 days or 3 mg (kg$day^{-1})^{-1}$ for 7 days. Results indicated that 87.5% of the patients originated from urban areas, while only 12.5% came from rural regions. This distribution aligns with existing literature, which highlights rural depopulation and the subsequent rise in infectious diseases within urban environments. The process of rapid and unregulated urbanization facilitates the spread of infectious disease vectors, contributing to the growing incidence of these diseases in cities (Miranda et al., 2022).

The mean age of the study population ranged from 0.6 to 52 years. Evidence suggests that VL disproportionately impacts the youngest population. In our cohort, 77.28% of the patients were younger than 10 years old, aligning with reports that characterize VL as a childhood disease. This increased vulnerability in children is associated with the immaturity of their immune system, making them more prone to infections. Additionally, immunosuppression caused by malnutrition and starvation in some cases exacerbates the risk, facilitating the onset and progression of the disease (Costa et al., 2023).

Among the study participants, there was a predominance of males, with 19 cases (70.4%). This trend could be explained by increased exposure to high-risk areas through housing, work, or leisure activities (Cavalcante et al., 2020) as well as sex-related physiology differences. However, it is also known that VL infection can occur without a specific gender preference (Guerra-Silveira & Abad-Franch, 2013).

All patients across both treatment groups demonstrated satisfactory clinical and laboratory progress. Every patient achieved clinical cure by the end of treatment, with no cases of relapse. High cure rate for VL cases at the service can be attributed to several factors, including rapid diagnosis, early treatment, and the involvement of multidisciplinary teams at family health centers. These teams played a key role in ensuring patient care, monitoring adherence to treatment, and preventing abandonment, all of which are essential in avoiding relapses. Additionally, the consistent availability of medications to meet demand was crucial for maintaining the success of VL management (Mazire et al., 2022)

Regarding the clinical manifestations observed in our study, they were consistent with epidemiological reports, with the most frequent symptoms being fever, splenomegaly, hepatomegaly, edema, vomiting, diarrhea, and bleeding in both treatment groups. Notably, jaundice was absent in 100% of the cases, aligning with literature that indicates liver involvement may occur but is not a predominant symptom of VL (Sinani et al., 2022).

The [WHO] recommends a dose of 3-5mg(kg$day^{-1})^{-1}$ for 3 to 5 days with a maximum cumulative dose of 15 mg kg^{-1} or even a single dose of 10 mg kg^{-1}. Our study suggests that patients treated with liposomal amphotericin B for 5 days experienced clinical and laboratory outcomes comparable to those treated with the 7-day protocol. A low-dose therapy with amphotericin B has shown to be efficient and safer to patients due to less toxicity (Ren et al., 2021). But a study conducted by Ekram et al. (2021) investigated the efficacy and safety of a single-dose liposomal amphotericin B treatment for patients with visceral leishmaniasis (VL). The study administered a 10 mg kg^{-1} single dose, resulting in a 100% cure rate in children and an 89.47% cure rate in adults. One adult required a five-day intravenous infusion of 5 mg kg^{-1} amphotericin B to achieve cure. Overall, the study demonstrated an 96.66% cure rate for patients with mild adverse events.

In VL, hematological compromise is common, making continuous monitoring through laboratory analyses essential. Pancytopenia is the most frequent hematological abnormality, manifesting as anemia, leukopenia, and thrombocytopenia. These findings align with our study, where reductions in hemoglobin, leukocytes, and platelets were observed. Previous studies also report similar patterns, highlighting the clinical significance of these changes in VL (De Cerqueira et al., 2024).

However, in our study, both treatment protocols using liposomal amphotericin B restored hematological parameters to normal values. This improvement is attributed to the drug's mechanism of action, which involves binding to ergosterol, disrupting membrane permeability and osmotic balance, ultimately leading to parasite death (Frézard et al., 2022).

Parameters such as amylase and lipase are frequently evaluated in VL due to the potential toxic effects of drug therapy, which can elevate these enzymes and lead to pancreatitis. In our study, although a significant increase in amylase levels was noted for both treatment protocols, the values remained within the reference range. Lipase levels did not change significantly, although a tendency toward elevation was observed. Monitoring these markers is crucial, as disturbances in their levels have been linked to mortality in VL patients (Mengstie et al., 2021).

Amphotericin B is widely recognized for its effectiveness in treating resistant cases of VL by targeting both promastigote and amastigote forms. Additionally, it is the only therapeutic option recommended for pregnant women (O'Grady et al., 2023). However, its use is limited due to severe side effects, including fever, chills, blood pressure fluctuations, renal impairment, and electrolyte imbalances (Salehzadeh et al., 2020; Khosravi et al., 2022). The development of the liposomal formulation has mitigated these toxic effects, allowing better absorption and metabolism with reduced toxicity (Seify et al., 2023). These findings align with our study, which demonstrated a reduction in TGP levels in response to therapy (Pelloso et al., 2020). Furthermore, plasma electrolyte levels were restored without evidence of renal damage, underscoring the importance of kidney function monitoring due to potential drug toxicity.

This study represents a pioneering effort to compare recommended VL treatment protocols using liposomal amphotericin B. However, the conclusions drawn are limited by the conditions and sample size of our study. Future research should involve a larger number of participants to enable stratification by severity, age, and sex, offering further perspectives for the clinical management of VL.

Conclusion

The results of this study show that patients who used liposomal amphotericin B for 5 days had similar clinical and laboratory outcomes compared to the 7-day group, there were no records of recurrences, in addition to having a shorter hospital stay. The relevance of the study lies in its pioneering spirit in making this comparison and attesting to the equity of effectiveness for the two treatment groups.

Acknowledgments

We would like to express our sincere gratitude to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for their financial support throughout this study. We also extend our appreciation to FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais). Additionally, we are grateful to CNPq (Concelho Nacional de Desenvolvimento Científico e Tecnológico) for their valuable contributions to our research.

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