

Intestinal parasitosis in AIDS and/or HTLV patients: findings from an infectious disease reference hospital

Parasitoses intestinais em pacientes com AIDS e/ou HTLV: resultados de um hospital de referência em doenças infecciosas

Parasitosis intestinal en pacientes con SIDA y/o HTLV: hallazgos de un hospital de referencia en enfermedades infecciosas

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ABSTRACT

Background and Objectives: Human Immunodeficiency Virus (HIV) and Human T-Lymphotropic Virus (HTLV) infections precipitate immunological deficiencies, predisposing afflicted individuals to opportunistic diseases and exacerbating clinical symptoms. A prevalent health concern among these patients is enteroparasitosis. This research delineates the intestinal parasitosis profile in patients diagnosed with AIDS and/or HTLV at a specialized infectious disease hospital in Alagoas. **Methods:** this quantitative, cross-sectional analysis was conducted from August 2021 to May 2022, encompassing patients diagnosed with AIDS and/or HTLV. The study employed various copro-parasitological assessments, including the Hoffman, Pons, and Janer, Baermann-Moraes, and Safranin-methylene blue techniques, complemented by macroscopic examination of fecal consistency. These assessments were carried out at the Infectious-Parasitic Diseases Laboratory of the *Universidade Estadual de Ciências da Saúde de Alagoas*. Data processing was performed utilizing the Statistical Package for the Social Sciences®. Ethical clearance was obtained from the *Universidade Estadual de Ciências da Saúde de Alagoas* Research Ethics Committee. **Results:** from the 77 participants recruited, 44 provided fecal specimens. All participants were HIV-positive, with a male predominance. Enteroparasites were detected in 27.27% of the samples. Notably, *Entamoeba histolytica/Entamoeba dispar* and *Strongyloides stercoralis* emerged as the most prevalent protozoan and helminth, respectively. The Hoffman, Pons, and Janer technique successfully identified parasites in all positive samples. Treatment was administered to all patients with positive findings. **Conclusion:** the significant prevalence of opportunistic parasites observed underscores the criticality of routine copro-parasitological screening in immunocompromised patients. Such proactive measures are essential to mitigate the risk of heightened morbidity and mortality within this vulnerable population.

Keywords: Acquired Immunodeficiency Syndrome. Human T-lymphotropic virus 1. Human T-lymphotropic virus 2. Opportunistic Infections. Parasitic Intestinal Disease.

RESUMO

Justificativa e Objetivos: as infecções pelo Vírus da Imunodeficiência Humana (HIV) e pelo Vírus Linfotrópico T Humano (HTLV) desencadeiam deficiências imunológicas, predispondo os indivíduos afetados a doenças oportunistas e exacerbando sintomas clínicos. Uma preocupação de saúde prevalente entre esses pacientes é a enteroparasitose. Esta pesquisa delinea o perfil de parasitose intestinal em pacientes diagnosticados com AIDS e/ou HTLV em um hospital especializado em doenças infecciosas em Alagoas. **Métodos:** esta análise quantitativa e transversal foi realizada de agosto de 2021 a maio de 2022, abrangendo pacientes diagnosticados com AIDS e/ou HTLV. O estudo empregou diversas avaliações coproparasitológicas, incluindo as técnicas de Hoffman, Pons e Janer, Baermann-Moraes e azul de metileno safranina, complementadas por exame macroscópico da consistência fecal. Essas avaliações foram executadas no Laboratório de Doenças Infecto-Parasitárias da Universidade Estadual de Ciências da Saúde de Alagoas. O processamento dos dados foi realizado utilizando o Pacote Estatístico para as Ciências Sociais®. A aprovação ética foi obtida do Comitê de Ética em Pesquisa da Universidade Estadual de Ciências da Saúde de Alagoas. **Resultados:** dos 77 participantes recrutados, 44 forneceram amostras fecais. Todos os participantes eram HIV-positivos, com predominância masculina. Enteroparasitas foram detectados em 27,27% das amostras. Notavelmente, *Entamoeba histolytica/Entamoeba dispar* e *Strongyloides stercoralis* emergiram como o protozoário e helminto mais prevalentes, respectivamente. A técnica de Hoffman, Pons e Janer identificou com sucesso parasitas em todas as amostras positivas. Tratamento foi administrado a todos os pacientes com achados positivos. **Conclusão:** a significativa prevalência de parasitas oportunistas observada sublinha a criticidade da realização rotineira de exames coproparasitológicos em pacientes imunocomprometidos. Tais medidas proativas são essenciais para mitigar o risco de aumento da morbidade e mortalidade nessa população vulnerável.

Descritores: Síndrome da Imunodeficiência Adquirida. Vírus Linfotrópico de Células T Tipo 1 Humano. Vírus Linfotrópico de Células T Tipo 2 Humano. Infecções Oportunistas. Doenças Parasitárias.

RESUMEN

Justificación y Objetivos: las infecciones por el Virus de Inmunodeficiencia Humana (VIH) y el Virus Linfotrópico Humano de Células T (HTLV) provocan deficiencias inmunológicas, predisponiendo a los individuos afectados a enfermedades oportunistas y exacerbando los síntomas clínicos. Una preocupación sanitaria prevalente entre estos pacientes es la enteroparasitosis. Esta investigación delinea el perfil de parasitosis intestinal en pacientes diagnosticados con SIDA y/o HTLV en un hospital especializado en enfermedades infecciosas en Alagoas. **Métodos:** este análisis cuantitativo y transversal se llevó a cabo de agosto de 2021 a mayo de 2022, abarcando a pacientes diagnosticados con SIDA y/o HTLV. El estudio empleó diversas evaluaciones coproparasitológicas, incluyendo las técnicas de Hoffman, Pons y Janer, Baermann-Moraes y azul de metileno safranina, complementadas con el examen macroscópico de la consistencia fecal. Estas evaluaciones se realizaron en el Laboratorio de Enfermedades Infecto-Parasitarias de la Universidad Estadual de Ciências da Saúde de Alagoas. El procesamiento de datos se llevó a cabo utilizando el Paquete Estadístico para las Ciencias Sociales®. Se obtuvo la aprobación ética del Comité de Ética en Investigación de la Universidad Estadual de Ciências da Saúde de Alagoas. **Resultados:** de los 77 participantes reclutados, 44 aportaron muestras fecales. Todos los participantes eran VIH positivos, con una predominancia masculina. Se detectaron enteroparásitos en el 27,27% de las muestras. Notablemente, *Entamoeba histolytica/Entamoeba dispar* y *Strongyloides stercoralis* emergieron como el protozoo y helminto más prevalentes, respectivamente. La técnica de Hoffman, Pons y Janer identificó con éxito parásitos en todas las muestras positivas. Todos los pacientes con resultados positivos recibieron tratamiento. **Conclusión:** la prevalencia significativa de parásitos oportunistas observada subraya la importancia de la evaluación coproparasitológica rutinaria en pacientes inmunocomprometidos. Tales medidas proactivas son esenciales para mitigar el riesgo de aumentar la morbilidad y mortalidad en esta población vulnerable.

Palabras clave: Síndrome de Inmunodeficiencia Adquirida. Virus Linfotrópico Tipo 1 de Células T Humanas. Virus Linfotrópico Tipo 2 de Células T Humanas. Infecciones Oportunistas. Enfermedades Parasitarias.

INTRODUCTION

The Acquired Immunodeficiency Syndrome (AIDS) caused by the Human Immunodeficiency Virus (HIV) is considered a pandemic, with approximately one million cases detected in Brazil from 1980 to June 2018.¹ The definition of AIDS is associated with viral infection, high viral load, immunological deficiency, especially in CD4+ T-cells,

which can lead to neoplasms and opportunistic infections.²

Another retrovirus that represents a public health concern is the Human T-Lymphotropic Virus (HTLV), which induces symptoms in a minority of patients (around 3% to 5% of infected individuals), whereas HIV causes clinical disease in all infected individuals.³ Currently, two subtypes capable of infecting humans have been identified: HTLV I and HTLV II, both with tropism for T-lymphocytes.⁴

Despite having a considerable national average prevalence (0.41%) and possessing immunosuppressive potential, HTLV infection is not subject to mandatory reporting, and the disease is neglected in the country.¹

Among the opportunistic infections that can affect these immunocompromised patient groups, intestinal parasitosis stands out as one of the challenges faced by healthcare systems in developing countries like Brazil.⁵ Controlling these parasitic infections requires widely available sanitation facilities, high access to clean water, and relatively adequate personal and domestic hygiene.⁶ Furthermore, understanding the extent of intestinal parasitic infections in a community is crucial for planning efficient intervention programs.^{7,8}

Understanding the prevalence of intestinal parasitosis in AIDS and/or HTLV patients is crucial to develop effective preventive measures and targeted treatments to improve their overall health outcomes. By exploring the presence and types of parasites in this vulnerable group, healthcare professionals can optimize diagnostic strategies and implement appropriate interventions, thereby reducing morbidity and mortality associated with enteroparasitosis.

Within the framework of this investigation, the primary objective is to delineate the epidemiological profile of intestinal parasitosis among patients hospitalized with AIDS and/or HTLV, specifically in a hospital renowned for its expertise in managing infectious diseases in the Alagoas State.

METHODS

The present study analyzes the prevalence of parasitic infections in immunocompromised patients. We specifically examined the hypothesis that there may be a correlation between parasitic infection and stool consistency in immunocompromised patients. To this end, the research adopted a quantitative and cross-sectional approach, utilizing medical records, questionnaires, and fecal samples from patients diagnosed with AIDS and/or HTLV. These patients were admitted to the *Hospital Escola Dr. Helvio Auto* (HEHA), a center of excellence for treating infectious diseases, located in Maceió, Alagoas. The timeframe for data collection spanned from August 2021 to May 2022.

Upon obtaining their consent to participate in the study, patients received a printed copy of the Informed Consent Form (ICF) from researchers, providing accessible and concise information about the purpose of the research and the manner in which they would participate. They were also informed that they could withdraw from the study at any stage.

Subsequently, a structured questionnaire related to socioeconomic and epidemiological aspects was administered to identify potential risk factors for parasitic infection, such as food preparation methods, hygiene habits, and living conditions.

Following this, fecal samples (approximately 25g)

were collected from patients using wide-mouth containers with lids and examined using three parasitological methods: Hoffman, Pons, and Janer (HPJ); Baermann-Moraes (BM); and Safranin-methylene blue technique (SBMT).

The HPJ method aims to increase the concentration of eggs, larvae, or cysts and isolate fats from most of the debris through spontaneous sedimentation by gravitational or centrifugal force. As a result, the cysts, eggs, and larvae settle at the bottom of the container while the debris remains suspended at the surface.⁹

The BM technique identifies nematode larvae, particularly *Strongyloides stercoralis* and hookworms, based on the larvae's hydro- and thermotropism properties. It takes advantage of their attraction to warm water for isolation.¹⁰

The SBMT method uses differential staining to visualize oocysts separately from the rest of the fecal material. Consequently, the oocysts acquire a reddish color, whereas fecal artifacts appear blue under the microscope, allowing for a quick and easy diagnosis.¹¹

These exams were performed at the Laboratory of Infectious-Parasitic Diseases (LaDIP), located in the Ib Gatto Research Pavilion within the headquarters of *Universidade Estadual de Ciências da Saúde de Alagoas* (UNCISAL). Biosafety measures were strictly observed for all these methods. Additionally, the data from the questionnaires, medical records, and coproparasitological sheets of each patient were individually entered into an Excel spreadsheet for subsequent tabulation.

The study included patients with confirmed diagnoses of AIDS and/or HTLV who were admitted to HEHA between August 2021 and May 2022. Only patients who willingly provided fecal samples for coproparasitological analysis were considered for inclusion. Patients with incomplete medical records or data on their AIDS/HTLV status, those who declined to participate, and individuals with known allergies or contraindications to the exam procedures were excluded from the study.

The data collected were subsequently tabulated and analyzed using the Statistical Package for the Social Sciences®. A percentage analysis of the results was performed and Fisher's exact test was applied, which assesses the association between two independent variables.¹² This test was used to establish the significance between stool consistency and presence of parasites as well as presence of mucus in stools and presence of parasites. A p-value of < 0.05 was used to determine significance between the two variables in question.

The study was approved by the UNCISAL Research Ethics Committee, under registration CAAE (*Certificado de Apresentação para Apreciação Ética* - Certificate of Presentation for Ethical Consideration) 70057017.2.00005011. Acceptance report 4.728.414 was approved on May 24, 2021. It included the main researcher's commitment statement and ethical statements as well as absence of conflicts of interest declarations from all researchers. The research was conducted in accordance with the ethical standards required - Ministry of Health Resolutions 466/2012, 510/2016, and 580/2018.

RESULTS

From August 2021 to May 2022, 111 patients with AIDS and/or HTLV admitted to HEHA were approached. Out of these, 77 agreed to participate in the research, granting access to their medical records and expressing interest in responding to the questionnaire and providing a fecal sample for intestinal parasite investigation. The reasons for refusal encountered were: a) discharge from the hospital; b) refusal to participate; c) bedridden patient without an accompanying person; d) psychiatric patient without isolation.

A total of 44 fecal samples were collected for coproparasitological examination. The numerical difference

between the research participants and collected fecal samples occurred due to some patients either not providing the fecal sample for examination (due to hospital discharge or loss of the sample) or not being eligible for inclusion in the study. All participants in the research were HIV-positive, and none had HTLV infection.

Table 1 describes the sample socioeconomic profile. The majority of those infected with parasites were male (22.7%), aged between 35 and 39 years (9.1%), lived in the municipality of Maceió (15.9%), had incomplete elementary school (13.6%), had an income equal to or less than 2 minimum wages (25%) and were single (11.4%).

As shown in Table 2, parasitic infection was confirmed through coproparasitological exams in 27.27% (12) of patients. Among these, 83.33% (10) were infected with a single parasite species (monoparasitism), whereas 16.67% (2) were infected with multiple parasite species (polyparasitism).

Table 1. Independent variables related to sociodemographic data according to the presence or absence of intestinal parasites in HIV-positive patients admitted to a reference hospital for infectious diseases, Maceió, Alagoas.

Variable	Parasitized		Not parasitized	
	Absolute	%	Absolute	%
Sex				
Female	2	4.5	4	9.1
Male	10	22.7	28	63.6
Age range				
<18	0	0	0	0
18-24	1	2.3	1	2.3
25-29	0	0	1	2.3
30-34	0	0	4	9.1
35-39	4	9.1	6	13.6
40-44	0	0	6	13.6
45-49	2	4.5	8	18.2
50-54	1	2.3	1	2.3
55-60	2	4.5	3	6.8
>60	2	4.5	2	4.5
Municipality				
Maceió	7	15.9	19	43.2
Others	5	11.4	13	29.5
Income				
Up to 2 minimum wages	11	25	29	65.9
2-4 minimum wages	0	0	2	4.5
4-6 minimum wages	1	2.3	1	2.3
6-8 minimum wages	0	0	0	0
8 or more minimum wages	0	0	0	0
Education				
No education	3	6.8	5	11.4
Incomplete elementary school	6	13.6	22	50
Complete elementary school	0	0	0	0
Incomplete high school	0	0	0	0
Complete high school	1	2.3	3	6.8
Incomplete higher education	1	2.3	1	2.3
Complete higher education	1	2.3	1	2.3
Marital status				
Single	5	11.4	17	38.6
Married	4	9.1	4	9.1
Divorced	2	4.5	8	18.2
Widower	1	2.3	3	6.8

Table 2. Occurrence of enteroparasites in patients with AIDS assisted at a reference hospital for infectious diseases in Maceió, Alagoas, from August 2021 to May 2022.

Coproparasitological test		
Positive (%)	Negative (%)	Total (%)
12 (27.27)		
10 monoparasitized (83.33%)	32 (72.73)	44 (100)
2 polyparasitized (16.67)		

Table 3. Enteroparasites identified and quantity of positive results by parasitological technique performed in patients with AIDS assisted at a reference hospital for infectious diseases in Maceió, Alagoas, from August 2021 to May 2022.

Enteroparasites	HPJ*	BM**	SMBT ***
Protozoa			
<i>Entamoeba histolytica/Entamoeba dispar</i>	3	-	-
<i>Endolimax nana</i>	2	-	-
<i>Cystoisospora belli</i>	1	-	1
<i>Giardia lamblia</i>	1	-	-
Helminths			
<i>Strongyloides stercoralis</i>	4	4	1
<i>Ancylostomatidae</i>	2	-	-
<i>Schistosoma mansoni</i>	1	-	1

*Hoffman, Pons, and Janer; **Baermann-Moraes; ***Safranin-methylene blue technique.

Table 3 shows the distribution of positive results for each parasite concerning the parasitological methods used: HPJ; BM; and SBMT. The findings revealed that under the protozoa category, *Entamoeba histolytica/Entamoeba dispar* was identified in three instances exclusively through the HPJ method, whereas *Endolimax nana* was detected in two cases, also solely with HPJ. Additionally, *Cystoisospora belli* was observed in one case each by HPJ and SBMT, but not by BM. *Giardia lamblia* was detected in a single case, again only through HPJ. In

Table 4. Relationship between stool consistency and the results of parasitological exams in patients with AIDS assisted at a reference hospital for infectious diseases in Maceió, Alagoas, from August 2021 to May 2022.

Stool consistency	Parasitological exam (N/%)		Total (N/%)	Odds Ratio	p-value
	Negative	Positive			
Formed	22 (73.30)	8 (26.70)	30 (100)	1.1	1.0
Diarrheic	10 (71.40)	4 (28.60)	14 (100)		
Total	32 (72.70)	12 (27.30)	44 (100)		

the helminths category, *Strongyloides stercoralis* showed a higher prevalence, being identified in four cases each with HPJ and BM, and in one case with SMBT. The family *Ancylostomatidae* was detected in two cases, but only through HPJ. Lastly, *Schistosoma mansoni* was identified in one case each by HPJ and SMBT, but not by BM.

These outcomes indicate that the HPJ method demonstrated a broader detection capability for a range of parasites in the sample. In contrast, the BM method showed a specific efficacy in identifying *Strongyloides stercoralis*. SMBT, while showing limited overall detection, was effective in identifying *Cystoisospora belli* and *Schistosoma mansoni*, highlighting its potential utility in diagnosing specific parasitic infections.

The macroscopic examination of the stools allowed the identification of 14 (31.81%) diarrheal samples, as shown in Table 4. Among them, only four (28.70%) were parasitized. Furthermore, mucus was found in five (35.71%) of the diarrheal samples and in seven (15.90%) out of the 44 samples collected. The relationship between the analyzed stool consistency and parasitized individuals, according to Fisher's exact test, was not statistically significant ($p=1.0$). The odds ratio of 1.1 suggests a slight, but not substantial, increase in the odds of testing positive for parasites in individuals with diarrheic stool compared to those with formed stool. However, this is not statistically significant.

DISCUSSION

Opportunistic infections by enteroparasites are found in 30-60% of HIV-positive patients in developed countries and 90% in developing countries. In particular, infections caused by agents such as *Cystoisospora belli*, *Cryptosporidium sp.*, and *Giardia duodenalis* have been frequently reported in individuals with AIDS.¹³ For instance, *Cystoisospora belli* is an obligatory intracellular protozoan exclusive to humans, belonging to the phylum *Apicomplexa*, which leads to diarrhea, commonly reported in opportunistic infections, especially in individuals with CD4+ T lymphocyte count below 200 cells/ml.¹⁴

Cryptosporidium, on the other hand, is an intracellular extracytoplasmic protozoan that can cause diarrhea of varying severity in both immunocompetent and immunocompromised individuals. For instance, in individuals with AIDS or children with nutritional deficits, condition tends to be more severe.¹⁵ Additionally, the protozoan

Giardia lamblia and the nematode *Strongyloides stercoralis*, also found in immunocompetent individuals, are frequently identified in the parasitological profile of immunosuppressed individuals.¹⁶

Therefore, coproparasitological exams should be frequently requested for immunocompromised patients to determine the presence of any opportunistic agent or to ensure criteria for intestinal parasitic cure.¹⁷

The predominance of monoparasitized individuals among those with AIDS is consistent with findings in the literature,¹⁸ although polyparasitism is more common in people with HIV than in immunocompetent individuals.¹⁹ However, due to the small sample size, it is not possible to determine the relevance of this information. Nevertheless, it is necessary to mention that individuals with HIV/AIDS are 11.42 times more likely to develop parasitic infections than healthy individuals. In addition to environmental contamination, host nutritional and immunological factors are essential in the establishment of parasitic diseases.²⁰

According to Table 1, the most frequently identified parasites in the exams were the protozoa species *Entamoeba histolytica/Entamoeba dispar* (25%) and the helminth species *Strongyloides stercoralis* (33.33%). Additionally, *S. stercoralis* is capable of causing severe gastroenteropathy and significant weight loss in HIV-positive individuals.²¹ In immunocompromised individuals, hyperinfection syndrome may occur, with a mortality rate exceeding 80%.²²

Two polyparasitized patients were detected, one infected with two species of protozoa (*Endolimax nana* and *Entamoeba histolytica/Entamoeba dispar*) and the other with two helminth species (*Ancylostomatidae* and *Schistosoma mansoni*). Polyparasitism can exacerbate the severity of infectious diseases, such as AIDS.²³ Moreover, in immunocompromised individuals, schistosomiasis is more likely to lead to portal hypertension, hepatic fibrosis, digestive bleeding, and severe liver failure in the chronic phase of the disease, resulting in death.²⁴

Each fecal sample was analyzed using three different parasitological methods, as applying different techniques to the same individual's sample allows for greater accuracy and confidence in the results obtained. The BM technique, specific for identifying helminth larvae, only yielded positive results for *S. stercoralis* larvae. The Modified Ziehl-Neelsen technique identified *Cystoisospora belli* oocysts, *S. stercoralis* larvae, and *Schistosoma mansoni* eggs.

All parasites were detected using the HPJ method after analyzing the same fecal material. Thus, it is inferred

that this technique was the most sensitive and comprehensive in diagnosing these enteroparasites, making it the most effective.²⁵

Research participants are more vulnerable to opportunistic parasitic infections due to their immunocompromised status. Therefore, persistent symptoms, such as prolonged diarrhea, may be present, which, if not treated promptly, can lead to unfavorable outcomes for patients.²⁶

All coproparasitological exams were performed promptly, and the reports were attached to patients' medical records so that positive cases could be treated by the hospital's technical staff. Treatment for HIV and/or HTLV-positive individuals is essential as symptoms can become chronic, given that the gastrointestinal tract is an organ significantly affected by HIV infection.²⁷

A factor that can worsen parasitic infections affecting this population is poor adherence to antiretroviral therapy, which has significant implications for the immunosuppression of these individuals. Inadequate hygiene conditions, lack of basic sanitation, and consumption of contaminated water and food can also contribute to their increased vulnerability.²⁰

Coccidia are also resistant to conventional water treatment.²⁰ This favors their viability and dissemination in the environment. Therefore, preventive measures for intestinal parasitic infections are the responsibility of both the individuals – through adherence to antiretroviral treatment and proper hygiene conditions – and the State, which should guarantee the constitutional right to basic sanitation.

The study has certain limitations that should be acknowledged. Firstly, the sample size of 77 participants, with only 44 providing fecal samples, may be considered relatively small, potentially affecting the study's statistical power and generalizability. Secondly, the cross-sectional design limits the ability to establish causal relationships between intestinal parasitosis and disease progression. Additionally, missing data on AIDS/HTLV status and other variables might introduce bias into the results. The sensitivity of the coproparasitological exam methods used could influence parasite detection accuracy.

Despite these limitations, the study provides valuable insights into the prevalence and profile of intestinal parasitosis in AIDS and/or HTLV patients, offering a foundation for further research and clinical implications while recognizing the need for cautious interpretation. At the end, it was possible to identify opportunistic parasites, which are frequently described in immunocompromised populations. As a result, measures to combat the transmission of these parasites should be implemented, and policies should encourage antiretroviral treatment maintenance. Among preventive measures is the performance of parasitological exams and immediate treatment for positive cases to avoid significant morbidity and mortality.

Based on the findings from the work, the recommendations include the need for frequent coproparasitological examinations in immunocompromised patients, particularly those with HIV/AIDS, to promptly identify and treat opportunistic parasitic infections. Emphasis should be placed on enhancing adherence to antiretroviral the-

rapy and improving hygiene and sanitation conditions to mitigate the risk of parasitic infections. For future studies, it is suggested to expand the sample size and possibly employ a longitudinal study design to better understand the causal relationships between intestinal parasitosis and the progression of HIV/AIDS and HTLV infections. Further research could also explore the impact of varying antiretroviral adherence levels on the prevalence and severity of parasitic infections and the effectiveness of different water treatment methods in controlling the spread of resistant parasites.

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Victória Christine de Almeida Santos contributed to bibliographical research, abstract writing, introduction, methodology, discussion, interpretation and description of results, preparation of tables, conclusions, review, statistics and final approval of the version to be published. **Fernando Luiz de Andrade Maia** contributed to project administration and supporting patients' medical treatment. **Lucas Emanuel de Oliveira Silva** contributed to article writing, review and final approval of the version to be published. **Anna Amélia de Paula Moraes** contributed to data collection and processing in the laboratory. **Josenildo Francisco da Silva** contributed to data collection and processing in the laboratory. **Flaviana Santos Wanderley** contributed to project administration and supervision, interpretation of results, review and final approval of the version to be published.

All authors approved the final version to be published and are responsible for all aspects of the work, including ensuring its accuracy and integrity.