Leishmaniose visceral

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ABSTRACT

Introduction: Visceral Leishmaniasis is a parasitic disease transmitted by sandflies, causing fever, weight loss and inflammation of the spleen. Identified in 1903, its global spread is influenced by socioeconomic and environmental factors. The parasites responsible for this disease are trypanosomatid protozoa that belong to the genus Leishmania. The vectors are sandflies, mainly of the species Lutzomyia longipalpis infected by Leishmania chagasi. Popularly known as sand flies, they reproduce in areas rich in organic matter. The evolutionary cycle of Leishmania chagasi is heteroxenic, involving vertebrate hosts such as canids, marsupials or humans, and invertebrate hosts, which are sandfly insects. Initially, Visceral Leishmaniasis may be asymptomatic or present with prolonged fever, weight loss, weakness, and anemia. Then, symptoms such as enlargement of the spleen and liver, pallor, intermittent fever and impairment of the immune system appear. In the advanced stage of infection, serious complications such as hemorrhages, secondary infections, profound anemia, and hepatosplenic insufficiency occur. The diagnosis of Visceral Leishmaniasis involves a series of clinical, laboratory and epidemiological approaches. The treatment of Visceral Leishmaniasis in Brazil is done with pentavalent antimonial compounds, such as the antimoniate N-methyl glucamine, administered intravenously or intramuscularly. Some contraindications include renal or hepatic failure, pregnancy in the first two trimesters, and patients taking beta-blockers or antiarrhythmics. Preventive measures must be carried out in individual and collective environments. These measures include the use of repellents, avoiding exposure at times when the vectors are most active, environmental management, tree pruning, cleaning of pet shelters, among others. Visceral Leishmaniasis represents a complex public health challenge. Understanding the protozoan cycle, diagnostic methods, and treatment options is crucial for effective disease management.

Keywords: Visceral Leishmaniasis, *Leishmania chagasi*, Sandflies, Reservoirs.

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INTRODUCTION

In the year 1903, the first revelations about the agent responsible for Visceral Leishmaniasis (VL) emerged, courtesy of William Boog Leishman and Charles Donovan. Leishman, in examining the preparations of the spleen of a soldier coming from DumDum, India, made a remarkable discovery, while Donovan found these parasites in the splenic aspirates of a young Hindu. At the same time, Charles Louis Alphonse Laveran and Félix Étienne Pierre Mesnil gave birth to the agent under the name *Piroplasma donovani*, later corrected by Ronald Ross to *Leishmania donovani*.

In 1911 and 1912, Carlos Chagas, exploring the Amazon River valley and its tributaries, came across patients with unexplained splenomegaly, fueling initial suspicions of Human Visceral Leishmaniasis (HVL) in South America. Then, in 1913, Migone and his team identified the parasite during an autopsy on a native of Boa Esperança, now part of Mato Grosso do Sul. This event marked the first record of the disease in humans in the Americas.

Visceral leishmaniasis (VL) involves a complex host group with some species of mammals, including man and winged vectors. However, the occurrence of VL in a region does not depend only on these elements; it is also shaped by the geographical changes promoted by human activity. Physical, social, and biological factors play crucial roles in this context. Such factors alter the relationship between the parasite and the host, either favoring the proliferation of vectors due to environmental transformations, such as the accumulation of organic matter in the soil, or enabling the migration of infected animals to areas previously free of VL.

When examining the risks associated with the occurrence of VL in certain geographic regions, it is clear that exposure to sandflies plays a central role, as well as co-infection with HIV, malnutrition, the high prevalence of infected dogs, and the socioeconomic precariousness of the population. These are all ingredients that make up the intricate enigma of Visceral Leishmaniasis, which continues to challenge the medical and scientific community.

ETIOLOGIC AGENT

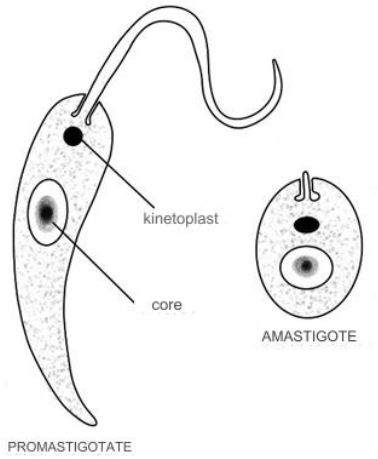
Visceral leishmaniasis is frequently identified in patients with visceral leishmaniasis in South America. The responsible for this disease are trypanosomatid protozoa that belong to the genus *Leishmania*. These protozoa are obligate parasites that inhabit the interior of the cells of the mononuclear phagocytic system of vertebrate hosts. Many authors have considered *L. chagasi* and *L. infantum* to be the cause of infection. However, it is the same species, and therefore both denominations are accepted. In this chapter *Leishmania chagasi* will be adopted. During the biological cycle, the protozoan can be observed in the following forms:

Promastigote: flagellated form found in the intestine of the vector insect. It is an elongated, spindle-shaped cell, about 15 to 20 µm in size, with a kinetoplast anterior to the nucleus and a free

flagellum from the anterior portion of the cell. Its development is characterized by two stages: procyclic (non-infectious) and metacyclic (infectious).

Amastigote: an oval-shaped cell, 1 to 3 µm in size, with a single acentric nucleus and flagellum restricted to the flagellar pouch. Figure 1 shows a schematic drawing of the promastigote and amastigote forms.

Figure 1 – Schematic drawing of the promastigote (left) and amastigote (right) forms of *Leishmania* sp. Source: Prepared by the authors.



VECTOR

Also known as sand flies, sandflies of the species *Lutzomyia longipalpis* are considered the most common vectors in cases of Visceral Leishmaniasis in Brazil. In the region of Mato Grosso, *Lutzomyia cruzi* has also been associated with the protozoan cycle. Such insects are small diptera, measuring between 1 and 3 millimeters and holometabolous, presenting in their evolutionary cycle the phases of egg, larva, pupa, and adults, males and females, with only the females involved in the transmission of *Leishmania*. The immature stages of this insect develop in areas rich in organic matter and do not depend on water for their hatching. Sandflies are nocturnal insects and have a type of leaping and short flight.

RESERVOIRS

Visceral leishmaniasis (VL), also known as kala-azar, is a zoonosis of wide distribution in Brazil and has a complex ecology, involving multiple hosts and vectors. The transmission cycle of the disease involves vertebrate hosts, which act as reservoirs, and sandflies (sandflies) which are the vectors. The presence and dynamics of reservoirs are crucial for the maintenance and dissemination of the transmission cycle of this zoonosis, which is widely distributed in Brazil. In the Brazilian context, these reservoirs can be classified as wild and domestic and each plays a crucial role in the

epidemiology of the disease, as they maintain the parasite cycle and facilitate its transmission to humans. In addition, among the various reservoirs, the domestic dog plays a fundamental role, especially in Brazil, where visceral leishmaniasis is a serious public health problem.

WILD RESERVOIRS

Wild reservoirs, present in forest areas and rural regions, include a variety of mammals. Studies have identified wild canids, including the hoary fox (*Lycalopex vetulus*) and the wild dog (*Cerdocyon thous*) as important natural reservoirs of the parasite. These animals are usually asymptomatic, allowing the parasite to persist in nature and maintain the enzootic cycle. Concomitantly, ecological studies suggest that habitat fragmentation and the proximity of urbanized areas to forest areas favor contact between vectors, wild reservoirs, and humans, increasing the risk of VL transmission.

DOMESTIC RESERVOIRS

In the Brazilian urban and peri-urban context, domestic dogs (*Canis lupus familiaris*) are recognized as the main reservoirs of VL. Epidemiological and molecular studies have shown the high prevalence of *Leishmania chagasi infection* in canine populations, and these animals are considered the main responsible for the maintenance and dissemination of the parasite in urbanized areas (Ministry of Health, 2020). This situation occurs due to multiple sociocultural factors rooted in the Brazilian territory, such as contact with this domestic animal, the structure of large urban centers, the ineffectiveness or absence of public policies, among others.

WAYS OF CONTAMINATION

According to the Ministry of Health, visceral leishmaniasis is endemic in 76 countries. Brazil, in particular, is one of the most affected, corresponding to 90% of Latin American cases among the more than 12 countries that register cases of this disease. The transmission of the protozoan that causes visceral leishmaniasis occurs primarily through the bite of the infected female sandfly. Other forms of infection have already been described, such as the use of intravenous drugs, transfusion of infected blood, organ transplantation, congenital infection and laboratory accidents, without however presenting any epidemiological importance.

BIOLOGICAL CYCLE

The protozoan cycle, to be complete, is composed of the vertebrate host (canid, marsupial or man) and the invertebrate host (insect), being classified as a heteroxenic biological cycle.

The disease is transmitted to the vertebrate host through the bite of the female infected sandfly insect, since during feeding the insect regurgitates the aspirated material; inoculating the protozoan in the metacyclic promastigote form, which penetrates the healthy victim. At the bite site, the parasite can invade a range of cells, such as dendritic cells, fibroblasts, neutrophils, and especially macrophages, when they adhere to the membrane and initiate the internalization process through phagocytosis.

When phagocytosed, the parasite is internalized in the parasitophore vacuole, where it differentiates into the amastigote form, which is the tissue form of the protozoan and has no apparent flagellum. In the cytoplasm of macrophages, lysosomes fuse with the vacuole, discharging their lysosomal enzymes, which do not affect the parasite.

Thus, the amastigote form multiplies by successive binary fissions, until it breaks the macrophage membrane, releasing the amastigote form into the intercellular space. Once in the tissue, the amastigote forms can infect new cells, repeating binary divisions and increasing the number of protozoa, or reach the circulatory stream, being carried to other organs and tissues.

When a female feeds on an infected host, she ingests amastigote forms. In the middle portion of the insect's digestive tract, there is a membranous compartment responsible for digesting the ingested content, called the peritrophic matrix. In this place, the amastigotes transform into promastigotes, moving with the help of the flagellum and attach themselves to the intestinal epithelium of the insect, where they undergo a series of morphological and biochemical transformations. The last of these reactions is called metacyclogenesis, which promotes important changes in the cell structure, which starts to express new membrane proteins and adds the ability to infect a new host. At this time, after several mitotic multiplications, the protozoan passes to the metacyclic promastigote form and migrates to the pharyngeal region of the vector, called the proboscis. These metacyclic promastigotes will be transmitted to new hosts at the next blood meal.

CLINICAL FORMS AND DIAGNOSIS

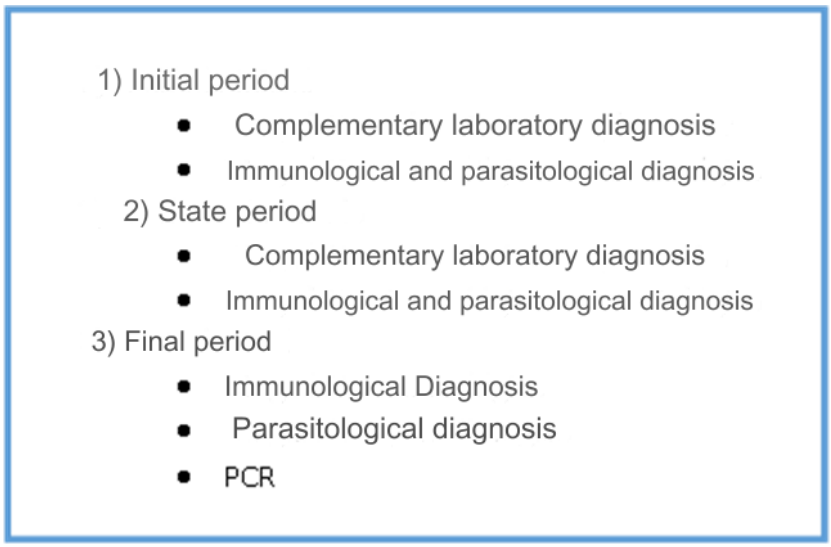
The diagnosis must be made accurately and as early as possible, since it is a notifiable disease with serious clinical characteristics. It is essential that diagnoses, treatment, and patient follow-up routines are mandatorily implemented in all areas with transmission or at risk of transmission. Whenever possible, parasitological confirmation of the protozoan should precede treatment, but in situations where serological and/or parasitological diagnosis is not available or there is a delay in releasing the results, the start of treatment should not be postponed.

The infection caused by *L*. chagasi has a wide clinical spectrum, ranging from completely asymptomatic individuals, discrete manifestations (oligosymptomatic), to moderate and severe forms. If left untreated, these manifestations can lead to the patient's death. Therefore, it is crucial to

suspect visceral leishmaniasis when in endemic areas, the patient presents fever and splenomegaly, associated or not with hepatomegaly, and anemia.

PERIODS OF THE DISEASE/CLINICAL FORMS AND THEIR DIAGNOSIS:

In this topic, for didactic purposes, there will be a discussion of diagnostic methods related to the three different periods of Visceral Leishmaniasis.



Initial period

The initial phase of the disease, also known as "acute" by some authors, marks the beginning of symptoms, which can vary from patient to patient. In most cases, these symptoms include fever lasting less than four weeks, paleness of the skin and mucous membranes, and hepatosplenomegaly. The patient's general condition is usually preserved, and the spleen usually does not exceed 5 cm from the left costal margin. Often, these patients arrive at the medical service after having used antimicrobials without obtaining a clinical response, and, in some cases, report a history of cough and diarrhea. It is important to note that during clinical examination, especially in children, the stethoacoustic maneuver is quite useful to check for the presence of hepatosplenomegaly.

In areas where the disease is endemic, a small proportion of individuals, usually children, may present with a mild clinical picture lasting approximately 15 days, which often results in spontaneous cure (oligosymptomatic form). These patients present with milder clinical symptoms, such as low-grade fever, mild paleness of the skin and mucous membranes, diarrhea, and/or nonproductive cough, along with a small hepatosplenomegaly. This clinical presentation can be easily confused with other infectious processes of a benign nature. The combination of clinical manifestations and laboratory abnormalities, which seems to better characterize the

oligosymptomatic form, is composed of the following findings: fever, hepatomegaly, hyperglobulinemia, and high blood sedimentation rate.

Complementary laboratory diagnosis

The blood count usually reveals mild anemia, with hemoglobin levels above 9g/dl. The white blood cell count does not show significant changes, with a predominance of lymphomonocyte cells, and the platelet count may remain normal. The erythrocyte sedimentation rate is often high, exceeding 50mm. Total proteins and their fractions may present discrepancies. In the oligosymptomatic form, laboratory tests usually remain unchanged, with the exception of erythrocyte sedimentation rate, which tends to be elevated, and hyperglobulinemia.

Immunological and parasitological diagnosis

Serological tests, such as Indirect Immunofluorescence (IFA) and Enzyme-Linked Immunosorbent Assay (ELISA), usually show positive results. Analysis of bone marrow aspirate and spleen usually reveals the presence of amastigote forms of the parasite. In the case of the oligosymptomatic form, bone marrow aspiration may or may not show the presence of the protozoan and, in principle, it is not indicated.

Status Period

The hallmark feature is the presence of intermittent fever, typically accompanied by progressive weight loss, paleness of the skin and mucous membranes, as well as an increase in hepatosplenomegaly. This clinical form develops in a dragged manner, usually with more than two months of evolution, and is often associated with a compromise of the patient's general condition.

Complementary laboratory diagnosis

Complementary tests reveal abnormalities, such as anemia, thrombocytopenia, leukopenia with a marked predominance of lymphomonocyte cells and an inversion in the relationship between albumin and globulins. Biochemical changes may also be observed, including an increase in aminotransferase levels (two to three times above normal values), an increase in bilirubins, and a slight increase in urea and creatinine levels.

Immunological and parasitological diagnosis

Levels of specific *anti-Leishmania* antibodies are increased. At this stage of the disease, the amastigote forms of the parasite can be seen on smears of bone marrow aspirate, spleen, liver, and lymph nodes.

End period

If diagnosis and treatment are not carried out, the disease progresses continuously to an advanced stage, characterized by constant fever and a more marked impairment of the patient's general condition. At this stage, malnutrition develops, evidenced by brittle hair, elongated eyelashes, and dry skin. Edema of the lower limbs can arise and, in severe cases, progress to anasarca. Other significant manifestations include hemorrhages, such as epistaxis, gingivorrhagia, and petechiae, jaundice, and accumulation of fluid in the abdominal cavity (ascites). In these patients, death usually occurs due to bacterial infections and/or bleeding.

Immunological diagnosis

In Brazil, the most common immunological test for visceral leishmaniasis is indirect immunofluorescence (IFA) and enzyme-linked immunosorbent assays (ELISA). Indirect Immunofluorescence results are usually expressed in dilutions, and are considered positive from 1:80. If the titers are equal to 1:40, it is recommended to take a new sample after 30 days. The ELISA test presents results in units of light absorbance, with fixed dilutions or, more commonly, simply as a reagent or not.

Parasitological diagnosis

Aspiration puncture of the spleen is the method that provides the highest sensitivity (90-95%) for the detection of the parasite, although it presents some procedural restrictions. Next, in terms of sensitivity, comes bone marrow aspirate, liver biopsy, and lymph node aspiration. Due to its safety, it is recommended to perform bone marrow aspiration. The collected material should be examined following the following sequence: Direct examination, followed by isolation in *in vitro culture medium*.

PCR

The PCR (Polymerase Chain Reaction for parasite DNA amplification) method represents a new approach in the diagnosis of Visceral Leishmaniasis (VL), with a sensitivity of approximately 94%. However, the results of this method can vary depending on several factors, including the endemic region where the test is performed, the type of sample collected, and the specific DNA target used for amplification. Another limiting factor for performing the technique is the high cost and the need for specific structure and skilled labor.

TREATMENT

In Brazil, antimonial compounds, in the form of trivalent salts, were initially used in the treatment of tegumentary leishmaniasis in 1913, by Gaspar Vianna. The treatment of visceral leishmaniasis with these drugs began two years later, in Italy. Pentavalent derivatives (Sb+5) were introduced in the 40s and, since then, have been considered the drugs of choice in the treatment of visceral leishmaniasis.

Currently, there are two formulations of Sb+5 available on the market: sodium stibogluconate and antimoniate-N-methyl glucamine. There do not appear to be significant differences in therapeutic efficacy between these formulations. In Brazil, the only formulation available is the antimoniate N-methyl glutamine (Glucantime), which is distributed by the Ministry of Health in 5 ml ampoules. The ampoules should be stored in a cool place and protected from light to avoid problems with the stability of the drug.

The mechanism of action of antimonials is not completely understood, but it is known that they act on the amastigote forms of the parasite, inhibiting its glycolytic activity and the oxidative pathway of fatty acids. In recent years, the use of progressively higher doses of antimonials has been recommended by the World Health Organization (WHO) and the Center for Disease Control (CDC) of the United States due to the emergence of primary resistance of the parasite to these drugs, especially in countries such as Sudan, Kenya and India.

In Brazil, although there is no record of *L. chagasi* strains resistant *in vitro* to antimonials, the treatment of visceral leishmaniasis is recommended with a dose of 20 mg of Sb+5 per kg/day, administered intravenously (E.V) or intramuscularly (I.M) for a minimum of 20 and a maximum of 40 days, with a maximum limit of 2 to 3 ampoules/day of the product, resulting in high cure rates.

In advanced cases of the disease, in which the clinical response is not evident within the first 20 days, the minimum treatment time should be extended to 30 days. This recommendation is based on the need for longer treatments to achieve satisfactory cure rates.

Before starting treatment, some precautions should be observed, including the evaluation and stabilization of the patient's clinical conditions and the treatment of concomitant infections. In situations where follow-up is feasible, treatment may be administered in an outpatient setting.

In cases of hospital treatment, it is essential to carefully observe the following signs and symptoms: severe anemia (hemoglobin less than 5g%), severe or prolonged diarrhea, generalized edema, severe malnutrition, bleeding, concomitant infections, associated diseases (heart disease, nephropathy, liver disease, hypertension), lack of response to treatment (primary refractoriness), recurrence, jaundice, patients less than 6 months old or over 65 years old.

Intramuscular injections of pentavalent antimonials should be administered in areas with adequate muscle mass, such as the gluteal region. In malnourished patients with low muscle mass

and thrombocytopenia, the preference should be for intravenous administration. It is important to note that there is no difference in serum drug levels in relation to the route of administration. When administered by IV, the infusion should be slow, over 5 to 7 minutes, and the dose may be diluted in 5% glucose solution to facilitate administration.

In cases of disease recurrence, a second treatment with the same dose, but for a longer period (maximum 40 days), should be initiated before labeling the case as refractory to treatment with pentavalent antimonials. Only after this attempt should alternative regimens with second-line drugs be considered. With the therapeutic options currently available, splenectomy is no longer recommended as a therapeutic measure in visceral leishmaniasis.

At the time of diagnosis, an electrocardiogram (ECG) is indicated in all cases of visceral leishmaniasis, and it is mandatory in patients over 50 years of age, during and after treatment. Because of the arrhythmogenic potential of the drug, pentavalent antimonials are contraindicated in patients using beta-blockers and antiarrhythmic drugs. They are also contraindicated in patients with renal or hepatic impairment, pregnant women in the first two trimesters of pregnancy, and in cases where the electrocardiogram shows a QTc interval greater than 400 ms (men) and 450 ms (women).

Alternative treatment options include amphotericin B sodium deoxycholate and its liposomal formulations (liposomal amphotericin B-liposomal and amphotericin B-colloidal dispersion), pentamidines (sulfate and mesylate), and immunomodulators (interferon gamma and GM-CSF). With the exception of the first two drugs, the others are still in the investigation phase. All these drugs should only be administered in referral hospitals. Amphotericin B is the drug of choice for the treatment of visceral leishmaniasis in pregnant patients, with a recommended dose of 1 mg/kg/day for 14 consecutive days.

PROFILEAXIA

There are several approaches to control visceral leishmaniasis. Today they are constituted in two main groups, namely: vector control and control of infected humans and animals, in order to reduce reservoirs of infection. It is also noteworthy that the entire Brazilian territory is considered potentially endemic. Thus, prophylaxis and disease control depend on epidemiological surveillance developed by a national control program.

REGARDING VECTOR CONTROL

The Ministry of Health cites as examples of VL prevention several environmental hygiene practices, such as periodic cleaning of backyards, with removal of decomposing organic matter where *Lutzomyia* reproduces.

Barrier methods, such as screens along the windows and doors of the house or insecticide-treated mosquito nets, especially at the end of the night, the time corresponding to the peak of vector activity.

Avoid the construction of houses and camps in areas close to the forest or in regions eminently inhabited by the vector.

The use of insecticides. These should only be used when recommended by health authorities, as is the case of municipalities with intense, moderate transmission or in an outbreak of visceral leishmaniasis, according to the criteria of the Ministry of Health.

REGARDING THE REDUCTION OF INFECTION RESERVOIRS:

The main measure is, without a doubt, the rapid diagnosis and effective treatment of anthroponotic visceral leishmaniasis.

The use of repellents on the skin and clothing is indicated, especially when visiting endemic areas.

Attention to domestic animals, such as: regular testing according to the region of residence; the use of repellent collars on domestic animals.

The control of stray animals is also of fundamental importance for the control of this disease.

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