LEISHMANIASIS: A REVIEW

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Summary

Leishmaniasis, a parasitic disease manifests in mainly three forms: The cutaneous, the mucocutaneous and the visceral leismaniasis. All Leishmania species have two main developmental stages in their life cycle: the amastigote and the promastigote. The diagnosis of the visceral form is conventionally made by the demonstration of amastigotes of the parasite in the aspirated body fluids. Various therapeutic agents available for treatment are anti-monials, amphotericin-B, miltefosine, aminosidine, pentamidine and immunotherapy. Today, leishmaniasis has gained a relevant position worldwide among the causes of death by infectious diseases. This can be attributed to its risk of co-infection with HIV and rapid emergence of drug resistance. In the present article, an attempt has been made to investigate the present status and progresses in the treatment of this neglected disease.

Key Words: Leishmania, Leishmaniasis. Kala-azar, diagnosis, antimony

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Introduction

Leishmaniasis is caused by the protozoa belonging to the genus *Leishmania*. It is transmitted by the bite of the infected female *Phlebotomine* sandfly in the old world and *Lutzomyia* in the new world. Most leishmaniasis are zoonotic. The sandfly vector is usually infected with one species of flagellate protozoa belonging to the genus *Leishmania*. Hosts are infected humans, animals such as rodents, and domestic animals such as dogs.

Human infection is caused by about 30 species that infect mammals. These include *L. donovani*, *L. infantum*, *L. chagasi*, *L. mexicana*, *L. amazonensis*, *L. tropica*, *L. major*, *L. aethiopica*, *L. venezuelensis* and the subgenus *Viannia* with 4 main species *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, and *L. (V.) peruviana*.

Clinically, a patient with leishmaniasis may present with one of the three quite distinct clinical syndromes: cutaneous, mucocutaneous or visceral leishmaniasis.

**Cutaneous Leishmaniasis** (CL) (oriental sore) is caused by *L. major*, *L. tropica*, *L. mexicana*, *L. amazonensis*, *L. guyanensis*, *L. panamensis*. The cutaneous leishmaniasis produces large numbers of skin ulcers, as many as 200 in some cases, on the exposed parts of the body. In general, half of these lesions caused by *L. major* or *L. mexicana* heals in 3 months, those caused by *L. tropica* takes about 10 months and those due to *L. braziliensis* persists much longer.

**Mucocutaneous leishmaniasis** (MCL) (espundia or uta) is caused by *L. braziliensis*. It is an uncommon manifestation of cutaneous leishmaniasis that may present years after the initial skin ulcer has healed. This metastatic complication of the primary lesions result in disfiguring and ulceration of mucous membranes of the nose, mouth and throat cavities.

**Visceral leishmaniasis** (VL) (kala-azar) is produced by *L. donovani*, *L. infantum*, and *L. chagasi*. It is characterized by fever, substantial weight loss, hepatomegaly, splenomegaly, and anaemia. It is fatal without treatment and may be fatal despite of treatment.

**GEOGRAPHIC DISTRIBUTION:**

The leishmaniasis is endemic in 88 countries and a total of 12 million people are affected by leishmaniasis worldwide. A common estimate of the incidence per year is 1.5-2 million newly reported cases of cutaneous leishmaniasis and 500,000 cases of visceral leishmaniasis. In India, cutaneous leishmaniasis occurs mostly in Rajasthan. and some cases have been reported from Kerala and Assam, while, visceral leishmaniasis is rampant in Uttar Pradesh, West Bengal, Bihar, Assam, Sikkim and to a lesser extent in Tamil Nadu and Orissa.
LIFE CYCLE:

All Leishmania species have two main developmental stages in their life cycle: the amastigote, that reside inside the reticulendothelial cell of the vertebrate hosts, and the promastigote, that replicate in the gut of sandfly. Life cycle begin when the vertebrate host are bitten by the infected sandfly. The sandfly vector becomes infected when feeding on the blood of an infected host. Within the sandfly gut, the protozoa are carried as extracellular promastigotes. These parasites multiply in the gut. As the leishmania replicate, they create a blockage in the fly's esophagus and when the insect swallow the blood from the host, it expels the valve’s content, including the parasites. Parasites are phagocytosed into macrophages, where they shed their flagella and become amastigotes that multiply by binary fission. The parasite replicate in the macrophage and eventually burst free from the infected macrophage. The released amastigotes are taken up by additional macrophages and so the cycle continues. When the new insect bites the infected vertebrate host, it swallows the infected macrophage, the parasite differentiates into promastigote and the sandfly is ready to infect another vertebrate host8, 9.

PATHOGENESIS:

Molecular understanding:
During its time in sandfly gut, a biochemical modification of the parasite's glycolipid coat occurs. This important transformation protects the parasite from rapid lysis via the mammalian complement system when it enters a host. Once in the mammal, promastigotes are opsonized with complement component C3. Mac-1, the integrin receptor for iC3b, is present on the surface of macrophages. Surface bound C3 binds to Mac-1 and is followed by phagocytosis of the promastigote. Once internalized, the phagosome, which contains the promastigote, fuses with lysosomes to form a phagolysosome, where, despite a pH of 4.5-5.0 and activated proteinases, it survives 48 hours following phagocytosis. Amastigotes are formed within the macrophage. Amastigotes survive and proliferate in low pH until eventually the host macrophage lyses and releases amastigotes10, 11.

Leishmania’s virulence:

Leishmania’s virulence play a key role in leishmaniasis infection. Leishmaniasis cannot occur unless it is infected by intact leishmania parasite. After vertebrate infection, infective leishmania must resist the action of complement and within phagolysosome, it must resist the hydrolytic environment, avoid macrophage activation and differentiate to the amastigote stage. Leishmania promastigotes are covered with a dense surface glycoalx, composed largely of molecules attached by glycosylphosphatidylinositol (GPI)-anchored molecules which includes protein such as the parasite surface protease Gp63 and proteophosphoglycans, a large GPI-anchored phosphoglycan called lipophosphoglycan (LPG) and a GPI-anchored glycosylinositolphospholipids (GIPLs) 12. Gp63 is an ecto-metalloprotease that is especially abundant on the surface of promastigotes.
Gp63 is known to help promastigotes in evasion of humoral lytic factors by rendering them resistant to complement-mediated cytolysis. It also appears to act, (perhaps, together with LPG) in infection of macrophages by promastigotes via receptor-mediated endocytosis\textsuperscript{13}. LPG contributes to the establishment of infection inside macrophages possibly by creating conditions propitious for the promastigote to amastigote differentiation\textsuperscript{14}. One class of enzymes, cysteine proteinases (CP), localized in the megasomes in amastigote forms has been demonstrated to be present in *Leishmania* spp. and is implicated to be involved in mechanisms of survival and growth of amastigotes inside macrophages\textsuperscript{15}.

**IMMUNOLOGICAL UNDERSTANDING:**

**Humoral Response**

Infection of leishmania in human is characterized by the appearance of anti-leishmanial antibodies in the sera of the patients. The elevated antibody titres against promastigote or amastigote antigens, their fractions or recombinant antigens have been extensively exploited for specific serodiagnosis in last two decades. Generally, elevated levels of IgG, IgM, IgE and IgG subclasses are found during this disease. In CL, usually they are present at low levels during the active phase of the disease. Contrastingly, strong anti-leishmanial antibody titres are well documented in VL\textsuperscript{16}.

**Cell Mediated Immunity**

In leishmaniasis two functionally distinct T helper (Th) cell subsets, Th1 and Th2, will decide whether there will be cure or progression of the disease. The action of Th1 (IFN-\(\gamma\), IL-2, TNF-\(\alpha\)) results in cure while action of Th2 cytokines (IL-4, IL-5, IL-10) results in progression of the disease. In cutaneous leishmaniasis, Th1 cytokines (IL-2 and IFN-\(\gamma\)) predominate over Th2 cytokines (IL-4) while in visceral and mucocutaneous leishmaniasis, there is increase in Th2 cytokine\textsuperscript{17}. Other Th-2 cytokines like IL-13 and transforming growth factor-beta (TGF-\(\beta\)) have been reported to be produced in VL\textsuperscript{18,19}.

**Genetic understanding**

The pathology resulting from infection with *Leishmania* substantially depends on host genetic factors. Susceptibility to *Leishmania donovani*, *Salmonella typhimurium*, and *Mycobacterium bovis* is controlled by the same gene on mouse chromosome 1 which codes for natural resistance associated macrophage protein 1 (NRAMP1)\textsuperscript{20}. This gene may not play a major role in human leishmaniasis as no association has been seen in humans between allelic forms of NRAMP1 and leishmaniasis but an understanding of its action may still help explain the peculiar ability the parasite has of surviving the harsh environment of the phagolysosome\textsuperscript{21}. Humans with specific alleles of the NRAMP1 gene were significantly more likely to have tuberculosis\textsuperscript{22}. 
DIAGNOSIS

CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS:

Laboratory diagnosis of leishmaniasis can be made by the following: (i) demonstration of parasite in tissues of relevance by light microscopic examination of the stained specimen, in vitro culture, or animal inoculation. (ii) detection of parasite DNA in tissue samples, or (iii) immunodiagnosis by detection of parasite antigen in tissue, blood, or urine samples, by detection of nonspecific or specific antileishmanial antibodies (immunoglobulin), or by assay for Leishmania-specific cell-mediated immunity.

1) Demonstration and isolation of parasite:

In most of cutaneous leishmaniasis cases, microscopy can reveal the parasite. Slides are made from punch biopsy specimens obtained at the border of the lesion rather than from its surface and stained with Giemsa stain and examined for the presence of amastigotes. Culture of the organisms is also an option. The cultures normally used are Schneider Drosophila medium or Novy-MacNeal-Nicolle (NNN) medium. These cultures can produce positive results in 1-3 weeks. Culture based diagnosis of mucocutaneous leishmaniasis has very low sensitivity as the organisms are often scant.

Immunological method:

Leishmanin (Montenegro) skin test (LST): The leishmanin skin test is an immunological test which measures delayed hypersensitivity reaction to an antigen prepared from a culture of different species of Leishmania. In cutaneous leishmaniasis the LST will become positive 2-3 months after the appearance of the lesion.

VISCERAL LEISHMANIASIS

The diagnosis of visceral leishmaniasis is complex because its clinical features are shared by many other commonly occurring diseases for eg: malaria, typhoid, and tuberculosis (i.e. long-term unexplained fever, cachexia and hepatosplenomegaly etc.). Many of these diseases may be present with VL (co-infection cases) and sequestration of the parasite in the spleen, bone marrow, or lymph nodes may add to further complication.

1) Demonstration and isolation of parasite:

The commonly used method for diagnosing visceral leishmaniasis has been the demonstration of parasites in splenic or bone marrow aspirate. The presence of the parasite in lymph nodes, liver biopsy, or aspirate specimens or theuffy coat of peripheral blood can also be demonstrated. 90% of the active cases show parasites in splenic and liver aspirates, while bone marrow aspirates show parasites in 60% of cases and lymph node aspirate show parasites in 30% of cases. It is performed by using a sterile syringe containing sterile buffered saline and a 26-gauge needle. The needle is inserted under the outer border of the lesion and rotated several times, and tissue fluid is aspirated into the needle.
Part of the splenic aspirate can be used to make smears for direct microscopic examination and the rest should be cultured. In preparations stained with Giemsa or Leishman stain, the cytoplasm appears pale blue, with a relatively large nucleus that stains red. In the same plane as the nucleus, but at a right angle to it, is a deep red or violet rod-like body called a kinetoplast.

Culture of parasite improves the sensitivity of detection of parasite. The culture media used may be Schneider Drosophila medium or Novy-McNeal Nicolle (NNN) medium. Generally, NNN medium is preferred for primary isolation, and Schneider Drosophila insect medium is preferred to amplify parasite numbers.

The parasite can also be demonstrated after inoculation of laboratory animals (such as hamsters, mice or guinea pigs) with infected specimen. Golden hamster is the animal of choice for maintaining L. donovani complex. Intraperitoneal and intrasplenic routes are preferred for infection. Both amastigotes and promastigotes can infect the animal.

**DNA detection method:**

Polymerase Chain Reaction (PCR), which is used in this study, is a technique, which allows a sensitive, specific and fast detection of minute amounts of pathogen DNA. PCR is based on the amplification of a known, specific sequence using oligonucleotide primers, which specifically bind to the DNA flanking the region of interest. Then the target sequence is amplified using a heat-stable DNA polymerase isolated from Thermus aquaticus.

**Serological method:**

The Indirect Fluorescent Antibody Test (IFAT) is one of the most sensitive tests available. The test is based on detection of antibodies, which are present during early stage of infection. Titers above 1:20 are significant and above 1:128 are diagnostic. Cross reaction with trypanosomal sera can be overcome by using leishmania amastigotes as the antigen instead of the promastigotes.

The direct agglutination test (DAT) has proved to be a very important sero-diagnostic tool combining high levels of specificity and sensitivity. The test uses stained promastigotes either as a suspension or in a freeze-dried form. Problems such as the need of a cold chain for storage of antigen are avoided by using the freeze-dried antigen, which makes DAT very suitable for use under field conditions.

Enzyme-linked Immunosorbant Assay (ELISA) has been used as a potential serodiagnostic tool for almost all infectious diseases, including leishmaniasis. The technique is highly sensitive, but its specificity depends upon the antigen used. Several antigens have been tried like crude soluble antigen (CSA), fucose-mannose ligand which is a 36-kDa glycoprotein present throughout the life cycle of leishmania (amastigote and promastigote stages), a recombinant antigen, rK39.

Leishmanin skin test: The role of leishmanin skin test is limited for diagnosis of visceral leishmaniasis. It may not become positive until six to eight weeks following recovery from the disease and is negative in acute cases of visceral leishmaniasis.
TREATMENT:

In recent years, the treatment of leishmaniasis is far from satisfactory. Chemotherapy constitutes the main tool for the control of leishmaniasis, but it is usually slow, expensive and toxic. Most of the antileishmanial drugs are to be used parenterally for prolonged periods. The therapy is further complicated by large number of infected children and declining effectiveness of pentavalent antimonial compounds. Although the lipid formulations of amphotericin B are an important advancement in therapy, their high cost precludes their use. At such a point of time, availability of an affordable oral agent, miltefosine has benefited the patients suffering from Visceral Leishmaniasis, also in countries like India. The therapeutic agents available for treatment of leishmaniasis are described here.

1) Pentavalent Antimony

Organic salts of pentavalent antimony have been the cornerstone of treatment for all forms of leishmaniasis for more than 60 years. Antimonials are thought to act by inhibiting the enzymes of glycolysis and other metabolic pathways. Two major pentavalent antimonials are currently used: Sodium stilbogluconate and meglumine antimoniate. The recommended regimen consists 20 mg/kg/day IV or IM for 28 days. Disadvantages of antimonials include the parenteral mode of administration, the long duration of therapy and the adverse reactions. Adverse effects include fatigue, body ache, pancreatitis, cardiac toxicity (arrhythmias), and renal toxicity (tubular and renal insufficiency).

2) Amphotericin B and lipid formulation

(i) Amphotericin B deoxycholate:
The anti-fungal agent amphotericin B can also be used for treatment of leishmaniasis. It probably intercalates with the parasite episterol precursors of ergosterol. Its clinical use has been limited by difficulties in its administration and its toxicity. Only in areas where VL cases show a high level of resistance to antimonials has Amphotericin B been much used, with great effectiveness. However, infusion-related side effects (fever, chills and bone pain) and renal toxicity of conventional amphotericin B are still major problems.

(ii) Lipidic Amphotericin B
Lipidic Amphotericin B formulation increases the effectiveness and reduces the toxicity associated with amphotericin B treatment. Lipidic Amphotericin B formulations are taken up by tissue macrophages, especially those of the liver and spleen. Once taken inside a macrophage by endocytosis, the cell’s phospholipases break open the liposome, freeing the Amphotericin B. In this way, the Amphotericin B is targeted at the host cells of the leishmanial amastigote. Three commercial preparations of lipidic Amphotericin B have been used: Amphotericin B lipidic complex, Liposomal amphotericin B and Amphotericin B cholesterol dispersion. A five days long regimen consisting of daily infusion or a 10-day regimen consisting of infusions on days 1-5 and day 10 are considered to be remarkably active. Liposomal amphotericin B is effective in a dose of 6 mg/kg in India. Amphotericin B lipidic complex is effective in a dose of 2 or 3 mg/kg/day.
3) Miltefosine

Miltefosine is one of a series of alkylphosphocholines. Miltefosine has been shown to block the proliferation of *Leishmania* and to alter phospholipid and sterol composition. The dose of miltefosine is 2.5 mg/kg/day preferably in two divided doses or single dose orally for 28 days. Adverse effects include gastrointestinal symptoms such as vomiting and diarrhoea. Pregnancy is a contradiction to the use of miltefosine because it has been shown to be teratogenic in animals.

4) Aminosidine

Aminosidine is an aminoglycosidic antibiotic and is administered once daily, usually by the intra-muscular route. Combined with antimonials, aminosidine in a dose of 12-18 mg/kg for 21 days allows a reduction in the duration of therapy and may be more efficient than antimonials alone in areas with high levels of antimonial resistance. Aminosidine also appears to be active in India when used alone. However, its potential for causing ototoxicity and nephrotoxicity needs further evaluation.

4) Pentamidine

Pentamidine isothienate is used in the treatment of antimonial-resistant VL. It is given in a dose of 4 mg/kg intramuscularly thrice weekly for six weeks. The precise mode of action of pentamidine is unclear but there is considerable evidence for its direct interaction with the pathogenic genome. However, side effects such as myalgia, nausea, headache, dizziness and hypoglycaemia were common at this dose, with an exceptional risk of developing irreversible diabetes.

5) Immunotherapy

Multiple T cell and macrophage-activating cytokines likely interdigitate to mediate host defense in visceral infection. IFN-γ can be used as an adjunct to sodium stilbogluconate to directly stimulate tissue macrophages and trigger intracellular leishmanicidal mechanisms. Such a combination could improve outcome by accelerating the kinetics of parasite killing and reducing the duration of chemotherapy.

Conclusion

Leishmaniasis is a vector-borne disease caused by a kinetoplastid protozoan parasite. The parasite is transmitted from one host to another through the bites of female sandfly, or occasionally through non-vector routes including blood transfusion, congenital, laboratory, acquired, sexual or from person to person. It manifests in three clinical forms, of which visceral leishmaniasis (kala-azar) is most dangerous and fatal, if untreated. The diagnosis is usually based on the presence of the amastigote stage of the parasite in the bone marrow, spleen, liver or lymph node aspirates or by histopathological findings.
Recent biological understanding of leishmaniasis should be used for designing therapeutic agents in several ways. Further, parasite virulence genes identified as a part of Leishmania Genome project may be specifically targeted for the development of a vaccine or a novel drug design. Through human genome project, individuals genetically susceptible to leishmaniasis may be identified and targeted for vaccination in endemic areas, which saves both economic costs and the rate of side effects from vaccination. It may also be possible to reserve treatment for those infected individuals who are genetically susceptible. This will spare genetically resistant individuals from unnecessary, avoidable and potential toxic side effects. Further, people who are frequent travelers or working in endemic areas should undertake necessary measures to avoid the risk of exposure to the organism.

References

34. Silva ES, Schoone GJ, Gontijo CM, Brazil RP, Pacheco RS, Schalling HD. Application of Direct Agglutination Test (DAT) and Fast Agglutination Screening Test (FAST) for serodiagnosis of visceral leishmaniasis in endemic area of Minas Gerais, Brazil. Kinetoplastid Biology and Disease 2005; 4: 4.