

REVIEW ARTICLE

Leishmaniasis: A Menace

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ABSTRACT

Leishmaniasis is a disease caused by protozoan parasites of genus *Leishmania* which gets spread by the bite of female phlebotomine sandfly. This may be presented in patients in the form of either visceral leishmaniasis or post kala-azar or dermal leishmaniasis or cutaneous or mucocutaneous. More than 20 species of *Leishmania* are responsible for causing infections in humans. Unfortunately, the affected regions are often remote and unstable, with limited resources for targeting this deadly disease. The most effective diagnostic tests for leishmaniasis are invasive, potentially dangerous and samples are required from spleen, lymph nodes or bone marrow. Additionally, such diagnostic tests are not readily available in resource-poor and endemic areas. Different treatment options are available for treatment of this disease, with varying effectiveness and adverse effects. The paper herein highlights the problem of leishmaniasis along with diagnostic options available, currently used drugs and different agents in the stage of pre-clinical and clinical development.

Keywords: Leishmaniasis; Diagnosis; Treatment; Clinical Trials

INTRODUCTION

Leishmaniasis, a parasitic disease, caused by obligate intracellular protozoans of the genus *Leishmania* and transmitted by bite of a female phlebotomine sandfly is a neglected vector-borne tropical infection ^[1]. This is considered to be the disease of the poor ^[2]. This disease is known to be the second most common protozoal disease and one of the neglected diseases which has now received special focus from WHO ^[3]. It has been reported that these parasites are endemic in large areas of tropics, subtropics and Mediterranean basin, globally spanning more than 98 nations ^[4]. However, over 90% of the new cases occur in only 13 countries (Afghanistan, Algeria, Bangladesh, Bolivia, Brazil, Columbia, Ethiopia, India, Iran, Peru, South Sudan, Sudan and Syria)

^[5]. A report by WHO states that an estimated 900000-1.3 million new cases and 20000 to 30000 deaths occur annually. Major clinical manifestations include visceral leishmaniasis or kala-azar, post kala-azar dermal leishmaniasis, cutaneous and mucocutaneous or espundia ^[6, 7].

Visceral leishmaniasis is caused by chronic infection of the parasite *Leishmania donovani*, transmitted by *Phlebotomus argentipes* ^[8]. This results in prolonged fever, anemia, splenomegaly and is fatal if left untreated ^[9]. At present, this is a serious public health problem in Indian subcontinent particularly, in Bihar state ^[10]. Post kala-azar dermal leishmaniasis is a common complication of visceral leishmaniasis that usually follows apparently successfully treated visceral leishmaniasis ^[11, 12]. This has been reported to

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occur in up to 10% of the patients treated for visceral leishmaniasis in India [13]. This condition is characterized by a macular, maculopapular, and nodular rash. This rash generally starts around the mouth from where it spreads to other parts of the body [14]. Cutaneous leishmaniasis is known to be the most rampant form of leishmaniasis across the globe. 90% of the cases have been reported in seven countries namely, Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria [15]. This is however a neglected disease continuing to spread in endemic and non endemic areas secondary to environmental and human made changes [16]. Mucocutaneous leishmaniasis, a complication of cutaneous leishmaniasis is a chronic inflammatory process that involves nasal, pharyngeal and laryngeal mucosa which can result in extensive tissue destruction [17]. Mucocutaneous form caused by *Leishmania brasiliensis* is endemic in major part of South America [18].

Life Cycle of *Leishmania donovani*

Leishmania parasites are transmitted to the host by the bite of infected female sand fly. It needs a blood meal to produce its eggs. Sand fly vectors are infected when feeding on the blood of infected individual or a vertebrate reservoir host. Many mammal species act as a reservoir host *viz.* rodents or dogs. During feeding, host macrophages, containing amastigotes get ingested by the vector. These parasite forms are released into the posterior abdominal midgut of the insect. These forms get transformed into promastigotes and begin their extracellular life cycle in the vector. These motile, elongated and flagellated forms migrate to the anterior part of alimentary tract and multiply by binary fission. Around after seven days, promastigotes get converted into metacyclic promastigotes. These promastigotes are released into the host together with saliva. These metacyclic promastigotes get taken up by the host macrophage and get converted into amastigote form. Upon binary fission of amastigotes, cells eventually burst, infecting other phagocytic cells, thus continuing the cycle [19, 20]. An overview of the complete cycle has been shown in **Figure 1**.

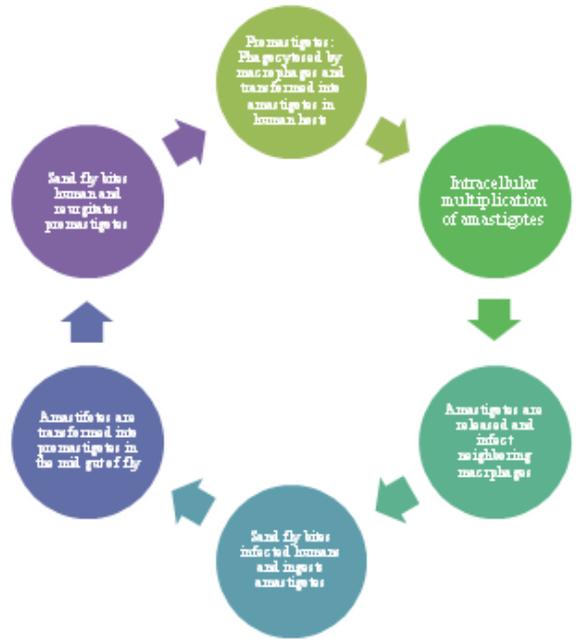


Figure 1: Life Cycle of *Leishmania donovani*

Diagnosis

Majority of the visceral leishmanial infections can be diagnosed clinically with observation of irregular fever, anemia, leucopenia and hepatosplenomegaly. There are several laboratory methods for the laboratory diagnosis of leishmaniasis. These methods include parasite detection by microscopic examination, culture and successive isoenzyme analysis for analysis for identification, or molecular biology-based assays for detection of parasite DNA [21, 22]. Several PCR techniques have been found to show superior sensitivity in comparison to microscopy smear and *in vitro* culture [23].

Culture and Microscopic Examination

Definitive cases of visceral leishmaniasis are supported by presence of parasites in clinical specimens and certain molecular methods [24]. Commonly used samples for examination include splenic or bone marrow aspirates. Other samples such as liver biopsies, lymph nodes and buffy coats of peripheral blood can also be used to demonstrate the presence of amastigotes. 60% and 85% sensitivity has been reported bone marrow and splenic aspirates in case of staining with Giemsa stain respectively [25]. However, fluorescent dye-conjugated antibodies can be employed to enhance sensitivity [26]. Low sensitivity has been reported in peripheral blood smears, particularly in patients with low parasitemia. To improve such results culture technique of the parasite can be used. However, this technique is tedious, time consuming and

costly. Hence, it is infrequently employed in clinical diagnosis. In the series of development, micro-culture method (MCM), is the one that has better sensitivity in case of buffy coat and peripheral blood mononuclear cells [27].

Detection of Antigen in Urine

A number of studies have reported detection of leishmanial antigen in urine of patients suffering from visceral leishmaniasis. De Colmenares and co-workers had reported the presence of two polypeptide fractions (72-75 kDa and 123 kDa) in the urine of patients suffering from visceral leishmaniasis [28]. In another study by Sarkari *et al.*, presence of 5-20 kDa carbohydrate based, heat stable antigen has been reported in the patients of visceral leishmaniasis [29].

Serological Diagnosis

Basic principle for specific serological diagnosis lies in the presence of a particular humoral response. Indirect fluorescent antibody (IFA), enzyme linked immunosorbent assay (ELISA), western blot and direct agglutination test (DAT) are the major tests included in this category.

Indirect Fluorescent Antibody Test

Indirect fluorescent antibody (IFA) has been reported to demonstrate considerable sensitivity and specificity [30]. Promastigote forms form the choice for diagnosis of visceral leishmaniasis [31].

Enzyme Linked Immunosorbent Assay

ELISA is the most preferred test under the category of serodiagnosis. It is a highly sensitive technique. Specificity of this technique is dependent upon the antigen used. One of the antigens used in this test is a crude soluble promastigote antigen (CSA), which is obtained by freezing and thawing live promastigotes. Sensitivity of this test using CSA lies in the range of 80% to 100% while specificity is in the range of 84% to 95% [32, 33]. rK39, a conserved portion of kinesin-related protein recombinant antigen from *Leishmania chagasi* has been reported to be highly reactive to sera from human and canine visceral leishmaniasis cases [34, 35]. rK9 and rK26, two hydrophilic antigens of *Leishmania chagasi* have been reported to be used in serodiagnosis of visceral leishmaniasis [36]. Additionally, heat shock proteins, HPS70 or histone proteins H2A, H2B, H3 and H4 have significant role in the serodiagnosis of visceral leishmaniasis [37].

Direct Agglutination Test (DAT)

This test is based on direct agglutination of *Leishmania* promastigotes which react specifically in the presence of anti-*Leishmania* antibodies in the serum specimen leading to agglutination of promastigotes [38, 39]. DAT offers benefits of high sensitivity and specificity. However, it has some limitations as well like relatively long incubation time and serial dilutions which have to be made. In order to overcome these drawbacks, a fast agglutination screening test (FAST) was developed by Schoone *et al* [40]. This technique requires only one serum dilution with three hours of incubation. This is very advantageous for screening of large populations [41]. DAT is the first real field test. Additionally, it remains the serological test of choice and first antibody detection test used in field settings, particularly, in many developing countries [42, 43].

Molecular Methods

Polymerase Chain Reaction

In spite of the number of molecular methods which have been evaluated for diagnosis, polymerase chain reaction (PCR) based assays have emerged as main molecular diagnostic tools, particularly in immunosuppressed patients [44, 45]. A number of samples like spleen, lymph node, bone marrow aspirates, whole blood and buffy coats can be taken to determine the presence of *Leishmania* DNA in cases of visceral leishmaniasis [46-48]. Specificity and sensitivity of the diagnostic test is based upon the sample used [49]. Highest sensitivity (nearly 100%) has been reported for spleen or bone marrow samples [50]. Different studies have been reported regarding the use of PCR in diagnosis of visceral leishmaniasis. The major drawback of PCR techniques is that these techniques are complex, expensive, and in majority of the endemic areas, these are restricted to certain few teaching hospitals and research centers.

Therapy Available

Classical treatment of this disease requires administration of certain toxic and poorly tolerated drugs. Pentavalent antimonials (Glucantime) and sodium stibogluconate (Pentostam) are the first line drugs employed in treatment of this disease. Pentamidine, amphotericin and certain other drugs are used in the treatment [51]. However, parasite resistance has significantly lessened efficacy of the conventional therapy [52]. Efficacy, adverse effects and cost also hinder the way of treatment of leishmaniasis [53].

Pentavalent Antimonials

For almost a century, antimonial have been in use for the treatment of this parasitic disease. Despite the fact that antimonials are first line drugs, they elicit several limitations like severe side effects, need for daily parenteral administration and drug resistance [54]. Though, the precise mechanism of action is not known, but they are known to cause inhibition of glycolytic enzymes and fatty acid oxidation in *Leishmania* amastigotes. Resultantly, there is a dose dependent inhibition of net formation of adenosine triphosphate (ATP) and guanosine triphosphate (GTP) [55]. Major side effects of the use of antimonials are abdominal pain, vomiting, nausea, fatigue, headache, and increase in the levels of liver enzymes, nephrotoxicity, arthralgia, fever, rash, pancytopenia and reversible peripheral neuropathy [56].

Pentamidine

Exact mechanism of action of pentamidine still remains unclear. However, it is known to exert its mechanism of action by interfering with DNA biosynthetics [57]. Immediate adverse reactions include hypotension, nausea and vomiting. Local pain at the site of injection, mild azotemia, leucopenia, abnormal findings from liver function tests, hypoglycemia and musculoskeletal pain have also been reported [58].

Miltefosine

Miltefosine, an alkylphosphocholine drug, has significant activity against the parasites [59]. It is effective *in vitro* against both promastigotes of different species of *Leishmania* [60]. Leishmanicidal activity of this drug is associated with perturbation of the alkyl-phospholipid metabolism and the biosynthesis of alkyl-anchored glycolipids and glycoproteins [61]. It interferes with cellular membranes without interacting with DNA. It leads to modulation of cell membrane permeability, membrane lipid composition, phospholipid metabolism, mitogenic signal transduction and inhibition of cell growth. It is also an inducer of apoptotic cell death [62].

Liposomal Amphotericin B

Amphotericin B is an efficacious antileishmanial agent, whose use is limited by drug toxicity [63]. However, over the past decade, liposomal amphotericin B has been increasingly employed to treat the cases of visceral leishmaniasis. Amongst the current antileishmanial drugs, it has the highest therapeutic index. However, the major

hitch in its wider use is high cost, which somewhere beyond the affordability of developing countries [64]. It is the treatment of choice for immunocompetent patients in the Mediterranean region [65]. Effectiveness of liposomal amphotericin B in the treatment of leishmaniasis is due to its ability to bind to ergosterol in the parasite membranes [66].

Candidates in Pre-clinical and Clinical Phase

Certain novel molecules in pre-clinical and clinical stage for the treatment of leishmaniasis have been mention in **Table 1** [67].

Table 1: Current Status of Potential Antileishmanial Agents

Candidate	Status
DNDI-0690 Nitroimidazole	Phase I Clinical Trials
DNDI-6148	Phase I Clinical Trials
Aminopyrazoles	Initial <i>in vitro</i> safety assays
DNDI-5421 and DNDI-Oxaboroles	Pre-clinical Stage
Fexinidazole	Phase IIIb

CONCLUSION

Leishmaniasis is the second most deadly infectious disease after malaria. In spite of the different therapeutic options available at the moment, there is a need for development of more novel agents to curb this menace.

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