

## Leishmaniasis

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Leishmaniasis is a vector-borne, protozoal disease caused by *Leishmania* species (Order Kinetoplastida). Geographic distribution of the disease spans across South-East Asia, Middle East, Southern Europe, Eastern Africa and Latin America but majority of the cases are from the Indian subcontinent, Sudan and Brazil. Measures of disease burden are far from precise as majority of the reporting is through passive case detection and the most susceptible groups living in poverty have no easy access to healthcare facilities; yet estimated global prevalence is around 12 million and around 350 million people are at risk of exposure to leishmania infection. The disease stands second only to Malaria & Trypanosomiasis in terms of global burden among vector-borne diseases. Clinical manifestations of the disease range from incidental detection to life-threatening visceral leishmaniasis. Clinical presentation can be classified as - i) Mucocutaneous leishmaniasis, ii) Visceral leishmaniasis and iii) Post- kala azar dermal leishmaniasis (PKDL).

### Life Cycle:

There are numerous species of *Leishmania* (genus) that can infect humans; of which the two most common are *Leishmania donovani* (prevalent in the Indian subcontinent) and *Leishmania infantum* (prevalent in Latin America and Mediterranean region). Likewise there is a tropic variation in the species of disease transmitting vector mosquito *Phlebotomus* (sandfly) e.g. *Phlebotomus argentipes* in India, *P. orientalis* in Africa and *Lutzomyia* in New World. Disease transmission occurs in zoonotic form (human-canine-human) in South America whereas in India it is predominantly anthroponotic (human-human). Motile, flagellar forms of leishmania, called as Promastigotes are inoculated into the skin of mammalian hosts during the blood meal of female mosquito and phagocytosed by Polymorphonuclear neutrophils, Macrophages and Dendritic cells. Here it transforms into non-motile, aflagellar forms – Amastigote and further multiplies by binary fission until the infected cell ruptures. Released amastigotes are picked up by other recruited cells and further spread takes place via regional lymphatics. The organism shows organ tropism and preferentially colonizes the reticuloendothelial tissue (Liver, Spleen and Lymph nodes) and bone marrow. Circulating amastigotes are ingested by vector sandfly while feeding off infected hosts, transform back into promastigote forms in its gut and migrates to proboscis of the vector.

### Immunopathogenesis:

Immune response to leishmania infection is characterized by bipolar cytokine response i.e. immunosuppressive cytokines such as IL-4, IL-10 and IFN- $\gamma$  are significantly raised while that of pro-inflammatory cytokine like IL-12 reduced; a milieu that favors persistence of the parasite inside the phagosomes. *Leishmania* specific antibodies are produced in early stages of infection and persist for years after successful therapy but are non-protective in nature. The molecular pathways of inflammatory response are currently being investigated as potential targeted immunotherapy in view of emerging resistance to standard drugs used for its treatment.

**Clinical features:**

The incubation period can range from 2 weeks to 18 months. Demographic factors such as poor housing conditions (Mud house, inadequate sanitation) and herd rearing increases the chances of exposure to infection. Depending upon the interplay between pathogen and host variables such as given species of leishmania, age & nutrition of the host and immune status (more common in children, immune-compromised ones) etc. infection may go unnoticed or may manifest as localized mucocutaneous lesions and/or disseminated visceral disease.

**Visceral leishmaniasis:**

The most florid presentation is Visceral leishmaniasis (aka Kala-azar i.e. Black Fever in India), with mortality rates up to 75-95% if left untreated. It is characterized by triad of fever, splenomegaly and anemia. Patient usually runs high grade spikes of intermittent fever in an irregular trend and there is stark absence of any significant localizing symptoms. Constitutional symptoms such as malaise, easy fatigability, anorexia and weight loss may be prominent. In due course patient develops rapidly progressive non-tender splenomegaly that may reach beyond umbilicus. Often, there is marrow infiltration by the protozoa with consequent secondary marrow failure that manifests as anemia which is often compounded by hypersplenism. One may develop mucocutaneous bleeding tendencies from thrombocytopenia. Leucopenia and Leishmaniasis-induced immunosuppression may also increase susceptibility to super-added chest infections and viral exanthemata. Mild hepatomegaly and peripheral lymphadenopathy is also frequently encountered. Prolonged anorexia may cause hypoalbuminemia which acts as a poor prognostic factor. The presentation must be differentiated from diseases such as Malaria, Enteric fever, Brucellosis and Tuberculosis.

**Mucocutaneous leishmaniasis & PKDL:**

On the other end of the spectrum are mucocutaneous leishmaniasis and post-kala azar dermal leishmaniasis and are endemic in Middle East region and Latin America. The predominant infecting species are *L. tropica* and *L. major* in Old World while it is *L. maxicana* Viannia subgenus in New World. Mucocutaneous leishmaniasis presents as a papulo-nodular lesion at the insect bite site and regional lymphadenopathy. Satellite lesions may develop from local spread through lymphatics. Parasitic load within the lesion may vary as chronic solitary lesions on the face are oligoparasitic in nature and termed as *Leishmania Recidivans* while the lesions are multiple, disseminated, non-ulcerative nodular & polyparasitic in Diffuse Cutaneous Leishmaniasis. Close differentials include *Lupus vulgaris*, Leprosy, sarcoidosis and infiltrating malignancies. Mucosal leishmaniasis develops as a metastatic lesion from cutaneous leishmaniasis or as a direct contiguous spread of it and involves nasopharyngeal mucosa. The geographic distribution is restricted to New World and major causative species being *L. braziliensis*, *L. amazonensis* & Viannia subgenus. Cutaneous and Mucosal leishmaniasis may co-exist but frequently development of mucosal lesions lag behind by months to years after resolution of cutaneous lesions. Clinical manifestations include nasal obstruction, epistaxis or progressive, ulcerative lesions causing nasal septum or palatal perforation.

*Post-kala-azar dermal leishmaniasis (PKDL)* is the term coined for occurrence of cutaneous leishmaniasis during or after treatment for visceral leishmaniasis. Likelihood of developing PKDL is low in Indian subcontinent (10%) as compared to Sudan (50%). The lesion tends to be papulo-nodular and the most commonly affected site is face. Again, there is a geographic variation in the natural evolution of the lesion; spontaneous resolution is the rule in Sudan while it requires treatment in Indian subcontinent.

### Diagnosis:

**Light Microscopy:** The definitive test to establish the diagnosis of Visceral Leishmaniasis is demonstration of amastigote forms of the protozoa by light microscopy using Geimsa stain. Samples such as peripheral blood film buffy coat films are easy to obtain but have low detection rates. It can also be detected in bone marrow aspirate or imprint smear, lymph node aspiration but the highest yield is from splenic aspirate; however it carries the risk of splenic rupture. Culture of the organism from tissue samples is also a sensitive method but requires special culture techniques as temperature and nutrient requirements are strain specific. Also, culture facilities are not routinely available.

For Mucocutaneous leishmaniasis and PKDL, samples for microscopic examination are collected from respective sites of lesion. Nodular lesions have better diagnostic yield as compared to macular ones.

**Serologic diagnosis:** It is the most widely used indirect method of diagnosis. Among the array of serologic test methodologies Direct Agglutination Test (DAT) and Immunochromatographic Test (ICT) using rK-39 antigen are most widely accepted because of their high sensitivity, use of standardized recombinant antigen and convenient dipstick format for field applicability. However, significant proportions of population residing in endemic areas have false positive results. Further, rK-39 antigen is encoded by a kinesin-related gene derived from a particular strain of *L. infantum* and the protozoan is known to exhibit significant diversity for the related gene. Hence a negative result does not rule out the diagnosis and serves only as supportive evidence to clinical diagnosis. Recently a new diagnostic test to detect heat-stable carbohydrate moiety in the urine of patients with visceral leishmaniasis has been developed based on latex agglutination methodology. The test is well adopted for field conditions but has low sensitivity.

Other test methodologies such as Immunofluorescence assay (IFA), ELISA and Western blot require equipments not suitable for field conditions and there are no standardized protocols for their performance. Montenegro or Leishmanin skin test is mentionable for its historic interest only in view of its poor sensitivity (14%) and specificity.

**Molecular diagnosis:** PCR-based nucleic acid amplification techniques have the advantage of higher sensitivity and also require peripheral blood as test specimen rather than invasive tissue sampling such as bone marrow aspirate or lymph node biopsy for light microscopy; however the facility is available only in referral centers and not suitable for field diagnosis as it requires specialized equipment.

**Treatment:**

Pharmacotherapy for leishmaniasis is influenced by the clinical syndrome of presentation and disease trend in a given geographic area. The treatment has evolved from parenteral therapy with pentavalent antimonial compounds to various formulations of amphotericin-B to oral agents like mifepristone; guided by the quest for better drug response and fewer side-effects.

**Visceral leishmaniasis :**

Pentavalent antimonial (Sb V) compounds: Once a first-line treatment for visceral leishmaniasis, these drugs are now obsolete in India because of rising incidence of drug resistance, particularly the northern states like Bihar where up to 60% of the cases may be have primary drug resistance. However they still are used as first-line treatment in New World region. Recommended dosage is 20 mg/Kg/day for 28-30 days. It is likely that use of subtherapeutic dosage for fear of cardiotoxicity and shorter duration of treatment course from poor compliance resulted in drug resistance. Although effective against the New World strains, the drug is losing ground in view of cardiac side-effects, nephrotoxicity and availability of safer alternatives.

Amphotericin B: It emerged as an effective and safer anti-leishmania drug. Amphotericin B Deoxycholate is the first formulation used. Cure rates are in the range of 98-100% as first-line treatment and around 92% for antimonial resistant cases. Administered as slow intravenous infusion in dosage of 0.75-1.0 mg/Kg/day for 28 doses, it requires hospitalization to watch for their side-effects; predominantly nephrotoxic. With the development of formulations like Amphotericin B Lipid Complex and Liposomal Amphotericin B, the incidence of nephrotoxicity has fallen considerably but at the monetary cost of treatment. A potential advantage of liposomal amphotericin B could be short course treatment; ranging from single dose infusion to total dose administered over 4-5 days. Various clinical trials have confirmed their efficacy to the tune of 93-98% with dosage regimen ranging from single dose infusion to daily administrations for 4-5 days. No clinical drug resistance has been documented against any of the formulations.

Miltefosine: This phospholipid-derivative anti-leishmania drug has cure rates comparable to amphotericin B and has the added advantage of ease of oral administration. For the same reason, it was introduced as the backbone of visceral leishmaniasis elimination program in India. Recommended dosage is 50 mg twice daily for 28 days. Because of unsupervised domiciliary treatment, poor compliance has resulted in emergence of drug resistance and the drug has potential teratogenicity.

Sitamaquine: This is another oral drug undergoing evaluation in clinical trials. Initial experience with the drug against various species of leishmania from Kenya and Brazil studies has been promising but the drug has been associated with methemoglobinemia and nephrotoxicity.

**Drug resistance:** It is speculated that WHO treatment guidelines are followed by medical practitioners only in 26% of cases and majority of patients do not follow up regularly or terminate

treatment prematurely on their own will. This has led to progressive development of drug resistance and treatment failure. Viable options to tackle the issue of drug resistance could be Direct Observation Therapy as for Tuberculosis (as recommended by WHO), Combination or Multidrug therapy and withdrawal of drug from open market and supply through health care providers only.

**Combination therapy:** In an attempt to protect the limited pharmacologic armamentarium from emerging drug resistance, it has been advocated to administer these drugs in various combinations. It has added benefit of reducing drug toxicity and treatment duration. This may improve patient compliance and reduce treatment cost as well. Numerous clinical trials have reported successful treatment with combination therapy achieving the cure rates up to 91-98%.

**Immunotherapy:** Drugs modulating the immune response against leishmania infection to help in reducing parasite load are under investigation. Potential target sites are anti-IL-10 & anti-IL-4 therapy, dendritic cell surface receptor modulation, MAP kinase pathways for oxidative burst in leishmania infected neutrophils. These modalities are in preliminary stages of conception and yet to be evaluated in human subjects.

#### **Mucocutaneous leishmaniasis and PKDL:**

Treatment recommendations for Mucocutaneous leishmaniasis and PKDL follow similar principles as that for visceral leishmaniasis albeit shorter duration of treatment with Sb V. In certain regions the cutaneous lesions tend to run self-limiting course where expectant watchful observation would suffice. Severe involvement of nasopharynx with upper airway obstruction may require concomitant steroid therapy.

**Leishmaniasis & HIV co-infection:** Estimated incidence of co-infection is around 2-5%. Challenges with co-infection include atypical presentation, poor response to therapy especially if the CD4 count is less than 200/ $\mu$ L, higher incidence of drug toxicity and frequent relapses after treatment. Currently, co-infected patients are treated on similar lines as for immunocompetent ones. Few clinical trials conducted in Europe with *L. infantum* have suggested best response rate and tolerability to lipid formulations of amphotericin B. Experience with antimonial compounds and miltefosine has been disappointing with low cure rates and high relapse rate. There is a dire need for rigorous evaluation through large scale clinical studies to determine preferred drugs, their dosages and treatment duration. There is no universally accepted recommendation regarding primary and secondary prophylaxis in this subset of patients.

#### **Prevention and Control:**

To stop disease transmission cycle, interventions can be aimed at reducing vector density and animal reservoir like insecticide spray, animal culling; human protection measures like use of bed nets, indoor residual spray. Though a reasonable approach, these interventions have failed to deliver unequivocal evidence for any effective decline in human transmission in field trials. Attempts for vaccine development have been futile so far on account of genetic diversity and polymorphism exhibited by the protozoa and inability to maintain quality control & proper standardization.

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