GENUS: LEISHMANIA

Species parasitic in man:

Under the genus Leishmania, there are 2 subgenus:

1. Leishmania
2. Viannia

SPECIES PARASITIC IN MEN

Under subgenus Leishmania, there are following species:

LEISHMANIA DONOVANI COMPLEX

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>DISEASE CAUSING</th>
<th>VECTOR</th>
<th>RESERVOIR HOSTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Leishmania donovani</td>
<td>*Kala-azar (Visceral leishmaniasis)</td>
<td>1. P.argentipes (India)*</td>
<td>Man*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. P.chinesis (China)</td>
<td></td>
</tr>
<tr>
<td>*Leishmania infantum</td>
<td>1. Infantile kala-azar*</td>
<td>1. P.perniciosus (Mid asia,</td>
<td>Dog, Fox, Jackal.</td>
</tr>
<tr>
<td></td>
<td>2. ZVL (Zoonotic visceral</td>
<td>Middle east, China)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>leishmaniasis)*</td>
<td>2. P.chinesis and P.alexandri</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(China)</td>
<td></td>
</tr>
<tr>
<td>*Leishmania chagasi</td>
<td>ZVL in new world*</td>
<td>Lutzomyia longipalpis (New</td>
<td>Dog, Fox.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>world)</td>
<td></td>
</tr>
<tr>
<td>Leishmania</td>
<td>Visceral leishmaniasis in Africa</td>
<td>1. P. Orientalis</td>
<td>Rodents, carnivorous animals.</td>
</tr>
<tr>
<td>archibaldi</td>
<td></td>
<td>2. P.martini (Africa)</td>
<td></td>
</tr>
</tbody>
</table>

*Only remember the vector of kala-azar in India (P.argentipes).

LEISHMANIA TROPICA COMPLEX

Leishmania tropica: It causes a dry type of cutaneous lesion in urban areas of North-West India.
LEISHMANIA MAJOR COMPLEX

Leishmania major: It causes a moist type of cutaneous lesion in rural areas of Central Asia.

LEISHMANIA MEXICANA COMPLEX

Leishmania Mexicana: It causes a single cutaneous lesion on the face, ear or hand in countries of South America. (Mexico, Venezuela, Columbia etc.)

Other species in this group are:

1. Leishmania amazonensis
2. Leishmania venezuelensis
3. Leishmania garnhami etc. (All of them cause local cutaneous lesion)

Under subgenus Viannia, there are following species:

Leishmania braziliensis complex

Leishmania braziliensis:

It causes a malignant type of lesion as a papulopustular swelling of skin localised on the nostrils, mouth, eyes, face, ears, elbow and knee, which with time causes destructive and mutilating erosions. It commonly occurs in countries of South America.

Other complexes are (not needed):

1. Leishmania guyanensis complex
2. Leishmania lainsoni complex
3. Leishmania naiffi complex
CLINICAL CLASSIFICATION OF LEISHMANIASIS

1. VISCERAL LEISHMANIASIS (KALA-AZAR)

Indian kala-azar is caused by Leishmania donovani with a human reservoir.

2. CUTANEOUS LEISHMANIASIS

• The classical lesion is characterised by a nodule at the site of inoculation followed by formation of a central crust which may fall away forming a wet type of ulcer.
• Or it may be present as a papulonodular lesion covered by superficial scales forming a dry type of ulcer.
• So, a depressed scar and altered pigmentation on healing of the nodules at the edge of lesion is a characteristic feature of CL.

It is of 3 types as discussed below:

1. Post kala-azar dermal leishmaniasis: (PKDL)
   • It occurs mainly in India.
   • It is caused by Leishmania donovani.
   • It is a late sequel to visceral leishmaniasis. It occurs typically 1-2 years after recovery from VL.
   • It is characterized by non ulcerating skin lesion, which is of 3 types:
     1) Macular and hypopigmented lesion: on the trunk and extremities.
     2) Erythematous patch: on the nose, cheek and chin. Also called “butterfly erythema”.
     3) Nodules: Soft, painless, yellowish pink nodules appear mainly on the face. There is absence of ulceration which can distinguish them from oriental sore (dermal leishmaniasis).
   • They do not heal spontaneously.
2. Old world CL:

- The main causative agents are:
  1) Leishmania major
  2) Leishmania tropica
  3) Leishmania aethiopica.

- It is of 3 types:
  1) Urban/ lupoid/ tuberculoid leishmaniasis
  2) Rural leishmaniasis
  3) Diffuse cutaneous leishmaniasis

- Urban leishmaniasis is caused by L.tropica in North India. It is characterized by a self healing ulcer starting as a small itching papule covered with fine whitish scale which gradually becomes dark and thick and finally falls away and a depressed scar is formed. It is present on the face, feet, legs and arms.

- Lupoid/ tuberculoid leishmaniasis is caused by L.tropica. It is characterized by a non-self healing ulcer with peripheral activity, which mainly occurs on the face, which is a result of an incomplete immune response of an earlier episode of oriental sore.

- Rural leishmaniasis is caused by Rural leishmaniasis is caused by L.major in North India. It is characterized by multiple painless lesions on the nose, lips and limbs, which heals rapidly but in non-immune person may result in disfigurement of face.

- DCL of old world is caused by L.aethiopica. It is a result of specific deficiency of CMI to Leishmania antigen. It is characterized by appearance of nodular infiltrative lesions mainly over the face, ears, extremities and buttocks.

- But there is no ulceration or mucosal involvement. Histologically the nodes consist almost entirely of histocytes with a relative absence of lymphocytes and plasma cells.
• It has a strong resemblance to lepromatous leprosy, because of the absence of CMI as in leprosy and that the leishmanin skin test is negative. Amastigote form is found both in blood and bone marrow.

3. New World CL:
• It is seen mainly in South and Central America.
• Causative agents:
  ➢ L.peruviana
  ➢ L.braziliensis
  ➢ L.mexicana
  ➢ L.guyanensis
  ➢ L.panamensis
• New world DCL is caused by:
  ➢ L.amazonensis
  ➢ L.mexicana

3. MUCOCUTANEOUS LEISHMANIASIS

• It is mainly caused by L.braziliensis and occasionally by L.panamensis.
• It is seen in south and central America.
• It consists of two stages: the primary cutaneous lesions, sometimes followed by secondary mucosal involvement, which occurs after a variable time of latency.
• It is seen in nasal mucosal membrane, pharynx, larynx and upper lip, sometime whole nasal septum is destroyed.
• Granulomas develop at mucocutaneous junction followed by gross destruction of soft tissue and cartilage causing disfigurement of nose and mouth.
• Death may occur from severe **respiratory infections due to acute obstruction of respiratory passages.**

**MORPHOLOGY OF LEISHMANIA DONOVANI**

The parasite exists in mainly two stages-

1. **Amastigote forms**: Aflagellar state, appears in man.
2. **Promastigote forms**: Flagellar state, appear in gut of sandfly and in artificial culture.

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**AMASTIGOTE FORM**

**PROMASTIGOTE FORM**

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*1st row is of Giemsa stain, 2nd row is of SEM. (4th photo- dividing promastigote)*
**GENERAL STRUCTURE OF LEISHMANIA (WITH FLAGELLA)**

<table>
<thead>
<tr>
<th>FEATURES</th>
<th>AMASTIGOTE FORM</th>
<th>PROMASTIGOTE FORM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size and shape</td>
<td>Round/ oval measuring 2-4 ( \mu m ).</td>
<td>Earlier stage: Short and oval, <strong>pear shaped</strong>.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature stage: Long, slender, <strong>spindle shaped</strong>.</td>
</tr>
<tr>
<td>Cell membrane</td>
<td>Delicate.</td>
<td></td>
</tr>
<tr>
<td>Nucleus</td>
<td>Oval/ round and <strong>situated in the middle of the cell/ along the side of the cell wall.</strong></td>
<td><strong>It is situated centrally.</strong></td>
</tr>
<tr>
<td>Kinetoplast (It comprises of a DNA containing body.)</td>
<td><strong>It is situated tangentially/ at right angle of nucleus.</strong></td>
<td><strong>It is situated transversely near the anterior end.</strong></td>
</tr>
<tr>
<td>Axoneme</td>
<td>A delicate filament extending from the kinetoplast to the margin of the body, it <strong>represents</strong></td>
<td><strong>Same as that.</strong></td>
</tr>
<tr>
<td>Flagellum</td>
<td>Not present.</td>
<td>Present, projecting from the front.</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Vacuole</td>
<td>A clear unstained space lying along the axoneme.</td>
<td>A light eosinophilic vacuole is situated infront of the kinetoplast.</td>
</tr>
</tbody>
</table>

### CULTURE OF LEISHMANIA

- The cultivation of Leishmania is done in a special culture medium named “NNN medium”, which contains salt agar and defibrinated rabbit’s blood in 2:1 ratio.
- In this medium, the material is inoculated at water of condensation at 22-24°C and intracellular growth can be maintained at 37°C for around 32 days.
- In the culture, the amastigote form is converted to promastigote form, which is divided by longitudinal fission and gives rise to numerous flagella.
- There should be special measures to control bacterial contamination because it may cause degeneration and death of L.donovani.

### LIFE CYCLE AND PATHOGENESIS

- Leishmania parasite exists in two forms- amastigote and promastigote.
- When an infected sandfly bites an individual, there is inoculation of promastigote form into the skin.
- After inoculation into skin by a sandfly, promastigotes are phagocytosed by dermal macrophages, where they convert to amastigotes and multiply within acidic vacuoles.
- Additional mononuclear phagocytes are attracted to the site of the initial lesion and become infected.
- The host cells are enlarged by the burden of multiplicating amastigotes, and are eventually ruptured.
- Amastigotes then disseminate through the regional lymphatics and the vascular system to infect mononuclear phagocytes throughout the RE system.

![Bone marrow aspirate from a patient suffering from VL showing amastigotes in the macrophages.](image)

- Progressive recruitment of amastigote-infected mononuclear phagocytes and inflammatory cells within organs results in distortion of the native tissue architecture and often, massive hepatosplenic enlargement.
- Parasitized reticuloendothelial cells can be found in bone marrow, lymph nodes, skin and other organs.
- A blood sucking insect draws these free amastigote forms as well as those in the monocytes during its blood meal.
- In certain species of sandfly, these ingested amastigote forms develop into promastigote forms which divides by binary fission in the midgut of sandfly giving rise to numerous flagellates.
- These flagellates tend to spread to the anterior part of the alimentary canal (buccal cavity and pharynx and finally proboscis) after 6-9 days of ingestion of blood. This type of development is called as "Anterior station development".
- As salivary glands of sandfly are not affected, the transmission is by bite, not the saliva.

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>RESERVOIR OF INFECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>INDIA</td>
<td>HUMAN</td>
</tr>
<tr>
<td>CHINA AND BRAZIL</td>
<td>DOG</td>
</tr>
<tr>
<td>EAST AFRICA</td>
<td>RODENT</td>
</tr>
<tr>
<td>RUSSIA</td>
<td>JACKAL</td>
</tr>
</tbody>
</table>
CLINICAL FEATURES OF LEISHMANIASIS

- Infection with the *Leishmania* species causing visceral leishmaniasis can manifest as a progressive fatal disease or as an asymptomatic form.
- The incubation period typically varies from 3 to 8 months, but can be weeks or years.
- Typical, symptomatic VL is associated with heavily infected mononuclear phagocytes throughout the reticuloendothelial system and suppressed cellular immune responses.
- VL patients, if left untreated in 75-95% of cases, may die within 2 years.
- The onset of disease is insidious in most cases and marked by: progressive development of:
  - fever,
  - weakness,
  - anorexia,
  - weight loss and
  - abdominal enlargement from hepatosplenomegaly.
• **Fever**, accompanied by **chills** is usually **intermittent or remittent** with **twice-daily temperature spikes**.

• During the less common acute cases, fever can be of abrupt onset and have a periodicity similar to that of malaria.

• **Progressive and massive hepatosplenomegaly is characteristic of VL.**

• Infected individuals in the Sudan often also develop lymphadenopathy.

• **In India patients with VL commonly develop hyperpigmentation of extremities, face and abdomen.**

• Hemorrhage can occur from various sites.

• **Severe cachexia is a prominent feature of VL, driven in part by high levels of TNF-α.**

• **Death from VL** occurs either from the:
  - primary, multisystem disease causing malnutrition and bone marrow suppression and/or,
  - from secondary bacterial infections such as tuberculosis, dysentery, pneumonia and measles.

• Important laboratory findings in advanced visceral disease include profound pancytopenia (due to bone marrow suppression), eosinopenia, hypoalbuminemia and hypergammaglobulinemia (mainly IgG).

• **The cause of anemia in kala-azar is now thought to be due to splenic hemolysis of RBC.***

• The ESR is usually elevated.

• Kidneys may show evidence of immune complex deposition, but renal failure is rare.

• **Several infectious and hematologic diseases can mimic visceral leishmaniasis.** These include:
  1) Malaria,
  2) Schistosomiasis,
  3) Miliary tuberculosis,
  4) African trypanosomiasis,
  5) Typhoid fever,
  6) Brucellosis,
  7) Histoplasmosis,
  8) Bacterial endocarditis,
9) Lymphoma and Leukemia.

DIRECT EVIDENCES (Demonstration of L. donovani)

- **Peripheral blood smear**: A microscopical examination of a stained blood film has to be done to identify the amastigote forms present in the PBS. Because of the small number of parasites present in the blood, there is often a negative result obtained. The chances of finding a Leishmania parasite is greatly increased if any of the following methods are applied:
  1. By making a thick blood film.
  2. By centrifuging citrated blood.
  3. By producing a thick leucocytic edge (when a thick blood film is drawn and just before the blood is totally exhausted, the spreading slide is abruptly lifted off.)

- **Blood culture**: It is the least sensitive method for diagnosis. The only disadvantage is that the result is obtained slow and after a long time (almost a month). 1-2 ml of blood is taken from a vein aseptically and mixed with 10 ml citrated saline solution. The cells are then allowed to settle in a cold incubator (22°C) overnight. The cellular deposit is then inoculated in NNN medium and incubated.
at 22°C for 1-4 weeks. At the end of each week, a drop of condensation fluid is examined for promastigote forms.

**Biopsy material:**

1) **SPLENIC PUNCTURE:** When spleen is enlarged, it is the most important clue to diagnosis. Amastigote forms are found in the stained culture and promastigote forms are found in culture. The only risk of a splenic puncture is that bleeding may continue from the punctured wound in patients of leukaemia and hemorrhagic diathesis.

2) **BONE MARROW PUNCTURE FROM STERNUM/ILIAC CREST:** It offers a method of diagnosis particularly in early cases, when spleen is not so enlarged as to be punctured. Its disadvantage is that parasites are scanty. As in the splenic puncture, the amastigote forms are found in the stained culture and promastigote forms are found in culture.

**INDIRECT EVIDENCES**

**Blood count:**

1) Neutropenia with a relative lymphocytosis and monocytosis is revealed.

2) Average TC is <3000/mm3, may fall upto below 1000/mm3.

3) RBC is also decreased, the RBC:WBC ratio becomes 1:2000, when the normal being 1:750.

**Serological tests:**

1) **Aldehyde test:**

   - It is the test for rise of γ-globulin.
   - 1-2 ml of serum is taken in a glass test tube and 1-2 drops of 40% formalin is added to it.
   - A positive result is obtained by the observation of jellifying/ milk white opacity like a hard-boiled egg in 2-20 minutes.
   - It should be remembered that the result will not be positive until 3 months of disease progression.
   - False positive result- *African trypanosomiasis, multiple myeloma and cirrhosis.*
   - False negative result- *cutaneous leishmaniasis.*
2) Complement fixation test with WKK antigen:

- The antigen used in this reaction is prepared from human tubercle (by Witebsky, Klingenstein and Kuhn, hence WKK antigen) because Leishmania and Mycobacteria share a common antigen.
- The test is based on the presence of certain immune bodies in the sera of kala-azar patients.
- This test has distinct advantage of early detection of the case, becoming positive within 3 weeks of the disease.
- False positive results:
  1. Leprosy
  2. Pulmonary TB
  3. Tropical pulmonary eosinophilia.

*Other serological tests:
  1. IFA (Immuno fluorescence assay) - Most commonly used.
  2. ELISA
  3. Direct agglutination test
  4. Latex particle agglutination test
  5. Immunoblotting
  6. Countercurrent immunoelectrophoresis etc.

TREATMENT OF LEISHMANIASIS (FOR PHARMACOLOGY)

The following classes of drugs are used in treatment of leishmaniasis:

- **ANTIMONY COMPOUND**
  - SSG (Sodium stibogluconate)

- **DIAMIDINE**
  - Pentamidine

- **ANTIFUNGAL DRUGS**
  - AMB (Amphotericin B)

- **OTHER CHOICES**
  - Paromomycin
SODIUM STIBOGLUCONATE (SSG)

It is a first line drug against kala-azar but now it is not used in Bihar (India) due to extensive resistance. It is an pentavalent antimonial compound, which is water soluble and containing 1/3 rd of antimony by weight.

**Mechanism of action:**
- The drug is in pentavalent form and water soluble.
- **It is converted to active trivalent form by an enzyme present in Leishmania amastigote form.**
- This active trivalent form causes efflux of Glutathione from the parasite and oxidative damage to the parasite.

**Dose and route of administration:**
- 20 mg/ kg daily by i.m/ i.v route (Maximum 850 mg) for 20-30 days or more.
- Relapsing cases should immediately be retreated with same dose.

**Preparations:**
- Abnate
- Stibo.

**Adverse effects:**
- Nausia
- Vomitting
- Metallic taste
- Cough
- Pain abdomen
- Pain and stiffness of injected muscle
- Sterile abscess
- Mental syndromes
PENTAMIDINE

**Spectrum:** It has a relatively wide spectrum against:
- L. donovani,
- Trypanosoma,
- Pneumocystis jiroveci,
- Some bacteria and
- Some fungi (Blastomyces).

**Mechanism of action:**
This drug interacts with **kinetoplast DNA and inhibits Topoisomerase 2.**

**Dose and route of administration:**
4mg/kg deep i.m/ slow i.v infusion over 1 hour on alternate days till no parasite is found in 2 splenic aspirates.

**Adverse effects:**
The toxicity of pentamidine is high. Because of its highly basic nature, it causes massive histamine release, which causes anaphylactic reactions:
- Acute fall in BP
- Cardiovascular collapse
- Dyspnoea
- Palpitation
- Fainting
- Vomiting
- Rigor
- Fever
- Hypoglycemia due to cytolysis of pancreatic β cells.
Use: **Only for salvage therapy of antimonial failure cases.**

**Amphotericin B (AMB)**

**Mechanism of action:**
Like fungi, *Leishmania* also has high percentage of ergosterol. So, antifungal agents are highly effective in kala-azar.

**Use:**
It is extensively used in antimonial resistance. In bihar, it is the standard treatment due to extensive SSG resistance. But high toxicity and prolonged hospitalization limits its application.

*Liposomal AMB* is particularly suitable for treatment of kala-azar because it delivers the drug directly inside the RE cells of liver and spleen where the amastigote live.

**PARAMOMYCIN**

**Type:** An aminoglycoside antibiotic.
**Dose:** 10-15 mg/ kg/ day for 21 days.
**Side effects:** Ototoxicity, elevated serum transaminase levels, pain at injection site.

**LOCAL TREATMENT OF DERMAL LEISHMANIASIS/ ORIENTAL SORE**

1. **SSG:** 2 ml solution round the sore.
2. **Paramomycin ointment:** Applied locally.