Leishmania

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Introduction

- *Leishmania* is a hemoflagellate, different species of causes the disease leishmaniasis.

- Clinically leishmaniasis are manifested as cutaneous, mucocutaneous and visceral lesions. Of its three clinical forms, Visceral leishmaniasis is the most severe one.

- The different forms of Leishmaniasis constitute severe public health problems. Above 12 millions peoples are infected and some 350 million people are at risk throughout the world.

- All of these parasitic diseases are transmitted by biting of different species of sand flies.
  - Visceral Leishmaniasis (VL) is fatal while left untreated.
  - Mucocutaneous Leishmaniasis (MCL) is a mutilating disease.
  - Diffuse cutaneous Leishmaniasis (DCL) is a disabling disease.
Epidemiology

- Leishmaniasis is prevalent throughout the tropical and subtropical regions of Africa, Asia, the Mediterranean, Southern Europe (old world) as well as South and Central America (new world).

- Annual incidence of leishmaniasis is estimated to be 1 to 1.5 million cases of Cutaneous leishmaniasis and 500,000 cases of Visceral leishmaniasis.

- About 350 million people are at risk with an overall prevalence of over 12 million people worldwide.
Host factors

- **Age and sex**: In East Africa and India the peak age group is 5-9 years. In Southern Europe and China, people of all age groups are affected. Males are affected twice as often as female.

- **Socioeconomic status**: VL is usually more prevalent in the under-developed countries than developed countries. Poor people are affected most by VL.

- **Population movement**: Recently, the population movement is regarded as the important factor in epidemiology. Movement of the population from endemic to non-endemic areas results in the spread of infection.
Environmental Factors

✓ **Season:**
  - There is a seasonal variation of VL incidence in the endemic and hyper-endemic regions. Usually there is high prevalence during and after rainy season. There are two peaks of VL incidence in endemic regions of Bangladesh. One takes place in June and the other in November-December, which coincides, respectively to usual pre and post-monsoon seasons.

✓ **Rural area:**
  - The disease is usually confined to rural areas where conditions for breeding of sand flies is favourable compared to urban areas.

✓ **Altitude:**
  - Plains are the homeland of VL. It does not occur in altitude over 2000 feet as the sand flies are not found above this altitude due to unfavorable climatic conditions.
Bangladesh situation

- It affects 88 countries of the world of which 72 are developing and 13 of them are among the least developed countries.

- In Bangladesh, India, Nepal and Sudan, 90% cases of Visceral Leishmaniasis are reported. Conversely, 90% cases of Cutaneous leishmaniasis occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria.

- In India, VL has been continuing as endemic in the states of Bihar, Uttar Pradesh and West Bengal. More than 1 lakh new cases occur in India every year and state of Bihar accounts for more than 90% of those.

- In Bangladesh, about 18% of the total population (20 million) live in areas with active transmission of VL.
  - Highest number of VL cases were recorded in the district of Mymensingh followed by Pabna, Tangail and Gazipur. The incidence was assumed to be in the range of 15000-30000 per year.
Leishman and Donovan first described VL in 1903. Both of these physicians separately but simultaneously demonstrated parasites in stained smears from the spleen of patients suffering from a malaria-like illness, which became known as Visceral Leishmaniasis and its causative agent was named *Leishmania donovani* as per the names of two discoverers.

Swaminath *et al.*, 1942, proved using human volunteers that the *Leishmania* parasite could be transmitted by the Phlebotomine sand flies.
Phylum - Euglenozoa,

Class - Kinetoplastea,
  – Order - Kinetoplastida,
  – Family - Tyopanosomatidae,
  – Genus – Leishmania, Subgenus - Leishmania sensu stricto & Viannia,

• Species - L. donovani, L. infantum, L. tropica, Complexes: L. donovani, L. tropica, L. major, L. ethiopica, L. maxicana, L. braziliensis, L. guyanensis
Clinical Classification

1. **Visceral leishmaniasis or Kala-azar** is caused primarily by *L. donovani*, *L. chagasi*, or *L. infantum*. Dogs (feral or domesticated) are a reservoir for *L. chagasi*.

2. **Cutaneous leishmaniasis** usually divided into
   1. Old World leishmaniasis caused primarily by *L. tropica*, *L. major*, *L. aethiopica*, and
   2. New World leishmaniasis caused primarily by *L. Mexicana* or *L. braziliensis*.
   3. Diffuse cutaneous leishmaniasis is caused primarily by *L. aethiopica* or *L. mexicana*.

3. **Mucocutaneous leishmaniasis** (*espundia*) is caused primarily by *L. (viannia) braziliensis*.

Altogether, there are about 21 leishmanial species that are transmitted by about 30 species of sand flies.
Clinical classification...

- **Visceral leishmaniasis or kala-azar** on the basis of biological reservoir as follows:
  - African Kala-Azar - Rodent reservoir.
  - Mediterrarean Kala-Azar or infantile kala-azar - Canine reservoir.
  - Indian Kala-Azar - Human reservoir.

- **Cutaneous leishmaniasis** can also be divided further as follows:
  - Oriental sore - caused by *L.tropica*.
  - Espundia or Mucocutaneous leishmaniasis - caused by *L.braziliensis*.
  - Dermal leishmanoid or PKDL, a late sequel to visceral infection.
Morphology

- Exist in both amastigote and promastigote forms.
- **Amastigote (Aflagellar stages)** form resides in the reticulo-endothelial system of the vertebrate hosts:
  - It is usually short oval or pear shaped or slender spindle shaped bodies, about 5-10 µm in length. Cell membrane is delicate and can be demonstrated in fresh specimens only. Nucleus lies centrally. Kinetoplast (DNA containing rod shape body) lies transversely near the anterior end. Flagellum may be of same length as the body or even longer, projecting from the front. Nucleus is less than 1 µm in diameter. It is oval or rounded and situated in the middle of the cell or side of the cell wall.
- **Promastigote (Flagellar stages)**: this stage of the parasite is only encountered in cultures and insect vectors (sand flies):
  - It is usually rounded or oval body, about 2-5 µm in diameter
life cycle
Life cycle

The vertebrate host is infected with promastigotes when bitten by the vector.

The amastigotes are released in the vector’s gut, and the parasite reproduces as promastigotes.

The promastigotes enter circulating macrophages and reproduce as amastigotes.

The vector (a sand fly) ingests macrophages when it ingests blood.

The macrophage dies, the amastigotes are

The "type" of leishmaniasis (i.e., cutaneous, visceral, etc.)
Life cycle at a glance

• Life cycle stages: Amastigote, promastigote
• Host: Two hosts, man (definitive) and sandfly (Intermediate)
• Vector: Sand fly
• Reservoir host: Varies with area wild or domestic mammals (such as the fox, jackal, rodents) or human beings
• Infective form: Promastigotes
• Pathogenic form: Amastigotes
• Route of infection: Penetration through skin by the bite of female sandflies.
• Site of localization: Mononuclear phagocyte system
Transmission

- **Vector borne transmission**: VL is transmitted to man by the bite of infected female sand fly. Once the vector is infected it remain infective throughout its life time and capable of infecting several persons.

- **Direct transmission**: Due to contamination of the bite wound. Accidental inoculation of parasite during laboratory work results in leishmaniasis. Transmission by blood transfusion is also recorded.

- **Congenital transmission**: Rarely occurs. Transmission of infection to fetus in uterus may occur due to placental defect.
Virulence factors

1. Intracellular parasitism: Most important ***
2. Resistance to serum complement components
3. Glycoprotein 63 (gp-63)
4. Acid phosphatases (ACP)
5. Scavanger enzymes: Superoxide dismutase, catalase & glutathione peroxidase
6. Cysteine proteininases and megasomes
Pathogenesis of VL

✔ Visceral leishmaniasis is also known as Kala-azar (Hindi: kala=black, azar=sickness). The etiological agents belong to the *Leishmania donovani* complex, *L.d donovani*, *L.d infantum* and *L.d archibaldi* in the old world and *L.d chagasi* in the new world.

✔ Visceral leishmaniasis or Kala azar has many features in common with chronic malaria. Kala-Azar or Dum Dum fever is characterized by a low degree of fever with hepatoplenomegaly and severe progressive cachexia (wasting), swollen lymph glands, leucopeania, thrombocytopenia with relative monocytosis and loss of hair.

✔ The parasites (*Leishmania donovani*) are mainly found in bone marrow, liver and spleen. If left untreated this manifestation of leishmaniasis is fatal in more than 90% of cases.
Leishmaniasis is a slowly progressive disease that can take up to 7 years to become clinically apparent. Even then, signs are frequently nonspecific and a diagnosis of Leishmania is seldom considered.

One of the most consistent findings (~100%) with Leishmania sp. is the presence of hyperproteinemia due to hyperglobulinemia, often in conjunction with hypoalbuminemia (94%). Serum protein electrophoresis commonly reveals a polyclonal gammopathy consisting primarily of IgG immunoglobulins and some acute phase proteins.

Indirectly, increased antibody production and resultant immune-complex disease are responsible for many of the clinical and laboratory abnormalities encountered.
Pathogenesis of VL...

- Immune-mediated vasculitis leads to loss of proteins and cells resulting in hypoalbuminemia (94%) and thrombocytopenia (50%), whereas deposition of immune complexes into joints results in polyarthritis and lameness.

- Proteinuria (85%) and azotemia (45%) are associated with glomerulonephritis which is reported to be the leading cause of death in untreated animals.

- Liver failure occurs less commonly than renal failure, but elevations in liver enzyme activities, such as alanine aminotransferase (61%) and alkaline phosphatase (51%), are not uncommon.

- Hemostatic disorders such as epistaxis (15%), hematuria and disseminated intravascular coagulation (DIC) are not uncommon.
Cutaneous leishmaniasis or "oriental sore"

- The cutaneous manifestation of leishmaniasis is found mainly in North Africa, and in the Middle East and Central Asia, where it is caused by *L. tropica* and *L. major*. In the New World cutaneous leishmaniasis is caused by *L. mexicana*.

- At the location of the bite of the sandfly a papule develops which enlarges and then starts to necrotize centrally. The ulceration which may be acute leading to a moist ulcer, or slow in the case of a typical dry lesion is invaded with macrophages that serve as the host cells for the parasite and that permit the amastigotes to multiply.

- The ulceration may last for many months before healing occurs. Eventually granulomatous swelling subsides, and the ulceration heals, generally leaving a large scar and major mutilation, regarded as characteristic in highly endemic areas.

- The lesion is painless and secondary infection is restricted to the dead tissue.

- In the case of the Old World disease, when cure has occurred a solid immunity has been acquired against re-infection by the same parasite.
Mucocutaneous leishmaniasis or "espundia"

- *L. d. braziliensis* causes in man a slow healing often very extensive oriental sore, which self-cures after a variable sometimes extended time. Following the cure of the initial lesion the infection may metastasize, reappearing on the mucosal surfaces of the oronasal region, causing the disfiguring and eventually often fatal disease of espundia.

- The mucocutaneous lesions do not self heal but continue to invade the tissues which become blocked with granulomatous infiltration, eventually necrotizing and eroding insidiously.

- An early manifestation is often the end of the nose, which becomes expanded, so producing the syndrome of 'tapir nose'.

- Death frequently results as an indirect consequence, due to bacterial super-infection or obstruction of airways or the food passage.
Post Kala-azar DermaL Leishmaniasis (PKDL)

✔ Post Kala-azar dermal leishmaniasis (PKDL) Usually follows recovery from a Kala-azar infection.

✔ The disease begins with small measles-like lesions (hypo pigmented macules, papules or nodules) appearing on the face and gradually increase in size (rarely greater than 1cm in diameter).

✔ Eventually the lesions spread to the upper trunk, arms, forearms, thighs, legs, abdomen, the neck and the back.

✔ The multiple lesions can coalesce to form larger lesions and can lead to the gross enlargement of facial features such as the nose and lips, giving an appearance similar to leprosy.

✔ The disease is particularly severe if the lesions spread to the mucosal surfaces of the nasal septum, hard and soft palate, oropharynx, larynx or the eye lids and the cornea leading to blindness.
Post Kala-azar DermaL Leishmaniasis (PKDL)

- The lesions are usually self-limiting, however those that do not heal spontaneously within six months have to be treated.
- Pentavalent antimonials remain the drugs of choice for treating PKDL.
- Sodium stibogluconate at a dose of 20 mg/Kg of body weight administered intramuscularly for 4-5 months is recommended. In addition Ketoconazole and allopurinol can be given orally to improve response.
- In antimony resistant cases amphotericin B is an effective replacement. Indian PKDL appears any time between 1-7 years after apparent cure of Kala-azar, although longer periods of up to 20-30 years have been reported.
- The African form of the disease usually appears within a few months after cure, in most cases within 6 months, on average within 56 days.
- However it can develop during the treatment of Kala-azar, in which case the term Para Kala azar dermal leishmaniasis would seem more fitting.
Immunity

- *Leishmania* is an intracellular parasite that escapes from the humoral immune response by hiding as an amastigote in the lysosomes of the host cell.
  - This has important implications for the immunological response of the host towards the parasite, because: the intracellular parasite is not exposed to the host's humoral response, the circulating antibodies have no effect on the infection and may even be harmful. So, the immunity is entirely cell-mediated.

- T cells of the CD8 subtype seem to play an important role in controlling the infection by *Leishmania*. Only the cutaneous form of leishmaniasis is self-curing. This indicates that it is possible to develop a certain degree of immunity against the parasite, resulting in healing of the ulcers.

- However, the parasites probably never disappear completely from the body, since in situations where the immune system is compromised, such as in AIDS, or is suppressed by cancer chemotherapy or in cases of organ transplantation, leishmaniasis may suddenly reappear.

- The fact that the immunological response plays an important role in controlling cutaneous leishmaniasis indicates that vaccination is possible.
Laboratory Diagnosis

✓ Principle:

- Leishmaniasis can be diagnosed by demonstration of the parasite (LD bodies) from the cutaneous, mucosal lesions and incase of visceral leishmaniasis from Bone marrow, Spleen or Lymph node aspirate.

- It can also be diagnosed by detection of antigen or antibody by immunological tests and isolation of the organism by culture if facilities are available. Nucleic acid techniques are also helpful.
Laboratory Diagnosis

- **Microscopy**: Demonstration of the parasite (LD bodies) from the Bone marrow, Spleen or Lymph node aspirate and buffy coat preparation is the hallmark of diagnosis in case of Kala-azar. Leishman stained smear is very helpful.

- **Immunological tests**: Immunological diagnosis is based on the presence of specific humoral antibodies in blood or Leishmanial antigen in urine. There is a range of serological methods available for the diagnosis of VL varying in accuracy and specificity.
  - *Indirect fluorescent antibody tests (IFAT)*,
  - *Direct agglutination test (DAT)*,
  - *Enzyme linked immunosorbent assay (ELISA)*,
  - *Immunochromatography (ICT)* is commercially available.
  - Aldehyde test is a non-specific serological test, which detects marked increase in IgG.

- **Nucleic acid based techniques**: PCR has 90% sensitivity and 100% specificity and can be used where facilities are available.
Treatment: Traditional drugs

- **Pentavalent antimonials**: Sodium Stibogluconate and meglumine antimoniate were first introduced in the 1940's and have since been used as first-line chemotherapeutic agents against all forms of leishmaniasis including visceral leishmaniasis. Sodium stibogluconate is usually administered at a dose of 20 mg per Kg body weight for 20-40 days, however due to wide-spread antimony resistant cases of Indian PKDL and kala azar, treatment over **four months** is recommended.

- **Pentamidine**: Pentamidine isethionate (Pentamidine) or pentamidine dimethane sulphonate (Lomidine) is used. Both of these compounds are very effective in the treatment of Kala-Azar but because of their toxicity and potential side effects they are used as drugs of second choice.

- **Amphotericin B**: Amphotericin B is another drug of second choice. It is very active, in vitro, killing extracellular and intracellular forms of leishmania at concentrations of 1 mg per millilitre of medium. Amphotericin B was up to 400 times as potent as sodium stibogluconate.
Treatment : New drugs

✓ Lipid associated Amphotericin B currently under trial for treatment of leishmaniasis.

✓ Miltefosine
  – First oral drug for visceral leishmaniasis. This new therapy for “Kala-azar is 95% effective. Miltefosine is likely to cost less and is much easier to deliver than all current therapies.

✓ Immunotherapy
  – Interferon gamma (IFN-gamma)
Prevention

- Early detection by serological diagnosis and treatment of infected persons, especially in areas where humans are the only or important reservoirs of infection.

- **Personal protection from sandfly bites by:**
  - Using insect repellants.
  - Avoiding endemic areas especially at times when sandflies are most active.
  - Use of pyrethroid impregnated bednets and curtains.

- Vector control by the use of insecticide spraying of houses such as DDT, Malathion, Fenitrothion, Propoxur, and Diazinon.

- **Destruction of stray dogs and infected domestic dogs in areas where dogs are the main reservoir hosts.**

- Elimination and control of rodents in areas where these are the sources of human infection.
Summary

1. Leishmania is a hemoflagellate, that causes Leishmaniasis affecting more than 12 million people of the world with annual incidence rate of 1 to 1.5 million cases of Cutaneous leishmaniasis and 500,000 cases of Visceral leishmaniasis.

2. The genus Leishmania has many species, of which Cutaneous leishmaniasis is caused by – L. tropica, Diffuse leishmaniasis by L. amazonensis, Mucocutaneous leishmaniasis by L. braziliensis and Visceral leishmaniasis by L. donovani.

3. Leishmania has two stages in their life cycle, Amastigote and Promastigote of which Promastigote is the infective stage found in sand fly and in culture. Amastigote (LD body) stages are found in the tissue macrophages of man and are responsible for pathogenicity. It is also diagnostic form.

4. Leishmaniasis are manifested as cutaneous, mucocutaneous and visceral lesions. Of its three clinical forms, Visceral Leishmaniasis is the most severe one. Mucocutaneous leishmaniasis (MCL) is a mutilating disease. Diffuse cutaneous Leishmaniasis (DCL) is a disabiling disease.
Summary...

4. Microscopic detection of LD bodies from particular site of lesions is the gold standard for definitive diagnosis. Culture of parasites aid in the definitive diagnosis. Antigen and antibody detection also practiced reliably through out the world. Antibody detection against rK 39 antigen by Immunochromatography (ICT) is now popular method of non-invasive diagnosis.

5. Treatment of leishmaniasis is done by 1st line injectable drug pentavalent antimony compounds. In resistant and treatment failure cases, pentamidine and amphotericin B is given. Recently, miltefosine has shown higher cure rate and safely being used as an oral drug in India.

6. Prevention depends on successful control of sand fly vectors by insecticides, adequate treatment of Kala-azar cases and generating health consciousness among illiterate and poor population. Vaccine is not yet available.
Study Questions

1. What is leishmaniasis? Name its different forms with causative agents.
2. Briefly describe the world situation of leishmaniasis.
3. Name different vectors of leishmaniasis as per geographic location.
4. What are the possible modes of transmission of leishmaniasis?
5. Name the reservoir hosts for leishmaniasis.
6. Outline different virulence factors of *leishmania*.
7. Briefly describe the pathogenesis of visceral leishmaniasis.
8. What are the immunologic events related in leishmaniasis?
9. How can you diagnose different form of leishmaniasis?
10. What do you know about treatment and new drugs of leishmaniasis?
11. How can we prevent leishmaniasis?