Medical Laboratory Techniques Department
Lecture 5 : *L.donovani*
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L. donovani

Pathogenicity

L. donovani causes visceral leishmaniasis or kala-azar.

- Kala-azar is a reticuloendotheliosis resulting from the invasion of reticuloendothelial system by *L. donovani*.
- The parasitized macrophages disseminate the infection to all parts of the body.
- In the spleen, liver, and bone marrow particularly, the amastigotes multiply enormously in the fixed macrophages to produce a 'blockade' of the reticuloendothelial system. This leads to a marked proliferation and destruction of reticuloendothelial tissue in these organs.

Clinical Features of Kala-Azar

- The onset is typically insidious يزداد تدريجيا. The clinical illness begins with fever, which may be continuous, remittent متقطع, or irregular.
- Splenomegaly starts early and is progressive and massive.
- Hepatomegaly and lymphadenopathy also occur but are not so بارزprominent.
- Skin becomes dry, rough, and darkly pigmented (hence, the name Kala-azar).
- The hair becomes thin and brittle هش.
- Cachexia هزال with marked anemia, emaciationارهاق, and loss of weight isseen.
- Epistaxis رعاف and bleeding from gums are common.
- Most untreated patients die in about 2 years, due to some inter current disease such as dysentery, diarrhea, and tuberculosis.

Post Kala-azar Dermal Leishmaniasis

About 3–10% cases of patients of visceral leishmaniasis in endemic areas develop PKDL, about a year or 2 after recovery شفاء from the systemic illness.

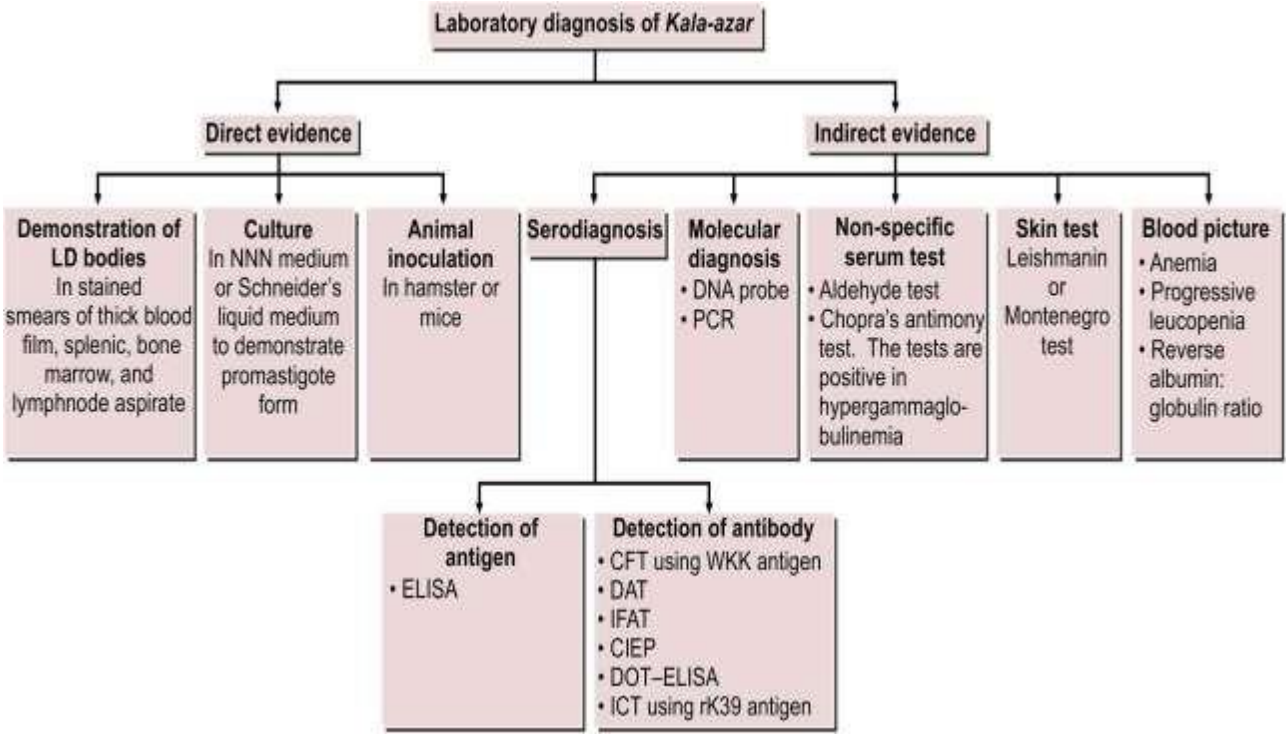
- PKDL is seen mainly in India and East Africa and not seen elsewhere. The Indian and African diseases differ in several aspects; important features of PKDL in these two regions.
- PKDL is a non-ulcerative lesion of skin. The lesions are of 3 types.
  - Depigmented macules بقع بدون صبغة : These commonly appear on the trunk and extremities and resemble tuberculoid leprosy الجذام السلي.
  - Erythematous patches البقع ال حْمامية: These are distributed on the face in 'butterfly distribution'.

- Nodular lesion عقيدية انة: Both of the above mentioned lesions may develop into painless yellowish pink no ulcerating granulomatous nodules.

- The parasite can be demonstrated in the lesions.

Immunity

- The most important immunological feature in Kala-azar is the marked suppression of cell mediated immunity to leishmanial antigens. This makes unrestricted intracellular multiplication of the parasite possible.
  Cellular responses to tuberculin and other antigens are also suppressed and may be regained some 6 weeks after recovery from the disease.
- In contrast, there is an overproduction of immunoglobulins, both specific anti-leishmanial antibodies as well as nonspecific polyclonal IgG and IgM.
  Circulating immune complexes are demonstrable دليل وجوده in serum.



Comparison of aspiration biopsies

Although splenic aspiration is the most sensitive method (98% positive), bone marrow puncture (50–85%, positive) is a safer procedure when compared to spleen puncture ثؤب, as there is risk of hemorrhage in splenic puncture particularly in patients with advanced stage of disease with soft enlarged spleen. Splenic aspiration is contraindicated in patients with prolonged prothrombin time or if platelet count is less than 40,000/mm3. Liver biopsy is also not a safe procedure and carries a risk of

hemorrhage. Lymph node aspiration is positive in 65% of cases of African Kala-azar, but not useful in cases of Indian Kala-azar.

Culture

Different tissue materials or blood are cultured on NNN medium. This is a rabbit blood agar slope consisting of 2 parts of salt agar and 1 part of defibrinated rabbit blood. The material is inoculated into the water of condensation and culture is incubated at 22°–24°C for 1-4 weeks. At the end of each week, a drop of culture fluid is examined for promastigotes under high power objective or phase contrast illumination. Other biphasic medium, like Schneider's drosophila tissue culture medium with added fetal calf serum can also be used.

Serodiagnosis

- **Detection of antigen:** The concentration of antigen in the serum or other body fluids is very low. ELISA and PCR have been developed for detection of leishmanial antigen.
- **Detection of antibodies:** CFT was the first serological test used to detect serum antibodies in visceral leishmaniasis. The antigen originally used, was prepared from human tubercle bacillus by Witebsky, Kliengenstein, and Kühn (hence, called WKK antigen). CFT using WKK antigen becomes positive early in the disease, within weeks of infection. Positive reaction also occurs in other conditions, including tuberculosis, leprosy, and tropical eosinophilia.
- Specific leishmanial antigens prepared from cultures have been used in a number of tests to demonstrate specific antibodies. These tests include:

- Indirect immunofl uroscent antibody test (IFAT)
- Counter immunoelectrophoresis (CIEP).
- ELISA and DOT-ELISA
- DAT
- A specific rapid immunchromatographic dipstick (ICT) method for antibody has been developed using a recombinant leishmanial antigen rk 39 consisting of 39 amino acids conserved in kinesin region of L. infantum. The sensitivity of the test is 98% and specificity is 90%.

Leishmania Tropica Complex

It includes 3 species:

- *Leishmania tropica*
- *Leishmania major*
- *Leishmania aethiopica*

- All these species cause old world cutaneous leishmaniasis. The disease is also known as oriental sore, Delhi boil, Bagdad boil, or Aleppo button حلب حبة.

Morphology

Morphology of *L. tropica* complex is indistinguishable from that of *L. donovani.*

Mode of transmission

- The most common mode of infection is through bite of sandflies.
- Infection may also sometimes occur by direct contact.
- Infection may be transmitted from man-to-man or animal-to-man by direct. inoculation of amastigotes.
- Infection may also occur by autoinoculation.
  - The amastigotes are present in the skin, within large mononuclear cells, neutrophils, inside capillary endothelial cells, and also free in the tissues.

Clinical Features *L. tropica*

Causes Old World Cutaneous leishmaniasis.

- Features of the disease vary with epidemiological pattern from region to region.
- Three distinct patterns of old world cutaneous leishmaniasis have been recognized.
- The anthroponotic urban type النوع الحضري الناتج عن الانسان causing painless dry ulcerating lesions, leading to disfiguring scars مشوهة ندبات, caused by the species *L. tropica*.
- This is prevalent from the Middle East to north-western India. The most important vector is *P. sargenti*.
- It is seen mainly in children in endemic areas and is called as oriental sore or Delhi boil.
- It begins as a raised papule, which grows into a nodule that ulcerates over some weeks.
- Lesions may be single or multiple and vary in size from 0.5 to more than 3 cm. Lymphatic spread and lymph gland involvement may be palpable and may precede the appearance of the skin lesion.
- The margins of the ulcer are raised and indurated متصلبة.
- The ulcer is usually painless unless secondary bacterial infection occurs.
- There may be satellite lesions, especially in L. major and *L. tropica* infections.
- The dry ulcers usually heal spontaneously in about

- The zoonotic rural type causing moist ulcers which are inflamed, often multiple, caused by *L. major*.
- The incubation period is usually less than 4 months.
- Lesions due to L. major heal more rapidly than *L. tropica*
- This is seen in the lowland zones of Asia, Middle East, and Africa.
- Gerbils, rats, and other rodents are the reservoirs.
- *Phlebotomus papatasi* is the most important vector.

Laboratory Diagnosis

- Microscopy
- Culture
- Skin Test
- Serology

New World Leishmaniasis *L. braziliensis* complex and *L. mexicana* complex

Habitat

These occur as intracellular parasite. The amastigote form is seen inside the macrophages of skin and mucous membrane of the nose and buccal cavity الذجويف الفموي. The promastigote form occurs in vector species *Lutzomyra*.

Clinical Features

L. mexicana complex leads to cutaneous leishmaniasis which closely resembles the old world cutaneous leishmaniasis. However a specific lesion of caused by *L. mexicana* is chiclero ulcer which is characterized by ulcerations in pinna الصيوان.

- Chiclero ulcer is also called as **self-healing sore of Mexico**.
  L. brazilensis complex causes both mucocutaneous leishmaniasis and cutaneous leishmaniasis.
- *L. braziliensis* causes the most severe and destructive تدميري form of cutaneous lesion.
- It involves the nose, mouth, and larynx.
- The patient experiences يتعرض a nodule at the site of sandfly bite with symptoms consistent تتفق with oriental sore. Subsequent mucocutaneous involvement leads to nodules inside the nose, perforation ثقب of the nasal septum الحاجز الانفي, and enlargement of the nose and lips (espundia).
- If the larynx is involved, the voice changes as well.
- Ulcerated lesions may lead to scarring and tissue destruction that can be disfiguring.
- The disease occurs predominantly in Bolivia, Brazil, and Peru.

Laboratory Diagnosis

- **Microscopy**: Amastigotes are demonstrated in smears taken from lesions of skin and mucous membrane. L mexicana amastigotes are larger than those of *L braziliensis* and their kinetoplast is more centrally placed.
  Biopsy Amastigotes can also be demonstrated from slit-skin biopsy.
- **Culture:** Culturing material obtained from ulcers in NNN medium demonstrates promastigotes. *L. mexicana* grows well in comparison to *L. brazilensis*, which is a grows slowly.
- **Serology:** Antibodies can be detected in serum by IFA test, which is positive in 89–95% of cases. ELISA is also a sensitive method to detect antibody; being positive in 85% of cases.
- **Skin Test**: Leishmanin test is positive in cutaneous and mucocutaneous leishmaniasis.