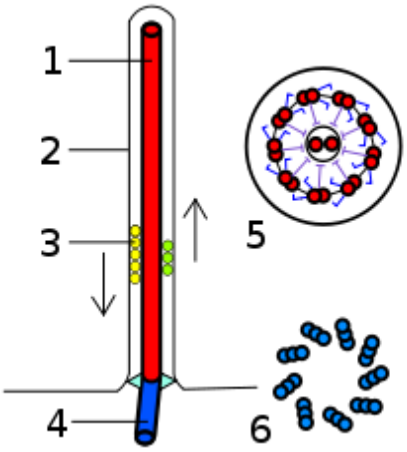
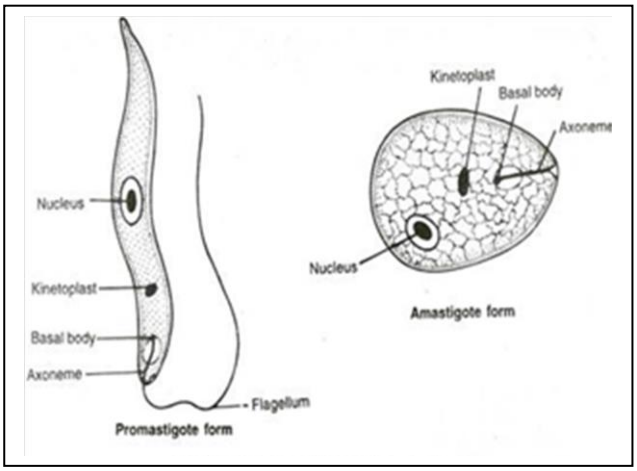
HAEMOFLAGELLATES

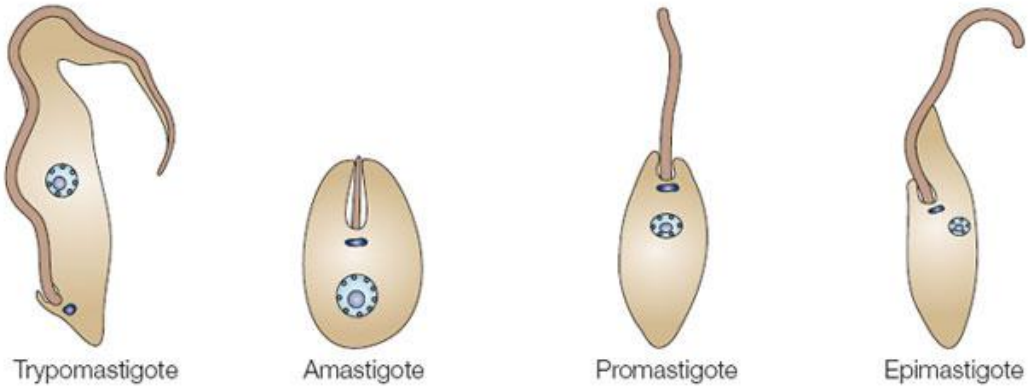
hemoflagellate protozoan of the Sarcomastigophora phylum, Mastigophora subphylum, Kinetoplastida order, Trypanosomatidae family, and is characterized by the presence of one flagellum and a single mitochondrion, which contains the kinetoplast, a specialized DNA-containing organelle. Medically important haemoflagellates require two hosts to complete their life cycle; some are called (digenetic or heteroxenous). They live in the blood and tissue of human and other vertebrate hosts and also in the gut of insect vectors. Members of this family have a nucleus, kinetoplast and single flagellum. A **kinetoplast** is a network of circular DNA (called kDNA) inside a large mitochondrion that contains many copies of the mitochondrial genome. The most common kinetoplast structure is a disk, but they have been observed in other arrangements. Kinetoplasts are only found in protozoa of the class Kinetoplastida. The variation in the structures of kinetoplasts may reflect phylogenic relationships between kinetoplastids. During the trypanosome life cycle, the position of the kinetoplast changes relative to other cell organelles, but it always remains close to the basal body. **kinetoplast** consists from dot-like blepharoplast and parabasal body

beside it. The flagellum arises from basal body (blepharoplast), **is a protein structure found at the base of a eukaryotic (cilium or flagellum). It is formed from a centriole and several additional protein structures,** The basal body serves as a nucleation site for the growth of the axoneme microtubules the position of flagellum which is inside the body of parasite named the axoneme or axial filament.



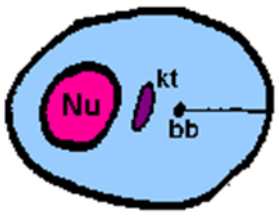


Haemoflagellates exist in two or more of four **morphological stages**, these are



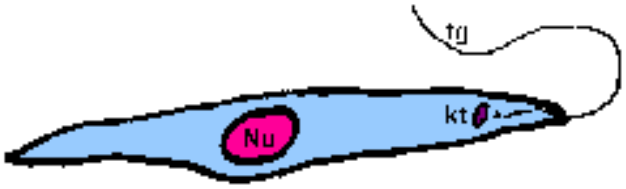
1. Amasitgote (leishmanial form):

This stage is rounded or oval shaped without flagellum. The nucleus, kinetoplast and axial filament can be seen. 2-5 microns in diameter, surrounded by delicate cell membrane, have single vesicular nucleus with large central karyosome, This is the stage in which T. cruzi and leishmania are found intracellularly in vertebrate hosts.



2. Promasitgote (leptomonal form):

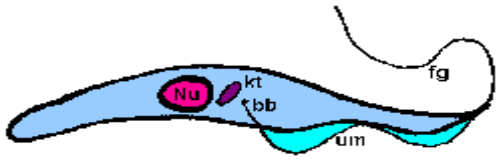
This stage is elongated (spindle in shape), the kinetoplast is anterior to the nucleus, From blepharoplast, single free flagellum projects from the anterior end, equal or longer than the body length.This form has no undulating membrane



this is the infective stage of leishmania found in the mid-gut of insect.

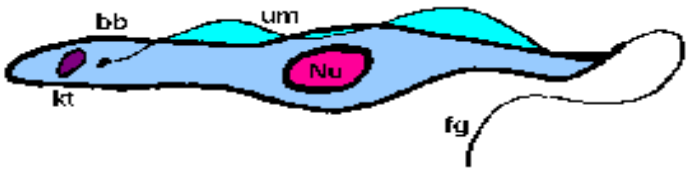
3. Epimastigote (crithidial form):

This stage is elongated with the kinetoplast placed more posteriorly and in front the nucleus. The flagellum extends alongside the body as a short undulating membrane. Before protruding from the anterior end. This is the stage in which T. gambiense & T. rhodesiense occur in the salivary gland of tsetse fly, and T. cruzi in the mid-gut of the vector reduviid bug (triatomate). This stage lacking in leishmania.



4. Trypomastigote (trypanosomal form):

This stage is more elongated, spindle-shaped with central nucleus and the kinetoplast posterior to the nucleus, situated at the posterior end of the body. The flagellum extends alongside the entire length of the cell to form long undulating membrane before protruding from anterior end. This is the infective stage of trypanosomes found in the vector arthropod and the stage found in the blood of infected vertebrate. This stage lacking in leishmania.



Haemoflagellates infecting human belong to two genera, in the family trypanosomatidae (trypanosome and leishmania).

LEISHMANIA

All members of this genus are obligatory intracellular parasites that pass their life cycle in two hosts, the mammalian host and insect vector (female of sand fly). In the human and other mammalian hosts, they multiply within the macrophages, in which they occur exclusively as the amastigote form while in the sand fly, they occur as promastigote form.

Leishmaniasis is considered to be zoonotic disease, the infection being maintained in the endemic areas in dogs, wild rodents or other mammals as reservoir hosts.

Leishmania

It includes parasites cause three diseases in human:

1- Cutaneous Leishmaniasisor oriented sore.

2- MucocutaneousLeishmaniasisor Espondia

3- Visceral Leishmaniasis or Kala-azar

Clinical classification of Leishmaniasis:

Leishmania parasitic in human classified in to two broad groups:-

1-Visceral leishmaniasis: is the most severe form of leishmaniasis caused by *L. donovani* and transmitted by *Phlebotomus* sand flies. This disease is the second-largest parasitic killer in the world (after malaria), responsible for an estimated 500,000 infections each year worldwide. The parasite migrates to the internal organs such as liver, spleen and bone marrow, and, if left untreated, will almost always result in the death of the host

2-**Cutaneous leishmaniasis**:

Oriental sore:- (Old world leishmaniasis),transmitted by Phlebotomus sand fly and caused by *L. tropica* :

a-L.tropica minor---------- dry type, causes urban cutaneous leishmaniasis, reservior host is dog.

b-L.tropica major---------- wet type, cause rural cutaneous leishmaniasis, reservior host is rodent.

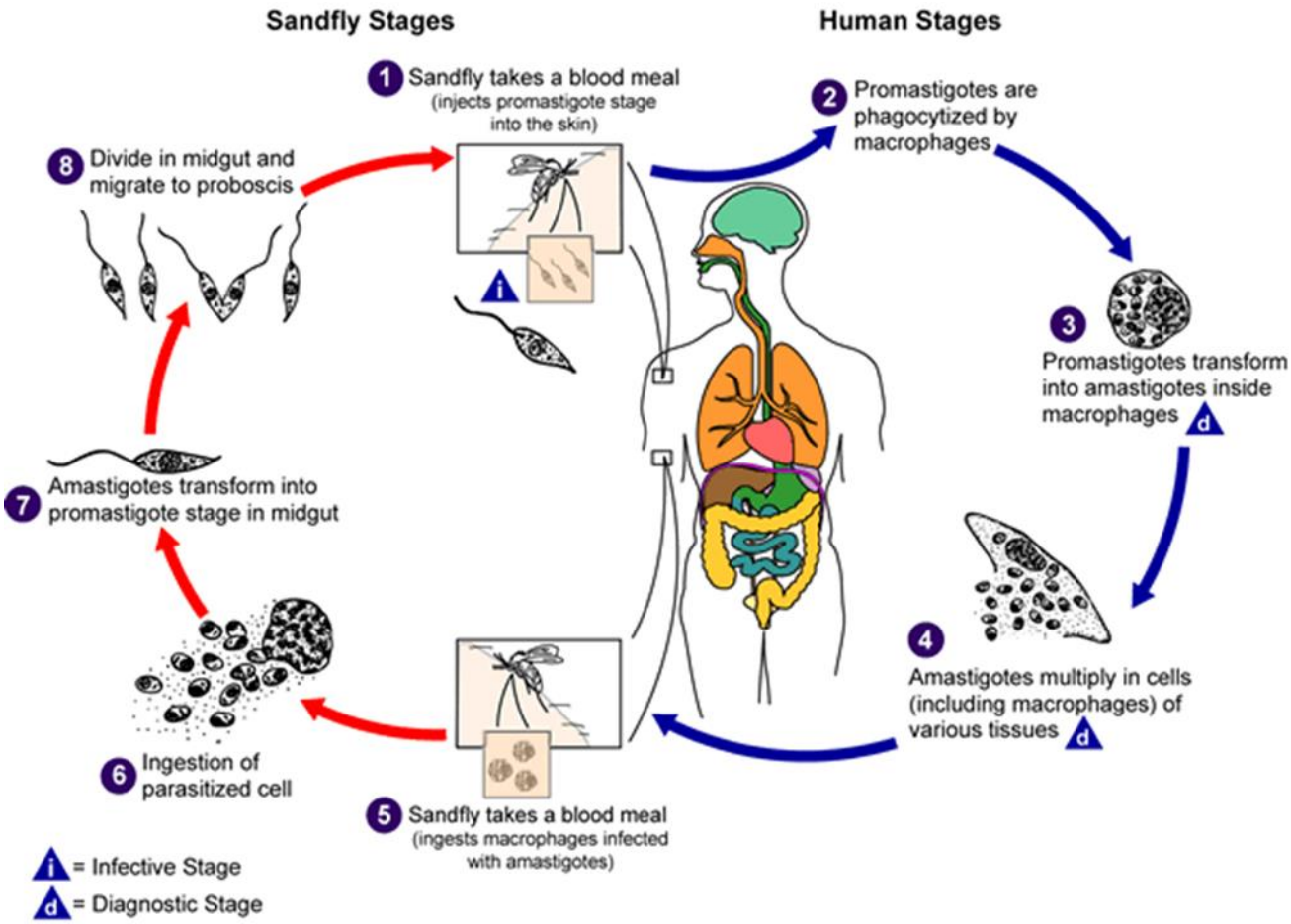
3-**Mucocutaneous Leishmaniasis:** no visceral manifestation, these include:

Espundia or nasopharyngeal leishmaniasis: (New world leishmaniasis), transmitted by Lutzomyia spp. And caused by L.braziliensis, caused cutaneous and mucocutaneous leishmaniasis.

Post kala-azar dermal leishmaniasis (PKDL), also this named as dermal leishmanoid, it is a late sequel of visceral leishmaniasis infection caused by L.donovani.

Life cycle

Involves an alternative existence in a vertebrate (man,…ect) and an insect (sand fly).The flagellated **promastigote** enter the body (skin) of the final host through infected sand fly bite → the parasites engulfed by macrophage and endothelial cells of skin capillaries →promasitgote develops into amastigote (**Leishman-Donovan (LD) bodies**) →amastigote multiply inside macrophages by binary fission → cell burst → free amastigote either infect other cells (macrophages) in skin as in cutaneous leishmaniasis or other cells in skin and the adjacent cells in mucous membrane as in mucocutaneousleishmaniasis or pass to different organ by blood stream (spleen, liver, bone marrow and lymph nodes) as in visceral leishmaniasis, then amastigotes engulfed by new reticuloendothelial cells →a blood sucking sand fly (female) draws amastigotes (L.D bodies) with its blood meal (by bites of proboscis) →amastigotes develop in promastigote forms in the mid gut of sand fly → multiply by longitudinal binary fission → solid mass of promastigotes fill up the anterior end of the mid gut and the esophagus , extending up to the pharynx → a heavy pharyngeal infection of the sand fly is known as anterior station development , which may block esophagus → at the time of sucking blood , vomiting of promastigotes from their buccal cavity in the skin puncture by proboscis (biting organ) → infection of man.



Life Cycle of leishmania

General characters of genus Leishmania

1- Life cycle is indirect and completed in tow hosts, vertebrate (human, dog, rodent) as a final host and invertebrate; blood sucking insect (female of sand fly) as an intermediate host (vector).

2- Tow developmental forms are found, amastigote and promastigote ,amastigote in the final host (human) and promastigote in the vector (sand fly).

3- The vector is sand fly of genus Phlebotomus in Old World and genus Lutzomyia in New World.

4- Promastigote is the infective stage to final host (man) and amastigote is infective stage to sand fly (vector).

5- The parasite infects the reticuloendothelial cells of skin, mucus membrane or viscera (as liver, spleen and bone marrow) of the final host (man).
6- The parasite multiplies by binary fission (asexual).

1-Cutanous Leishmaniasis

Leishmania tropica:-

Disease: - Oriental sore, Delhi ulcer, Aleppo boil, Delhi boil or Baghdad boil, cutaneous leishmaniasis.

Morphology and life cycle:-

The morphology of *L. tropica* is similar to that of typical leishmanian parasite except large sized forms. In man, the leishmanian forms occur intracellularly. In the margins of the cutaneous lesions, the intracellular forms are in large mononuclear leukocytes, polymorphonuclear leukocytes, neutrophils and epithelial cells also free in the tissues, released by ruptured cells. They are ingested by sand flies feeding near the skin lesions. In the midgut of insect, the amastigote develop into promastigote (this leptomonal form similar to that of L. donovani). These are in turn transmitted to the skin of persons bitten by sand fly. In the skin, the promastigotes are phagocytosed by macrophage in which they become amastigotes and multiply, however they remain limited to the skin without being transported to the internal organs .The common mode of infection is through biting of sand fly, but 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.

Pathogenecity and symptoms:-

The parasite lives in the reticuloendothelial cells and lymphoid tissues of the skin and in the endothelial cell of the local capillaries and neighboring lymphatic glands, but it rarely metastasized to other sites and does not invade the viscera.

Tissue reaction is initiated with the introduction of promastigote into the dermis, macrophages in the surrounding area pick up the parasite which rapidly transform into amastigote and multiply, destroying the host cells, soon there is a dense concentration of macrophages in the invaded area, all of which are prone to infection and destruction.

The lesion then becomes necrotic at the center and the margins containing parasitized macrophages, the lesion appears first as a macule, then as a papule with a slightly raised center covered by a thin blister like layer of epidermis, then the lesion breaks down with discharge of a small amount of clear or purulent exudate, at its crater -like base in the dermis a granulating layer is formed. In the urban disease, the ulceration is slow and may not occur. There is comparatively little surrounding tissues reaction.

Healing time is one year or more, but if no bacterial interfering (secondary bacterial infection), takes place, the healing time may occur within 6 months.

The incubation period usually between 2-6 months, but may be shorter as 2 weeks or longer as 3 years. Also it is a self-limiting disease in the case of the absence of any complications and no systemic manifestations. But in cases of pyogenic complications, local ulcer, disfiguring, pain, neutrophilia, leukocytosis, fever, and sometimes septicemia.

Types of cutaneous and mucocutaneous leishmaniasis :-

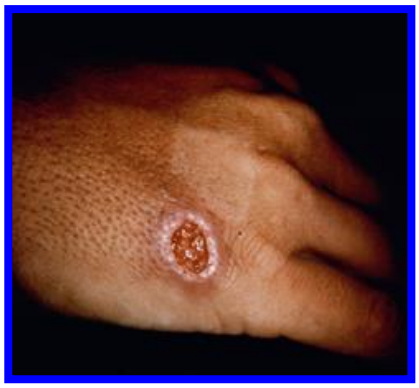
1- Old world C.L.:- in the old world, is also known as oriental sore, Baghdad boil, little sister, Delhi boil. This type caused by L. tropica major, L. tropica minor, and L. aethiopica. This is usually as self-limited, ulcerative skin leison of the uncovered parts of the body especially the face.

2- New world C.L.:- in the new world, CL is caused by species of L, mexicana and L. brazilinsis complex. The first is normally uncomplicated infection and self-limited. If the ear is infected, this is called (Chiclero`s ulcer) characterized by destruction of the cartilage and disfigurement of the ear. While the infection with the second primarly present as cutaeous leishmaniasis but are related to high risk for later progression to mucocutaneous disease.

3- Chronic forms of C.L. :-

* Patients lacking adequate cell mediated immunity may develop (**diffuse C.L.)** which characterized by numerous nodular non-ulcerating lesions develop, particularly on the face and limbs, which resemble the lesion of lepromatous leprosy.

* **Leishmaniasis recidivans** is the type of lesion seen in the persons with high degree of cell-mediated immunity to the parasite; the lesions are chronic with alternative periods of activity and healing, characterized by a central scar with peripheral activity. The lesions resemble those of lupus or tuberculoid leprosy. Parasites are very scanty in the lesions, and the leishmanian skin test is strongly positive.



Diagnosis:-

1. Direct skin aspiration :- smears stained with giemsa stain to demonstration the amastigotes of parasites in the materials obtained by puncturing the edges of the lesion .

2. (LST) leishmanian skin test and other serological methods.

3. Culture on NNN medium.

Treatment:

- Pentavalent antimonial are the drug of choice for treatment.
- Aminosidine ointment has been found to be useful as local treatment.

2-Mucocutaneous leishmaniasis

- *L. brazilinensis* produces a disease in humans known as **espundia, uta,** or mucocutaneous leishmaniasis. Morphologically, *L. braziliensis* cannot be differentiated *from L. tropica, L. mexicana, or L. donovani*.
- Life cycle is similar to other type, except, the vector is *Lutzomyia*.

- In some times the lesions appear as flat, ulcerated plaques that remain open and oozing.
- *L. braziliensis*, the parasites have a tendency to metastasize , or spread directly from the primary lesion to mucocutaneous zones.
- The secondary lesion often involves the nasal system and buccal mucosa, causing degeneration of the cartilages and soft tissues
- Necrosis and secondary bacterial infection are common. Espundia and uta are the names applied to these condition
- The ulceration may involve the lips, palate, and pharynx, leading to great deformity.
- Invasion of the infection into the larynx and trachea destroys the voice.

The condition may last for many years, and death may result from secondary infection or respiratory complications.

Diagnosis and treatment

- Diagnosis is established by finding L-D bodies in affected tissues
- Treatment is similar to kala-azar and tropical sore, antimonial compounds applied on the lesions or injected intravenously or intramuscularly .

Secondary bacterial infections should be treated with antibiotics

3-Visceral Leishmaniasis

Leishmania donovani:

This parasite causes visceral leishmaniasis in addition to post kala-azar dermal leishmaniasis.

The disease visceral leishmaniasis was first characterized in India, where it was known under the names kala-azar (meaning black sickness), Dum Dum fever, Burdwan fever or tropical splenomegaly.

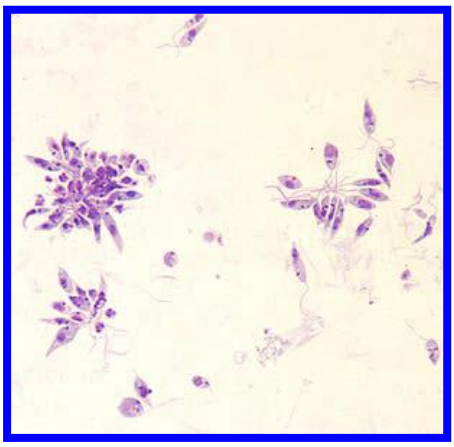
The infection is transmitted by the bite of the sand fly (phlebotomus argentipes) . Transmission of the disease by blood transfusion, sexual contact, inoculation and congenitally have been recorded, but these are extremely rare and with no epidemiological significance.Morphology and life cycle:

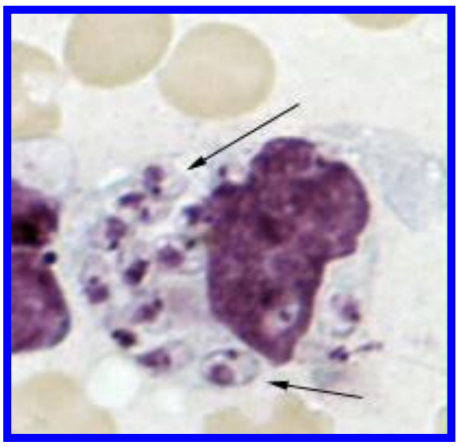
The parasite exists in two morphological forms in their life cycle, amastigote form in human and other mammals and the promastigote form in the sand fly and in artificial cultures.

The amastigote forms of the parasite as seen in smears from patients are called leishman-donovan bodies (LD bodies). (LD body) is ovoid or rounded cell, about 2-4 µm in size. It is typically intracellular, found inside **macrophages, monocytes, neutrophils or endothelial cells**. Smears stained with leishman, Giemsa, or wright stains show a pale blue cytoplasm enclosed by a limiting membrane, the nucleus is stained red. Laying at right angle to the nucleus the purple stained kinetoplast The axoneme arising from the blepheroplast to the anterior tip of the cell.

The habitat of the amastigote is the **reticulo-endothelial system**. They are found mostly within the macrophages in the spleen, liver, and bone marrow and less often in other locations such as the skin, intestinal mucosa and mesentric lymph nodes. They multiply by binary fission producing numerous cells that distend the macrophage and rupture it. The liberated cells are in turn phagocytosed by other macrophages and histiocytes. Small number of LD bodies can be found in peripheral blood inside polymorphnuclear leukocytes or monocytes. Rarely may be seen in feces, urine and nasal secretions.

When the vector feeds on an infected person, the amastigotes present in peripheral blood and tissue fluids enter the insect during its blood meal. In the midgut (stomach) of the sand fly the amastigote develops in to promastigote form. The promastigotes multiply by longitudinal binary fission and reach enormous numbers. In the sand fly they migrate from the midgut to the pharnyx and hypostome, where they accumulate and block the passage. Such blocked sandflies have difficulty in sucking blood. When they bite a person and attempt to suck blood, plugs of adherent parasites may get dislodged from the pharynx and deposited in the punctured wound, the promastigotes are phagocytosed by macrophages inside which they change in to amastigotes and start multiplying.





Clinical features:

Most infections are inapparent or subclinical and only about 3% of cases develop the typical kala-azar syndrome. The incubation period usually from **2-6 months**, though occasionally it may be as short as 10 days or as long as two years.

The onset is typically insidious. The clinical illness being with fever, which may be continuous, remittent or irregular. Splenomegaly starts early and is progressive prominent, the disease progresses for several months, with periods of pyrexia followed again by emaciating and anemia. The skin becomes dry, rough and darkly pigmented (hence the name kala-azar), the hair becomes thin and brittle. bleeding gums are common. Most untreated patients die in about 2 years due to some inter-current disease such as dysentery or tuberculosis.

Pathogenesis:

Kala-azar is a reticuloendotheliosis resulting from the invasion of reticulo-endothelial system by *L. donovani*. Parasitised macrophages disseminate the infection to all parts of the body. In the spleen, liver and bone marrow particularly, the amastigotes multiply enormous in the fixed macrophages to produce an (obstruction) of the reticuloondothelial

system. This leads to a marked proliferation of the reticuloendothelial tissue in these organs.

The spleen is the organ most affected, it is grossly enlarged and the capsule is frequently thickened, it is soft and friable and cuts easily without resistance, due to the absence of fibrosis. The cut section is red or chocolate in colour due to the dilated and engorged vascular spaces. The trabeculae are thin and atrophic.

The liver is enlarged, the kupffer cells and vascular endothelial cells are heavily parasitised . liver functions are not seriously affected, though prothrombin production is commonly decreased.

The bone marrow is heavily infiltrated with parasitised macrophages which may crowd in the haemopioetic tissues. Peripheral lymph nodes and lymphoid tissues of the nasopharynx and intestine are hypertrophic due to infiltration with parasitised cells.

Anaemia occurs as a result of infiltration of the bone marrow as well as by the increased destruction of erythrocytes due to hypersplenism; auotoantibodies to red cells may contribute to haemolysis. Leucopenia with marked neutropenia and thrombocytopenia are frequently seen.

PKDL

About 10-20% of patients who recover develop (PKDL) **post kala-azar dermal leishmaniasis**. The dermal lesions usually develop about a year or two and sometimes 20 years after recovery from the systemic illness. The lesions are of two types: -

* depigmented macules, which appear commonly on the trunk and extremities.

* Erythematous patches appearing on the face (butterfly patches), both of which develop into Painless yellowish-pink non ulcerating granulomatous nodules.

The parasites can be demonstrated in the lesions, the PKDL is seen mainly in India, it is rare in East Africa and China and not seen elsewhere.

Laboratory diagnosis:

Methods employed in laboratory are as follows:-

2- Demonstration of the parasites in materials obtained from patients by microscopy, culture, and animal inoculation. These material are:-

A- peripheral blood :-

Peripheral blood contains the amastigotes inside the circulating monocytes and less often in the neutrophils, but the numbers are so scanty that a direct blood smear may not show them. Chances of detecting them by examination of thick blood film or examine buffy coat smears though even these are not often found positive.

B- Bone marrow aspirates :-

It is the most common diagnostic specimen collected; generally the sternal marrow is aspirated by puncturing the sternum at the level of the 2nd or 3rd intercostal space, using a sternal puncture needle. This consists of a short stout needle with a stylet; it has a movable guard which is fixed at 1-2 cm from the tip, depending on the thickness of the chest wall over the sternum. After disinfecting and anaesthetising the skin, the needle is introduced into the sternal marrow and about 0.5 ml of marrow fluid aspirated using a syring. Bone marrow samples can be obtained also by puncturing the iliac crest.

C- Spleen aspirates :-

These are richer in parasites and valuable for diagnosis, but this procedure can sometimes cause dangerous bleeding. To guard against bleeding, prothrombin time and platelets count should be checked before the procedure.

These materials collected can be tested by:-

**Microscopy: - Smears are stained by leishman, giemsa or wright stains and examined under the oil immersion objective, ammastigotes can be seen in the large number within the macrophages.

** Cultures :- are made on (NNN) Novy-McNeal-Nicole medium , incubated at 24 C for 7 days .

** Animal inoculation: - Is not used for routine diagnosis, the materials are inoculated intraperitoneally or intradermally in to the skin of the nose and feet, the inoculated animals are kept at 23-26 C but takes several weeks to become positive.

3- Demonstration of antibodies or antigens (serological methods) by numbers of tests include :-

- Complement fixation test.

- Counter immunoelectrophoresis.
- Enzyme linked immunosorbent assay (ELISA).
- Immunofluorescent antibody test (IFAT).

4- Molecular diagnosis:- by using polymerase chain reaction (PCR) technique, this method based on the ampilification of the leishmania DNA by using specific primer which depend on the sequence of nitrogen bases in the leishmania genome .

Treatment :-

Pentavalent antimonial sodium stibogluconate is the drug of choice, given intravenously 600mg daily for 6 days.