Diagnosis of kala-azar

1- Microscopic detection of amastigotes (LD bodies) in Giemsa stained smear of bone marrow, spleen and s.t lymph node aspirates. Also, the specimens may be liver biopsy, buffy coat or thick blood film.

2- Cultivation of aspirates in specific culture medium as NNN medium → promastigotes seen.

3- Serological method (detection of specific anti-leishmanial antibodies):
   • IFAT (indirect immunofluorescent antibody test)
   • Immunochromatography strip test (rapid test)
   • DAT (Direct agglutination test)
   • ELISA (enzyme linked immunosorbent assay)
   • Immunoblotting (western blotting)

4- Molecular method (PCR polymerase chain reaction) the most sensitive and specific diagnostic method.

5- Leishmanin skin test (Montenegro test): in kala azar this test is negative.

6- Aldehyde test: it is non specific test, indicate reversal albumin / globulin ratio due to hypergammaglobulinaemia in VL.

Treatment

1- Sodium stiboyluconate (pentostam)
2- Pentamidine isothionate
3- Amphotericin B
Genus Trypanosoma

1-Trypanosoma cruzi

(American trypanosomiasis, Chagas’ disease)

Life cycle of Trypanosoma cruzi

- In vector (reduviid bug)
  1- Trypomastigotes ingested in blood meal of bug from infected vertebrate (man).
  2- Trypomastigotes transform to epimastigotes in midgut.
  3- Epimastigotes multiply by binary fission in midgut and migrate to hindgut.
  4- Epimastigotes transform to trypomastigotes in hindgut.
  5- Slender, infective (metacyclic trypomastigotes) formed in rectum (posterior station).

- In vertebrate (man)
  6- Transmission occurs by penetration of trypomastigotes in bug feces through skin abrasions or mucosa of vertebrate (man).
  7- Trypomastigotes do not multiply, they invade tissue cells of vertebrate.
  8- Trypomastigotes transform within tissue cells to amastigotes, which multiply forming pseudocyst.
  9- Amastigotes in pseudocyst transform to epimastigotes and trypomastigotes, then pseudocyst ruptures to release trypomastigotes which either re-enter other cells or enter the blood stream and ingested by the bug.

*Vector: Reduviid (kissing) bug of genera Triatoma and Panstrongylus.
*Final host: Man, dog, Cat and rodent.
life cycle of *Trypanosoma cruzi*

**Posterior station transmission in *T. cruzi***
After multiplication in the midgut of the insect vector, the parasites pass posteriorly and produce the infective forms (metacyclic trypomastigotes) in the rectum and the introduction of infective forms in bug feces to new hosts via skin abrasions or mucous membrane (especially conjunctiva).

Life cycle in bug need about 8-10 days to excrete infective metacyclic trypomastigotes in feces.

The amastigote forms are intracellular, within the cells of virtually every organ or tissue but cells of RES, cardiac, skeletal, smooth muscles and neuroglia cells are preferentially parasitized, while trypomastigotes are extracellular (present in peripheral blood).
**Modes of transmission**
1- Bite of reduviid bug (commonest mode).
2- Blood transfusion in endemic areas.
3- Congenital transmission.
4- Through contaminated syringes and needles.

At night the bugs emerge from the cracks in the walls and painlessly extract a blood meal from the sleeping people. These bugs defecate during or soon after biting. The bite wound is readily contaminated. The sleeping victim may also inadvertently scratch or rub the affected area, thereby helping the entry of the parasite into the body.

**The clinical aspect**
American trypanosomiasis (Chagas’ disease) is caused by *trypanosoma cruzi*.
This disease is distributed in central and south America. The disease is seen in most commonly and in its most severe form in children under 5 years, in whom symptoms of CNS involvement may be prominent, in older children and adults the disease is usually occurs in a milder subacute or chronic form which generally follows an acute attack.

**Diagnosis of Chagas’ disease**
1- Microscopic detection of trypomastigotes in peripheral blood:
   a) Wet mount preparation: trypomastigotes are only faintly visible, but their rapid progressive snake like motion among erythrocytes make their presence apparent.
   b) Fixed preparation by Giemsa stained smear (thick or thin blood smear): the parasites are typically C or U shaped. In young children the parasites may be detected in blood with ease, particularly at acute stage. In older children and adults, the parasites are frequently very difficult to find.
   c) Examination of concentrated blood smear.
2- Culture : such as on NNN medium.
3- Xenodiagnosis: at least 6 clean uninfected laboratory bred reduviid bug are allowed to feed on the suspected patient and two weeks later the hindgut of the bugs is examined for epimastigotes.

4- Biopsy examination: lymph node or skeletal muscle biopsy is examined for amastigotes.

5- Serological diagnosis: IFAT, RIA (radioimmunoassay), ELISA.

6- PCR (polymerase chain reaction).

7- Animal inoculation.

Amastigotes from biopsy of cardiac muscle

C – shape trypomastigotes in blood smear
2-Trypanosoma brucie

(African Trypanosomiasis, Sleeping sickness)

Life cycle of T. brucie

A- In vector (Tsetse fly)
1- Short stubby and long trypomastigotes ingested by tsetse fly during blood meal by proboscis and go to midgut of the fly.
2- The trypomastigotes transform to procyclic trypomastigote and multiply in midgut.
3- Then procyclic trypomastigotes migrate forward to the oesophagus → proboscis → to reach the salivary gland.
4- In fly's salivary gland, procyclic trypomastigotes transform into epimastigotes and multiply.
5- Epimastigotes in salivary gland transform to infective metacyclic trypomastigotes.

B- In vertebrate (man).
6- The infective metacyclic trypomastigotes pass from fly’s salivary ducts and injected into the bite wound of the vertebrate (man) at the time of blood meal and transform to blood trypomastigotes.
7- Trypomastigotes multiply inside bite asexually for 1-2 days before entering the blood circulation by lymphatics.
8- Trypomastigotes multiply extracellularly in blood at the site of the bite and/or in other tissue fluids (lymph and CSF) and the cycle repeated.

*Final host: man, antelope and other domestic and wild cattle.
*Vector (intermediate host): Tsetse fly (genus Glossinia), Glossinia morsitans for T. rhodesiense and Glossinia pallipidis for T. gambiense.

Life cycle of T. Brucie (T. gambiense and T. Rhodesiense)

Tsetse fly, vector of sleeping sickness
**Antigenic variation of T. brucie**
Parasitaemia in African trypanosomal infection persists inspite of strong antibody response because the parasite shows antigenic variation every week to 10 days. This phenomena occurs because the parasites shed thier outer varient surface glycoprotien (VSG) coat and replace it with another one. Each VSG is immunogenic but antigenically different from the previous sheded VSG → Thus the parasite escapes the host immune response.

**Diagnosis of African trypanosomiasis**
1- Microscopic detection of trypomastigotes in:
   a- Trypanosomal chancre. b- Peripheral blood. c- Bone marrow. d- Lymph node aspiration. e- CSF, by:
   2) Fixed preparation by Giemsa stained smear.
   3) Concentrated method.
2- Serological diagnosis:
   - IFAT (indirect fluorescent antibody test).
   - IHT (indirect haemoagglutination test).
   - ELISA.
   - CFT (complement fixation test).
   - Card indirect agglutination test.
3- Aldehyde test: positive due to reverse albumin/globulin ratio.
4- Serum and spinal fluid IgM measurement: are of diagnostic value, because in many cases the total serum IgM exceeds eight (8) times the normal amount.
5- Animal inoculation.
*Trypanosoma gambiense* or *rhodesiense* trypomastigote forms in blood smear