Tikrit University

College of Nursing

Basic Nursing Sciences

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Microbiology

Parasitology

Pathogenic amoeba

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Pathogenic amoeba

Laboratory Diagnosis of Entamoeba histolytica:

1. **Stool examination:** Examine fresh stool. Can use direct stool smear and concentration method as zinc sulfate floatation or formalin-ether technique.
    1. Find motile trophozoites in the fresh warm stool or trophozoites and cysts in the fixed stool specimen.
    2. Use stool examination for three executive days unless positive results are found.
2. Can see trophozoites that measure 15 to 20 µm with one small central nucleus. There may be the inclusion of RBC in the cytoplasm. Occasionally bacteria may be seen.
    1. With the help of a wire, the loop takes a small amount of blood and mucus on one side of the slide.
    2. Cover with cover glass without saline addition and press gently to make a thin smear.
    3. Make another slide with the eosine drop. Mix and cover with a cover glass.
        1. Eosin does not stain living amoeba, but the motile amoeba is in the pink background.
        2. Examine the preparation immediately.

Flagellates

Flagellates are unicellular microorganisms. Their locomotion is by lashing a tail-like appendage called a flagellum or flagella and reproduction is by simple binary fission.

There are three groups of flagellates:

1- Luminal flagellates: Giardia lamblia
2- Hemoflagellates: Trypanosoma species. Leishmania species.
3- Genital flagellates: Trichomonas vaginalis

***Trypanosoma Brucei Gambiense* (West African Trypanosomiasis)**

Diagnosis of African Trypanosomiasis

- Light microscopy of blood (thin or thick smears) or other fluid sample

Diagnosis of African trypanosomiasis is made by identifying trypanosomes in fluid from a chancre, lymph node aspirate, blood, bone marrow aspirate, or, during the late stage of infection, cerebrospinal fluid (CSF). Preferred sources are blood smears for *T. b. rhodesiense* and fluid aspirated from an enlarged lymph node for *T. b. gambiense*. Wet preparations should be examined for motile trypanosomes, and smears should be fixed, stained with Giemsa (or Field) stain, and examined. The concentration of trypanosomes in blood is often low, and concentration techniques (eg, centrifugation, miniature anion-exchange centrifugation, quantitative buffy coat technique) enhance sensitivity.

Antibody detection assays are not very useful clinically because seroconversion occurs after the onset of symptoms. However, a card agglutination test for *T. b. gambiense* is useful in mass screening programs to identify candidates for microscopic examination.

A lumbar puncture should be done in all patients with African trypanosomiasis. When CSF is involved, opening pressure may be increased, and CSF has elevated levels of lymphocytes (≥ 6 cells/mcL), total protein, and nonspecific IgM. In

addition to trypanosomes, characteristic Mott cells (plasma cells with cytoplasmic vacuoles that contain immunoglobulin [Russell bodies]) may be present.

Other, nonspecific laboratory findings include anemia, monocytosis, and markedly elevated serum levels of polyclonal IgM.

Leishmania

Laboratory Diagnosis
Laboratory diagnosis of Kalaazar depends upon direct and indirect evidences.

Microscopy

*Demonstration of amastigotes in smears of tissue aspirates is the gold standard for diagnosis of visceral leishmaniasis. For microscopic demonstration of the parasite, the materials collected are:
* Peripheral blood
* Bone marrow
* Splenic aspirate
* Enlarged lymph node.
*The smears are stained by Leishman, Giemsa, or Wright's stains and examined under oil immersion objective.
*Amastigote parasite can be seen within the macrophages, often in large numbers. A few extracellular forms can also be seen.

Peripheral blood smear:

* Peripheral blood contains the amastigotes present inside circulating monocytes and less often in neutrophils, but the numbers are so scanty that a direct blood smear may not show them.
* Chances of detecting them are somewhat improved by examination of a thick blood film.
*It is best to examine Buffy coat smear, although ven these are not often found positive.
* Buffy coat smears show a diurnal periodicity, more smears being positive when collected during the day than at night.

Bone marrow aspirate:

* Bone marrow aspirate is the most common diagnostic specimen collected.
*Generally, the sternal marrow is aspirated by uncturing 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 anesthetizing the skin, the needle is introduced into the sternal marrow and about 0.5 mL of marrow fluid is aspirated using a syringe. The puncture wound is sealed with celloidin or tincture benzoin. Bone marrow samples can also be obtained by puncturing the iliac crest.

Splenic aspirates:

* Splenic aspirates are richer in parasites and therefore, are more valuable for diagnosis.
*But the procedure can sometimes cause dangerous bleeding and therefore, should be done carefully and only when a marrow examination is inconclusive.

Lymph node aspirates:

*Lymph node apsirates are not useful in the diagnosis of Indian Kala-azar, although it is employed in visceral leishmaniasis in some other countries.

Comparison of aspiration biopsies

Although splenic aspiration is the most sensitive method (98% positive), one marrow puncture (50–85%, positive) is a safer procedure when compared to spleen puncture, 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 fl uid 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.

Animal inoculation

Animal inoculation is not used for routine diagnosis.

When necessary, Chinese golden hamster is the animal employed.

*The material is inoculated intraperitoneally or intradermally into the skin of nose and feet.

*The inoculated animals are kept at 23°–26°C.

*In positive cases, the amastigote can be demonstrated in smears taken from ulcers or nodules developing at the sites of inoculation or from the spleen.

*Animal inoculation is a very sensitive method, but takes several weeks to become positive.

Indirect Evidences

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 Kuhn (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 immunofluroscent antibody test (IFAT)

*Counter immunoelectrophoresis (CIEP)

LD body in spleen smear of experimentally infected animal (Giemsa stain)
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%.

Molecular diagnosis

A number of molecular diagnosis methods have been developed, which help in species identification of Leishmania. The methods include Western blot and PCR. The use of PCR is confined to specialized laboratories and is yet to be used for routine diagnosis of visceral leishmaniasis in endemic areas.