Entamoeba histolytica

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Introduction

Amoebiasis, the third most common cause of parasitic death in the world is caused by *Entamoeba histolytica* protozoa with pseudopodia.
Epidemiology

✔ Occurs worldwide. more common in tropics and subtropics than temperate zones, most in developing countries like Bangladesh, India, Pakistan and Nepal.

✔ About 500 million people are infected every year of which 50 million (10%) suffers from disease and 100,000 dies.

✔ Common in adults, rare in children, and Amebic liver abscess is more common in male
Taxonomy

- **Phylum** - Amoebozoa,
- **Class** – Entamoebidae,
  - **Order** - Amoebida,
  - **Family** - Entamoebidae
    - **Genus** – Entamoeba,
    - **Species** – E. histolytica,
      - E. dispar,
      - E. hartmanii,
      - E. coli,
      - E. gingivalis.
Morphology

*E. histolytica* has two stages:
- trophozoite and
- cyst with an intermediate
- precyst.

The trophozoite is the actively metabolizing, motile stage, and the cyst is dormant and environmentally resistant.
Morphology

- **Trophozoites** vary remarkably in size—from 10 to 60 µm or more in diameter, actively motile by pseudopodia.
- Amebas are anaerobic organisms and do not have mitochondria.
- Shape changes constantly due to pseudopodial movement.
- Cytoplasm is divided into clear outer **ectoplasm** and inner granular **endoplasm**.
- Endoplasm contains the nucleus and **food** vacuoles.
- The nucleus has a distinctive central karyosome and a rim of finely beaded chromatin lining the nuclear membrane.
- The food vacuoles contain may contain bacteria or red blood cells.
Morphology of cyst

- The cyst is spherical, 10-20 µm in diameter, with a thin transparent wall.
- Fully mature cysts contain four nuclei. The nuclei have fine evenly distributed uniform granular peripheral chromatin, with small discrete central karyosome.
- **Chromatoidal bars**, crystallized ribonucleoproteins, are present variably, and are more common in immature cysts. They are elongated bars with bluntly rounded ends.
- **Inclusions in the form** of glycogen masses also may be present. Usually diffuse, concentrated masses often present young cysts. They stain reddish brown with iodine.

*E.histolytica* cyst: cysts measure 10-15 µm in diameter and are spherical. (Iodine stain)
Morphology of *E. histolytica* at a glance

1. Has two stages - Trophozoite and Cyst
2. Trophozoites are 20-60 µm, motile with pseudopodia.
3. Cytoplasm of trophozoite is divided into clear ectoplasm and granular endoplasm often contains food granules and RBC.
4. Cysts are non-motile, round, 10-20 µm with thin transparent cyst wall.
5. 4 nuclei in mature cyst (immature cyst contains variable numbers but less than 4)
6. Chromatoidal bars are characteristic but presence is variable.
Variation of *E. histolytica*

✓ *Entamoeba Dispar*

- It is now established that *E. dispar* (dispar = different) (the name proposed by Brumpt in 1925 but recognized only in 1997 by WHO) is the nonpathogenic species of *Entamoeba* which is morphologically identical with *E. histolytica*.
- *E. dispar*, is generally agreed to be about ten times as common worldwide as *E. histolytica*. 
### Comparison of *E. histolytica* & *E. dispar*

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<th><em>E. dispar</em></th>
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<td><strong>Microscopy</strong></td>
<td>No RBC seen within cytoplasm of trophozites</td>
<td>Ingested RBC may be seen &amp; is diagnostic</td>
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<td><strong>In vitro culture</strong></td>
<td>Xenic (requires bacteria for growth in culture)</td>
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1/7/2014 Prof. Muhammad Akram, Entamoeba
Life cycle of *Entamoeba histolytica* and the clinical manifestations of infection in humans.
Life cycle at a glance

- **Host**: Man is the only host (Definitive)
- **Infective stage**: Mature quadrinucleate cyst (Metacyst)
- **Route of infection**: Fecal – oral
- **Site of lesion**: Large intestine
- **Diagnostic stage**: Cyst & trophozoite
- **Pathogenic stage**: Trophozoite
**life cycle**

- *E. histolytica* passes its life cycle in single host, the man and
- it has 2 stages, 1. trophozoites 2. cysts.
- Mature Cysts containing four nuclei (metacyst) is the infective form, which spread via the ingestion of faecally contaminated food or water.
- When mature cysts are ingested via food and drinks their excystation occurs within the lumen of the small intestine. During excystation, nuclear division is followed by cytoplasmic division, giving rise to eight young trophozoites known as amebula.
- These young trophozoites, reside in the lumen of the cecum and large intestine, where they adhere to the colonic mucus and epithelial layers.
- Approximately 90% of individuals infected with *E. histolytica* are asymptotically colonized. Re-encystation of the trophozoites occurs under certain unfavorable conditions like dehydration within the lumen of the colon, resulting in the excretion of cysts in the faeces and continuation of the life cycle.
- Alternatively, the trophozoites can invade the colonic epithelium, causing amebic colitis (in ~10% of infected people).
- *E. histolytica* can spread in the bloodstream (hematogenously) after it has penetrated the colonic epithelium and can establish persistent extraintestinal infections, most commonly amebic liver abscess.
Virulence factors

1. **Lectin**: Galactose and N-acetyl-D-galactosamine (Gal/GalNAc) specific lectin by which the young trophozoites binds with colonic mucin glycoproteins.

2. **Apoptotic killing** of host cells - Occurs by a novel pathway

3. **Pore forming protein** (Amoebopore) - Kills endocytosed bacteria and purified protein also capable of causing necrotic death of host cells.

4. **Anti complement activity** - Invasive *E. histolytica* resist destruction by lectin mediated complement activation.

5. **Motility** - Invasion is probably also promoted by the cytoskeleton-induced motility

6. **Enzymes** - Invasive parasite secrete enzymes like proteases, Collagenases, elastase, hyaluronidase that degrade the extracellular matrix and antibody.
Intestinal amoebiasis:

- The trophozoites are pathogenic form which colonize the intestine by adhering to colonic mucin glycoproteins, via a galactose and N-acetyl-D-galactosamine (Gal/GalNAc)–specific lectin.
- Host cells are killed via the induction of an apoptotic cascade. Apoptotic killing occurs by a novel pathway that is not blocked by bcl-2 and does not require fas, or the TNF-α receptor.
- Recently the amebas have been demonstrated to activate “effector” caspases immediately before destruction of the host cell. Inhibition of these human caspases blocks killing by the amebas.
- Trophozoites also contain a pore-forming protein that is probably involved in the destruction of endocytosed bacteria.
- The purified protein is also capable of inducing necrotic death of eukaryotic cells.
- Motility and resistant to destruction by lectin mediated complement activation also play a role.
- Enzymes like proteases destroy extracellular matrix.
Features of amoebic ulcer:

- Trophozoites establish themselves in cecum and proximal colon, and then invade tissues. This is frequently mild and self-limiting, with minimal symptoms. However, can cause extensive destruction of the colonic mucosa, and invade other organs.

- Initially produces small, discrete erosions or minute crypt lesions. These then extend through the mucosa, into the submucosa, and expand laterally to produce flask-shaped lesions. These may merge and cause denudation of overlying mucosa, producing a minute hole of communication. These flasks shaped ulcer usually have ragged margins, and bases due to digesting effect of proteases.

- The floor remains full of altered blood from oozing of the sidewalls. Base of the ulcer usually remains limited to muscularis mucosa, rarely reach to submucosa, and up to muscularis externa.
Extra intestinal amoebiasis

- *E. histolytica* can metastasize in any organ which hepatic amoebiasis is the commonest.

- **Hepatic amoebiasis** – Ameba can reach the liver via the portal vein from which they extend progressively in all directions, producing liver cell necrosis and liquefaction, to form an amebic liver abscess, which will extend eventually into adjacent structures. They stay small or continue to grow – center of abscess is full of necrotic fluid, outer wall full of trophozoites. If abscess ruptures, organisms are available to eat other organs. The necrotic fluid is well comparable to ancovy sauce as per its colour and consistency. Microscopically, this fluid contains nil to very few pus cells, trophozoites can be found but never cysts.

- **Pulmonary amoebiasis** - develop generally when liver abscess ruptures through diaphragm.

- Other ectopic sites include **brain, skin, penis** (possibly acquired sexually). Rare sites include **kidneys, spleen, male and female genitalia** and **pericardium**.
Pathogenesis of intestinal amoebiasis

- Young trophozoites released from cyst binds with colonic mucosa by lectin (Gal/GalNAc)
- Colonization follows binding and then destruction by contact dependent cytolysis.
- Invasion is facilitated by many factors like – pore forming proteins, proteases, motility and phagocytosis.
Immunity

✓ Immunity to infection with *E. histolytica* is associated with a mucosal IgA response against the carbohydrate recognition domain of the Gal/GalNAc lectin.

✓ These IgA molecules act as the receptor blocking antibodies, and interfere attachment of trophozoite on mucosal epithelial surface.

✓ Over a one-year period, children with this response had 86 percent fewer new infections than children without this response. Cell-mediated responses have been described in patients with amebic liver abscess, characterized by lymphocyte proliferation and lymphokine secretion that is amoebicidal in vitro.
Clinical Features

- Individuals who are infected with *E. histolytica* can either remain asymptomatic or present clinically with dysentery or extraintestinal disease.

- **Asymptomatic**: Asymptomatic infection with *E. histolytica* is defined as the presence of *E. histolytica* in stool in the absence of colitis or extraintestinal infection. Colonization with the morphologically identical parasite *E. dispar* is more common in developing countries. *E. histolytica* colonization is frequently observed in high-risk settings. Colonization with *E. histolytica* carried a low but definite risk of development of invasive amoebiasis.

- **Amebic colitis**: Amebic dysentery occurs gradually, with symptoms (such as abdominal pain and tenderness, painful sudden bowel evacuation (tenesmus), and intermittent diarrhoea, vomiting and general malaise) developing over a period of one to several weeks. Amebic diarrhea is marked by 4-6 loose stools per day. Fever occurs in only a minority of patients with amebic colitis, and only microscopically detectable blood is present in a majority. Because of the chronicity of the illness, weight loss is common.
Clinical Features

Amebic liver abscess:

Liver abscess is overwhelmingly the most common manifestation of amoebiasis occurring outside the intestine (extraintestinal).

This complication is 10 times more common in adult men than in adult women. Children rarely develop amebic liver abscess.

Most of the patients, who present clinically with an amebic liver abscess, do not have coexistent dysentery, although a past history of dysentery is common. Multiple cysts or cysts >10 cm in size usually occur in superior right liver lobe involvement. Spontaneous resolution by 6 months in 66% and Persist >1 year in 10%.
 Isoenzyme patterns

Isoenzyme patterns are known for four amebic enzymes which helps in differentiating pathogenic strains from non pathogenic strains and also has epidemiological importance.

The isoenzymes patterns are as follows:
- glucose phosphate isomerase (GPI),
- hexokinase (HK),
- malate:NADP+ oxidoreductase (ME), and
- phosphoglucomutase (PGM).

The isoenzyme patterns of three of these, GPI, HK, and PGM, can be used to define >20 zymodemes of *E histolytica*. The enzyme markers associated with pathogenicity are the presence of a b band and the absence of an a band for PGM.

Zymodemes II, VI, VII, XI, XII, XIII, XIV, XIX, and XX are pathogenic.

Zymodemes II and XI are responsible for liver abscesses. Zymodeme patterns are of epidemiologic and research interest but their limited availability makes them less useful clinically.
Laboratory Diagnosis

Principle:

- Laboratory diagnosis of intestinal amoebiasis is based on microscopic demonstration of hematophagous trophozoite or cyst of *E. histolytica* and antigen detection from stool. Nucleic acids techniques are also helpful. Culture followed by isoenzyme analysis is the ‘gold standard’ though it is time consuming and highly technical.

- Laboratory diagnosis of hepatic amoebiasis is practically done by antigen detection from blood, saliva and pus from liver.
Laboratory Diagnosis Stool ME

- Diagnosis in most laboratories is still by microscopic examination of fresh, warm, liquid faeces for hematophagous trophozoites (i.e. trophozoites that have ingested red blood cells).

- Sensitivity is only 30-50%. This may be improved by merthiolate iodine formalin concentration and staining of multiple stool specimens by modified Ritchie formalin-ether concentration and examination of wet mounts and preparations stained by iron haematoxylin or trichrome for trophozoites and cysts, or by examining sigmoidoscopic swabs and scрапings from large bowel ulcers and biopsies of rectal mucosa.

- Pathogenic strains of E. histolytica can only be differentiated by indirect immunofluorescence with monoclonal antibodies.

- Delays in the processing of stool samples affect the sensitivity of light microscopy, which under the best of circumstances is only 60% of that of the stool culture method.

- The young cyst has a single nucleus but this rapidly divides twice to form firstly a two- and then four-nucleus stage.
Laboratory Diagnosis M/E Stool

✔️ In semifomed stool it is possible to find precysts and cysts with 1-4 nuclei but quadrinucleate (4 nuclei) cysts, the metacyst, is most common in formed stool.

✔️ Culture of stool samples followed by isoenzyme analysis can accurately distinguish E. histolytica from E. dispar, and is considered to be the 'gold standard' for diagnosis.

✔️ However, one to several weeks required for this test and also requires special laboratory facilities, making it impractical for use in the developing world.
Lab diagnosis

- Antigen detection by ELISA on stool is the most sensitive and specific method. The sensitivity of this method is >85%, and its specificity is >90%.

- Serological methods for antibody detection can also be used to distinguish accurately between prior infection with *E. histolytica* and *E. dispar*.

- Levels of anti-amebic antibodies remain elevated in the serum for years, so detection of immunoglobulin M (IgM) antibodies prove to be useful for the diagnosis of acute disease.

- PCR is helpful for the differentiation of *E. histolytica* from *E. dispar* from faeces, pus and culture. The sensitivity and specificity of PCR-based methods for the diagnosis of *E. histolytica* infection is similar to stool culture followed by isoenzyme analysis, and is more sensitive than antigen detection tests.
Hepatic amoebiasis is best diagnosed by ELISA detection of lectin antigen. The test on serum has sensitivity of 96% and specificity of 75-100%, while that on saliva has specificity of 70%. When performed on abscess fluid or pus, the specificity is 100%.

Other extraintestinal complications include splenic abscess, empyema and pericarditis. Diagnosis in these cases is similar to that for liver abscess.
Treatment

✔ **Intestinal amoebiasis**
  - **Metronidazole** is the drug of choice for treatment of symptomatic intestinal amoebiasis 750 mg t.i.d., 5-10 days or 50 mg/kg, single dose for adults, cure rate 90%. Or **Tinidazole** can be used 50 mg/kg q.d. 3 days, cure rate 86%.

✔ **Amoebic liver abscess**
  - **Metronidazole** 750 mg t.i.d., 5-10 days or 2.4 g q.d., 1-2 days, cure rate is 90%. Or **Tinidazole / Ornidazole** 2 g orally single dose.

✔ **Asymptomatic cyst passers**
  - Diloxanide furoate, 500 mg t.i.d., 10 days, cure rate 87-96%. Or **Iodoquinol (Diiiodohydroxyquin)** 650 mg t.i.d., 20 days, cure rate 95%. Or **Paromomycin** 500 mg t.i.d, 10 days or 30 mg/kg/day in 3 doses, 5-10 days, cure rate 85-90%.
Prevention

Non-Pathogenic Ameba

1. Entamoeba coli
2. Entamoeba hartmanni:
3. Endolimax nana
4. Iodamoeba bütschlii
5. Entamoeba dispar
Summary

1) **Entamoeba histolytica** causes amoebiasis, the third most common cause of parasitic death in the world, which kills 100,000 people every year.

2) **E. histolytica** has two stages in its life cycle, Cyst and Trophozoites with an intermediate Precystic stage of which cysts are the infective stage and trophozoites are the pathogenic stage and both the stages found in the stool of patient with amoebiasis. It is transmitted by fecal oral route. It passes its life cycle in a single host, the man.

3) **E. histolytica** causes intestinal and extraintestinal amoebiasis. Intestinal amoebiasis is manifested by dysentery. Clinical manifestation of extraintestinal amoebiasis varies with site of involvement. Hepatic amoebiasis is commonest extraintestinal amoebiasis.

4) Diagnosis of intestinal amoebiasis is based on identification of cysts or trophozoites of **E. histolytica** by microscopic examination of stool and also by detection of antigen from stool. Direct fluorescent antibody test, EIA and PCR are also helpful. Extraintestinal amoebiasis is diagnosed by microscopy of aspirated pus from the site of lesion and detection of antigen and antibody.

5) Amoebiasis can be effectively treated by metronidazole or tinidazole.
Study Questions

1. What is the mortality and morbidity of amoebiasis? What is its clinical significance?
2. Describe morphology of *Entamoeba histolytica*.
3. Write about the laboratory diagnosis of amoebiasis? Which test is most sensitive?
4. Write about the pathogenesis of amoebiasis.
5. What are the virulence factors of *Entamoeba histolytica*.
6. What is *Entamoeba dispar*? Why it is so called? How it can be differentiated from *E. histolytica*.
7. What are the non-pathogenic intestinal amebas? How *E.histolytica* can be differentiated from *Entamoeba coli*.
8. Enumerate important non-pathogenic amebas? What is their clinical significance?