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Amoebiasis: An Infectious Disease Caused by *Entamoeba histolytica*

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ABSTRACT

Amoebiasis is an infectious disease caused by *Entamoeba histolytica* (*E. histolytica)*, the common symptoms are cramping, abdominal pain, watery or bloody diarrhea, and weight loss. Sometimes patients suffer amoebiasis as an asymptomatic behavior. In 1859 scientist W.D. Lambl first described the amoebiasis vector *E. histolytica*. In developing countries, millions of people die due to amoebiasis. A doctor diagnosed the disease detection of *E. histolytic* antigen in stool or antibodies against the parasite in serum. Metronidazole, tinidazole, lodoquinol, diloxanide furoate are given for amoebiasis patients. In this review, we have summarized the statistics, pathogenesis, diagnosis, prevention, and treatment strategy of amoebiasis disease. It also gives information about the life cycle of *E. histolytica*.

Keywords: *Amoebiasis, E. histolytica, Asymptomatic, Parasite, Infectious disease.*

Introduction

Amoebiasis is an infection caused by the pseudopod-forming, non- flagellated protozoan parasite *Entamoeba histolytica (E. histolytica)***.** Synonyms include entamoebiasis, amoebiosis, amoebic dysentery, or bloody flux [1]. Entamoeba is derived from the Greek words Entos: within and Amoeba: Various species of Entamoeba viz., *Entamoeba histolytica, Escherichia coli, E. gingivalis, E. dispar, E. hartmanni, E. Poleck* [2],[3]. From these bacterial strains, only *Entamoeba histolytica* is pathogenic to man. Sometimes the infection is asymptomatic, but the invasive intestinal disease may occur manifesting with several weeks of cramping, abdominal pain, watery or bloody diarrhea, and weight loss [4]. The taxonomic hierarchy of *E. histolytica*describe in table 1. *E. histolytica*was first described by W.D. Lambl 1859 in the colonic autopsy of a child who died of diarrhoea. 1875-Fedor Loschin described the pathogenic nature of *Entamoeba histolytica*. He inoculated the parasite through the rectum of a dog and found that the dog had developed dysentery, thus proving the pathogenic nature of the parasite [5].

Table 1. The taxonomic hierarchy of *Entamoeba histolytica*

Kingdom	**Protista**
Class	Lobosea
Order	Amoebida
Family	Entamoebidae
Genus	Entamoeba
Species	*Entamoeba histolytica*

E. histolytica gets nutrients from the gut microbiota by phagocytosis of the host cell. *E. histolytica* gets nutrients from the gut microbiota by phagocytosis of the host cell. A fundamental component of *E. histolytica is actin*, which

involves changing cell shape and other pivotal processes like motility, phagocytosis, and parasite-substrate interaction**.** Two proteins Ehvps20 and Ehvps24, are essential for transport in erythrophagocytosis. During colonization, *E.histolytica* triggers an acute inflammatory process by releasing reactive oxygen species, nitric oxide, and cytokines from active cells of the immune system. *E. histolytic* possesses 10 phosphatidylinositol (PI) kinases and 23 phosphatidylinositol phosphatises [6],[7].

Epidemiology

Amoebiasis is a worldwide disease, mostly seen in developing countries (India, China, Mexico, Africa, and South America). It is the third most common cause of death from parasitic disease. Around 500 million people are infected every year, and around 0.1 million people are dying due to amoebiasis globally. Fifteen percent of the Indian population gets affected due to *E.histolytica*. The distribution of luminal amoebiasis as measured by a cyst in stool is a significant case throughout the world. Amoebiasis is mainly related to the sanitation and socioeconomic status of the country. Depending upon the age and body's immunity, this bacterial infection also varies. Children who are less than five years of age are affected mainly by amoebiasis. Approximate death rate of children in a year is represented fig.1. The rate of mortality found higher in males in comparison to females [8]-[10].

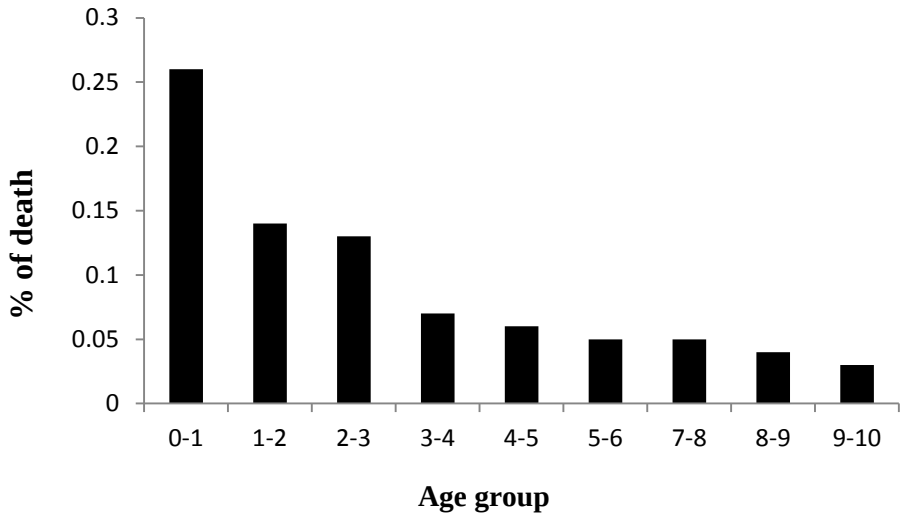

Fig.1. Death rate of amoebiasis between 0-15 year age group

The infection begins when the host swallows the mature quadrinucleate cyst along with the contaminated food or water. As the cyst wall is resistant to the acidic content of the stomach, the quadrinucleate cyst passes unaltered into the small intestine, where excitation takes place. In the intestine, the cyst wall is digested by the action of trypsin in an alkaline medium at a temperature of 37°C. The cytoplasmic body retracts and loosens from the cyst wall during this process. Pseudopodia are formed at various points, and vigorous amoeboid movements occur within the cyst. Frequently, the pseudopodia press against the wall at certain spots as though the imprisoned organism were searching for the exit. Eventually, a tetranucleate amoeba known as metacyst (with four nuclei) emerges. Immediately on emergence, four nuclei of the metacyst undergo division to form eight nuclei. Each nuclei gets surrounded by a bit of its cytoplasm and leads an independent existence. Thus, eight amoebulae are formed. These are known as metacystic or metacyclic trophozoites which are actively motile [11],[12]. The metacystic

trophozoites move down to the caecum and ileocloacal region of the intestine. The young amoebulae being actively motile invade the tissues and finally lodge themselves in the mucosa and submucosa of the large intestine-its final abode. They prefer this site as the organic material (food), pH and gases in this part of the large intestine are more stable and ideal for *E. histolytica* trophozoites. Here, the trophozoites grow at the expense of living tissues and multiply by simple binary fission. The trophozoites secrete histolysin, which causes necrosis and destruction of the host's tissue and helps the parasite derive nourishment from the dissolved dead tissues [13].

However, some non-invasive trophozoites remain in the lumen of the large intestine and multiply by binary fission. These trophozoites feed on the host's nutrients from the surrounding medium. Some of the trophozoites from the cells of the mucosa and submucosa after repeated binary fission move back into the lumen of the intestine. When the conditions become unfavorable for the trophozoites in the lumen of the large intestine, they start to develop a cyst wall. A pre-cyst is first formed, which soon becomes a uninucleated immature cyst. The nucleus within the cyst divides first to create a binucleate cyst. The nucleus divides again for the second time to form a quadrinucleate/ tetranucleate mature cyst (infective stage). The transformation of trophozoite into a mature quadrinucleate cyst is called encystations and is a means of protection of a species from extinction [14],[15].

Encystation does not occur in man's tissues, neither in the intestinal mucosa nor in the liver, lungs, etc. Thus, the metastatic invasion of the trophozoite for all biological purposes is a dead-end for the parasite. Encystation takes only a few hours, and the mature quadrinucleate cyst can remain viable in the lumen of the large intestine for only two days. Mature quadrinucleate cysts are passed out along with the feces of the host. About 45 million cysts may be voided out from one infective person in a day. The cysts are resistant to environmental conditions and can live for a few weeks to a few months depending on the temperature (thermal death occurs at 50°C). Moisture is essential for the long existence of the cyst. They can live up to 10 days in a moist stool. The human feces also expel trophozoites, but they cannot survive outside the host's body for more than one hour, and another human ingests even them before one hour they are killed in the host's body by acid juice in the host stomach [16],[17].

Pathogenesis

The infection due to *E. histolytica* typically occurs with the injection of mature, quadrinucleated cysts found in physically contaminated food or water. Excystation begins at the small intestine by releasing motile trophozoites, then migrating to the large intestine. Through binary fission, the trophozoites form a new cyst. Both the stages are found in feces, but only cysts can cause disease due to the protection conferred by their wall [18]. The trophozoites can adhere to the colonic epithelium, lyse it, and subsequently spread through the portal vein system to distant sites such as the liver, brain, lung, and peritoneum [19],[20].

This adherence is through the Gal/GalNAc lectin, which targets galactose and L-acetyl-D-galactosamine residues found on mucins' O-linked sugar side chain. Mammals that do not have N-acetyl-D-galactosamine or N-terminal galactose are resistant to the adherence, providing some degree of immunization against the disease. Specific enzymes have been found to increase the risk factor for invasive disease [21],[19]. Glycosidades enzyme like sialidase, N-acetylglucosaminidase, N-acetylgalactosaminidase are needed to remove the branched polysaccharides from the mucin cells and causes the trophozoites to degrade the protective mucus layer and

subsequently to penetrate the colonic epithelium increasing the risk of transfer to distant sites. The production of mucosal immunoglobulin, mostly IgA, plays a significant role in the host gut immune system by preventing pathogens from adhering and entering the mucosal barrier. Mucosal anti-Gal/GalNAc lectin IgA plays a critical role in resistance against pathogen colonization and invasion. Pathogenesis of *E. histolytica* is representing fig.2 [19].

Other Effects of *Entamoeba Histolytica*

Dephosphorylation: *E. histolytica* alters host cell tyrosine phosphorylation by dephosphorylating it, and host phosphatases appear to be responsible for it. This mechanism is responsible for killing because pre-treatment of the host cell with a protein tyrosine phosphatase inhibitor prevents death [22].

Calcium Influx: Intracellular calcium influx is critical for target cell killing because it is found that the treatment of inhibitors and chelators of calcium blocks the cytotoxicity. After parasitic contact, intracellular calcium in the host cell increases tremendously and causes death. Pretreatment with a calcium channel blocker prevents killing. Calcium-regulated transcription factor, URE3-BP, modulates expression of known virulence factors, and URE3-BP mutant that binds DNA results in enhanced parasite invasion in vivo [23].

Apoptosis: Caspase-3 dependent apoptosis is the principal mechanism both in vivo and in vitro. Caspase-3, activated in the host cell in a contact-dependent manner, that killing is blocked by the caspase-3 inhibitor AC-DEVD-CHO. *E. histolytica* induces apoptosis in SW-480 cells [24].

Formation of amoebapores: Cause cytolysis of infected cells. These are pore-forming proteins having sequence similar to the mammalian membrane permeabilizing proteins NK-lysin and granulysin [25],[26].

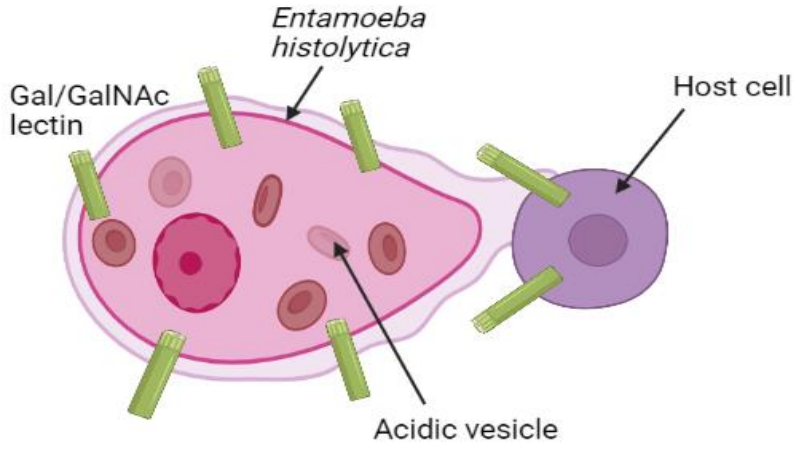

Fig.2. Pathogenesis of *Entamoeba histolytica*

Clinical Manifestation

Mostly clinical manifestations are insidious and intermittent, occurs with abdominal discomfort, bloating, irregular bowel habit, cramping, bloody stool, fever, abdominal tenderness, intermittent dysentery with or without blood/mucous., appetite disturbance, weight loss, breathlessness occur. In case of complication, there may be toxic megacolon, liver abscess, ameboma, perianal rectal pistulas, amebic dysentery, and rupture of hepatic abscess. The

fatality rate for amebic dysentery is 2%, and overall complication is 3-4%.The extraintestinal form of manifestation is primarily of hepatic involvement. The extraintestinal indication involves pain in the right lower chest related to respiration, fever, breathlessness, cough, and appetite disturbance [27],[28].

Diagnosis

Diagnosis is mainly confirmed by detecting *E. histolytica* antigen or DNA in stool or antibodies against the parasite in serum. Fresh liquid stools have to show hematophagous trophozoites with Charcoat-Leydon crystals in characteristics [29]. Cyst of *E. dispar* is non-invasive and harmless but ten times common than *E. histolytica*, so three consecutive days test is advocated. Ultrasound scan of the abdomen helps in the delineation of hepatic abscesses. X-ray of the chest helps detect whether the infection is spread to the pleura, lung, or pericardium. X-ray of abdomen detects peritonitis and toxic megacolon. X-ray, Computer tomography/magnetic resonance imaging help diagnose intracranial spread. For antibody detection IHA (indirect hemagglutination assay) ELISA and confirmatory PCR can also occur. PCR in advance centers is confirmatory [30],[31].

Treatment

Two drug categories, luminal amebicides and tissue amebicides have been used to treat amoebiasis (Table 2). Luminal amebicides drugs are iodoquinol, and diloxanide furoate. Tissue amebicides drugs are metronidazole, tinidazole, chloroquine, emetine, and dehydroemetine. Symptomatic patients required electrolytes with metronidazole and iodoquinol [32]. Chloroquine is mostly useful for amoebic liver abscesses. For extra intestinal combined therapy, mostly metronidazole, dehydroemetine, and chloroquine have been given [33]. Surgery is sometimes required to treat massive gastrointestinal bleeding, toxic megacolon, liver abscess [34]. In the case of home remedies, we must increase fluid intake, coconut water, garlic, apple cider vinegar, cloves, also used for the amebiasis. A home remedy of a combination of 1-2 tablespoons of raw apple cider vinegar to a glass of warm water can be an effective solution. Also, mixing raw honey with lemon juice in warm water and drinking it twice a day can fight against infection and maintain good health [35].

Table 2. Drug used and dosing formula for amoebiasis

Nature of amoebiasis	Used drug	Dosing (Adult)	References
Asymptomatic	Paromomycin	25 to 35 mg PO	36
	Iodoquinol	650 mg PO	37
	Diloxanide furonate	500 mg PO	38
Intestinal disease	Metronidazole	500 to 750 mg PO	40
	Tinidazole	2g PO	41
Liver abscess or severe intestinal disease	Metronidazole	750mg PO	42
	Tinidazole	2g PO	43

Prevention

Public education about personal hygiene and sanitary disposal of feces is essential. Testing of drinking water resources is necessary. Advice infected individuals to avoid food preparation. Educate about the risk of sexual practices that permit fecal-oral contact. Advice infected people not to use the public swimming pools; contaminated water can be a source [36]-[44].

Conclusion

E. histolytica continues to be a threat to human health in many developing countries, mainly found in children. Since amoebiasis is a communicable disease so emigration from endemic area is one of the major factors which continues incidence and prevalence of amoebiasis. The asymptomatic cases are challenging to be diagnosed by clinicians, which leads to continuous spread of disease. Surprisingly, we could be able to know very little about the immune response underlying invasive amoebiasis. More evidence should be gathered to elucidate pathogenesis of *E. histolytica*, and the immune response working on it, so that more potential target proteins can be investigated for the development of vaccine. Asymptomatic patients can be able to mount partial immunity against intestinal infection can be a key for developing successful vaccine. A combination of target protein (galectin-which prevent adhesion of pathogen) and adjunct protein that harnesses both humoral and cell mediated immune response can facilitate development of successful vaccine. More research should be done on how immune response exactly works against the invasion of pathogen.

Abbreviations

URE3-BP = Upstream regulatory element 3-binding protein

DNA = Deoxyribonecleic acid

ELISA = Enzyme-linked Immunosorbent Assay

PCR = Polymerase chain reaction

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Competing Interests Statement

The authors declare no competing financial, professional and personal interests.

Consent for publication

Authors declare that they consented for the publication of this research work.

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