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A REVIEW OF *ENTAMOEBAHISTOLYTICA* GENOME, VIRULENCE FACTORS, PHYLOGENETIC TREE AND TREATMENT

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Abstract

The study in this review aims to know and study the parasite *Entamoebahistolytica* that causes Amoebiasis worldwide. During its life cycle, *E. histolytica* passes through several phases, trophozoite stage, precyst stage, the cyst stage, the Metacyst stage, and Metacystictrophozoite stage. In this study, the spread of the parasite globally and in the Arab world and methods of treating the disease were also discussed. The Genomic structureof *E. histolytica*, like other organisms, is characterized by diversity and heterogeneity in its genetic content, which is one of the most important reasons for its survival and its ability to infect. Interestingly, the genome of the *E. histolytica* contains a large amount of genes presumed to be of bacterial origin. The study of the genetic diversity of *E. histolytica* gives paths to the developmental change that resulted in the emergence of evolutionary features or traits. Understanding amoebic virulence is important. Several studies have shown that genetic factors influence the virulence of parasitic infections. Studying the virulence of *E. histolytica* by Serine-rich protein (REHP) gene is among the important issues used in molecular epidemiological studies.

Keywords: *E. histolytica*, Virulence factors,Phylogenetic tree, Genotypic diversity of *E. Histolytica.*

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Introduction

Morphology

There are several successive stages in the life cycle of the E. histolytica, which are the trophozoite stage, precyst stage, cyst stage, post-cyst stage and metacystictrophozoite [1]. In the following, a description of the trophozoiteand Cyst stages, which are considered to be the main stages of the life cycle:

1-Trophozoite stage

It is also known as vegetative cells, which are the active developing stage [2]. They are very sensitive to changes in pH and dehydration and cannot survive outside the host as they live for only 20 minutes outside the body of the organism making them unable to pass from one host to another [3], and they live in the colon and rectum and sometimes in the lower end of the small intestine of humans and other primates. The kinetochore has an average diameter of 25 µm (range, 15 - 60 µm) and is usually monopodial, producing one fingerlike pseudopodium. Pseudopodium are produced and re-used so rapidly that pseudopodium are rarely seen in activators [4].

In prepared microscopic slices, the cytoplasm is distinguished into two transparent outer regions called ectoplasm and a granular inner part called endoplasm that contains food vacuoles. These vacuoles may contain host erythrocytes, leukocytes, and epithelial cells as well as bacteria and other intestinal material. The trophozoitereproduce by direct mitosis (binary fission) within the alimentary canal of the host [4].

The trophozoites are uninucleateand the nucleus are of particular importance in differentiating between the E. histolyticaand most other intestinal amoebae [4], as it contains a large spherical nucleus with a centrally located karyosome, which is an important diagnostic trait for this species [5]. The nuclear envelope is hardly discernible in saline preparations, but in stained preparations the vesicular nucleus is clearly visible. Ideally the nucleus has a well-defined envelope and is lined on the inner surface of the nucleus by thin peripheral chromatin granules, the centrally located karyosome. This ideal description is not limited to E.histolytica frequently, other types of Entamoeba spp. - Especially the heterozygous mutant E. dispar - has the same phenotypic description of the nucleus [4].

E. histolyticadoes not have contractile vacuoles as in protozoan species (opposite to free-living species) [2].

Electron microscopy studies showed that the Trophozoite stagecontains a poorly developed endoplasmic reticulum, as it lacks the rough endoplasmic reticulum [6], and the cytoplasm contains lysosomes and glycogen particles bodies, and it lacks mitochondria and Golgi bodies [7].

-2 Cyst stage.

The trophozoitetake the precystic characteristics under some unsuitable environmental or physiological conditions or both together as they become more spherical, extruding the food vacuoles within them and shrinking in size. The formation of the pseudopodia, if formed, is slow and there does not appear to be any progressive movement [8].

An encystation begins with the secretion of a precysttrophozoite, a thin hyaline vitreous membrane that surrounds it to form the cyst wall. The cyst is usually globular at this stage, averaging 12 µm in diameter (range, 10-20 µm), with a single nucleus. Sometimes glycogen masses and chromatoidal bars can be observed, and the last structural stage is the formation of some nucleic acids such as RNA. The nucleus undergoes two direct divisions to produce four vesicular nuclei in the mature mature cyst of E. histolytica. These cysts represent the infectious form and come out of the host with feces. After that, metabolized glycogen and chromatoidal bars slowly and disappear, then the cell membrane in the mature cyst is covered with a thick cell wall [8], which consists of chitin (N-acetyl-glucosamine polymer), which gives the cyst its resistance for unfavorable conditions [9]. The cysts of the HM1-IMSS strain were examined by Atomic Force Microscopy (AFM). The cyst obtained is morphologically round with a rough outer surface (cyst wall) and in diameter between 12–15 µm [9].

E. histolyticacysts are highly resistant to dehydration and to some chemicals such as chlorinated compounds and fluorides. The cysts can stay alive in water for a month, while they can remain in feces on land for more than 12 days; It can withstand temperatures up to the thermal death point, which is 50℃[4].

Life cycle

E.histolytica has a simple life cycle that includes binary division in the vegetative cells called trophozoitesor a change between the trophozoitesand resistant cyst stages (Fig. 1) [10]. All Entamoeba spp.need to one host in which the trophozoiteslive; to multiply and form cysts [11]. An Interstage is required to complete the life cycle. In each of theE.histolytica, E. dispar, and E. invadens (a reptile parasite similar to E.histolytica) Transmission occurs when gastric cysts are ingested, usually with contaminated food or water, or through oral–anal sexual contact [12].

These non-invasive cysts of the parasite pass through the stomach to the small intestine, where bicarbonate, water and bile secretions lead to the exit from the excyst [13]. Tetranucleated amoeba begins to separate from the cyst wall and exit through tiny pore [14] and then nuclear division followed by cytoplasmic division that produces eight amoebic trophozoites. The trophozoitesmultiply by binary fission and spread throughout the host intestine, as they move down the intestine [15].

Trophozoitesuse the pseudopods in the colon to move, adhere, and invade the intestinal epithelium as well as trapping and ingesting red blood cells and food particles [6, 16]. In rare cases, trophozoitesbecome invasive by destroying intestinal tissue, causing ulceration. Tissue destruction can result in trophozoitesentering the host's bloodstream and causing subsequent injury to other organs, particularly the liver [15]. The trophozoiteenters the cystic stage after exposure to environmental factors, and these factors are galactose-terminated molecules such as mucin [17]. The precyst stage contains ribosome aggregates and chromatoid bodies as well as food vacuoles which it expels to shrink the cell to 10-16 µm. It is a mature cyst. The cyst divides its nucleus twice during the maturation process to become a tetranucleated, each nucleus containing approximately 25% of the total DNA [14, 12]. The cytoplasm becomes devoid of organelles with the completion of cyst maturation, and the wall surrounding the cyst is rich in chitin, making it osmotically resistant [18, 12]. Finally, the mature cyst is expelled from the host with the faeces to complete the life cycle and possibly contaminate food or water to start the life cycle over. Biochemical studies during the differentiation process of E. invadens have shown that chitin accounts for approximately 25% of the dry weight of the cyst wall and that chitinase enzyme activity increases under conditions that promote cyst formation [18, 12].

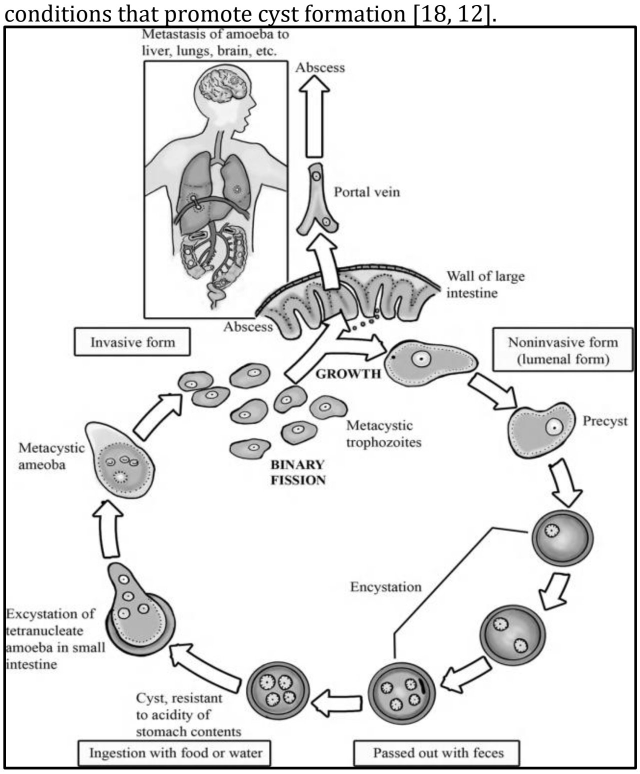

Fig. 1: The life cycle of *E. histolytica*. Image taken from Gino Barzizza [4].

Distribition of E.histolytica

A study in Switzerland, obtained an infection rate estimated at 5.4% when examining 37 faecal samples using PCR technique were positive microscopically [19]. In Poland, the infection rate for the E. histolyticaparasite was 0.6% in citizens who returned from travel, which is the lowest percentage that was obtained during the study, and the infection rate was (19%) in non-travelling citizens. When examining 340faecal samples with a wet swab, the percentage of infection reached Infection 8.1% when using PCR technique Myjaket al. (2000) [20].

It showed the highest rate of amoebiasisin Canada, as it appeared in the study conducted by Gonin and Tudel, (2003) [21], which recorded an infection rate of 71.5% when examining 95 samples microscopically, and the percentage reached29% using PCR technique.

In the study conducted by Pinheiroet al. (2004) [22], in Brazil, the infection was 4.1% when examining 1437faecal samples. In a study conducted in the Netherlands, Van et al. (2005) [23] mentioned an infection rate of 42% when examining 45 faeces samples were positive for microscopic examination out of 106 samples. Infection rate 18.8% for E. histolyticaand 25.3% for E. dispar in the study tried to distinguish between the types of the genus EntamoebaSamie (2006) [24].

Al-Nakkaset al. (2002) [25] the incidence of colitis was (21.9%), and the amoebae were treated in Kuwait when examining 912 stool samples, while in the study conducted by Youssef et al.(2006) [26]in Jordan, the infection rate was 4%, which included examination 265 children, as shown by AbdAlla, (2006) [27] in Egypt showed that the infection rate was 21%, while the study conducted by Al-Harithi and Jamjoom(2007) [28] in Saudi Arabia, where the infection was 76% when examining 615 stool samples. Of the infected who visited health centers in Makkah Al-Mukarramah, while the rate ofE. histolytica was 2.2% in Algeria when 2047 samples, which is the lowest recorded percentage in Arab Countries.

In Iraq, in the study conducted in Al-Qurna district within Basra governorate, the infection rate was 29%. Al-Shaheenet al., 2007 [29].

Virulence factors

1 Adhesion factor

An important adhesion molecule, a lectin known as Gal/GalNAc-lectin, is present on the cell surface of the trophozoiteof E. histolyticabecause it recognizes Galactose (Gal) and N-acetylgalactosamine (GalNAc), which are common components of mucosal and epithelial cells. The (Gal/GalNAc) lectin is a membrane complex (Fig. 2) consisting of a heavy (Hgl) subunit with a molecular weight of 170-KDa and a light (Lgl) subunit of a molecular weight of 31-35 KDa bound together by disulfide bonds, and they are covalently linked to an intermediate (Igl) subunit of molecular weight 150KDa [30]. Whereas, the carbohydrate recognition domain (CRD) is a region of lectins that binds to galactose (Gal) and N-acetylgalactosamine (GalNAc) and is confined to the heavy secondary unit (Hgl) [31].

Adhesion via Gal/GalNAc-lectin is a requirement for cell killing because in the absence of galactose or GalNAc, target cells are not killed by the E. histolytica. Target cell death occurs within 5 to 15 minutes, often followed by phagocytosis [32]. Resistance to cytolysis is mediated by the complement system. Lectin contains a CD59-like domain that likely helps protect trophozoiteof the complement system. CD59 is the surface antigen of many blood cells with this characteristic [33].

Gal/GalNAc-lectin stimulates plasma cells in the lamina propria to secrete IgA immunoglobulin which may prevent parasite attachment. Experimental experiments indicate that vaccination against the E. histolyticaGal/GalNAc-lectin can generate a protective immune response against the parasite [34], in addition, the parasite secretes cysteine proteases that break down IgA and render it nonfunctional [2].

Lectin is not only involved in adhesion but also plays a role in cytolysis. It has been shown that communication between parasite and host via Gal/GalNAclectin is required for cytolysis [35] and lectin itself directly contributes to lytic activity [36].

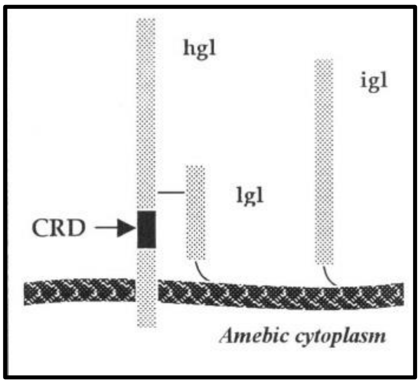

Fig. 2: Gal/GalNAcLectin consisting of three subunits with a carbohydrate recognition domain (CRD) located on the heavy subunit. Lectins are known to be involved in target cell adhesion, cystation and intercellular signaling [37].

2. Cysteine endopeptidases

This class of enzymes is present in all living organisms, and plays a major role in the pathogenesis of E. histolytica due to the degradative activity of this enzyme [38]. At least three types of cysteine protease have been found in E. histolytica, accounting for 90% of the enzymatic activity, but only one, known as (Entamoebahistolytica cysteine protease #5)EhCP5, is considered the most important. When the expression of the gene responsible for the production of EhCP5 was enhanced (by non-viral transfer of nucleic acid into cells) the ability of the parasites to invade the liver was increased in mice [39]. However, some studies have failed to make a clear relationship between the ability to phagocytosis and protease activity, and pathogenesis [40]. The glycolytic activity of the thiol-dependent protein (–SH) in E. histolytica is attributed first to the neutralizing sulphydryl proteinase [41] and then to the cytotoxic proteinase [42]. Other terms have been used to describe enzymes closely related or identical to this enzyme such as Cathepsin B [43], Neutral proteinase [44], Histolysin [45] (later changed to Histolysain [46]) and Amoebapain [47]. Cysteine endopeptidase enzymes secreted by E. histolytica [48] were found localized in lysosome-like vesicles or on the cell surface [49, 50]. Molecular cloning revealed a large number of Cysteine endopeptidase genes in the genome of E. histolytica [49, 51]. Interestingly, most of these genes are not expressed during culture of E. histolyticaIn vitro [51]. Because most current knowledge of the biology and pathogenesis of E. histolytica is based on the analysis of cultured cells, most of the functions of Cysteine endopeptidases and their exact role in the virulence of E. histolytica are largely unknown [5]. EhCP-A and EhCP-B are members of the subfamily of inactive Pre-pro enzymes that resemble the general structure of Cathepsin L-like enzymes [52].

The family of genes that encode for these surface proteins in the E. histolytica known as Ariel is lacking in the E. dispar [38].

Cysteine proteases are secreted to the extracellular environment of E. histolytica, where they have been identified as the major virulence factors of E. histolytica. Cysteine proteases have the ability to degrade the extracellular matrix, including Purified fibronectin, laminin, and type I collagen, that would allow the parasite to directly access target cells [53]. Furthermore, cysteine proteases interfere with both the complement pathway and the humoral response in the human immune system. Enzymes are able to cleave C3 in such a way as to activate the complement pathway, degrading E. dispar heterotrophs but not E. histolytica [54]. Gal/GalNAclectin inhibits the complement mediated complement mediated by E. histolytica mediated mechanisms, particularly via cross-reacting with CD59, and the C5b-9 membranous inhibitor is found in human blood cells [33]. In addition, cysteine protease is also primarily responsible for the decreased secretion of IgA and IgG, which may limit host humoral immune responses [55, 56].

3. Amoebic holes and related proteins

E. histolyticasecretes a pore-forming protein that is important in pathogenicity and is found in three types A, B, and C. This peptide can form ion channels in synthetic membranes and depolarize and may also depolarize cells in eukaryotic cells [57].

Type B and C amoebic holes are the most efficient in degrading erythrocytes and bacterial membranes and causing colitis. Genomic studies have shown that expression of amoebic hole type A in trophozoites of E. histolytica is required for amoebic liver abscess. However, trophozoites that do not express amoebic holetype A can still cause colitis [4]. Amoebic holes have been shown to be a critical component used by the parasite to kill host cells [5]. Relatives of these precursor polypeptides were also found in the cytotoxic lymphocytes of pig and human called NK-lysin and Granulysin, respectively. All of these polypeptides consist of 70–80 amino acids and are compact alpha-helical, contain disulfide bonds, and have a saposin-like fold [58, 59]. The biological activities of these peptides were measured in parallel [60, 61] to evaluate the similarities and differences of these effector molecules in organisms whose evolutionary paths differed at a very early stage. It was found that they are active against both prokaryotic and eukaryotic target cells, as they can be considered broad spectrum effector molecules [5].

Sixteen genes encoding saposin-like proteins (SAPLIPs) have been identified in the genome of E. histolytica. All of these genes were cloned in cells grown in axenic culture. Despite the similarity between amoebic holes and saposin-like proteins, it has been recently shown that these proteins have neither pore-forming activity in the cell membrane of the host cell nor bactericidal activity [62]. Therefore, it is likely that the three amoeboid holes are the only pore-forming molecules of the parasite [63]. Figure 3 shows some of the cell surface proteins and proteins secreted by E. histolytica.

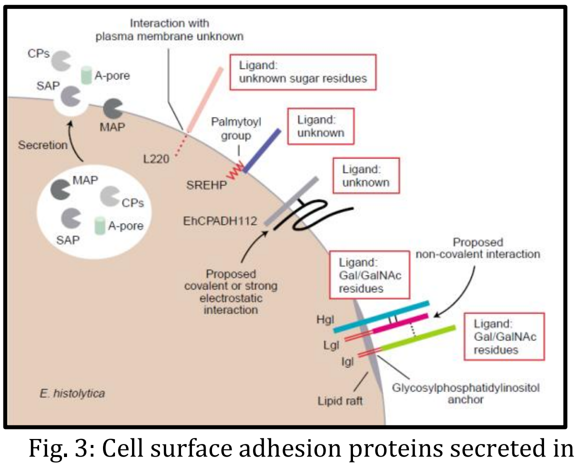

Fig. 3: Cell surface adhesion proteins secreted in E. histolytica [64].

It demonstrates E. histolytica surface proteins that help it pass to the surface of the host cell or the extracellular space and then attach to it and break down its components which is critical to parasite virulence. Adhesion is mediated by many proteins on the cell surface. One such protein is a complex protein that has a high affinity for binding to galactose (Gal) or N-acetyl-D-galactosamine (GalNAc). The complex consists of heavy (Hgl) and light (Lgl) secondary units linked by a sulfur and medium (Igl) bond, as the interaction between them is not covalent. The three subunits are located on the lipids of the plasma membrane of the parasite. Other adhesion molecules include transmembrane protein 220 KDa (L220), Serine-rich E. histolytica protein (SREHP), and cysteine heterodimer protease EhCPADH112. The mechanism by which L220 interacts with the membrane is unknown.

4. Antioxidants

The trophozoitesof E. histolytica are normally present and reproduce within the intestine of the host which forms an anaerobic or microaerophilic environment (up to 5% oxygen in the gas phase). However, the Entamoeba spp. is exposed. During tissue invasion, oxygen pressure increases due to toxic metabolites such as reactive oxygen species (ROS) or reactive nitrogen species (RNS) produced by activated phagocytes during a respiratory burst. E. histolytica lack the traditional respiratory electron transport chain that terminates in carbon dioxide and water [5], and accordingly toxic endogenous metabolites are not the only challenge facing E. histolytica [65].

To prevent parasite death from oxidative stress caused by reactive oxygen species generated during inflammation or by phagocytosis, many parasites have developed multi-level defense systems, including DNA repair systems and antioxidant enzyme systems. enzyme systems [66].

Respiratory blast is part of the immune system which is defined as the sum of ROS that are involved in the host's defense against pathogens as it imposes a great deal of oxidative stress. The optimal antimicrobial activity of neutrophils and other macrophages is based on the production of a wave of superoxide anions (O_2^-)by activating the enzymeoxidases NADPH and then this O_2^- is processed to give hydrogen peroxide (H2O2) and hydroxyl radical (OH), Hypochlorous acid (HOCl) and oxidized nitric oxide derivatives (NO^.) such as peroxynitrite(ONOO^-) [67]. Thus defense strategies against oxidative and nitrosative stresses include detoxification enzymes and repair systems that enable cells to resist ROS and RNS [65].

Within the genome of the E. histolytica, at least 32 genes encoding antioxidant enzymes have been identified. So far, only a few of them are known to participate in the protection against oxidation, and their interaction pathways have been clarified. The biochemical pathways of the majority of antioxidant enzymes are poorly understood [65].

Include antioxidant enzymes:Superoxide Dismutase (SOD), Rubrerythrin protein (Rbr), Peroxiredoxin (Prx), Thioredoxinreductase (TrxR) and Iron hydrogenases.

Genomic structure for parasite

E. histolytica, like other organisms, is characterized by diversity and heterogeneity in its genetic content, which is one of the most important reasons for its survival and ability to infect. The non-condensation of chromosomes during mitosis makes determining the exact number of chromosomes and ploidy very difficult. The genes are distributed on 14 chromosomes as well as many extra-chromosome elements. Most genes consist of only one axon, with introns present in 25% of genes. This genome is characterized by the presence of polymorphic internal repeat regions, and several gene families, one of these large families encoding transmembrane kinases, cysteine proteases (CP), SREHP protein, and others [68].

The genome sequencing project was published in 2005 [69] and this was the sequencing of the pathogenic strain HM-1: IMSS using the Whole-genome shotgun method. This genome consists of a 12.5-fold consisting of 9,938 genes which in turn consist of 23,751,783 base pairs (Bp) spread over 888 scaffolds. Genes have an average size of 1.17 kilobase pairs (Kb) and constitute 49% of the genome sequence, and it is predicted that approximately 25% of genes contain introns, with 6% possibly containing multiple introns. A prominent feature of the genome of E. histolyticais that it contains a huge amount of tRNA-encoding [70], which constitute about 10% of the entire genome and most of them are organized in linear arrays, of which 25 species have been identified, each of which contain up to five types of tRNA in a repeating unit. The unusual regulation of the rRNA-encoding genes is another feature of this parasite, as they are located on extrachromosomal plasmids where 200 copies can be found in each cell [71]. It constitutes about 20% of the total DNA in the cell.

Interestingly, the genome of the E. histolyticacontains a large amount of genes presumed to be of bacterial origin. Many important metabolic enzymes have been replaced by types of prokaryotic organisms. Like fructose-1,6 bisphosphatealdolase [72], malic enzyme, Acetyl-CoA synthetase [73] and Aldehyde-alcohol dehydrogenase (ADH2) [74], genes are believed to be involvedin the activation of metronidazole and pyruvate: ferredoxinoxidoreductase (PFOR) and ferredoxin (FD) [75].

Phylogenetic tree

It is an evolutionary tree living organism that is important to various departments of the bioiogyand other sciences [76].It is also a schematic diagram that shows the evolutionary relationship between different species. It consists of nodes and branches, the nodes represent the taxonomic units, and the branch represents the temporal estimate of the evolutionary relations between those taxonomic units, the nodes are related to the branches, each branch is connected to only two nodes, and the nodes are divided into:

Terminal nodes: called leaves, they represent taxonomic units such as species orpopulations, genes, or proteins.

Internal nodes: They are hypothetical taxonomic units, which represent a speculative unit and represent the last common ancestor (LCA) of the nodes that arise from this point. Individuals that separate from the same node are called sister groups. It separates from different nodes and is called outgroup.

Evolutionary trees may or may not have an outer shell, and covered trees are branches, and the length of the branch is proportional to evolutionary variation such as the number of nucleotides exchanged over time. As for the trees that are not covered here, the length of the branch is not proportional to the amount of differenceevolutionary, and the true number is indicated at a specific place on the branch [77].

Evolutionary trees may or may not have roots, and trees that have roots contain a root node from which other parts of the tree branch, the node denotes the last universal ancestor (LUCA), from which taxonomic groups originate and diverge through time.LUCA and LCA represent the sequence of DNA or protein. The ideal trees are rooted, but most genetic trees are non-rooted [78].

Genotypic diversity of E.histolytica

The term (genetic diversity) describes the relationship between determinants such as (species, genera, and microorganisms) [79]; It reflects the evolutionary history of the microorganism by observing the evolutionary characteristics and variations during the time stages, by comparing the similar characteristics of living organisms such as (the amino acid sequences of some specific genes). The study of genetic diversity gives a conclusion about the developmental change paths that resulted in the emergence of these features or Evolutionary traits [80].

Studies have shown that the structural features of the genome may play an important role in the genetic variance of E. histolytica and possibly with the virulence associated with the strain [80].

Understanding amoebic virulence is important. Several studies have shown that genetic factors influence the virulence of parasitic infections. The study of the virulence of E. histolytica by the Serine-rich protein (SREHP) gene is among the important issues used in molecular epidemiological studies [82].

Various types of genes associated with amoebic infection have been detected in the parasite E. Histolytica, including the SREHP gene. It is an important marker in identifying E. Histolytica parasite among other species, and its relationship to disease [83].

SREHP has antigenic properties, involving cellular phagocytosis of apoptosis of infected host cellsto inhibit the inflammatory response by the host [84]. Genetic diversity in E. Histolytica parasite in endemic regions reflects polymorphism of the Serine-rich protein gene and exhibits high genetic diversity [85].

Various types of genes associated with amoebic infection have been detected in E. Histolytica parasite, including SREHP gene, which is an important marker in the identification E. Histolyticaamong other species, its relationship to disease has been linked [83].

It is possible for the tissue-forming E. Histolytica isolated from the same region to show morphological diversity, as previous studies showed patterns of diversity of shapes for the SREHP gene for isolates from different geographical regions, as they found that the diversity of shapes is wide within a specific geographical area [86]. The morphological diversity of the SREHP gene may have a vital role in the process of immune evasion, and the diversity of the shapes of amino acids and nucleotides in the gene is important as a functional protein. [87, 88]

Treatment of Parasite

For several decades, metronidazole, ornidazole, and tinidazole (derivatives of 5-nitroimidazoles) have been the cornerstones of antiamoebic antagonists. These chemotherapeutic agents are generally addressed against anaerobic bacteria and parasites such as Trichomonasvaginalis, Giardia lambliaor Bacteroidesfragilis [89, 90]. The three drugs mentioned have almost the same efficacy, in controlling amoebic colitis, perhaps with a slight preference for tinidazole then ornidazole, but in some countries such as the United States of America, metronidazole is only available [91]. The natural therapeutic dose consists of 750 mg of metronidazole, three times daily for 5-10 days. After that, it is highly recommended to use the luminal agents paromomycin, Iodoquinol or Diloxanidefuroate as an additional treatment to eradicate the amoebae colonies, and Nitroimidazoles are not considered an effective agent against the luminal amoebae [92].

The treatment of amoebic liver abscess in most cases is the same as the treatment of amoebic colitis. Even large abscesses can be treated by taking metronidazole alone [93]. As in amoebic colitis, metronidazole should be followed by administration of luminal agent to eliminate colonies in the intestine [92].

One of the drugs that could become important in the future in the treatment of amebiasis is Nitazoxanide, a broad-spectrum antiparasitic drug that is also effective against Giardia and Trichomonas as well as parasitic worms [94]. Another interesting group is Alkylphosphocholines (Alkyl-PC), which was originally developed as an anticancer drug and was found to have potent antiamoebic activity [95]. Miltefosine is one of the Alkyl-PCs, which has already been used against visceral leishmaniasis (caused by Leishmaniadonovani) in India, but the Alkyl-PCs are effective against Trypanosoma spp. Also [96] and Acanthamoeba spp [97].

The side effects of metronidazole in the therapeutic dose include a metallic bitter taste and symptoms of the central nervous system (dizziness, headache, and sensory neuropathies). It also has a strong teratogenic effect [98].

Resistance to metronidazole is an important problem in mitochondrial parasites such as E. histolytica, G. intestinalis and T. vaginalis. No clinical isolates have been observed to possess a high level of resistance so far, although exposure to non-lethal levels of treatment can increase drug resistance [99]. However, resistance may occur in laboratory mutated strains up to a concentration of 40 µM to metronidazole [100], which is a lethal dose in non-modified strains. The expression of Superoxide dismutase and Peroxiredoxin is increased in the metronidazole-resistant line and the expression of Ferredoxin 1 is decreased while the expression of Pyruvate:ferredoxinoxidoreductase (PFOR) is slightly decreased [92].

All these data indicate the increasing problems of resistance to metronidazole in anaoerobic parasites, as there are only a limited number of chemotherapy available against and resistant to these organisms, it is clear that the search for new chemotherapy must intensify and continue [92].

Other parasites show similar developments with regard to resistance. For example, and perhaps the most important of these problems is the genus Plasmodium, as the burden of disease and the number of deaths increases greatly every year, and multi-resistant strains grow in different regions [101], until resistance against Artemisinin was observed [102, 103]. Therefore, the search for new chemical treatments is imperative and urgent. The mode of action of a widely used chemotherapy is often not known exactly [92].

Metronidazole in its original form is inactive, but it must be reduced to the nitro group to form the active nitroradical anion, which has cytotoxic properties [104]. This negative nitro radical is further reduced to Nitrosoimidazole [105] which is able to react with sulfhydryl groups [106] and with DNA [107]. Continuous reduction leads to the formation of hydroxylamine and finally to amine. This activation pathway can only occur in organisms that live in an anaerobic environment such as Giardia parasites, E.histolytica and T.vaginalis. In the aerobic environment, as in humans, the negative nitro radical ion is immediately re-oxidized in the so-called futile cycle [108], which leads to the formation of superoxide radical anions and oxidative stress, but severe cell damage does not occur. This reoxidation makes Nitroimidazoles safe for human use, although the risk of cancer due to oxidative stress cannot be definitively excluded as in a number of studies [109].

The enzymes responsible for reducing the nitro group have been extensively studied. Ferredoxin has been proposed as the major reducing agent. This iron-sulfur protein itself is reduced by pyruvate:ferredoxinoxidoreducase (PFOR) [110]. In T.vaginalis, all enzymes are located in the hydrogenosome, where metronidazole is activated [111]. However, ferredoxin did not appear in the parasites that were exposed to complete resistance to metronidazole, which indicates the existence of other pathways to activate metronidazole, possibly this pathway is the malic enzyme [112]. Recent discoveries have shown that the flavin enzyme thioredoxinreductase can reduce and activate metronidazole and other nitroimidazoles at significant rates in E. histolytica [113] as well as in T. vaginalis [114, 115].

The mode of action of metronidazole is still not fully understood In vivo. Which is supposed to interact with DNA [107], leading to damage, but no association with specific enzymes was found until recently, and the mechanism of cytotoxicity was also thought to be random [92].

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Conflict of Interest

No Conflict of Interest

Inform Consent and Ethical Statement

Not Required

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