Review

Biology

HIV/AIDS in Combination with *Enterocytozoon bieneusi* Infection

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Acquired immunodeficiency syndrome (AIDS) is a significant public health problem caused by infection with the human immunodeficiency virus (HIV). The most common cause of death in AIDS patients is opportunistic infection with various microorganisms. Intestinal protozoa, such as *Enterocytozoon bieneusi*, are prevalent yet difficult to treat. This review will provide an overview of HIV/AIDS mixed with *Enterocytozoon bieneusi* infection by discussing an introduction, epidemiology, detection methodology, and treatment strategies.

Keywords: Human Immunodeficiency Virus; Acquired Immunodeficiency Syndrome; Opportunistic Infection; *Enterocytozoon bieneusi*; Outcomes

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ONE of the top three global public health issues is acquired immunodeficiency syndrome (AIDS). HAART, also known as highly active antiretroviral therapy, is currently a popular treatment for human immunodeficiency virus (HIV) (1). Although there has been significant improvement in the treatment of AIDS, diarrhea is still a prevalent symptom of HIV/AIDS patients and has a negative impact on their quality of life and chance of survival. The body's immune system is destroyed after HIV infection, particularly the T cells, whose function is compromised, or their quantity falls significantly, making them extremely vulnerable to opportunistic intestinal protozoa like *Enterocytozoon bieneusi* (2). One of the major causes of death in HIV/AIDS-infected patients is chronic diarrhea, severe weight loss, malnutrition, and cachexia brought on by infection (3). Accordingly, opportunistic infections rather than AIDS itself were to blame for roughly 80% of AIDS-related fatalities (4). In underdeveloped nations, up to 90% of HIV/AIDS patients experience diarrhea symptoms, compared to 30%-60% in wealthy nations (5); among them, diarrhea is primarily brought on by co-intestinal protozoa infection (6). One of the most prevalent protozoa of HIV/AIDS co-infection, Enterosporidium, accounts for 11.8% of them (7), and *microsporidia enterica* infects the majority of them (8).

Enterosporidia

Brief Introduction

One type of obligatory intracellular parasite unicellular eukaryote is the microsporidia. According to phylogenetic study, microsporidia are closely related to fungi and may be a sister group or basal branch of fungi, most likely connected to Cryptomycota (9, 10). Cellular organisms, eukaryotes, fungi, and microsporidia were the divisions made by the National Center for Biotechnology Information (NCBI) in 2012 for

microsporidia (11). Microsporidia are common and can be found in terrestrial, marine, and freshwater ecosystems. They can infect a wide range of hosts, including protozoa, arthropods, fish, amphibians, reptiles, birds, mammals, and other invertebrates and vertebrates. In addition to invading human muscles, bile ducts, eyes, noses, livers, brains, and lungs, microsporidia infections can cause diarrhea as well as myositis, cholecystitis, keratoconjunctivitis, sinusitis, hepatitis, encephalitis, and lung infections (12). Microsporidia was identified as class B biocontainment pathogens and microbiological pollutants that can lead to waterborne outbreaks (13). Infectious spores are how microsporidia are spread. The villi's length and coverage are reduced as a result of the infectious spores' replication in the villi epithelial cells of the small intestine, which also affects the host's ability to absorb nutrients and results in diarrhea (14). Extremely resistant to their surroundings, infectious spores can survive in distilled water for more than ten years, at 56 ℃ for 60 minutes, and for 10 minutes in an autoclave at 120 ℃ (15). More than 200 genera and more than 1,500 species of microsporidia have been identified so far in a variety of hosts, and 9 genera and 17 species of them are capable of infecting people (16). More than 90% of individuals with microsporidia infection also had *Enterosporidium enterica* infection (17).

Enterosporidium pylori

Pichia enterica has a wide geographic distribution and can infect humans, domestic animals, wild animals, livestock, and poultry. Moreover, sexual, mother-to-child, and soil transmission of animal-human and animal-animal diseases can occur, which can result in symptoms like diarrhea, stomach pain, and malnutrition (18). At this time, 106 or more enteromicrospore genotypes have been discovered in humans, with more than 500 enteromicrospore genotypes divided into 11 genomes (19). Humans and animals are the main carriers of genotypes I and II. The genotype II has also been found in animals (20), indicating that genotypes II also has the risk of zoonotic infection, which should be taken into consideration.

People are typically exposed to *Enterosporidium pichia* through tainted water and food, and it can result in epidemics and significant public health crises. In China, a "dead pig discarding incident" reported in Shanghai Songjiang section of the Huangpu River in March 2013, and found that the water was contaminated by *Enterosporidium pichia*, and 17 genotypes were found there; the EbpC genotype, which was co-infected by humans and animals, was the predominant genotype (21), which suggests that there is a significant unrecognized risk of waterborne transmission of *Enterosporidioides enterica*. In a Danish staff canteen in 2020, there was an outbreak of foodborne microsporidiosis brought on by consuming contaminated sandwiches (22). It also found that gay men can contract *Enterosporidium pichia* from one another (23).

Humans are typically susceptible to *Enterosporidium pichia*, particularly those with immunosuppression or low immunity, including HIV/AIDS patients, transplant recipients of organs or bone marrow, cancer patients, homosexuals, the elderly, children, and others (24). These people frequently experience severe symptoms after contracting the infection, which can result in ongoing diarrhea and even death. It is unclear how Enterosporidium enters host cells, creates an infection, and progresses to illness. It is hypothesized that the host is kept in a subclinical state for a considerable amount of time by a delicate balance between the microorganisms and the host. When the host immune system is compromised, like when the CD4+ T lymphocyte count falls below 100 cells per liter of blood, clear clinical signs will manifest.

HIV/AIDS Combined with Enterosporidium pichia Infection

The course of several opportunistic parasite infections may be sped up following human infection with HIV, leading to more severe clinical symptoms, and complicating the management of AIDS. In contrast, certain intestinal protozoa (such as Cryptosporidium and Enterosporidium) infection can increase HIV susceptibility, boost HIV replication, and hasten the progression of HIV-infected patients to AIDS by causing mucosal destruction and changes in the immunological microenvironment (25, 26). The first instance of AIDS coupled with *Enterosporidium pichia* enterica infection was discovered in 1985 (27). Since then, there have been an increasing number of reports of AIDS cases that also had enterosporidium infections throughout the world, primarily in Southeast Asia (India and Thailand), the Middle East (Turkey), Europe, Africa, and Latin America (28). Enterosporidioides co-infection affects 2.5% to 51.0% of adults and 17.4% of children in underdeveloped countries, compared to 2% to 78% of HIV-infected people with diarrhea in developed nations (29).

There have been cases of HIV/AIDS co-infection with *Enterosporidium pichia* recorded from a total of 26 countries, with infection rates ranging from 0.8% to 42.8%. Portugal has the highest infection rate (42.8%), followed by Zimbabwe (31.8%), and Ghana has the lowest infection rate (0.8%) (30). The infection rate of Enterosporidioides changes as a result of many economic factors and detection techniques. High-income nations have lower infection rates than low-income nations, particularly in sub-Saharan Africa. The inability to carry out efficient prevention and control activities, which keeps the infection rate high, may be a result of the area's slow economic development and poor local sanitary conditions, drinking water quality, and health conditions.

Detection Methods of *Enterosporidium pichia*

Morphology, immunology, and molecular biology are the three main detection techniques for microsporidia currently used.

Morphological Detection

Microsporidia are extremely small, measuring just 1-3 μm in diameter, and their mature spores measuring 0.8-1.0 μm to 1.2-1.6 μm. The ideal method of detection is to see microsporidian spores under a light microscope. There are several staining techniques for microscopic investigation, including enhanced trichrome staining, improved acid-fast staining, Gibbs staining, methylene blue staining, acid-fast staining, hematoxylin-eosin staining, and Gram staining (31).

Ultrastructural analysis has become a method for the detection, diagnosis, and classification of Microsporidia since the first case of *Microsporidia enterica* was found by transmission

electron microscopy. The development cycle and nucleus structure of the microsporidian spores could be clearly seen under the transmission electron microscope after the sample was fixed with glutaraldehyde and processed by the transmission electron microscope's specialized embedding technique (32).

Microsporidia can also be found using fluorescence microscopy. By using fluorescent whitening chemicals like Calcofluor M2R, Uvitex 2B, etc. to stain the Microsporidia chitin components, brilliant blue Microsporidia spores can be seen under a fluorescent microscope (33). However, fungi and other microbes that include chitin components also have a vivid blue appearance, so inspectors must be knowledgeable about the morphological features of microsporidia and are able to distinguish them.

Although the morphological method is the gold standard for detection, it is impossible to type microsporidia because it takes a long time, has low detection effectiveness, and requires highly skilled detection workers.

Immunological Detection

Serum antigen and antibody detection are two immunological approaches for detecting microsporidia. Methods for detecting antigens include western blots, immunofluorescence assays, and enzyme-linked immunosorbent assays. Although created to fight human infections, anti-*enterosporidium pichia* antibodies have not been extensively employed in clinical trials (34). Enzyme-linked immunosorbent assays, lectin tests, and other procedures are used to identify serum antibodies. Although they are simple to use, they still have drawbacks, such as a high rate of false positives, and are not very popular.

Molecular Biological Detection

Because of its sensitivity, specificity, and high detection efficiency, PCR has been widely employed in the detection and identification of Enterosporidium. The identification and genotyping of pathogens can be accomplished in conjunction with the nucleotide sequence analysis of the amplified product. It can be found via nested PCR, multiplex PCR, real-time fluorescence quantitative PCR, loop-mediated isothermal amplification, and CRISPR/Cas12a detection methods. There are also techniques using DNA dot hybridization and oligonucleotide arrays, although the two have 100% sensitivity and specificity, there are also false positives, and the technology is still in its infancy and has not seen widespread adoption (35).

The most typical method for detecting, identifying, and genotyping Enterosporidium is nested PCR based on specific loci. Nowadays, the ribosomal small subunit rRNA gene is typically amplified using PCR, and the genotype is determined by the polymorphism of the transcribed spacer in the ribosome (36). The transcribed spacer within the ribosome only contains a small number of polymorphic sites; therefore, this method is unable to completely show the genetic diversity, genotype origin, and mode of transmission of Enterosporidium. To carry out high-resolution genotyping of M, the multi-locus sequence typing approach can be employed to amplify microsatellite loci 1, 3, and 7 as well as minisatellite locus 4 to clarify *Enterosporidium pichia*'s population structure and genetic variation traits.

Treatment of HIV/AIDS Combined with *Enterosporidium pichia* Infection

There is currently no medication specifically designed to treat microsporidiosis, which just inhibits the illness rather than curing it (37). Accordingly, HAART can successfully manage microspore infection. Adopting HAART can lower HIV viral loads and boost CD4+ T lymphocyte counts, which lowers mortality (38). Prior to the use of HAART, the infection rate of HIV/AIDS and microsporidia ranged from 2% to 70%; however, with the widespread use of HAART, the infection rate gradually fell, and the majority of those infected were HIV/AIDS patients with CD4+ T lymphocyte counts below 100/L of blood (39). Due to its unfavorable side effects, oral fumagillin (60 mg once day) is the only medication that is currently approved for use in South and North America to treat enterosporidioides infection (40). Nitazoxanide can treat chronic diarrhea brought on by enterosporidioides in the absence of HAART (41, 42), although the impact is less noticeable when the CD4+ T lymphocyte count is low (less than 100 cells/L blood).

Conclusion

The prevalence of HIV/AIDS and *Enterosporidium pichia* infections is influenced by regional, economic, and basic healthcare infrastructure. The infection rate in the same area will vary depending on the survey's timing, population, and location. In order to decrease the likelihood of co-infection with *Enterosporidium pichia*, extend the patient's life, and enhance their quality of life, HAART and other treatments should be actively administered to HIV/AIDS-infected people or patients especially when the count of CD4+ T lymphocytes is 100/L of blood. Clinicians should increase their knowledge of and proficiency in diagnosing and treating HIV/AIDS in conjunction with an *Enterosporidium pichia* infection, as well as strengthen routine examinations, in order to improve the prognosis of HIV/AIDS. Also, it is essential to practice good personal hygiene, including avoiding close contact with animals that can spread *Enterosporidium pichia* and maintaining good food, water, and sexual hygiene.■

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