REVIEW ARTICLE

Disseminated microsporidiosis: An underdiagnosed and emerging opportunistic disease

Putri Sabrina MOHAMED YUSOFF1, Emelia OSMAN* and Raja Zahratul Azma RAJA SABUDIN2

1Department of Parasitology and Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latiff, Bandar Tun Razak, 56000, Cheras, Kuala Lumpur, Malaysia**
Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latiff, Bandar Tun Razak, 56000, Cheras, Kuala Lumpur, Malaysia

Abstract

Disseminated microsporidiosis is a life-threatening disease resulting from the haematogenous spread of microsporidia species. The diagnosis is challenging owing to its subtle nonspecific clinical presentation, which usually reflects the underlying organ involved. Therefore, a high index of suspicion is required for early diagnosis. Besides, tools for confirmatory laboratory diagnosis are limited. Currently, there is no direct diagnostic method that can detect the infection without involving invasive procedures. Clinical confirmation of disseminated microsporidiosis is usually based on light and transmission electron microscopy of infected tissue specimens. These are then followed by species detection using polymerase chain reaction (PCR). Disseminated microsporidiosis shows the potential to be cleared up by albendazole or fumagillin if they are detected and treated early. Based on a series of case reports, this review aims to present a current update on disseminated microsporidiosis with emphasis on the clinical manifestations based on the organ system infected, diagnostic approach and treatment of this devastating condition.

Keywords: microsporidia, disseminated microsporidiosis, extraintestinal microsporidiosis

INTRODUCTION

Microsporidia is an obligatory intracellular eukaryote, known to cause opportunistic infection. A recent study has recognised this organism to be closely related to fungi.1 Before their status as opportunistic pathogens in humans was established, they were known to cause significant damage to economically important hosts, including silkworms.2 However, their medical importance in humans was only recognised during the acquired immunodeficiency syndrome (AIDS) pandemic.3 Considered as opportunistic pathogens, microsporidia have been noted to severely affect both immunocompromised and immunosuppressed individuals. There are at least 17 reported microsporidian species known to infect humans, Enterocytozoon bieneusi and Encephalitozoon intestinalis being the most common of them. While the E. bieneusi infection is mostly confined to the intestinal and biliary tract, the Encephalitozoon species are known to cause disseminated infection involving the kidneys, lungs, eyes, and other organs.2 The infecting stage of microsporidia is spores, in which the infectious and mature stages are typically shed with faeces or urine from the host, and relatively resistant to the environment. The purpose of this review is to describe the overview of this infection particularly focusing on the clinical presentation manifested, diagnostic approaches and chemotherapeutic agents applied for disseminated microsporidiosis.

1. Epidemiology

Microsporidiosis is a cosmopolitan disease. Data on prevalence have varied greatly depending on the geographical region, the population studied and the diagnostic methods used.5-9 The latest systemic and meta-analysis reviews reported that the worldwide prevalence of microsporidiosis in HIV patients was 11.8%, which ranges from 0.7% to 81.3%.9 Within this group of patients, there was a significant difference between those who were presented...
with diarrhoea in comparison to control. An earlier study by Lobo et al. (2012) exhibited the microsporidiosis prevalence of 13.9% and 8.5% in HIV positive patients and healthy individuals respectively in Portugal. Meanwhile, in China, the highest overall estimated prevalence of E. bieneusi in humans was 8.1%, which was observed in AIDS patients. In Malaysia, the reported prevalence of microsporidia in immunocompromised patients which includes HIV, cancer and transplant patients is as high as 29.2%. Although its medical importance was only recognised during the AIDS pandemic, microsporidia infection is not an indicator illness for the disease. Aside from HIV patients, this opportunistic parasite may also infect immunocompromised and immunocompetent individuals, but only causing self-limiting diarrhoea and are often asymptomatic in the latter. Although there is a lack of data on the prevalence of disseminated microsporidian infections among HIV-infected individuals, Zainudin et al. (2016) managed to report a 7% prevalence among this subset of patients through blood PCR. The lack of prevalence of disseminated microsporidiosis could be due to the unavailability of direct diagnosis tools. Often the infection of microsporidia is detected through a stool sample. However, microsporidia spore may be absence in the stool for disseminated microsporidiosis and their diagnosis required invasive procedure. Even so, it called for a high level of suspicion by the expert and they are likely to be underdiagnosed due to no specific clinical signs showed by disseminated infection.

The variability in microsporidiosis prevalence may be due to several factors including sanitation, intake of contaminated water, interaction with zoonotic host as well as the state of one immune system. Often manifested as self-limiting diarrhoea and asymptomatic in immunocompetent individuals, other groups at risk may suffer from chronic diarrhoea and other debilitating diseases for disseminated infection. This risk group consists of immunocompromised individuals such as HIV patients, organ transplant recipients and malignancies patients. HIV patients with CD4 T-lymphocyte counts of less than 100 cells/μl had are more susceptible to microsporidia infection. There are also increasing reports of microsporidia infection in an immunosuppressed patient who are exposed to chemotherapy for more than one year with a neutrophil count of fewer than 1500 cells/mm³. Asides from the immune status of the patient, microsporidia infection can be influenced by the economic status of a certain region. For instance, a study in Peru showed that people with greater wealth are more protected against microsporidia. A review by Wang et al. (2018) also indicated that the prevalence of microsporidiosis in HIV patient from low-income countries are significantly higher than in high-income countries.

2. Modes of Transmission and Life Cycle

The mode of transmission for microsporidia occurs mainly via the faecal-oral routes with the source of infection including other infected humans, animals as well as, contaminated water and food sources. Although not explicitly reported, there has been evidence that suggests these parasites are waterborne and can transmit through respiratory, ocular, sexual, congenital and zoonotic transmission. Spores are transmitted into the host cell via extrusion of polar tubule that pierces the host cell plasma membrane, initiating the infection of enterocytes. When the spore is transmitted into the host cell, their life cycles begin with merogony, followed by sporogony, and spore maturation phase. Polar tubule will pierce the host cell plasma membrane after germination, subsequently joining the host to the spore. The polar tubule act as a channel, aiding the sporoplasm to be inoculated into the host cell. During the next phase, merogony, the sporoplasm will divide to form the meront. In this phase, division cycles occurring once or a few times. In the following phase, the sporogony, the meronts will thicken producing sporonts that undergo division resulting in sporoblast that eventually develop into mature spores. Intracellular replication will produce more spore that causes the host’s cell to rupture and release these spores. A high intracellular parasite burden will eventually cause the host cell to rupture, released into a new environment and subsequently infecting adjacent cells. This subsequently causes huge amounts of spore to be produced in a single infection.

3. Clinical Manifestations of Extraintestinal Microsporidiosis

An early report of microsporidiosis in immunocompromised patient mainly discuss the infection on gastrointestinal, leading to diarrhoea. This happens due to the replication of microsporidia in the villous epithelium of the small intestine coupled with the decrement
of villus height and surface area.\textsuperscript{19} Aside from the gut, microsporidia can be observed to infect other organs and systems.\textsuperscript{18,20,21} After infection at the gastrointestinal tract, this disease can spread to the abdominal organs, followed by organs of the thoracic cavity and finally the brain (Figure 1).\textsuperscript{22} This migration from the commonly infected organ to other systems has led to the belief that this disease is disseminated.

Microsporidiosis usually cause malabsorption and diarrhoea in the infected patient. Yet, if another system aside from the gastrointestinal tract is infected, it may cause other symptoms.\textsuperscript{23} For instance, \textit{Encephalitozoon sp.} is reported to be the common species causing disseminated microsporidia infection in HIV positive patients in comparison to organ transplant recipients.\textsuperscript{24} Table 1 showed the reported cases of extraintestinal microsporidiosis from the year 2014 to 2019. Infection in other organs will give rise to symptoms specialised to that particular location.\textsuperscript{19}

3.1 Musculoskeletal

Microsporidia can cause myositis when a muscle is infected. Reported clinical manifestations include diarrhoea, muscle weakness, muscle tenderness, fever, weight loss, fatigue, generalised muscle weakness and pain, absent or reduce reflexes, dysphagia, glossitis, and peripheral oedema.\textsuperscript{25–28} This infection may lead to fatality if unsuccessfully treated using albendazole and itraconazole for a patient infected with \textit{Annacaliia algerae}, or incorrect treatment because of the undiagnosed microsporidiosis.\textsuperscript{25} Even with treatment, death may occur due to other complications such as pneumonia aspiration, a risk associated with microsporidia infection in muscle. Microsporidia species that were reported to cause myositis include \textit{A. algerae}, \textit{Tubulinosema acridophagus}, \textit{Pleistophora sp.}, as well as microsporidia closely related to \textit{Endoreticulatus} spp.\textsuperscript{25,26,28,29} Among the individual at risk of acquiring this type of infection are those on immunosuppressant, as well as a healthy individual with polyclonal gammopathy.

3.2 Ocular

Ocular infection of microsporidia is not limited to the immunodeficient individual but it is also reported in an immunocompetent individual. Patients with ocular microsporidia infection present symptoms such as blurring of vision, light sensitivity and lesion on corneal epithelial.\textsuperscript{30–32} It may be diagnosed as an allergic reaction for the immunocompetent patients and as graft rejection for a patient that underwent penetrating
keratoplasty. It also was thought to be Candida keratitis due to the previous medical history of the patient and stromal keratitis. Microsporidia species that reported for this infection include A. algerae, Nosema sp., Vittaforma corneae and Encephalitozoon sp. such as Encephalitozoon hellem.30,33,34

3.3 Renal

Dissemination of microsporidia to the kidney and urinary tract may manifest as an acute kidney injury. As reported by Shah et al. (2018), the parasitic infection if early treatment is not administered. Encephalitozoon sp. can disseminate to this area but recently, E. bieneusi was reported to be present in the urine sample of HIV patients.35 The species that have been detected for kidney and urinary tract infections include E. cuniculi, E. bieneusi, and E. intestinalis.24,35-37 E. intestinalis also present in urine specimens with a reported prevalence of 1.5%.36 According to Kicia et al. (2016), microsporidia DNA is detected more frequently in urine samples rather than the stool sample for renal transplant patient.6 This finding suggests the propensity of this parasite to infect the urinary tract aside from the gastrointestinal tract.

3.4 Pulmonary

Pulmonary infection by microsporidia can be detected using the bronchoalveolar lavage (BAL) and lung biopsy, and they tend to infect the immunosuppressed patient. Infection at this organ may be manifested as bronchitis and bronchiolitis obliterans. Dissemination of this organ indicated by symptoms such as fever, chronic diarrhea, dyspnea, cough, acute respiratory distress syndrome and altered mental status.24,38,39 In reported cases, microsporidia detection was only conducted after suspicion of infections with Chlamydia pneumoniae, Mycoplasma pneumoniae and Legionella pneumophila was ruled out. The reported species found in this system infection include E. bieneusi, E. cuniculi and Vittaforma-like species. A study by Lobo et al., (2012) and Ozkoc et al., (2016) exhibited a prevalence rate of 1% and 14.2% respectively, on the microsporidiosis in the lung by using BAL specimens. Although the infection may be localized because the spore can be transmitted through inhalation, dissemination could not be ruled out.36

3.5 Central Nervous System

Dissemination of microsporidia to the central nervous system has been reported among
immunosuppressed and organ transplant recipient. Microsporidia infection affecting this system may lead to encephalitis and cerebral atrophy. The possible cause of infection is due to the immunosuppressed state of the patient while the source of transmission may be an infected organ donor. Symptom recorded during the infection involves a central nervous system which includes fever, fatigue headache, tremor and blurry vision. Initial diagnosis of microsporidia infection at this site includes progressive multifocal leukoencephalopathy, medication side effect and viral encephalitis. Cerebrospinal fluid is typically used to detect the infection while urine is also used to monitor the clearance of the infection in cases where the microsporidia have disseminated. Undetected dissemination of microsporidia due to no specific clinical manifestation for this type of infection has resulted in the death of a patient.

4. Diagnostic Approach

Microscopy is the usual method used when first detecting microsporidia. Although transmission electron microscopy (TEM) is considered the gold standard for microsporidia detection, routine diagnosis using light microscopy on stool samples are more practical in diagnosing microsporidiosis. From these reported case studies, the common staining methods used were Ryan’s modified trichrome stain, and a series of staining method such as Ziehl-Neelsen stain, silver stain, periodic acid-Schiff, Giemsa as well as Gomori methenamine silver stain. As reported by Boileau et al. (2016), and Watts et al. (2014), light microscopic detection on muscle biopsy were poorly stained and the sample demonstrated necrotising myositis. Moreover, light microscopy may provide a false negative result as described by Wesolowska et al. (2019). Due to these limitations, a panel of diagnoses such as TEM and PCR are typically used to confirm the light microscopy result. The use of TEM for microsporidia detection is dependent on the observation of the microsporidia development phase and polar tubule coils. The quality of the electron micrograph, however, may be limited in identifying the species of microsporidia and even the presence of it. Meanwhile, a molecular method such as PCR is highly sensitive for species identification as reported by Sutrave et al. (2018). Sequence analysis is subsequently carried out after PCR analysis to further confirm the positive result by species identification, which may not be feasible in the microscopy method. Besides, the false-negative result is less in comparison to the light microscopy method in diagnosing disseminated microsporidiosis.

Indeed, microsporidiosis is difficult to diagnose as its clinical signs and symptoms may be absent or not associated with microsporidia. Underdiagnoses and late detection do occur because the method to identify them is not deliberately applied due to no suspicion of the infection. Unlike other parasites, isolation and identification techniques for microsporidia are not suitable and tedious as the culture system requires other host cells. Thus, tissue smear is required to observe the presence of spores making it an invasive procedure. The sample used for disseminated infection of microsporidia depends on the system affected. Therefore, the disease may be undetected if wrong biological specimens are used. In general, stool samples are often used to rule out gastrointestinal infection. However, Boileau et al. (2016) have described that in positive cases of disseminated microsporidiosis the spores were detected in the muscle biopsy rather than the stool.

Another systemic infection such as pulmonary and renal systems used the biopsy of the organ involves, but BAL, urine and cerebrospinal fluid were also used. By using tissue samples, all microsporidia’s phase of life can be observed, whereas the stool and urine samples only show the spore of microsporidia. In cases of keratoconjunctivitis, corneal scrapings are often used as a diagnostic sample. Diagnosis of disseminated microsporidiosis using blood samples by PCR also has been reported and thus may serve as a less invasive method. Frequently, the main reason for the underdiagnosed of disseminated microsporidiosis is because the infection is not considered in the differential diagnoses and contributes to the late detection of the parasite. Disseminated microsporidiosis often has a wide range of symptoms and chronic diarrhoea is the common indicator, but sometimes it may not be presented in the patient. This subsequently causes microsporidia to not be suspected nor included as prognosis. Moreover, microsporidia are only considered to be tested after other infection causes were excluded.

5. Patient Management

To date, microsporidia treatments are still limited. Often, the dose of immunosuppressant drugs such as prednisolone and corticosteroids is reduced in
The reduction of immunosuppressants is essential in managing the infection as displayed by HIV patients where immune reconstitution can clear the infection. High doses of steroids also cause a reduction in the proliferation of CD8+ T cells, which plays an essential role in the defence against microsporidia.43 Besides their direct cytotoxic effect, CD8+ T cell produce gamma interferon, IFN γ, which is one of the proinflammatory cytokines that help in the resistance of Encephalitozoon spp.44,45 This will then followed by drugs such as albendazole and fumagillin, the widely used drugs in treating microsporidiosis. These drugs are known to have the highest clinical specificity against microsporidia. Albendazole is an anti-tubulin polymerisation drug that mainly uses in treating parasitic worm. They have been reported to resolve the infection caused by Encephalitozoon spp. and A. algerae.25,39,46 However, the side effects of albendazole may include headache, an upset gastrointestinal system and elevated levels of transaminases.25 Of note, microsporidian such as E. bieneusi possessed β-tubulin genes containing amino acid residues making them resistant to this treatment. It causes an inhibitory effect on spore production and parasite malformations.46 There were reported case where a patient infected by E. bieneusi was clear of clinical symptoms, yet the spores were still present in the urine sample.38 For ocular infection by Nosema corneae, prescribed oral albendazole shows no improvement in the patient.33 Besides E. bieneusi, albendazole also is not effective against Vittaforma corneae (formerly known as Nosema corneae).47 Similarly to E. bieneusi, β-tubulin sequences of V. corneae also suggest the resistance toward Benzimidazole derivatives drugs.48

Besides albendazole, fumagillin and its analogues are effective for E. bieneusi and other microsporidia, as it inhibits methionine aminopeptidase.17 Fumagillin and nitazoxanide have been reported to successfully treat E. bieneusi.36 In ocular infections by microsporidia (E. hellem and Vittaforma corneae), the use of these drugs is able to clear up the infection in patients.33,49 A. algerae infection that was resistant to albendazole, can be treated using fumagillin.50 Apart from the mentioned drugs, a study by García-Torres et al. (2018) suggests that the thiol-reactive compound can also be used for microsporidiosis treatment. Their study focused on the inactivation of enzymes involved in glycolysis due to microsporidia’s lack of mitochondria by the thiol-reactive compound. Established potential drugs such as omeprazole, rabeprazole, and sulbutiamine have been able to inhibit enzymes that are crucial to the survival of microsporidia.

Highly active antiretroviral therapy (HAART) usage among HIV patients was reported to reduce the prevalence of microsporidia infection. This treatment restores the function of the HIV patient’s immune system which prevent or reduce opportunistic infections.51 Through this therapy, the patient’s CD4+ T-cell levels restored to 100 cells/mm³ or more. The immune reconstitution shown to improve the symptom and able to clear the infection by microsporidia. Consequently, this combination therapy has able to reduce the infection rate of microsporidia infection from 16.6% to 6.3% as compared to HAART-naïve HIV patients.52 Besides maintaining the patient’s immune system, HAART works by directly acting on the parasite via its HIV-protease inhibitors characteristic. However, this property has not been studied against microsporidia.53 Even though reported to reduce the incidence of microsporidiosis, this treatment may not always be accessible in developing countries.54 Plus, the HIV patient with CD4+ count below 200 cells/µl is still prone to opportunistic infection even when they are on HAART treatment.52

DISCUSSION

Despite being reported, disseminated microsporidiosis may be underdiagnosed due to a lack of suspicion of the infection. Particularly, its association with enteric infection has led them to be included as a differential test, only when a patient with diarrhoea is suspected to acquire microsporidiosis. As such, an accurate diagnosis is highly dependent on the clinician’s awareness and suspicion towards the presence of this infection.21 These underdiagnoses are also attributed to a wide range of clinical symptoms related to disseminated microsporidiosis and there are cases reported that detection of microsporidia does not happen until during autopsy.39,40 Misdiagnosing microsporidia as the fungal infection has also been reported and microsporidia were diagnosed only when the fungal cultures produce a negative outcome.18 Microsporidia have also been reclassified as a sister group of fungi. These misdiagnoses might happen due to some microsporidia size that measures up to 5µm, which is similar to
the size of small yeast and even toxoplasma. Reported cases have shown that the presumptive diagnoses of the microsporidia infection include fungi such as Histoplasma capsulatum, and toxoplasma.\textsuperscript{18,38,55} This further complicates the diagnosis of disseminated microsporidia infection because a high suspicion index was required before microsporidiosis can be considered.

Aside from the above, difficulties in the detection of microsporidia in the laboratory may also contribute to the parasite being underdiagnosed. Although the stool is typically used as the biological material for the detection of microsporidia microscopically or molecularly, the difficulty in diagnosis may happen due to a wrong specimen used for the detection of disseminated microsporidiosis. Some studies further reported the detection of microsporidia in other biological material aside from stool. For example, there is a higher prevalence of microsporidia found in patients’ urine rather than stool samples.\textsuperscript{6,38}

Notably, it is reported that the infection of disseminated microsporidia could not be detected in the stool sample and gut biopsy despite the existence of diarrhoea symptom.\textsuperscript{25} The absence of microsporidia in stool did not mean a negative result for disseminated infection. There was a case reported in a patient with a high burden of spores in a muscle biopsy but had a negative result for microsporidia in his stool sample.\textsuperscript{25,26} This shows that disseminated infection of microsporidia might be overlooked if the detection of microsporidia focuses only on the stool sample and the prevalence of the infection might be higher than the actual number reported. Likewise, the diagnosis may be time-consuming because experts and additional procedure are required to validate the result.\textsuperscript{18,20,50} Thus, delayed identification would lead to late in treatment which may cause fatality in the infected patient, particularly in those with immunocompromised states.\textsuperscript{56}

Microsporidiosis is hard to control disease because different drugs are required for the effective treatment of different species. Hence, early diagnosis of the infection is crucial to allow for prompt treatment. The immunocompromised patient has a high risk to be reinfected from this latent spore due to their weak immune system.\textsuperscript{25} Furthermore, the dissemination of this disease could cause death to the immunocompromised individual.\textsuperscript{24} The success in treating the disease will eradicate the symptoms of diarrhoea in the patient, but in some cases, not all spores were shed completely from the host. The degree of success in clearing the infection is also dependent on the immune state of the patient and the infected system organ. \textit{E. bieneusi} treatment is challenging due to its ability to resist albendazole, which is a commonly used drug for treating microsporidia. However, the use of fumagillin may cause an adverse effect on the patient and due to this, accessibility of fumagillin is limited.\textsuperscript{38,57} The management of microsporidial keratoconjunctivitis poses a difficulty as there was no definitive treatment exist. Even though HAART treatment has proven to decrease the infection of microsporidia, developing countries have less access to the treatment, exposing the HIV patient there to the risk of microsporidia infection. In addition, HAART regimes that do not include HIV-protease inhibitors may be futile against this infection.\textsuperscript{54,58}

\textbf{RESEARCH GAP}

Despite being reported, there are only a limited number of studies focusing on the detection and prevalence of disseminated microsporidiosis.\textsuperscript{6,59,60} In this review, we compared the clinical manifestations, diagnoses, and treatments for disseminated microsporidiosis based on case reports. The highlighted point discussed in these reports is mainly on the difficulty in diagnosing the said infection. This suggests that the actual prevalence of disseminated microsporidiosis may have been underestimated.\textsuperscript{21,61} Hence, more studies should be conducted to investigate the prevalence of this infection in immunodeficient patients. Besides that, the current detection methods for disseminated microsporidiosis is not standardised and involves invasive procedures. As microsporidiosis is not included in the differential test, often the detection is done using biopsy as a biological sample.\textsuperscript{21,25,26,50} Furthermore, the current method of detection causes delay in treatment due to multiple methods required for their detection. Perhaps, a direct detection method using blood or urine samples can be developed to aid in the diagnosis of disseminated infection and can be applied as a point of care test for patients.

\textbf{CONCLUSION}

Microsporidiosis has the propensity to disseminate, affecting many organs and systems of the infected patient which may lead to fatality. Hence it is crucial for the early detection and
clearing of the infection that can be caused by diverse species of microsporidia. They should be included as a differential test and considered when an aetiology could not be determined due to its non-specific and wide-ranging clinical presentations. Establishing a sensitive and effective assay, yet simple to use is important to avoid the possibility of errors in microsporidia detection. There is also a growing need to establish a drug that is safe and effective for microsporidiosis.

ACKNOWLEDGEMENT

This work was funded by the Fundamental Research Grant Scheme (FRGS/1/2019/SKK12/UKM/02/1) from the Malaysian Ministry of Higher Education (MoHE).

Authors’ contribution: Osman, E. conceived of the presented idea, design and implementation of the research. Mohamed Yusoff, P.S. took a lead to write the manuscript in consultation with Raja Sabudin, R.Z.A and Osman, E. All authors discussed the content and contributed to the final manuscript.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES

22. Kotkova M, Sak B, Kvac M. Differences in the intensity of infection caused by Encephalitozoon


44. Anane S, Attouchi H. Microsporidiosis: epidemiology, clinical data and therapy in the GASTROEN CLIN BIOL. 2010; 34(8-9): 450-64.


50. Boileau M, Ferreira J, Ahmad I, Lavallee C, Qvarnstrom Y, Dufresne SF. Successful Treatment of Disseminated Anncaliia algerae Microsporidial Infection With Combination Fumagillin and


