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Original papers

Dual infection of urinary tract with *Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* in HIV/AIDS patients

Maria Wesołowska[1], Bartosz Szetela[2], Marta Kicia[1], Żaneta Kopacz[1], Bohumil Sak[3], Weronika Rymer[2], Martin Kváč[3,4], Rusłan Sałamatin[5]

[1]Department of Biology and Medical Parasitology, Wrocław Medical University, ul. Mikulicza-Radeckiego 9, 50-367 Wrocław, Poland
[2]Department of Infectious Diseases, Hepatology and Acquired Immune Deficiencies, Wrocław Medical University, ul. Koszarowa 5, 51-149 Wrocław, Poland
[3]Institute of Parasitology, Biology Centre of the CAS, Branišovská 31, České Budějovice 37005, Czech Republic
[4]Faculty of Agriculture, University of South Bohemia in České Budějovice, Branišovská 31, České Budějovice 37005, Czech Republic
[5]Department of General Biology and Parasitology, Medical University of Warsaw, ul. Chałubińskiego 5, Warsaw, Poland

Corresponding Author: Maria Wesołowska; e-mail: maria.wesolowska@umed.wroc.pl

ABSTRACT. Microsporidia are emerging pathogens which cause an opportunistic infections in immunocompromised patients, especially those with AIDS. Intestinal microsporidiosis is the most recognized infection, whereas urinary tract infections caused by microsporidia are rarely paid attention to either due to their subclinical course or diagnostic difficulties. In this report dual microsporidial infection of urinary tract, caused by *Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* was described in HIV/AIDS patients under cART therapy. Since microsporidiosis can cause severe complications or even death in immunosuppressed patients, our results suggest that microsporidial infection should be included in routine investigation of HIV-positive patients, even asymptomatic.

Key words: AIDS, HIV, microsporidia, *Encephalitozoon cuniculi, Enterocytozoon bieneusi*, opportunistic parasites

Introduction

Microsporidia are important opportunistic intracellular pathogens, which can cause severe disease in immunosuppressed patients, such as those after organ transplantation, chemotherapy recipients and patients with HIV-infection. Recently, microsporidial infections have progressively been diagnosed also in immunocompetent persons [1]. To date, fifteen microsporidia species from eight genera have been shown to cause disease in humans. However, the most common infections are caused by four species: *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Enc. cuniculi* and *Enc. hellem* [2]. Since enterocytes are the cells primarily infected, the most common clinical symptom of microsporidiosis is chronic diarrhea and ensuing massive weight loss. Furthermore, *Encephalitozoon* species can disseminate to extraintestinal sites leading to nephritis, hepatitis, peritonitis, myositis, keratitis, sinusitis, cholangitis, cystitis, prostatitis, pneumonia, genital tract infection and central nervous system involvement [3–5]. Until recently it was believed that *Ent. bieneusi* infects only enterocytes of the small intestine, nevertheless spores were occasionally found also in urine. Although a reduction in the incidence of opportunistic parasitic infections, including microsporidiosis, has been seen since introduction of cART, such infections still can cause disease, sometimes even life-threatening [6,7]. We describe three cases of dual microsporidial infection of urinary tract in HIV patients despite cART.

Table 1. Clinical and laboratory results from confirmed cases

Questionnaire			Patient number		
			1	2	3
Sex			M	M	F
Age			50	38	60
HIV diagnosis (month/year)			10/2012	07/2013	06/2005
HIV transmission route			IDU	IDU	HTX
HIV/AIDS stage			C2	C2	C1
TCD4 at diagnosis/nadir/current (cells/mm^3)			533/300/344	208/208/208	75/75/875
Year cART was initiated			2012	2013	2005
Current cART			TDF/FTC/EFV	TDF/FTC/LPV/RTV	ETR/DRV/RTV
Current HIV viremia (copies/ml)			undetectable	81	undetectable
Clinical symptoms			none	none	none
Additional information			Currently PEG-IFN+RBV treated, liver cirrhosis, HBV/HCV co-infection	HBV/HCV co-infected, bad living conditions	Chronic nephropathy with proteinuria
Smoker			yes	yes	yes
Presence of microsporidia	stool	LM	ND	ND	ND
		PCR	ND	*E. bieneusi*	ND
	urine	LM	microsporidia spores	ND	microsporidia spores
		PCR	*Enc. cuniculi*/ *Ent. bieneusi*	*Enc. cuniculi*/ *Ent. bieneusi*	*Enc. cuniculi*/ *Ent. bieneusi*

Explanations: cART – combined antiretroviral therapy; DRV – darunavir; EFV – efavirenz; ETR – etravirine; FTC – emtricitabine; HBV – hepatitis B virus; HCV – hepatitis C virus; HIV – human immunodeficiency virus; HTX – heterosexual transmission; IDU – intravenous drug use; LM – light microscopy; LPV – lopinavir; ND – not detected; PCR –polymerase chain reaction; PEG-IFN – pegylated interferon; RBV – ribavirin; RTV – ritonavir; TDF – tenofovir.

Materials and Methods

Three HIV/AIDS patients, two men (nos.1 and 2) and one woman (no. 3), came from a group of patients from the Centre for Prevention and Treatment of Infectious Diseases and Addiction Therapy in Wroclaw in Poland. A structured questionnaire was used to collect data about age, sex, HIV infection route, current drug use, treatment and concomitant diseases. Each patient was asked to provide stool and urine samples. All stool samples were routinely examined for ova and cyst by light microscopy (LM). To identify the microsporidia presence, both stool and urine specimens were screened by LM using modified trichrome stain (MT) and fluorescent Uvitex 2B staining [8,9].

Genus-specific nested-PCR protocols amplifying the internal transcribed spacer (ITS) region of *Encephalitozoon* spp. and *Ent. bieneusi* were performed as previously described [10,11]. The study was approved by the Bioethics Committee of the Wroclaw Medical University (Poland) (protocol nr KB-345/2010) and all the participants signed an informed consent form.

A summary of clinical and laboratory findings, drug use history, current treatment and concomitant diseases among studied patients is shown in Table 1.

Results

From six stool and urinal diagnosed samples, four were positive for microsporidia infection: all urinal samples and one stool sample. Microsporidial spores were confirmed by LM in stained urinal

samples obtained from patients nos. 1 and 3 (Fig. 1). Moreover, phylogeny analyses of ITS sequences acquired from urine specimens revealed concurrent infection with *Encephalitozoon cuniculi* (genotype II) and *Enterocytozoon bieneusi* (genotype D) in all three patients (Table 1) and presence of *Ent. bieneusi* (genotype D) in one stool sample (patient no. 2).

Apart from the patient no. 2, who had mild watery diarrhea which subsided with cART commencement, no patient had any clinical symptoms of the urinary tract infection or other system involvement at the time of testing.

Discussion

Human microsporidiosis is largely underestimated because its course is often self-limited or even asymptomatic in most healthy individuals as well as in HIV patients as shown in this study [1]. People are frequently exposed to microsporidia, especially those who are in close contact with animals, because spores are excreted with feces, urine or respiratory secretions of infected organisms and can be acquired by humans after ingestion of contaminated water or food [2]. Seroprevalence studies confirm that microsporidia spores easily infect humans, both immuno-competent and immunocompromised, and cause asymptomatic infection. According to van Gool et al. [12] antibodies against *Enc. cuniculi* were found in 8% of blood donors and 5% of pregnant women. Sak and co-workers reported higher seroprevalence of *Ent. bieneusi* among healthy blood donors (10%) and animal keepers (33%) [13]. A study carried out on murine model revealed that a competent immune system is unable to eliminate fully microsporidial infection, even after treatment [14]. Recently, an infection with microsporidia through solid organ transplantation was reported [15,16]. The results of this investigation demonstrate that microsporidia can survive in organs of immunocompetent hosts and are able to cause disseminated infection in case of immunosuppression [17].

Microsporidia usually infect epithelial and endothelial cells, fibroblasts or macrophages and can exhibit diverse pathogenicity and symptoms. The symptoms of microsporidiosis depend on the immune status of the infected host and site of infection. As mentioned above, they can also reactivate from latent state [2,15]. As microsporidia more commonly cause intestinal infections with persistent diarrhea, the conventional diagnosis is currently based on identification of spores in fecal specimens [9–11]. As a consequence, most studies have revealed *Ent. bieneusi* exclusively in feces except for a few reports, which confirmed *Ent. bieneusi* in urine both in immunocompetent and immunosuppressed patients [18,19]. The presence of *Ent. bieneusi* in urine suggests the lack of tissue specificity of this parasite. Most of these reports are only case studies (including autopsies), so a large population study is still lacking. On the other hand, *Enc. cuniculi* has been reported as responsible for disseminated microsporidiosis mainly in renal transplant recipients and AIDS patients, especially those with CD4+ counts <50 cells/mm^3 [3,4,18]. Thus, performing urine tests for microsporidia in such cases can increase early detection of disseminated microsporidiosis.

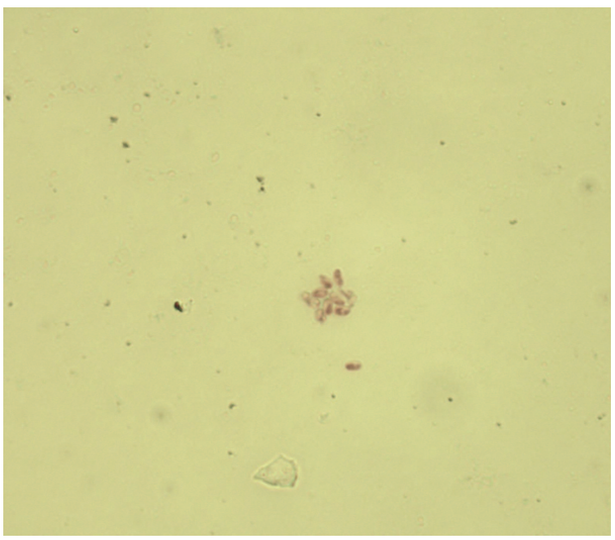

Fig. 1. Urine smear stained by modified trichrom. The microsporidian spores are oval, pinkish red in colour and show polar or central non-staining zone.

To our knowledge, this is the first report describing dual *Enc. cuniculi* and *Ent. bieneusi* infection of the urinary tract among HIV-infected patients. However, one report of dual microsporidial infection caused by *Enc. cuniculi* and *Ent. bieneusi* in HIV-positive patients has been documented up to date, but in stool specimen only and was based solely on LM and electron microscopy examinations [20].

None of the patients from our report had any symptoms of microsporidial infection, probably because of relatively high number of CD4+ T-lymphocytes (208–875cells/mm^3) at the time of examination resulting in low parasite burden due to self-limited infection. However, according to Fournier et al. [18], shedding of microsporidial

spores in urine may occur up to six months before the appearance of symptoms. The results of our study imply that HIV/AIDS patients receiving cART can remain latently infected with microsporidia and shed detectable amount of spores in their excretions despite being asymptomatic. Because microsporidial infection can reactivate, more often among patients with CD4+ T cell count <100 cells/mm^3, and cause clinical complications in the urinary system or potentially become disseminated causing severe morbidity, both stool and urine should be included in routine parasitological investigation of all HIV positive patients, even if asymptomatic.

Conflict of interest

All authors confirm that there are no conflicts of interest.

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