

SHORT REPORT

The first study on opportunistic intestinal microsporidiosis in IBD patients receiving immunosuppressive medications in Iran

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SUMMARY

Microsporida are known as opportunistic unicellular organisms and have recently been reclassified as fungi that have been frequently reported from patients with congenital and acquired immunity failure disorders, worldwide. However, use of immunosuppressive medications in inflammatory bowel disease (IBD) patients significantly decreases overall immunity, and increases their susceptibility to opportunistic infections. Totally, 71 stool samples were collected from IBD patients consisted of 69 ulcerative colitis (UC) patients and two Crohn's disease (CD) patients. All patients had taken immunosuppressive and/or immunomodulator drugs for at least 3 weeks. DNA was extracted from all stool samples and Nested PCR was performed using genus-specific primers based on small subunit ribosomal RNA (SSU rRNA) gene. Fisher's Exact Test was applied to evaluate statistical association between *microsporidia* infection and sex, age and types of IBD. Mean of age \pm s.d., women and men percentage of the attended patients were $36\cdot17 \pm 11\cdot93$, 60·6%, and 39·4%, respectively. A 440-bp fragment of SSU rRNA gene attributed to *Enterocytozoon bieneusi* was amplified from 12·7% of IBD patients. No *Encephalitozoon* DNA was detected in the samples. No microsporidia-positive sample was found in CD patients. Fisher's Exact Test showed that there was no statistically significant correlation between intestinal microsporidiosis and age, sex, and IBD types with *P* values: 0·389, 1·00, and 1·00, respectively. This study has shown IBD patients undergoing immunosuppressive/immunomodulators medications, which may be susceptible to intestinal microsporida infection. *E. bieneusi* is the commonest intestinal microsporidan reported from IBD patients.

Key words: *Enterocytozoon bieneusi*, IBD patients, immunosuppressive medication, Iran, opportunistic infection.

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Microsporida are known as opportunistic unicellular organisms and have recently been reclassified as fungi [1]. This microorganism has frequently been reported from both immunocompromised and

immunocompetent individuals, as well as a broad range of animals all over the world [2, 3]. Microsporidia infection in immunocompromised patients, particularly HIV/AIDS patients, is one of the most important concerns of healthcare systems. Reports of microsporidia infection have risen worldwide due to the increase in congenital/acquired immunity system disorders [4]. *Enterocytozoon bieneusi* is one of the most prevalent human-infecting microsporidan species reported from HIV/AIDS patients [5], patients undergoing chemotherapy [6], and individuals with immunity disorders.

Inflammatory bowel disease (IBD) is a complex gastrointestinal disorder and the etiological agent is not clearly known. Crohn's disease (CD) and ulcerative colitis (UC) are the two most important chronic IBD, frequently reported worldwide [7, 8]. Several genetic, environmental, and immunological factors have been suggested as triggers of immunological disorders and subsequent IBD. Nowadays, immunosuppressive/immunomodulatory agents such as corticosteroids and anti-inflammatory drugs are used to manage conditions of IBD patients [7]. However, the increasing use of immunosuppressive drugs in IBD patients, significantly decreases overall immunity and increases susceptibility to opportunistic infections. However, subsequent events of immunosuppressive therapy in IBD patients convinced the ECCF (European Crohn's and Colitis Foundation) to state that IBD patients on corticosteroids, biological agents, and immunomodulators are normally more susceptible to opportunistic pathogens [9].

Several studies have presented different opportunistic microbial pathogens from IBD patients [10]. Since intestinal microsporidiosis is considered as an opportunistic infection, frequently reported from immunocompromised patients, IBD patients undergoing immunosuppressive drugs could be more susceptible to this infection.

However, the current study is the first survey to investigate intestinal microsporidiosis in IBD patients undergoing immunosuppressive therapy.

Totally, 71 stool samples were collected from IBD patients consisted of 69 UC patients and two CD patients who had been referred to IBD clinic of Research Institute for Gastrointestinal and Liver Diseases in Shahid Beheshti University of Medical Sciences, Tehran during May 2015 to April 2016. A trained interviewer filled up a questionnaire consisted of age, sex, and type of IBD. All patients had taken immunosuppressive and/or immunomodulator drugs

for at least 3 weeks. Stool samples were immediately transferred to the Parasitology Laboratory of Foodborne and Waterborne Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases in Shahid Beheshti University of Medical Sciences and further investigations were carried out on the samples.

DNA extraction was performed for all stool samples using QIAamp DNA Stool mini Kit according to the manufacturer's instruction. Nested PCR was performed using genus-specific primers based on small subunit ribosomal RNA (SSU rRNA) gene according to the literature mentioned elsewhere [5]. Briefly, the first pair primers, PMicF (5' – GGTTGATTCTGCCTGACG – 3') and PMicR (5' – CTTGCGAGC(G/A)TACTATCC – 3'), amplified 779 bp of SSU rRNA gene of *Encephalitozoon* spp. and *Enterocytozoon bieneusi*. The second PCR employed primers EnbF (5' – GGTAATTTGGTCTCTGTGTG – 3') and EnbR (5' – CTAACTCCCTATCCGTTTC – 3') to amplify 440 bp as well as EncepF (5' – AGTACGATGATTTGGTTG – 3') and EncepR (5' – ACAAACTATATAGTCCCGTC – 3') to amplify 629 bp fragments for *E. bieneusi* and *Encephalitozoon* spp., respectively.

First, PCR reaction was performed in final volume 25 µl containing 12.5 µl of 2× Ampliqon PCR Master Mix (Denmark) 10 µM of each primers. Amplifications were carried out in Eppendorf thermocycler (Hamburg, Germany). The conditions for the first PCR reaction were: 95 °C for 5 min followed by 35 cycles of 94 °C for 40 s, 55 °C for 45 s, 72 °C for 45 s, and final extension of 72 °C for 4 min. The second PCR conditions were: 95 °C for 5 min followed by 25 cycles of 94 °C for 35 s, 57 °C for 35 s, 72 °C for 40 s, and 72 °C for 3 min as a final extension. The 10 µl of PCR products were electrophoresed on 1.5% of agarose gel and were visualized after ethidium bromide staining. A positive (sequenced isolates with accession no. KJ414444) and negative (Sterile Distilled Water) control were run together with all samples.

Fisher's Exact Test was applied to evaluate statistical association between microsporidia infection and sex, age, and type of IBD using IBM SPSS Statistics for Windows, v22 (Chicago, IL, USA). A *P*-value <0.05 was considered statistically significant.

Totally, 71 patients including 69 (97.18%) and 2 (2.82%) had UC and CD, respectively. IBD and type of IBD were proven in all patients using by colonoscopy and pathology evaluations. Mean of age ± s.d., women and men percentage of the attended patients were 36.17 ± 11.93, 60.6% and 39.4%,

Table 1. Demographic and summarized data of microsporidia infected patients among IBD subjects

		P-value
Microsporidia-positive patients	9/71	0
Microsporidia species		
<i>Enterocytozoon bieneusi</i>	9	
<i>Encephalitozoon</i> spp.	0	0
Age		
Mean of age \pm s.d. of enrolled patients	36.17 \pm 11.93	
Mean of age \pm s.d. of infected patients	36.44 \pm 8.56	0.389
Gender		
Male	3/28	
Female	6/43	1.00
IBDs type		
Ulcerative colitis	69/71	
Crohn's disease	2/71	1.00
Immunosuppressive/immunomodulator medication in infected patients		
Methyl-prednisolone	9/9	
Hydrocortisone	0/9	
Mesalamine	0/9	
Infliximab	0/9	—*

* P-value was not applicable.

respectively. All demographic data are summarized in Table 1. All enrolled patients had received one or several following drugs: Methyl-prednisolone or hydrocortisone, infliximab and mesalamine at least for 3 weeks. All patients had watery diarrhea or semi-form stool at the time of sampling.

PCR reactions were carried out on all DNA extracted samples and nine (12.7%) samples were identified as positive. All nine positive samples showed 440 bp fragment of SSU rRNA gene attributed to *E. bieneusi*, while no DNA amplification was found by specific primers for *Encephalitozoon* spp. Furthermore, all *E. bieneusi* positive-samples were seen in UC patients (13.4%), while no positive sample was found in CD patients. Statistical analysis was performed and Fisher's Exact Test showed that there was no statistically significant correlation between intestinal microsporidiosis and age, sex, and IBD types with P-values: 0.389, 1.00, and 1.00, respectively.

Microsporidia infection is one of the most important concerns and complications of physicians treating immunocompromised patients. Recently, gastrointestinal microsporidiosis was described as an emerging infectious disease reported from immunocompromised patients [5].

Nowadays, increase in the number of immune system disorders has led to opportunistic microorganisms being considered as one of the main secondary problems in immunocompromised patients. Several opportunistic

microorganisms have been reported from IBD patients on corticosteroids, immunomodulators, and biological agents. Although the co-existence of some viruses, bacteria, and fungi were represented, there are scarce data available on opportunistic parasites in IBD patients [10].

Studies have reported the opportunistic infection of intestinal microsporidia from almost all countries [2]. However, gastrointestinal microsporidiosis not only decreases the life-quality of IBD patients, but also increases the intricacy of the therapy process. The current study is the first to report gastrointestinal microsporidiosis in IBD patients undergoing immunosuppressive therapy. Recently, Andreu-Ballester *et al.* suggested an etiological role for microsporidia in CD patients before immunosuppression and stated that due to deficiencies of $\delta\gamma$ T lymphocytes and IL-7, CD patients are likely to be more susceptible to colonization of intestinal microsporidia. In contrast with our study, all enrolled patients in mentioned research had not been treated for CD and had not undergone immunosuppression therapy [11].

The prevalence rate of the microsporidia infection in the current study was 12.7%. In Iran, there are studies that described intestinal microsporidia from HIV/AIDS patients [5, 12], cancer patients under chemotherapy and transplant recipients [13]. A previous study from Iran by Mirjalali *et al.* showed high prevalence (30.86%) of *E. bieneusi* among HIV/AIDS patients, while some other studies reported

lower rates of the infection [5]. Agholi *et al.* found *E. bienersi* from 9.55% of HIV/AIDS patients and 6.81% of transplant recipient children in the south of Iran [12, 14]. In another study, Tabatabaie *et al.* reported prevalence rates of 5.3% and 4% for *E. bienersi* and *Encephalitozoon* sp., respectively, in healthy subjects and 0.7% and 5.7% for *E. bienersi* and *Encephalitozoon* sp., respectively, in various samples of immunocompromised patients [15]. In our study, only *E. bienersi* was found in 12.7% of IBD patients who underwent immunosuppressive/immunomodulator medications. The prevalence rate of our study is similar and in agreement with other studies showing microsporidiosis in immunocompromised patients in Iran.

In the current study, only *E. bienersi* was detected in all microsporidia-positive patients and no DNA of *Encephalitozoon* was amplified. Almost all previous studies in Iran showed that *E. bienersi* had a higher prevalence rate [3, 5, 12, 14]. On the other hand, the interesting point is that metronidazole consumption with or without prescription is common in IBD patients to prevent diarrhea. Although, He *et al.* showed that metronidazole could be effective against *Encephalitozoon* spp. [16], but *E. bienersi* is perceived as resistant to benzimidazoles such as metronidazole [17]. Therefore, the low prevalence of *Encephalitozoon* spp., compared with *E. bienersi* in IBD patients, as our results showed, could be predictable.

In this study, no positive sample was detected in CD patients. This finding is more likely related to the number of CD patients. In Iran, the prevalence rate of UC is more than the CD cases [18] and unfortunately, at the time of sampling the number of CD cases was very low. However, intestinal microsporidia, particularly *E. bienersi*, should be considered as one of the most important opportunistic microorganisms, capable of increasing the complexity of managing IBD patients.

IBD patients undergoing immunosuppressive, corticosteroid, and immunomodulator agents could be susceptible to intestinal microsporidia infection. In addition, *E. bienersi* is the commonest microsporidian species reported from this group of IBD patients.

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DECLARATION OF INTEREST

None.

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