

Study of *Blastocystis* frequency among IBD patients referred to a gastroenterology center

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Abstract:

BACKGROUND: *Blastocystis* is the most common anaerobic protozoa living in the large intestine of a broad spectrum of vertebrates. **OBJECTIVES:** The aim of this study was to investigate the *Blastocystis* infection rate in inflammatory bowel disease (IBD) patients. **METHODS:** A total of 80 stool samples were collected from IBD-proved patients. All stool samples were cultivated in Dulbecco's modified Eagle's medium and checked by light microscopy for detection of *Blastocystis*. The Correlation between demographic data of IBD patients and *Blastocystis* was calculated using SPSS 23. **RESULTS:** The enrolled patients comprised of 52 (65%) men and 28 (35%) women. The study showed *Blastocystis* in 16/80 (20%) of the samples by microscopic examination and culture method. The parasite was seen among 12 (23.08%) and 4 (14.29%) men and women, respectively. No statistically significant correlation was found between infection with the parasite and animal contact. Fisher's exact test represented that there was no correlation between gender and the presence of *Blastocystis* (p value= 0.397). Fisher's exact test denoted that there was no statistical correlation between age and the presence of the parasite (p value= 0.130). **CONCLUSIONS:** In this study, *Blastocystis* was found in 20% of enrolled patients who suffered from IBD. This infection rate was significantly higher than the studies have previously described *Blastocystis* in this group of patients.

Introduction

Blastocystis is the most common anaerobic protozoa in the large intestine of human

and many animals (Stensvold and Clark 2016, Mirjalali, Abbasi et al. 2017). The frequency of *Blastocystis* is worldwide with the highest rate in the countries with inap-

appropriate sanitation (Amin 2006, El Safadi, Gaayeb et al. 2014). Likewise, high prevalence of *Blastocystis* in the studied populations with close-contact to animals supports the importance of animal hosts as potential reservoirs of the parasite (Yoshikawa, Abe et al. 2004, Lee, Chye et al. 2012). Because of the frequent reports of *Blastocystis* from domesticated and pet animals like cattle, sheep, pig, dog, birds, etc., it is strongly suggested that the parasite can transmit to human subjects from animal sources (Cian, El Safadi et al. 2017). There are evidences of transmission of the parasite via unhealthy drinking water as well (Cian, El Safadi et al. 2017). The pathogenicity of *Blastocystis* is still unclear because of the frequent reports from both symptomatic and asymptomatic subjects (Roberts, Stark et al. 2014). The clinical symptoms associated with the parasite are often non-specific including diarrhea, bloating, nausea, abdominal pain (Stensvold, Nielsen et al. 2009, Alinaghizade, Mirjalali et al. 2017, Jalallou, Irvani et al. 2017). Although many studies have indicated potential linkage between *Blastocystis* and some clinical manifestations such as irritable bowel syndrome (IBS) (Jimenez-Gonzalez, Martinez-Flores et al. 2012), skin manifestations (Cassano, Scoppio et al. 2005) and gastrointestinal disorders (El Safadi, Meloni et al. 2013, Dagci, Kurt et al. 2014), the recent studies have shown a negative correlation between the presence of the parasite with some gastrointestinal disorders like inflammatory bowel disease (IBD) (Mirjalali, Abbasi et al. 2017).

IBD is a chronic inflammation including Crohn's (CD) and Ulcerative colitis (UC) with unknown etiology. CD is a chronic inflammation that may involve full thickness of the layers of upper and lower gastrointes-

tinal tract (GT) while UC is an inflammation restricted to colon and only affects surface epithelium and mucosal layer (Satsangi, Silverberg et al. 2006). Many factors are supposed to be potential causes of onset or relapse of IBD including genetic, climatic regions zones, vitamin D3 deficiency, high protein diet, diet high in omega 6 and microbial infections (Hunter 2014). However, some studies describe the significant role of microbiota at onset of the symptoms in IBD patients (Nourrisson, Scanzi et al. 2014). Furthermore, some researches proposed a protective role for some intestinal parasites against immunological disorders (Wang, Cao et al. 2008). Therefore, the aim of the current study was to determine the frequency of *Blastocystis* among IBD patients referred to the Research Institute for Gastroenterology and Liver Diseases during the period August 2016 to February 2017 and to evaluate the correlation of demographic data on the infection rate.

Material and Methods

Stool sampling: In the current study, a total of 80 stool samples were collected from IBD-proved patients who were referred to Gastroenterology Clinic of the Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences from August 2016 to February 2017. A well-trained interviewer filled a questionnaire that consisted of demographic data, drug type and dosage consumed through one month before the sampling. The consumption of metronidazole during the last month was considered as exclusion criteria. The stool samples were immediately transferred to Parasitology lab of Foodborne and Waterborne Diseases Research

Table 1. Overview of demographic and laboratory findings in IBD patients. aIBD: Inflammatory bowel disease; M: Male; F: Female.

	Age group, Patient age, Gender										Total no.
	I		II		III		IV		V		
	<25		26-35		36-45		46-55		>56		
	M	F	M	F	M	F	M	F	M	F	
IBDa	6	1	20	7	12	15	8	4	6	1	80
Positive patients close-contact to animal	3	0	4	0	3	2	2	1	0	1	16

Center located in the Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences for further investigation.

Microscopic examination and stool cultivation: All the stool samples were examined directly by Lugol’s iodine staining. Furthermore, the samples were concentrated with routine formalin-ethyl acetate and checked by light microscopy for detection of enteric parasites. A portion of the samples was immediately cultivated in Dulbecco’s modified Eagle’s medium (DMEM) with 20% inactivated calf serum (Zhang, Qiao et al. 2012). All the cultivated samples were incubated at 37 °c for 72 h and 10 µL of the sediment of the medium was examined by light microscopy with magnification X400 for growth of *Blastocystis*. The samples were checked for any growth of *Blastocystis* every 48 h and the samples without any growth after 10 days were considered negative.

Results

The enrolled patients consisted of 52 (65%) men and 28 (35%) women. In this study, *Blastocystis* was detected in 16/80 (20%) of the samples by microscopical examination and culture method (Fig 1, 2). The parasite was seen among 12 (23.08%) and 4 (14.29%) men and women, respectively. Fisher’s exact test represented that

there was no correlation between gender and the presence of *Blastocystis* (P value= 0.397). The mean age + SD of the IBD patients enrolled in this study was 38.07 + 11.11. Indeed, the mean age + SD of the IBD patients carrying *Blastocystis* was 37.06 + 12.35 while in the uninfected IBD patients it was 38.28 + 10.87. Fisher’s exact test denoted that there was no statistical correlation between age and the presence of the parasite (p value= 0.130). Likewise, the frequency of *Blastocystis* in the defined age groups was as followed: 37.5% (3/8) in less than 25 years-old, 15.38% (4/26) in 26-35, 18.51% (5/27) in 36-45, 25% (3/12) in 46-55 and 14.28% (1/7) in more than 56 years-old. Fisher’s exact test also showed that there was no significant correlation between age group and *Blastocystis* (P value= 0.699). All the demographic data are summarized in Table 1.

Furthermore, history of close-contact to animal was investigated and showed that there was no statistically significant correlation between the presence of the parasite and keeping pet or domesticated animals. Accordingly, although there was missing data from 10 IBD patients, only 3 of *Blastocystis*-positive patients stated history of close-contact to animals.

Discussion

In the current study, *Blastocystis* was de-



Figure 1. Various forms of Blastocystis in Lugol's iodine staining in direct method. (x400).

tected in 20% of IBD patients. No statistical correlation was found between age, sex and animal contact with the presence of the parasite. *Blastocystis* is a common enteric protozoan in fecal samples of human, worldwide. The pathogenic role of the parasite is still debated. In spite of evidence of the positive-correlation between IBS and *Blastocystis*, the association between IBD and *Blastocystis* has mostly been found negative (Nagel, Traub et al. 2015, Mirjalali, Abbasi et al. 2017). In this respect, a lower frequency of *Blastocystis* in IBD patients with active phase compared with controls was reported by Rossen and colleagues (Rossen, Bart et al. 2015). Coskun et al. investigated the presence of *Blastocystis* among UC patients and stated that patients with active phase had lower frequency of the parasite in comparison with those patients who were in the remission phase (Coskun, Malatyalı et al. 2016). The study by Dugruman et al. showed that IBD patients had lower frequency of *Blastocystis* in comparison with IBS patients (Dugruman-AI, Simsek et al. 2010). In another study, Peterson et al. reported lower prevalence of *Blastocystis* in IBD patients in comparison with the patients with other gastrointestinal disorders as well

as healthy subjects and suggested that this observation might be due to the unfavorable condition of GT in IBD patient for colonization of *Blastocystis* (Petersen, Stensvold et al. 2013). Recently, a case-control study performed by Mirjalali and colleagues showed that the prevalence rate of the parasite in IBD patients was significantly lower than healthy subjects. This observation was supported by previous studies assuming the role of IBD in dysbiosis (Mirjalali, Abbasi et al. 2017). According to the current findings contrasting mentioned studies, a high prevalence of *Blastocystis* 20% (16/80) was observed. These results are similar to the surveys conducted by Yamamoto-Furusho and Cekin et al. among UC patients (Yamamoto-Furusho and Torijano-Carrera 2010, Cekin, Cekin et al. 2012). Cekin et al. showed higher prevalence of *Blastocystis* among IBS and IBD patients than those subjects without significant gastrointestinal disorders (Cekin, Cekin et al. 2012). Yamamoto-Furusho described higher prevalence of *Blastocystis* among IBD patient who suffered from active phase than those patients who were in intermittent or remission phases (Yamamoto-Furusho and Torijano-Carrera 2010).

The current study showed that, although the prevalence of the parasite in age group under 25 years-old was higher than other groups, no significant correlation was found between age group and the presence of the parasite. Moreover, there was no significant correlation between the mean age of infected patients and uninfected subjects. Some studies have declared a noteworthy association between age group and the presence of the parasite in general population. This correlation was also seen in some studies that have been conducted among IBD pa-

tients. Against the current study, the correlation between age and the presence of the parasite was seen in the study conducted by Mirjalali et al. (Mirjalali, Abbasi et al. 2017). In agreement with our findings, in the study performed by Muttiucci and colleagues there was no significant association between age group and the presence of the parasite (Mattiucci, Crisafi et al. 2016). Similar results were seen in the study implemented by Cekin and colleagues, where there was no statistical correlation between age group and *Blastocystis* (Cekin, Cekin et al. 2012).

As the results illustrate, statistically significant relationship was not seen between gender and the presence of the parasite that is in accordance with the studies performed by Mirjalali and Muttucci (Mattiucci, Crisafi et al. 2016, Mirjalali, Abbasi et al. 2017).

Although no correlation was found between animal contact and infection with *Blastocystis*, it has been suggested that pet or farm animals could be considerable reservoirs of zoonotic transmission (Cian, El Safadi et al. 2017). Considering the possibility of zoonotic transmission of this parasite, keeping pets as well as close-contact to animals should be a concern, particularly in the human subjects with immunity disorders.

Conclusion: In this study *Blastocystis* was found in 20% of enrolled patients who suffered from IBD. This infection rate was significantly higher than the studies that have previously described *Blastocystis* in this group of patients. Therefore, these findings showed the importance of stool examination for surveying the common intestinal protozoans like *Blastocystis* in IBD patients particularly in different phases (Flare up and remission) of these groups.

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بررسی شیوع بلاستوسیسیتیس در بیماران مبتلا به IBD ارجاعی به مرکز گوارش

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چکیده

زمینه مطالعه: بلاستوسیسیتیس یکی از شایع‌ترین تک‌یاخته‌های بی‌هوازی موجود در روه بزرگ طیف وسیعی از مهره داران می‌باشد. **هدف:** این مطالعه با هدف بررسی شیوع بلاستوسیسیتیس در بیماران مبتلا به بیماری التهابی روده (IBD) انجام گردید. **روش کار:** جمع آوری نمونه مدفوع از ۸۰ فرد بیمار مبتلا به IBD انجام شد. همه نمونه‌ها در محیط کشت اختصاصی بلاستوسیسیتیس کشت داده شدند و جهت تشخیص بلاستوسیسیتیس تحت بررسی میکروسکوپی قرار گرفتند. ارتباط بین اطلاعات دموگرافیک بیماران مبتلا به IBD و تک‌یاخته بلاستوسیسیتیس توسط برنامه SPSS نسخه ۲۳ بررسی گردید. **نتایج:** از ۸۰ بیمار مبتلا به IBD تعداد ۵۲ (۶۵٪) مرد و ۲۸ (۳۵٪) زن بودند. آلودگی به بلاستوسیسیتیس در ۱۶ نمونه از ۸۰ نمونه (۲۰٪) با روش کشت و توسط میکروسکوپ مثبت گزارش شدند. تک‌یاخته بلاستوسیسیتیس در ۱۲ (۲۳/۰۸٪) مرد و ۴ (۱۴/۲۹٪) زن جدا شد. با تست دقیق فیشر هیچ ارتباط معنی‌داری بین آلودگی به بلاستوسیسیتیس و جنس نشان داده نشد ($p\text{value} = 0/۳۹۷$). همچنین هیچ ارتباط معنی‌داری بین آلودگی به بلاستوسیسیتیس و ارتباط با حیوان نیز یافت نشد. **نتیجه‌گیری نهایی:** در این مطالعه میزان آلودگی به بلاستوسیسیتیس در افراد مبتلا به IBD ۲۰٪ گزارش شد. این میزان آلودگی نسبت به سایر گزارشات قبلی که روی میزان شیوع بلاستوسیسیتیس و این گروه از بیماران انجام شده بود بصورت معنی‌داری بالاتر گزارش شد.

واژه‌های کلیدی: بلاستوسیسیتیس، اطلاعات دموگرافیک، بیماری روده التهابی، آزمایش میکروسکوپی، کشت مدفوع