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Blastocystis in ulcerative colitis patients: Genetic diversity and analysis of laboratory findings

Adil Coskun[1], Erdogan Malatyali[2*], Hatice Ertabaklar[2], Mustafa B. Yasar[1], Ali O. Karaoglu[1], Sema Ertug[2]

[1]*Department of Gastroenterology, Faculty of Medicine, Adnan Menderes University, Aydin, Turkey*

[2]*Department of Parasitology, Faculty of Medicine, Adnan Menderes University, Aydin, Turkey*

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ABSTRACT

Objective: To determine *Blastocystis* frequency and subtypes (ST) in ulcerative colitis (UC) patients and analyse some laboratory findings between *Blastocystis* positive and negative cases.

Methods: Faecal samples from 150 UC patients in Adnan Menderes University, Training and Research Hospital were examined by direct microscopy and cultivated in Jones medium. *Blastocystis* positive cultures were subjected to DNA isolation and subtypes were identified by sequencing of barcode region. A retrospective analysis was conducted on C reactive protein (CRP), leucocyte counts (WBC), neutrophil counts, and sedimentation rates.

Results: The overall positive rate of *Blastocystis* was 8% (12 patients) and the most abundant subtype was ST3 (eight isolates, 66.7%), followed by ST1, ST2 and ST7. Laboratory findings between *Blastocystis* infected and non-infected UC patients were not significantly different. *Blastocystis* frequency was 3.8% among the patients in active stage, while it was 11.8% among the patients in remission stage.

Conclusions: The present study confirms previous findings that have indicated the predominance of *Blastocystis* ST3 in humans and contributes additional evidence that suggests the low colonisation of *Blastocystis* infection in ulcerative colitis patients during active stage.

1. Introduction

Blastocystis is a common intestinal protozoon parasite, found in humans. Recent researches have shown contradictory findings about the pathogenesis of *Blastocystis*. In a great majority of human cases, *Blastocystis* infection is asymptomatic; however, nonspecific gastrointestinal and urticarial symptoms may be observed [1,2]. It was reported that *Blastocystis* has many different subtypes (ST) based on its small sub-unit ribosomal RNA (ssRNA) [3]. These subtypes can be detected by molecular methods such as sequencing, restriction fragment length polymorphism or sequence-tagged site-PCR [4]. There have been many attempts that offered a possible subtype related pathogenicity, nevertheless much uncertainty still continues about the relationship [5]. Ulcerative colitis (UC) is a chronic inflammatory disease of large intestine and is increasing in frequency throughout the world. There has been increasing evidence that suggests a possible link between gut microbiota and development of UC either by causing inflammation directly or indirectly through an altered immune system [6,7]. Despite being a common inhabitant of human intestinal tract, the role of *Blastocystis* in UC still needs to be investigated.

The primary aim of the present study was to determine *Blastocystis* subtypes in UC patients. Additionally, this study attempted to analyse C reactive protein (CRP), leucocyte counts (WBC), neutrophil counts, and sedimentation rates between *Blastocystis* positive and negative cases.

2. Methods

2.1. Samples

In the present study, faecal samples from 150 UC patients in Adnan Menderes University, Training and Research Hospital were collected from June 2013 to the end of February 2015.

2.2. Direct microscopy and culture

Faecal samples were examined by direct microscopy of native (0.9% serum physiological) and Lugol's iodine preparations as a part of routine parasitological examination. An

*Corresponding author: Erdogan Malatyali, Department of Parasitology, Faculty of Medicine, Adnan Menderes University, 09100 Aydin, Turkey.
Fax: +90 256 2120146
E-mail: erdogan.malatyali@adu.edu.tr
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approximate 50 mg of samples were inoculated in 3 mL of Jones' medium supplemented with 10% foetal calf serum. The cultures were checked for the presence of *Blastocystis* on third day of inoculation by direct microscopy.

2.3. PCR and sequencing

Prior to DNA isolation the cultures were pelleted by centrifugation at 12 000 *g* for one minute. Genomic DNA was isolated only from positive cultures by using a commercially available kit DNAzol (Invitrogen) according to manufacturer's instructions. A single PCR reaction was set with the primers RD5 and BhRDr as described by Scicluna *et al.* [4] for amplification of 'barcode region', an approximately 600 bp of SSU rRNA gene. The reaction was set in a 30-μL volume containing: 1–2 μL of template DNA, 0.4 pmol of each of the primers, 0.3 U of Taq DNA polymerase (Fermentas), 0.2 mM of each dNTP (Fermentas), 1 × Taq buffer with $(NH_4)_2SO_4$ (Fermentas). PCR amplicons were purified and sequenced by a commercial facility (MedSanTek, Istanbul) by using Applied Biosystems 377 DNA Sequencer.

2.4. Determination of subtypes

Subtypes were determined according to closest or exact match at GenBank nucleotide database using BLAST tool at the National Center for Biotechnology Information website [8]. Moreover, the sequences were queried against the *Blastocystis* Sequence Typing website database (http://pubmlst.org/Blastocystis/), curated by Stensvold and sited at the University of Oxford [9].

2.5. Analysis of laboratory findings

A retrospective analysis was conducted on the following laboratory findings: CRP, WBC, neutrophil counts, and sedimentation rates. The patients with endoscopic activity score below four were considered as in remission stage and the patients with equal or over four were considered as in active stage [10]. Data was analysed with non-parametric Mann–Whitney U test by using Statistical Package for the Social Sciences (SPSS).

3. Results

The overall positive rate of *Blastocystis* was 8% (12 out of 150) by culture, however the rate was 4.7% (7 out of 150) by direct microscopy of wet mounts. The most common subtype was ST3 (eight isolates, 66.7%), followed by ST1 (two isolates, 16.7%), ST2 (one isolate, 8.3%) and ST7 (one isolate, 8.3%). The sequences were deposited to Genbank with accession numbers: KU361317–323.

The mean values and standard deviations of laboratory findings between *Blastocystis* infected and non-infected UC patients were given in Table 1. The mean age of *Blastocystis* infected and non-infected cases were 54.5 ± 12.0 and 46.5 ± 14.0, respectively. *Blastocystis* frequency was 3.8% (2 out of 52) among the patients in active stage, while it was 11.8% (8 out of 68) among the patients in remission stage, data was available for 120 UC patients. The difference was not significantly significant ($\chi^2 = 2.41$, $P > 0.05$). Moreover, the infection rate was 7.8% (eight out of the 102) among male and 8.3% (four out of 48) among female patients ($\chi^2 = 0.11$, $P > 0.05$).

4. Discussion

In the present study, ST3 was found as the most common subtype in UC patients. ST3 is thought as human originated subtype and reported as predominant in a variety of study populations. Dogruman-Al *et al.* [11], found that ST3 was the most common among IBS, UC and patients with chronic diarrhoea. Additionally, ST1–3 are the usually reported subtypes from Turkey. Another subtype from Turkey is avian ST7 which rarely infects humans [12]. The use of cultures was suggested in many studies for the detection of *Blastocystis* both in epidemiological studies and routine laboratories, because of the low sensitivity of direct microscopy [13,14]. In the present study, the rate of positive cases was increased from 4.7% to 8.0% by the use of Jones medium. However, *Blastocystis* frequency could be higher than we detected, because of the fact that some *Blastocystis* strains might not grow in xenic culture [15]. In the present study, the rate of *Blastocystis* infection was not related to gender as previously reported [16].

The role of *Blastocystis* in certain gastrointestinal diseases and relations with symptoms are investigated. Nagler *et al.* [17] asserted that *Blastocystis* had no role in Irritable Bowel Disease (IBD); but the results were based on limited number of patients (five UC patients) and unable to encompass the entire picture. Mumcuoglu *et al.* [18] reported that the frequency of *Blastocystis* was higher among IBS patients and the symptoms declined after treatment of *Blastocystis*. In another study, *Blastocystis* positive 99 patients were compared with control group and none of the gastrointestinal symptoms were found to be related with *Blastocystis* infection [19]. It was noted that despite these controversies, *Blastocystis* should be screened in UC patients when the symptoms are refractory [20,21]. Tai *et al.*, [20] interestingly noted that the elimination of *Blastocystis* was supportive in the recovery of gastrointestinal symptoms and also confirmed their findings with colonoscopy. In a more recent study, Krogsgaard *et al.* [22] compared the frequency of *Dientamoeba fragilis* and *Blastocystis* between asymptomatic population and IBS patients in Denmark. The authors reported that the positive rate of *Blastocystis* was greater in asymptomatic population than in IBS patients (22% versus 15%) thus indicating that these parasites are not likely to have a direct role in the pathogenesis of IBS.

Table 1
Comparison of *Blastocystis* infected and non-infected UC patients in terms laboratory findings.

Blastocystis	*n*	WBC ($\times 10^3$/μL)	Neutrophil counts ($\times 10^3$/μL)	Sedimentation rates (mm/h)	CRP (mg/L)
Positive	11	7626.30 ± 1697.60	4791.80 ± 1065.10	37.18 ± 25.40	13.40 ± 23.60
Negative	112	8404.40 ± 2574.00	5338.30 ± 2241.30	35.10 ± 22.40	18.90 ± 40.60

The laboratory findings: WBC, Neutrophil counts, sedimentation rates and CRP between *Blastocystis* infected and non-infected cases was not significantly different in the present study. CRP is an important marker for the disease activity in UC patients; in active stage the level is high and in remission it is usually low [23]. In a previous study, haematological values were investigated between *Blastocystis* infected and control group and CRP and sedimentation rates were found to be significantly high in infected group [24]. Despite being statistically not significant, in our study the rate of *Blastocystis* was higher in patients who were in active stage than the patients in remission (11.8% versus 3.8%). This finding was in accordance with the previous study [25]. Additionally, Rossen *et al*. compared patients with active UC and healthy controls in a cohort, they reported that *Blastocystis* was significantly less frequent in UC patients (13.3%) as compared to healthy controls [26]. Nishikawa *et al*. compared mucosa-associated microbiota of patients with active UC and non-IBD controls using terminal restriction fragment length polymorphism (T-RFLP) analysis; they showed that diversity of microbial composition was significantly fewer in active stage [6]. In the present study, data from 120 out of 150 UC patients was available for the activity of diseases. It may be concluded that this inconsistency may be due to the number of patients. Furthermore, a study dealing with the CRP level of *Blastocystis* infected UC patients in remission stage would be worthwhile. Our study population was limited to 150 patients; increasing the number of patients in future studies would give more brief conclusions.

A limitation of this study is that data has not been supplemented with direct PCR of faecal samples which possibly could increase the positive rate and make comparisons of patient groups more valid. Additionally, the small number of *Blastocystis* positive UC patients (12 patients) prevented making parametric statistics between groups. Roberts *et al*. compared different methods and found PCR as the most sensitive at detecting *Blastocystis* [27]. Additionally, in another study, the positive rate of *Blastocystis* was 41.0% by culture and it was 44.6% by PCR [28].

In conclusion, the present study confirms previous findings that indicate the predominance of *Blastocystis* ST3 in humans and provides additional evidence that suggests low colonisation of *Blastocystis* infection in UC patients during active stage. Additionally, further studies investigating some other laboratory findings in *Blastocystis* infected UC patients will be worthwhile.

Conflict of interest statement

We declare that we have no conflict of interest.

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