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Blastocystis and irritable bowel syndrome: Frequency and subtypes from Iranian patients

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ABSTRACT

There are inconsistent findings about the role of *Blastocystis* infection in irritable bowel syndrome (IBS). The present study was aimed to determine the frequency of *Blastocystis* and their subtypes (ST) in patients with IBS. A total of 122 patients with IBS and 122 healthy individuals referred to the medical laboratory centers in Ahvaz (southwest of Iran) participated in the study. The frequency of *Blastocystis* was determined. *Blastocystis* genomic DNA was extracted from positive feces, and PCR was performed using seven primer pairs targeting the SSU rDNA gene. *Blastocystis* was detected in 19.67% of patients with IBS and 17.2% of individuals without IBS. The difference between two groups was not statistically significant ($P = 0.3$). Among the five subtypes of *Blastocystis*, ST3 was more common in patients with IBS and control group. However, there were no significant differences between two groups in terms of subtypes of *Blastocystis* ($P = 0.6$). It seems, the role of *Blastocystis* in the etiology of IBS should be further investigated. Furthermore, a model of study should be designed to investigate the role of host factors in severity of parasitic disease.

1. Introduction

Irritable bowel syndrome (IBS) is a chronic functional disorder of the gastrointestinal tract. It is a common healthcare problem with worldwide distribution that reduces the quality of life [1]. Young adults and people over the age of 50 years are more prone to IBS, and women are 2–3 times more frequently exposed [2]. The disease is characterized by abdominal pain, flatulence, irregular bowel movements and, diarrhea or constipation in the absence of organic etiology [1]. According to the widely used Rome II criteria, IBS sufferers can be clustered into three symptom groups based on the stool appearance, stool frequency, and defecatory symptoms: diarrhea (IBS-D), constipation (IBS-C), and mixed type (IBS-M) with alternating occurrence of both diarrhea and constipation [1,2]. More recently, the Rome III criteria, which focused on the symptom frequency over persistence, have been issued [2].

IBS is one of the most common complaints that disturbs the socio-economical life of patients [3]. The disease has a high prevalence in western countries ranging between 12–15% [4]. In Asian countries, some studies have shown the prevalence of IBS between 3.5–25% [5]. Several epidemiological studies have recorded the prevalence of IBS in Iran to vary between 4% to 12.6% [6]. The causes and pathophysiology of IBS are unknown. Several factors, including visceral hypersensitivity, irregular gut motility, and autonomous nervous system dysfunction are reported as probable causes of the signs and symptoms of IBS. Other studies have shown that genetic factors, chronic stress, and chronic immune activation can predispose people to developing IBS [7,8]. There is reliable evidence showing that IBS may be the result of an acute infectious gastroenteritis, post infection (PI_ IBS); the infectious agents involved in the development of IBS include pathogenic bacteria, viruses, and parasites [9]. One of the parasites that may be involved in the development of IBS is *Blastocystis;* an enteric cosmopolitan protist and one of the most common parasites found in stool samples [9]. The prevalence of *Blastocystis* has been reported in the variable range from 1.5% to 10% and 30 to 50% in the developed and developing countries, respectively [10].

There has been debate concerning the pathogenicity of *Blastocystis* in several studies [35]. Some of these studies specified an association between the parasite and IBS [11–14], while others did not report

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association that have been attributed with IBS [10,15]. Molecular and phylogenetic studies suggested that humans can involve with multiple subtypes of *Blastocystis*, as well as a number of factors such as intensity of infection, host immunity and parasite subtypes (STs) are considered for its pathogenicity [16,17]. The presence of sufficient information on the subtypes of *Blastocystis* found in IBS patients would be of interest in understanding the importance and role of *Blastocystis* infection in chronic illness. Thus, this study aimed to determine the frequency and identify *Blastocystis* subtypes in patients with IBS, in Ahvaz, southwestern Iran.

2. Materials and methods

2.1. Study area

Ahvaz city, capital of Khuzestan province which is located in the southwest of Iran (31°50′N and 49°11′E), is ranked as the 7th largest city throughout the country and based on the latest census, its population was calculated 1,395,184 in 352,128 families. Weather temperature is highly variable throughout the year so that in summer temperature exceeds 50 °C whereas in winter it falls to 5 °C. Also, annual average rainfall is approximately 230 mm [33].

2.2. Study design and population

This descriptive study was conducted over a period of 24 months from 2012 to 2014. The sample of the study consisted of 244 subjects (122 patients diagnosed with IBS and 122 individuals without IBS, with the age range of participants was 20–50 years) who were referred to the medical laboratory centers of Ahvaz, southwest of Iran by a gastroenterologist. Ethical approval was obtained from the Committee of Research, Publications and Ethics of the Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences. Before collecting stool samples, a written informed consent was obtained from participants.

2.3. Inclusion criteria

Patients were classified according to Rome III criteria as IBS category. According to these diagnostic criteria, IBS was defined by recurrent abdominal pain or discomfort at least 3-day per month in the last 3-month, associated with two or more of the following: improvement with defecation, onset associated with a change in frequency of stool, and onset associated with a change in form (appearance) of stool. Information about their medical history was obtained.

2.4. Exclusion criteria

Patients with pregnancy, the presence of thyroid disorders, chronic diseases, e.g., chronic liver or kidney diseases; intestinal surgery such as appendectomy and choledochotomy; being under antibiotic treatment during last month, being under chemotherapy.

2.5. Stool collection and examination

Fecal specimens were obtained from both IBS and control groups. The consistency of all stool samples, including formed, soft, loose, and watery was noted. All samples were examined through wet and lugol smears. Stool examination was performed and *Blastocystis*-positive stools were stained by Trichrome method.

2.6. DNA extraction

Genomic DNA of each isolate was extracted using a DNA extraction kit according to the manufacturer's protocol (QIAamp, QIAGEN Inc., Germany). Stool specimens that were co-infected with other parasites were excluded. Eventually, the extracted DNA was stored at −20 °C until PCR amplification.

2.7. Subtyping

PCR was performed using specific *sequence-tagged site* (STS) primers to identify subtypes of *Blastocystis*, including SB83 (351 bp) for ST1, SB340 (704 bp) for ST2, SB227 (526 bp) for ST3, SB337 (487 bp) for ST4, SB336 (317 bp) for ST5, SB332 (338 bp) for ST6, and SB155 (650 bp) for ST7 [18]. PCR reaction mixtures (25 μl of total volume) consisted of PCR buffer 1× [10 mM Tris-HCl, pH 8.8, and 50 mM KCl], 1.5 mM $MgCl_2$, 2.5 U/μl of *Taq* polymerase (Fermentas), 1.25 μM of each dNTPs (Fermantas), 0.5 pmol of each primer, and 5 μl of the DNA sample. The PCR conditions consisted of one cycle denaturing at 94 °C for 5 min, 40 cycles, including annealing at 57 °C for 30 s, extending at 72 °C for 60 s, denaturing at 94 °C for 30 s, and additional cycle with 5-min chain elongation at 72 °C (MyCycler, Bio-Rad, Hercules, CA, USA). The amplification products were electrophoresed on 2% agarose gels and Tris-borate-EDTA buffer. Gels were stained with ethidium bromide and photographed using an ultraviolet gel documentation system (Uvidoc, Gel Documentation System, Cambridge, UK) [33,34]. The PCR amplification for each primer pair was done thrice for each isolate.

2.8. Sequence analysis

PCR amplification of sequences from 23 randomly selected *Blastocystis* isolates was performed by MWG (Biotech AG, Germany), and were analyzed and graphics were generated using Chromas 2.23 software (Technelysium Pty Ltd. Australia). Sequences were individually compared with *Blastocystis* SSU-rRNA gene sequences available in GenBank using the basic local alignment search tool (BLAST) algorithm. Each sequence was then aligned with a panel of reference sequences from GenBank using the ClustalW program to determine sequence similarity.

2.9. Statistical analysis

Data were analyzed using Chi-square test and Fisher's exact test. A P value < 0.05 was considered as significant. Statistical analysis was carried out using SPSS v18.

3. Results

Among patients with IBS, 55% ($n = 67$) were male and 45% ($n = 55$) were female. The mean age of the patients was 38 years. The control group consisted of 64 (52.45%) males and 58 (47.55%) females; the mean age of this group was 31 years. There was no significant difference between the two groups in age and gender ($P > 0.05$). Individuals' chief complaints were abdominal pain, diarrhea, and constipation. Frequency of participants with different symptoms of IBS are listed in Table 1. *Blastocystis* was detected in 19.67% ($n = 24$) of patients with IBS and 17.2% ($n = 21$) of individuals without IBS. There were no significant differences in *Blastocystis* between two groups ($P = 0.3$). Prevalence of *Blastocystis* in participants with different stool consistency was reported in Table 2.

Table 1
Frequency of symptoms in IBS and control group.

Symptoms	IBS group	Control group	*P*-value
Abdominal pain	73 (59.8%)	13 (10.6%)	$P < 0.05$
Diarrhea	27 (22.1%)	16 (13.1%)	$P < 0.05$
Constipation	30 (24.5%)	17 (13.9%)	$P < 0.05$
Alternating diarrhea/constipation	36 (29.5%)	9 (7.3%)	$P < 0.05$