Research Article

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Blastocystis sp.: frequency and typing among Irritable Bowel Syndrome (IBS) patients

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ABSTRACT

Blastocystis is an intestinal protozoan that has been evaluated for its possible pathogenicity role in IBS disease. In Syria, no previous studies have focused on the presence of *Blastocystis* among IBS patients; therefore, this study aims to specify the frequency of *Blastocystis* parasite and its subtypes (STs) among IBS patients. Fecal samples were obtained from 70 patients; 35 IBS and 35 non-IBS patients. Samples were subjected to direct microscopic examination, and then studied by conventional PCR. Genotyping using seven specific primers targeting the *SSU rRNA* gene was employed. No significant correlation between gender was found (Chi-Square; *P*= 0.212); however, significant difference was found in ages among both groups (Mann-Whitney Test; *P*= 0.011). *Blastocystis* was detected in 71.4% (25/35) of IBS patients and in 77.1% (27/35) of non-IBS patients; nonetheless, the difference between the two groups was not statistically significant (*P*=0.299). Interestingly, a high proportion of single *Blastocystis* infection (88%; 22/25) was found among IBS patients' samples compared to those of the non-IBS. Three different subtypes were identified, where ST1 subtype was the dominant in IBS patients and ST3 was found only in the non-IBS participants. In addition, the patients suffering from IBS had approximately a six-time increase risk of having flatulence symptoms compared to the non-IBS patients (OR:5.712; *P*= 0.001), and it was the main clinical symptoms found among IBS patients with ST1 subtype. In conclusion this study is the first in Syria to highlight the prevalence of *Blastocystis* sp. among IBS patients. Further studies are needed to investigate *Blastocystis* pathogenic role causing IBS syndrome.

Keywords : *Blastocystis* sp., subtypes; IBS syndrome; clinical symptoms; Syria

INTRODUCTION

Blastocystis sp. is the most common unicellular parasite living in the lower intestinal tract of humans and a wide range of vertebrates [1, 2]. Different routes of transmission were observed, namely zoonotic, anthroponotic and waterborne transmission [3, 4]. Genetic diversity is common among *Blastocystis* sp. with more than 41 subtypes being identified so far [5]. The pathogenic role of *Blastocystis* is controversial; recent studies have indicated its association with gastrointestinal disorders as inflammation bowel diseases (IBD) and irritable bowel syndrome (IBS), [6, 7].

IBS is a common healthcare problem with worldwide distribution [8]. The prevalence of IBS varies between developed and developing countries ranging between 5-24% and 35-43% respectively [9, 10]. *Blastocystis* has never been examined in IBS patients in any previous study in Syria; therefore, this study aimed to identify the frequency of *Blastocystis* and its subtypes (STs) among Syrian IBS patients.

MATERIALS AND METHODS

Ethical approval

This study was approved and funded by Damascus University (approved No. 4031; funder No. 501100020595). Patients and their families were informed about the study and they signed a written informed consent.

Selection criteria and sample characterization

IBS along with non-IBS patients were recruited in this study. All patients were attending the Al-Mouwasat University Hospital and the Syrian Specialty Hospital for diagnosis and therapy. Patients and their families were informed about the

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study, and a written consent was signed. A form about the clinical and epidemiological data was filled for each patient containing information about gender, age and presence of gastrointestinal symptoms. The study was conducted from November 2021 to February 2022.

Stool collection and examination

Fecal specimens were obtained from both IBS and non-IBS individuals and collected in labeled and sterile containers. Stool smears were done as described by Manser et al. [11] and examined directly using light microscope (×40 magnifications). The diagnostic criterion adopted in determining the positivity of *Blastocystis* sp. infection was the detection of 2 to 5 vacuolar forms [12].

Molecular detection and genotyping

Approximately, a fecal sample of 250–300 mg from each patient was used for genomic DNA extraction using QIAamp DNA stool mini kit (QIAamp DNA Stool Mini Kit, QIAGEN) as described by Rebih et al. [13]. Molecular detection of *Blastocystis* sp. was applied using the small subunit ribosomal RNA *(SSU-rRNA)* gene by a pair of diagnostic primers (b11400 FORC and b11710. REVC), as mentioned by Stensvold et al. [14]. Samples were further subjected to typing using seven subtype-specific sequence-tagged-site (STS) primers [15]. The PCR cycling conditions were as described by Darwish et al. [16]. A negative control (4 µl of nuclease-free water) was used for contamination detection in all PCR experiments. PCR reactions were carried out using Eppendorf Master Cycler. The PCR products were electrophoresed in 1.5–2% agarose gel stained with ethidium bromide (Sigma Aldrich, USA) along with a 100 bp DNA ladder (GeneDirex Inc, Taiwan ROC) as a standard size.

Statistical analysis

The data were processed using the Statistical Package for Social Sciences (SPSS) version 23. Descriptive analyses were conducted for variables and were presented as frequencies and percentages. Pearson chi-square test and Fisher's exact tests were used to study the association between IBS and qualitative variables, namely gender and clinical symptoms. In addition, the Mann-Whitney U test was used to assess whether there is a statistically significant difference between the age of IBS and non-IBS patients. A *P*-value ≤ 0.05 was considered statistically significant.

RESULTS

The study included of 35 untreated IBS and 35 non-IBS patients. The participants' ages ranged from 3 to 83; the mean age of IBS patients was higher than that of the non-IBS individuals (43.3±18.8) and (31.3±20.9) respectively. Significant difference was found between the two groups (*P* =0.011). Furthermore, IBS was highly detected among female patients (25/35; 71.4%), whereas the ratio between male and female among non-IBS group was closely similar (15/35, 43%; 20/32, 57% respectively). No significant association was found between gender of both groups (OR: 1.87, 95% CI=0.69–3.06, *P*=0.212).

When comparing clinical symptoms between IBS and non-IBS patients, our data revealed that patients suffering from IBS had approximately a six-time increase risk of having flatulence symptoms compared with the non-IBS patients, (OR: 5.7, 95% CI=2.008-16.244, *P*= 0.001). In addition, significant differences were found between the two groups of patients regarding anorexia/weight loss and nausea/vomiting symptoms respectively (OR: 0.337, 95% CI= 0.125-0.907, *P*= 0.029; OR: 0.367, 95% CI= 0.134-1.003, *P*=0.050) (Table 1).

Table1. Evaluation of clinical symptoms between IBS and non-IBS patients

Symptoms		Non-IBS		IBS		OR (95% CI)	*P*-value*
		N	%	n	%		
Diarrhea	No	17	48.6%	17	48.6%	1.01 (0.392 - 2.553)	0.998
	Yes	18	51.4%	18	51.4%		
Constipation	No	31	88.6%	27	77.1%	2.296(0.622 - 8.480)	0.205
	Yes	4	11.4%	8	22.9%		
Constipation/Diarrhea	No	28	80.0%	27	77.1%	1.185(0.378 - 3.720)	0.771
	Yes	7	20.0%	8	22.9%		
Abdominal pain	No	13	37.1%	9	25.7%	1.707(0.614 -4.744)	0.303
	Yes	22	62.9%	26	74.3%		
Abdominal spasm	No	17	48.6%	23	65.9%	0.493(0.188 -1.290)	0.147
	Yes	18	51.4%	12	34.3%		

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Flatulence	No	22	62.9%	8	22.9%	5.712 (2.008- 16.244)	0.001*
	Yes	13	37.1%	27	77.1%		
Anorexia/Weight loss	No	16	45.7%	25	71.4%	0.337 (0.125- 0.907)	0.029*
	Yes	19	54.3%	10	28.6%		
Nausea/Vomiting	No	18	51.4%	26	74.3%	0.367 (0.134 - 1.003)	0.050*
	Yes	17	48.6%	9	25.7%		

*Chi-Square Test; **P*-value ≤ 0.05

The microscopic study results showed the presence of Blastocystis sp. in 71.4% (25/35) of IBS patients' samples and in 77.1% (27/35) of those of the non-IBS group. No significant difference was found between the two groups (*P*= 0.299). In addition, co-infection was found in eleven samples of the two groups (3 in IBS and 8 in non-IBS samples). Interestingly, high proportion of single *Blastocystis* infection (88%; 22/25) was found among IBS patients' samples compared to those of the non-IBS (Table 2).

Table2. The frequency of *Blastocystis* with/without other intestinal parasites

	IBS	**Non-IBS**
Single infection (*Blastocystis* sp)	22 (88%)	19 (70.4%)
Protozoa Co-infection	3 (12%)	8 (29.6%)
Co-infection		
Blastocystis sp + *Ent. histolytica complex**	2	3
Blastocystis sp + *Ent. coli*	0	2
Blastocystis sp + *Ent. histo.complex** + *Ent.coli*	0	2
Blastocystis sp + *Ent. histo.complex*+ Giardia*	0	1
Blastocystis sp + *Ent. coli* + *Chilomastex*	1	0

**Entamoeba histolyticacomplex = Ent. histolytica/Ent. dispar/Ent. moshkovskii*

Molecular detection and genotyping was pursued for all positive samples (Figure 1: A, B). Three different subtypes were identified (ST1, ST2, and ST3); however, six samples remained undefined (Table 3). Our results detected higher proportion of ST1 subtype among IBS samples compared to the non-IBS (72.7% vs 26.3%). Mixed STs (ST1 and ST2) was found in two samples from IBS patients. ST3 was only detected in non-IBS samples (Table 3).

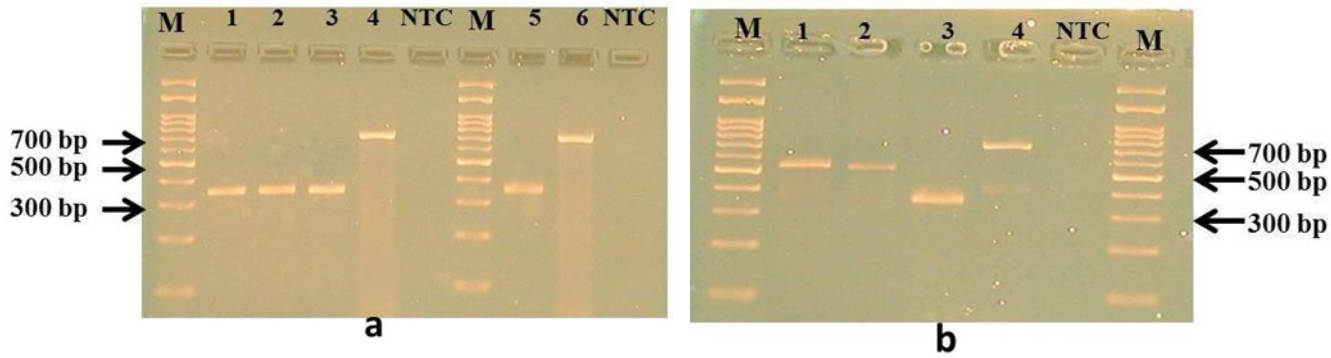


Figure 1 . Gel electrophoresis for the different subtypes detected in IBS (A) and non-IBS (B) patients.
a- M (100 bp DNA ladder marker). Single infection: lanes 1-3: ST1 (351 bp); lane 4: ST2 (704 bp). Mix-infection ST1+ST2: lanes5, 6. NTC: negative control for contamination detection.
b- M (100 bp DNA ladder marker). Lanes 1, 2: ST3 (526 bp); lane 3: ST1 (351 bp); lane 4: ST2 (702 bp). NTC: negative control for contamination detection.

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Table 3. The prevalence of *Blastocystis* subtypes in IBS and Non-IBS patients

IBS	Different subtypes				
	ST1	ST2	ST3	ST1+ST2	STs?
N=25 (%)	17 68%	2 8%	-	2 8%	4 16%
Non-IBS					
N=27 (%)	9 33.3%	3 11.1%	12 44.4%	1 3.7%	2 7.4%

STs?: unknown subtypes

Furthermore, the IBS clinical symptoms associated with *Blastocystis* ST1 subtype (single infection) were analyzed. Although no significant correlation was found, our results showed that flatulence and abdominal pains were the most frequent symptoms detected (81.3%, 68.8%; OR: 2.167 and 1.10 respectively) followed by nausea/vomiting (43.8%; OR: 1.556) (Table 4).

Table 4. The clinical symptoms related to the IBS patients with *Blastocystis* ST1

Symptoms		ST1				OR (95% CI)	*P* value*
		No		Yes			
		N	%	N	%		
Diarrhea	No	4	66.7%	10	62.5%	1.200 (0.166 - 8.659)	0.998
	Yes	2	33.3%	6	37.5%		
Constipation	No	4	66.7%	13	81.3%	0.462 (0.056 - 3.811)	0.585
	Yes	2	33.3%	3	18.8%		
Constipation/ Diarrhea	No	5	83.3%	11	68.8%	2.273 (0.208 - 24.882)	0.634
	Yes	1	16.7%	5	31.3%		
Abdominal pain	No	2	33.3%	5	31.3%	1.10 (0.149 - 8.125)	0.988
	Yes	4	66.7%	11	68.8%		
Abdominal Spasm	No	5	83.3%	12	75.0%	1.667 (0.147 - 18.874)	0.997
	Yes	1	16.7%	4	25.0%		
Flatulence	No	2	33.3%	3	18.8%	2.167 (0.262 - 17.892)	0.585
	Yes	4	66.7%	13	81.3%		
Anorexia/weight loss	No	6	100.0%	11	68.8%	-	0.266
	Yes	0	0.0%	5	31.3%		
Nausea/Vomiting	No	2	33.3%	9	56.3%	1.556 (0.218 - 11.086)	0.998
	Yes	4	66.7%	7	43.8%		

*Fisher's exact test

DISCUSSION

Irritable Bowel Syndrome (IBS) is a disorder where the development and the appearance of clinical symptoms can be attributed to multiple factors [10, 17]. Recently, it was found that *Blastocystis* sp. can promote a mild inflammatory environment within the intestinal mucosa that consequently can be positively associated with IBS clinical symptoms [18]. In Syria, to the best of our knowledge, no study highlighting the relation between IBS and enteric parasites has been conducted. Thus, the present study focused on the presence of *Blastocystis* as an intestinal parasite among IBS patients.

Our findings revealed that single *Blastocystis* sp. infection was more prevalent among IBS patients compared to those of the

non-IBS patients. This result is consistent with some previous studies from Europe and the Middle East which found that approximately 30-40% of IBS patients were *Blastocystis* carriers [19-21].

The pathogenicity of *Blastocystis* is still doubtful due to its existence in both asymptomatic and symptomatic patients [22, 23]. In humans, the pathogenic potential of *Blastocystis* sp. has focused on subtyping [10, 24]. Still, subtype prevalence seems to vary from country to country and even among communities within the same country [25]. However, subtypes ST1-ST4 and ST7 are common in the Middle East and Europe; they constitute 90% of all human cases [10, 26]. Some studies suggested a potential association between IBS disease and particular *Blastocystis* subtypes including ST1, ST3, ST4 and ST7 [9, 27-29], while other studies have not shown any correlation between the two [18, 23, 30]. Based on our molecular results, the ST1 genotype was predominant among IBS patients, whereas ST3 was only found in the non-IBS individuals. This finding is consistent with previous studies conducted in Pakistan [31, 32].

Furthermore, in this study, the risk of flatulence symptoms was about 6 times higher in IBS than in the non-IBS patients; additionally, flatulence and abdominal pains were the most common symptoms found in IBS patients with *Blastocystis* ST1. These findings may lead us to suggest that *Blastocystis* ST1 is likely to associate with these clinical manifestations. Moreover, our result revealed a high infection rate among IBS females than males (2.5:1). This finding is in agreement with previous studies, which reported that females are twice likely to suffer from IBS than males [33-35]. In addition, females are frequently reported as a risk factor for developing post-infected IBS (PI-IBS) [10, 29].

Furthermore, our results indicated that adults and the elderly are highly likely to be at risk of IBS more than the other age groups. This finding is consistent with the results of previous studies [33, 36, 37]. This study is the first in Syria to highlight the high prevalence of *Blastocystis* sp. among IBS patients. Among the three identified subtypes, ST1 was the predominant. However, the pathogenic potential of this subtype could not be elucidated in the study.

CONCLUSION

The identification of *Blastocystis* subtypes may help in understanding their possible role in IBS disease. Further studies are needed to clarify the influence of other pathogens and host factors in IBS patients, since the interaction between them might lead to phenotypic changes and different levels of virulence.

Author Contributions

Data curation : Buthaina Darwish, Ghalia Aboualchamat, Samar Al Nahhas.

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Writing – original draft : Buthaina Darwish, Ghalia Aboualchamat, Samar Al Nahhas.

Writing – review & editing : Buthaina Darwish, Ghalia Aboualchamat, Samar Al Nahhas

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Ethics approval and consent to participate

This study has been approved by the ethical committee of Damascus University (approved number: 4031). Informed written consent was obtained from the patients or a member of their family in case of children. Confidentiality of the details of the participants was assured. All methods, used in this study, were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable

Availability of data and materials

The datasets used in the current study are available without restriction

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Competing interests

The authors have no conflicts of interest associated with this publication

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