Parasitic infections in irritable bowel syndrome patients: evidence to propose a possible link, based on a case–control study in the south of Iran

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Abstract
Objectives: The current study aimed to evaluate the prevalence of parasitic infections and their possible association with irritable bowel syndrome (IBS), through a case–control study. Stool samples were collected from patients with IBS and healthy subjects and were examined microscopically to detect intestinal parasites.

Results: A total of 200 subjects were enrolled in the study including 100 patients with IBS and 100 healthy controls. The patients were selected based on the Rome III criteria. Of the 100 patients with IBS, 65 (65%) were female and 35 (35%) were male, with a mean age of 42.57 (±4.07) years. Of these, 30 (30%) were infected with at least one intestinal parasite; the most common ones were Blastocystis hominis and Giardia lamblia. Of the control cases, 64 (64%) were female and 36 (36%) were male, with a mean age of 41.82 (±11.75) years. Of these, 16 (16%) were infected with at least one intestinal parasite; the most common were B. hominis and Endolimax. There was a significant difference between the rate of parasitic infections between the patients with IBS and the control in particular, B. hominis and G. lamblia.

The findings of the study support a possible link between parasitic infections and IBS.

Keywords: IBS, Parasitic infection, Blastocystis spp., Giardia lamblia

Introduction
Irritable bowel syndrome (IBS) is a group of diseases of the gastrointestinal tract that is associated with altered bowel movement and abdominal pain, without any known causes [1]. IBS has a profound negative impact on the lives of patients suffering from this syndrome and imposes significant economic and social costs on these patients [2, 3].

The prevalence of IBS is different in various regions of the world, based on the diagnostic criteria. In American and European countries, the prevalence of the disease is reported to be between 9 and 22 percent, respectively [4]. Countries such as India and Thailand [5, 6] have reported a low prevalence of 4.2% and 4.4%, respectively.

In Iran, Mahmoudi et al. [7] reported an IBS prevalence of 4.2% in a study, based on the Rome I criteria, among Tehran University students while Pourshams et al. [8] reported the prevalence of this syndrome as 4.7% in a population of students, based on Rome II criteria.

Numerous studies have been conducted to evaluate the association between parasitic, bacterial, and viral infections and IBS. Blastocystis and Dientamoeba fragilis, which are considered to be non-pathogenic parasites, have been linked to the etiology of IBS, in particular, the diarrhea-predominant IBS [9, 10]. While some studies
have reported a significant association between the parasitic infections, especially Blastocystis, and IBS [9–11], in other studies no link has been found [12–14].

In a recent systematic review and meta-analysis focusing on the role of Blastocystis and D. fragilis in IBS, Blastocystis infection, but not D. fragilis infection was found to have a positive association with IBS [11]. Moreover, the meta-analysis reported subtypes 1 and 3 of Blastocystis as potential risk factors for IBS [11].

Considering the existing discrepancy in the association of parasitic infection and IBS, the current study was conducted to evaluate the prevalence of parasitic infections and their possible association with IBS, through a case–control study in Iran.

Main text
Methods
Study design
The IBS patients were selected from patients referred to internal clinics in university-affiliated hospitals in Shiraz, southern Iran. The inclusion criterion was having IBS, based on Rome III criteria, when examined by two gastroenterologists. The exclusion criteria were having celiac disease, gastrointestinal malignancies, or inflammatory bowel disease. The control subjects were recruited from a healthy population who referred to health centers for a routine check-up without having any complaint of gastrointestinal problems. A predesigned questionnaire was used to obtain sociodemographic, medical history, physical examination, and data related to IBS and parasitic infections. A fresh stool sample was collected from each participant for parasitological evaluation.

Parasitological evaluation of the fecal samples
The physical consistency of fresh fecal samples was classified as being formed or semi-formed. The fecal samples were grossly examined for the presence of blood, mucus, or adult helminthes. The samples were microscopically evaluated for intestinal parasites, using a wet preparation as well as a formalin/ethyl acetate concentration technique [15].

Statistical analysis
Statistical analysis of the data was performed, using SPSS (version 18; SPSS Inc., Chicago, IL, USA). The independent sample t-test was used to compare the means of different variables in two studied groups. Chi square, as well as Fisher’s exact tests, was used for comparing the discontinuous or categorical variables. P-value < 0.05 was considered statistically significant.

Results
Features of IBS patients and their parasitic infection
A total of 200 subjects were enrolled in the study including 100 IBS patients and 100 healthy controls. Of the 100 patients with IBS, 65 (65%) were female and 35 (35%) were male, with a mean age of 42.57 ± 14.07 years (range 14-80 years). The mean duration of disease in the patient group was 4.33 (±4.68) years with the lowest duration being 3 months and the longest 25 years.

Out of the 100 patients, 37 (37%) had an underlying disease, the most common of which were hypothyroidism (5%), hyperlipidemia (5%), coronary artery diseases (5%), and psychiatric disorders (4%).

Of the patients with IBS, 30 (30%) were infected with at least one intestinal parasite. The most common parasitic infections were B. hominis (15%) and G. lamblia (8%). Polyparasitism was found in 5 cases, including one (1%) with G. lamblia and B. hominis, two (2%) with B. hominis and E. nana, and 2 (2%) with B. hominis and Entamoeba coli.

With respect to daily stress and anxiety, 72 (72%) of the patients reported high levels of stress and anxiety. It should be noted that none of the patients with IBS had celiac disease, gastrointestinal malignancies, or inflammatory bowel disease, based on ultrasound, colonoscopy, and endoscopic examinations.

Features of control subjects and their parasitic infection
In the control group, 64 (64%) were female and 36 (36%) were male. The mean age of the control group was 41.82 (±11.75) years, ranging from 17 to 75 years. The differences between the age and sex of the patients and controls were not significant when statistically analyzed.

In the control group, 16 (16%) cases were infected with at least one intestinal parasite; the most common were B. hominis and E. nana. In the control group in 86 (86%) cases, stool consistency was formed and in 14 cases (14%) it was semi-formed.

Comparison of the rate and diversity of parasitic infection in patients with IBS and controls
Analysis of data by independent sample t-test revealed a significant difference between the rate of parasitic infections in the patients with IBS and the control group (p = 0.019, df = 198, F = 23.64). There was also a statistically significant difference between patients with IBS and the control group regarding underlying diseases where the rate of these diseases was higher in patients with IBS. Furthermore, there was a statistically significant difference between the patients and the control group regarding the level of daily stress and anxiety, as well as the level
of psychiatric disorders (p < 0.05). There was no statistically significant difference in the fecal specimen consistency between the patients and the control group.

With regard to the type of parasitic infection, there was a significant difference between the two groups concerning *G. lamblia* and *B. hominis* infections. In terms of polyparasitism, there was a significant statistical difference between the patients with IBS and the control group. Table 1 shows the demographic characteristics and differences between the control and Patients with IBS in the current study.

| Variable                      | Patients with IBS | Control | P-value |
|-------------------------------|-------------------|---------|---------|
|                               | Frequency | Percent | Frequency | Percent |         |
| Sex                           |           |         |           |         |         |
| Male                          | 35        | 35      | 36        | 36      | 0.883   |
| Female                        | 65        | 65      | 64        | 64      |         |
| Age (year)                    |           |         |           |         |         |
| ≤ 20                          | 6         | 6       | 3         | 3       | 0.683   |
| 21–30                         | 15        | 15      | 17        | 17      |         |
| 31–45                         | 37        | 37      | 41        | 41      |         |
| 46–60                         | 31        | 31      | 36        | 36      |         |
| ≥ 61                          | 11        | 11      | 3         | 3       |         |
| Parasitic infection           | 30        | 30      | 16        | 16      | 0.019   |
| Type of parasitic infection   |           |         |           |         |         |
| *Giardia lamblia*             | 8         | 8       | 0         | 0       | 0.004   |
| *Blastocystis hominis*        | 15        | 15      | 6         | 6       | 0.038   |
| *Endolimax nana*              | 5         | 5       | 6         | 6       | 0.758   |
| *Chilomastix mesnili*         | 2         | 2       | 0         | 0       | 0.157   |
| *Entamoeba coli*              | 2         | 2       | 4         | 4       | 0.410   |
| *Egg of Enterobius vermicularis* | 1  | 1     | 0         | 0       | 0.319   |
| *Dientamoeba fragilis*        | 1         | 1       | 0         | 0       | 0.319   |
| Living larvae                 | 1         | 1       | 0         | 0       | 0.319   |
| Mixed parasitic infections    | 5         | 5       | 0         | 0       | 0.024   |
| Stool consistency             |           |         |           |         |         |
| Formed                        | 84        | 84      | 86        | 86      | 0.694   |
| Semi-formed                   | 16        | 16      | 14        | 14      |         |
| Educational level             |           |         |           |         |         |
| Uneducated                    | 11        | 11      | 0         | 0       | 0.001   |
| High school or less           | 43        | 43      | 5         | 5       |         |
| High school diploma           | 21        | 21      | 11        | 11      |         |
| Undergraduate (AA or BA)      | 22        | 22      | 36        | 36      |         |
| Graduate (MSc or Ph.D.)       | 3         | 3       | 48        | 48      |         |
| Occupation                    |           |         |           |         |         |
| Unemployed                    | 2         | 2       | 1         | 1       | 0.001   |
| Housewife                     | 53        | 53      | 21        | 21      |         |
| Self-employed                 | 18        | 18      | 8         | 8       |         |
| Student                       | 5         | 5       | 13        | 13      |         |
| Clerk                         | 22        | 22      | 57        | 57      |         |
| Level of stress and anxiety   |           |         |           |         |         |
| High                          | 72        | 72      | 26        | 26      | 0.001   |
| Low                           | 28        | 28      | 74        | 74      |         |
| Psychiatric disorders         | 4         | 4       | 0         | 0       | 0.044   |
Discussion
The present study investigated the prevalence of intestinal parasites in patients with IBS and healthy controls. Of the 100 patients studied, 30 (30%) were infected with at least one intestinal parasite and G. lamblia and Blastocystis spp. were the most common. Differences in the rate of parasitic infection, in particular B. hominis and G. lamblia, between the patients with IBS and controls were statistically significant.

*Blastocystis* spp. is a common intestinal protozoan and its role as a parasitic pathogen is still controversial. The contribution of *Blastocystis* to IBS has been proposed in several studies [9–11]. The findings of the current study are consistent with previous studies, in that a higher rate of parasitic infection in patients with IBS was presented, in comparison with the healthy controls [9–11]. In Jimenez-Gonzalez et al. study [9], a significant association between Blastocystis infection and IBS was reported in the Mexican patient population, where the rate of infection with this parasite was higher in patients with IBS than in the control group.

In concordance with our findings, other reports from Europe, Mexico, India, the Middle East, and Pakistan, proposed for the role of *Blastocystis* in the pathogenicity of IBS [11, 16, 17]. A systematic review and meta-analysis in Iran supported the existence of a positive association between Blastocystis infection and IBS [11]. The yield of infection in patients with IBS has been reported to be higher than that of healthy counterparts, where patients with IBS had significantly more *B. hominis* per microscopic field [18].

On the other hand, several studies have not found a positive association between Blastocystis infection and IBS [12–14, 19]. Krogsgaard et al. [20] showed that both *D. fragilis* and Blastocystis spp. parasites are more prevalent in healthy subjects than in patients with IBS. Yakoob et al. [10] evaluated the rate of *B. hominis* and *D. fragilis* infections in IBS-diarrhea patients and controls. Although the rate of both infections was higher in patients with IBS, the author concluded that a prospective study with a larger population was needed to support any association between IBS and these parasitic infections. In another study by Khademvatan et al. [12] in Khuzestan, south of Iran, 122 patients with IBS and 122 healthy individuals were evaluated for Blastocystis infection where no statistically significant difference was found between the two groups. In another study in Iran, Beiromvand et al. [21] demonstrated that Blastocystis spp. was more prevalent in the control group in comparison with the patients with IBS. In a study in Thailand, [13] no significant difference was found in the incidence of Blastocystis in patients with IBS and healthy controls. In Morgan et al. [22] study no association was found between IBS and parasitic infections in a developing nation environment, Leon Municipality, where the rate of parasitic infection is substantial.

In the current study, *G. lamblia* was more prevalent in stool samples of patients with IBS than in the controls. This is in agreement with previous studies that reported a higher rate of *G. lamblia* in patients with IBS [16, 23].

The association between the genotypes of *B. hominis* with IBS is controversial. In a study by Yakoob et al. [17] in Karachi, Pakistan, type 1 of Blastocystis was predominant in the patients with IBS whereas type 3 was more common in the control group. Also researchers from Egypt reported genotype I as the dominant genotype of *B. hominis* in patients with IBS [24]. Such association was not found in the study of Khademvatan et al., [12] in Iran. In our study, the genotypes of *B. hominis* were not determined.

Conclusion
In this case-control study, concerning the potential role of parasitic infection in IBS status, a significant difference in the prevalence of Blastocystis spp and *G. lamblia* was seen in patients with IBS in comparison with the control group. The findings are in line with those researchers who reported a link between parasitic infections and IBS. Whether this is a causative, risk factor or an association between parasitic infection and IBS deserve further studies. Results of the present study also support an association between psychiatric disorders and IBS. Further studies with a larger sample size are needed to clarify the role of parasitic infections in the pathogenesis of IBS.

Limitations
There are several limitations to this study. First, the sample size of the study is relatively small. Second, the genotypes of *Blastocystis* have not been assessed to investigate the possible association of the Blastocystis subtypes and IBS. Also, the literacy levels of the two groups were somewhat different, which may, to some extent, have affected the rate of parasite infection in the two studied groups.

Abbreviation
IBS: Irritable bowel syndrome.

Acknowledgements
The technical assistance of Dr. N. Arefkhah and Mrs. Fariba Ghorbani is acknowledged.

Authors’ contributions
BS, ZS, ZR, and FE conceived and designed the study; ZS, FE, and ZR collected the samples and performed the laboratory works; MRF and SKHA contributed to diagnosing, selecting, and referring the patients with IBS. FE and BS prepared the first draft of the manuscript. All authors read and approved the final manuscript.
Funding
The study was financially supported by the office of vice-chancellor for research of Shiraz University of Medical Sciences (Grant No. 93-01-01-8586). The study was the subject of Dr. Z. Shafiei MD dissertation.

Availability of data and materials
Any further requested information regarding the experimental and data analysis during the current study is available from the corresponding author on request.

Ethics approval and consent to participate
The protocol of this study was approved by the Ethical Committee of Shiraz University of Medical Sciences (Sums). Written informed consent was provided by all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 31 March 2020 Accepted: 27 May 2020 Published online: 01 June 2020

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