

## Original paper

# The possible link between intestinal parasites and irritable bowel syndrome (IBS) in Diyala Province, Iraq

Wisam Faisal WADI<sup>1</sup>, Munther Hamza RATHI<sup>2</sup>, Abdul-Lateef MOLAN<sup>2</sup>

<sup>1</sup>Department of Biology, College of Sciences, Diyala University, Diyala, Iraq

<sup>2</sup>Department of Biotechnology, College of Sciences, Diyala University, Diyala, Iraq

Corresponding Author: Abdul-Lateef Molan; email: molanal99@gmail.com

**ABSTRACT.** The objective of this study was to investigate the prevalence of intestinal parasites and the possible association between irritable bowel syndrome (IBS) and parasitic infections. The study included 100 IBS patients and 100 healthy control subjects. All study subjects filled a structured questionnaire, which covered demographic information and clinical data. Fresh stool samples were collected from patients and control subjects and processed during the same day of collection. Iodine wet mounts and trichrome stained smears prepared from fresh stool and sediment concentrates were microscopically examined for intestinal parasites. Patients attended private gastroenterology clinics and those found to have IBS (45 males and 55 females) were then selected in this study. The healthy subjects (50 males, 50 females) were recruited as controls. In the IBS patients, *Blastocystis* sp. was detected in 57% and *Giardia* sp. cysts were observed in 43%. In the control subjects, *Blastocystis* sp. was detected in 12% and *Giardia* sp. cysts were observed in 20%. These parasites were found either alone or with other parasites. Only the differences in the presence of *Blastocystis* ( $P=0.0001$ ) and *Giardia* ( $P=0.0006$ ) between IBS patients and controls were statistically significant. Abdominal pain and bloating were the leading symptoms in IBS patients and controls. Prevalence of *Blastocystis* and *Giardia* was higher in IBS patients than in controls. These parasites are likely to have a role in the pathogenesis of IBS. The findings of the study support a possible link between parasitic infections and IBS.

**Keywords:** *Blastocystis*, *Giardia*, protozoan parasites, irritable bowel syndrome

## Introduction

Irritable bowel syndrome (IBS) is a common medical disorder and represent a group of diseases of the gastrointestinal tract that is characterized by chronic abdominal pain, bloating, passage of mucus or straining with bowel movements, a sense of incomplete evacuation after bowel movements or a sense of urgency to move the bowels [1]. Although IBS is rarely debilitating, it imposes significant economic and social costs and in some cases, it restricts the ability to attend school or social functions, go to work or even travel short distances [2]. The etiology and pathogenesis of IBS are still not well known and a wide range of factors such as genetic, physical, mental health problems and environmental factors have been suggested [3,4]. A wide range of triggers have been proposed such as certain foods, hormones, medications and stress

[5,6]. According to Rome III criteria, IBS has been divided into four subtypes, Diarrhea (IBS-D), Constipation (IBS-C), Mixed (IBS-M) and Unclassified (IBS-U) types [7].

Regarding the epidemiology of IBS, the prevalence is ranging between 9 and 22% in American and European countries, while in some Asian and Mediterranean countries it is between 4 and 33% [8–10]. In Iraq, and as far as we know, only two studies have been conducted in order to investigate the prevalence of IBS. Zedan [8] reported that 30% of the general populations in Tikrit Province were found to have IBS. Hazaa and Lami [10] investigated the prevalence and identified the potential determinants of IBS among high school students in Baghdad Province, during 2017, and reported that 29.7% of the students were found to have this syndrome. The authors concluded that IBS is a common health problem among high school

students and among the hereditary, environmental, and psycho-social factors, stress was the most important determinant.

Numerous studies have been conducted to evaluate the association between parasitic infections and IBS [11]. *Blastocystis* and *Dientamoeba fragilis* have been linked to the etiology of IBS [12,13]. In his systematic review which focuses on the role of *Blastocystis* and *D. fragilis* in IBS, Rostami et al. [11] reported that *Blastocystis* infection, but not *D. fragilis* infection was found to have a positive link with IBS. In a recent case-control study, Shafiei et al. [1] evaluated the prevalence of parasitic infections and their possible association with irritable bowel syndrome (IBS) and found that 30% of Iranian IBS patients were infected with at least one intestinal parasite and the most common ones were *Blastocystis hominis* and *Giardia lamblia*. About 16% of healthy control individuals were found infected with at least one intestinal parasite; the most common were *B. hominis* and *Endolimax*. There was a significant difference between IBS patients and healthy controls regarding the prevalence of both *B. hominis* and *G. lamblia*. The authors concluded that their results support a possible link between parasitic infections and IBS.

Although some studies have reported a significant association between the parasitic infections and IBS [1,11], the results of some studies did not find such a positive link [14,15].

Regarding the prevalence of *Blastocystis* sp. in healthy people, a recent study conducted in Africa showed that 80.4% of healthy school children living in 10 villages of the northwestern region of Senegal were found infected with *Blastocystis* sp. as tested by real-time polymerase chain reaction (PCR) [16]. The authors concluded that the prevalence of *Blastocystis* sp. in Senegal was the highest worldwide.

The objective of this study was to evaluate the possible association between the IBS and the infection with intestinal parasites in Diyala Province, Middle of Iraq.

## Materials and Methods

### *Study design and microscopic examination for intestinal parasites*

Patients attended private gastroenterology clinics in Baquba City, Diyala Province, Iraq, and those found to have IBS and agreed to provide stool sample were then selected for this study. The

exclusion criteria were having celiac disease, gastrointestinal malignancies, inflammatory bowel disease, and those who had been recently taking drugs that might interfere with complaints and laboratory investigations, based on the information obtained from the participants. 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 socio-demographic, medical history, physical examination, and data related to IBS and parasitic infections. To make it easy for the patients and control subjects, the questionnaire was translated into Arabic. Moreover, the participants received written information on the objective of the study and written consent was obtained from each individual. Fresh stool samples were collected from patients and control subjects and processed during the same day of collection. Lugol's Iodine wet mounts and trichrome stained smears prepared from fresh stool sample and formalin-ethyl acetate sediment concentrates were microscopically examined for intestinal parasites. It has been reported that trichrome staining is more sensitive compared with other staining methods, and the morphology is more clearly defined with this method.

### *Statistical analysis*

SPSS (version 18; SPSS Inc., Chicago, IL, USA) was used for the analysis of the data. The comparison between the IBS patients and the control subjects regarding the prevalence of intestinal parasites was evaluated with Chi-square test. In addition, odds ratio (OR) and 95% confidence intervals (95% CI) were estimated using the On-line MEDCALC Statistical Software ([https://www.medcalc.org/calc/odds\\_ratio.php](https://www.medcalc.org/calc/odds_ratio.php)). P-value < 0.05 was considered statistically significant.

### *Ethics approval*

The Human Ethics Committee at the College of Sciences, University of Diyala has approved this study (Protocol 2/2020).

## Results

Patients attended private gastroenterology clinics and those found to have IBS (45 males and 55 females, with a mean age  $\pm$  SE of 39.93  $\pm$  1.48 years) and agreed to provide stool samples were

Table 1. Demographic features and differences between the control subjects and the patients with IBS

Variables	IBS patients No. (%)	Controls No. (%)	OR	P-value
Gender				
Males	45	50	0.82	0.4791
Females	55	50	1.22	0.4791
Overall % prevalence	94 (94%)	44 (44%)	23.5	0.0001
Polyparasitism	39 (39%)	8 (8%)	7.4	0.0001
Types of parasitic infections				
<i>Blastocystis</i> sp.	57 (57%)	12 (12%)	9.7	0.0001
<i>Giardia</i> sp.	43 (43%)	20 (20%)	3.02	0.0006
<i>Cryptosporidium</i> sp.	13 (13%)	7 (7%)	1.99	0.2530
<i>Entamoeba coli</i>	7 (7%)	1 (1%)	7.4	0.0626
<i>Enterobius vermicularis</i>	2 (2%)	1 (1%)	2.02	0.5684
<i>Isospora belli</i>	1 (1%)	2 (2%)	0.5	0.5684
Overall frequency of clinical symptoms				
Abdominal pain	77	39	5.2	0.0001
Bloating	72	16	13.5	0.0001
Constipation	56	17	6.2	0.0001
Diarrhea	36	19	2.4	0.0079
Joint pain	53	17	5.5	0.0001
Fatigue	60	13	6.7	0.0001
Vomiting	20	9	2.5	0.0309
Rash	12	12	1.0	1.000

then recruited in this study. The healthy subjects (50 males and 50 females) with a mean±SE age of 34.08±0.15 years were recruited as a control group. Of the patients with IBS, 94 (94%) were infected with at least one intestinal parasite, while 44% of the healthy subjects in the control group were found infected with one or more intestinal parasites and the statistical analysis showed that IBS patients had 23.5 times higher risk of getting parasitic infections than controls (OR=23.5; P<0.0001) (Tab. 1). In the IBS patients, *Blastocystis* sp. (mostly the vacuolar forms) was detected in 57% and *Giardia* sp. cysts were observed in 43%. In the control subjects, *Blastocystis* sp. (mostly the vacuolar forms) was detected in 12% and *Giardia* sp. cysts were observed in 20% of controls. Only the differences in the prevalence of *Blastocystis* (OR=9.7; P=0.0001) and *Giardia* (OR=3.02; P=0.0006) between IBS patients and controls were statistically significant (Tab. 1). The comparison between the IBS patients and healthy controls regarding the prevalence of

other parasites such as *Cryptosporidium* sp., *Entamoeba coli*, *Isospora belli*, and *Enterobius vermicularis* showed no significant differences between the two groups (Tab. 1). Thirty nine (39.0%) IBS patients were co-infected with two and more parasites such as *Giardia* sp., *Cryptosporidium* sp., *Entamoeba coli*, and *Enterobius vermicularis*. In control group, 8 subjects (8%) were found infected with two and more parasites. The statistical analysis showed a significant difference (OR=7.4; P=0.0001) between IBS patients and the control group regarding the polyparasitism (Tab. 1).

Although the abdominal pain was the leading clinical symptoms in both the IBS patients and the control group (Tab. 1) based on the total number of subjects in each group (77 in IBS patients and 39 control individuals, respectively), according to the OR values, the leading symptom was bloating (OR=13.5; P=0.0001), followed by fatigue (OR=6.7; P=0.0001), constipation (OR=6.2; P=0.0001), joint pain (OR=5.5; P=0.0001), abdominal pain (OR=5.2;

Table 2. Comparison between patients with irritable bowel syndrome (IBS) and healthy individuals (control group) who were infected with *Blastocystis* sp. regarding the frequency of clinical symptoms. The Odds ratio (OR) and 95% Confidence interval (95% CI) have been calculated to clarify the statistical differences between the IBS patients and controls.

Symptoms	No. with symptoms IBS		Odds ratio (OR)	95% Confidence interval (95% CI)	P-value
	Patients (57)	Controls (12)			
Abdominal pain	44	5	4.7	1.3–17.5	0.0194
Bloating	44	5	4.7	1.3–17.5	0.0194
Constipation	33	3	4.1	0.93–15.7	0.0486
Diarrhea	19	3	1.5	0.36–6.2	0.5752
Joint pain	34	3	4.4	1.1–18.2	0.0384
Fatigue	33	1	15.1	1.8–125.2	0.0118
Vomiting	10	2	1.1	0.2–5.6	0.9419
Rash	7	3	0.4	0.1–1.9	0.2656

P=0.0001), vomiting (OR=2.5; P=0.0309), and diarrhea (OR=2.4; P=0.0079). The statistical analysis showed significant differences between the IBS patients and the control group in all the selected clinical symptoms, except for the rash (Tab. 1).

The comparison between the control group and the IBS patients who were found infected with *Blastocystis* sp. showed that the leading symptom was fatigue (OR=15.15; P=0.0118), followed by abdominal pain (OR=4.7; P=0.0194), bloating (OR=4.7; P=0.0194), joint pain (OR=4.4; P=0.0001), constipation (OR=4.1; P=0.0486) and the differences between the two groups regarding the frequency of these symptoms were statistically significant (Tab. 2). No significant differences were observed between the two groups regarding the frequency of diarrhea (OR=1.5; P=0.5752), vomiting (OR=1.1;

P=0.9419) and rash (OR=0.4; P=0.2656).

Table 3 shows a comparison between patients with IBS and healthy individuals (control group) who were infected with *Giardia* sp. regarding the frequency of clinical symptoms. In IBS patients and control subjects who were found infected with *Giardia* sp., the leading symptom was bloating (OR=14.6; P=0.0002), followed by constipation (OR=11.3; P=0.0026), fatigue (OR=7.2; P=0.0048), abdominal pain (OR=5.7; P=0.0033), joint pain (OR=5.4; P=0.0154), and the differences between the two groups regarding the frequency of these symptoms were statistically significant (Tab. 3). Although the difference was not significant between the IBS patients and control individuals who were infected with *Giardia* sp. regarding the frequency of vomiting (OR=11.3; P=0.1009), the IBS patients

Table 3. Comparison between patients with irritable bowel syndrome (IBS) and healthy individuals (control group) who are infected with *Giardia* sp. regarding the frequency of clinical symptoms. The Odds ratio (OR) and 95% Confidence interval (95% CI) have been calculated to clarify the statistical differences between the IBS patients and controls.

Symptoms	No. with symptoms IBS		Odds ratio (OR)	95% Confidence interval (95% CI)	P-value
	Patients (43)	Controls (20)			
Abdominal pain	34	8	5.7	1.8–18.03	0.0033
Bloating	31	3	14.6	3.6–59.2	0.0002
Constipation	24	2	11.3	2.3–55.2	0.0026
Diarrhea	16	5	1.8	0.5–5.8	0.3417
Joint pain	21	3	5.4	1.4–21.2	0.0154
Fatigue	24	3	7.2	1.8–28.1	0.0048
Vomiting	9	0	11.3	0.6–204.3	0.1009
Rash	3	1	1.4	0.1–14.6	0.7656

Table 4. The relationship between the prevalence of the infection with the intestinal parasites that belong to the genera *Blastocystis* and *Giardia* in IBS patients and age groups in comparison with the healthy subjects in the control group. The Odds ratio (OR) and 95% Confidence interval (95% CI) have been calculated to clarify the statistical differences between the IBS patients and controls.

Age group (years)	IBS patients infected	Control subjects infected	Odds ratio (OR)	95% CI	P-value
	with <i>Blastocystis</i> sp. (N=57)	with <i>Blastocystis</i> sp. (N=12)			
14–30	16	6	0.39	0.11–1.4	0.1466
31–40	16	2	2.0	0.4–10	0.4199
41–50	9	1	2.1	0.2–18	0.5127
>50	16	3	1.2	0.28–4.9	0.8288

  

Age group (years)	IBS patients infected	Control subjects infected	Odds ratio (OR)	95% CI	P-value
	with <i>Giardia</i> sp. (N=43)	with <i>Giardia</i> sp. (N=20)			
14–30	16	9	0.72	0.25–2.1	0.5569
31–40	10	5	0.9	0.3–3.1	0.8798
41–50	7	2	1.8	0.3–9.2	0.5114
>50	10	4	0.61	0.15–2.4	0.4811

were 11 times more prone to have this clinical symptom than the healthy controls (Tab. 3).

Table 4 shows the relationship between the prevalence of the infection with *Blastocystis* sp. and *Giardia* sp. in IBS patients and age groups in comparison with the subjects in the control group. The age groups 14–30 and 31–40 years in IBS patients infected with *Blastocystis* sp. showed the highest prevalence rate (28.1% each) followed by the age group 51–60 (17.5%) and no significant differences have been observed between the IBS patients infected with *Blastocystis* in comparison with the subjects in the control group infected with the same parasite. Similarly, no significant

differences were found between the IBS patients infected with *Giardia* in comparison with the subjects in the control group infected with the same parasite regarding the distribution of the infection in age groups. Briefly, the prevalence of *Giardia* infection was 37.2% in IBS patients aged 14–30 years, 23.3% in 31–40 years, and 16.3% in the age groups 41–50 and 51–60 years (Tab. 4).

## Discussion

The current study investigated the prevalence of the intestinal parasites in Irritable bowel syndrome (IBS) patients in comparison with apparently

Table 5. Prevalence of *Blastocystis* sp. in Iraq and neighboring countries

Country	Number of patients	Method of detection	Prevalence %	References
Iraq	608	Direct-light microscopy	33.7	[18]
Iran	100	Direct-light microscopy	15	[1]
Iran	122	PCR	19.7	[15]
Turkey	105	Direct-light microscopy	38	[20]
Turkey	877	Direct-light microscopy	5.8	[21]
Turkey	617	Direct-light microscopy	1.8	[22]
Jordan	109	Direct-light microscopy	14.7	[19]
Saudi Arabia	631	PCR	13.5	[18]
Iraq: Present study	100	Direct-light microscopy	57	



healthy control individuals and our hypothesis was that the prevalence of some intestinal parasites would be higher in IBS patients than control subjects and this was supported by our results, which showed that the prevalence of the parasites which belong to the genera *Blastocystis* and *Giardia* was significantly higher in IBS patients than the control individuals. The information regarding the prevalence of *Blastocystis* sp. in Iraq is very scanty. In fact, only one previous study has been conducted in Iraq [17], in order to investigate the possible role of *B. hominis* in the development of IBS and reported that 33.7% of IBS patients were found infected with *B. hominis* in comparison with 12.3% among healthy control group and the differences were statistically significant. The authors concluded that *B. hominis* plays a crucial role in IBS development.

Interestingly, the prevalence of 57 % reported in the current study using the same diagnostic methods was higher than that reported by Hammood et al. [17]. By comparison, the prevalence reported in a few other neighboring countries was 19.7% and 15% in Iran [1,15], 13.5 % in Saudi Arabia [18], 14.7 % in Jordan [19], and 1.8%, 5.8%, and 38% in Turkey [20–22] (Tab. 5). On the other hand, Ozcakir et al. [23] reported that the prevalence rates of *Blastocystis* infection range between 1.05% and 80.0% among the symptomatic patients in different countries of the world. Yakoob et al. [12] studied the prevalence of *B. hominis* and *D. fragilis* in patients with IBS-diarrhea (IBS-D) and reported that 49% of Pakistani IBS patients were found positive for *B. hominis* by stool microscopy while 24% of the control groups were found positive and the difference between the two groups was significant.

The differences in the prevalence of *Blastocystis* sp. between the present study and studies conducted in the neighboring countries and other countries may be attributed to the differences in the diagnostic tools used (direct-light microscopy, *in vitro* culture and PCR) with a wide range of differences in their sensitivities [24]. Indeed, El-Safadi et al. [24] confirmed that the direct-light microscopy showed only 45.8% sensitivity in comparison with the PCR assay. Accordingly, the prevalence rate of *Blastocystis* sp. reported in the present study is very high and alarming because it does not represent the actual prevalence rate of this parasite due to the fact that the direct-light microscopy method is less sensitive than the culture and molecular methods such as the PCR [1,19,24].

*Giardia* species (syn. *Gardia duodenalis*, *Giardia lamblia*, *Giardia intestinalis*) are intestinal flagellated protozoan parasites that live in the upper part of the human small intestine with a worldwide distribution and recently have been included in the World Health Organization's Neglected Disease Initiative [25,26]. Halliez and Buret [27] conducted an excellent review on the long-term consequences of *Giardia* infections and stated that "in addition to its classical intestinal presentation, the infection with this parasite may cause ocular complications, arthritis, skin allergies or myopathy, in addition to failure to thrive, stunting and growth retardation in, diminished cognitive functions, chronic fatigue, and may lead to post-infectious functional gastrointestinal disorders such as irritable bowel syndrome".

Grazioli et al. [28] evaluated the prevalence of *G. lamblia* infection in Italian IBS patients and reported that 9 patients out of 137 IBS patients (6.5%) were infected with this parasite. Hanevik et al. [29] reported for the first time that 80.5% of the patients with persisting abdominal symptoms caused by *Giardia* infection had symptoms consistent with IBS according to Rome II criteria. The authors concluded that acute *Giardia* infection may cause functional gastrointestinal syndromes such as IBS and functional dyspepsia.

It has been reported that *Giardia duodenalis* has been previously involved in the appearance of post-infectious irritable bowel syndrome (PI-IBS) after the 2004 outbreak of giardiasis in Norway [30]. Jadallah et al. [19] investigated the presumed role of protozoan parasites in the development of IBS and reported that *Giardia* cysts were observed in 11%, while in the control subjects, *Giardia* were detected in 1% and the difference between IBS patients and controls was statistically significant. Recently, Shafiei et al. [1] evaluated the prevalence of parasitic infections and their possible association with IBS and reported that *Giardia* cysts were detected in 8% of IBS patients, while none of the control subjects (0%) were found positive for this parasite and the difference between IBS patients and controls was statistically significant. On the other hand, some studies have failed to find a positive link between *G. lamblia* and IBS [31,32].

In the present study, *Giardia* sp. was significantly more prevalent in the stool of Iraqi IBS patients than in the stool of the subjects in the control group. The figure reported in the present study is higher than that reported in the results of

previous studies conducted in Iran [1], Jordan [19], and in Norway [29]. The differences between the results of the present study and the previous studies may be related to differences in control measures including hereditary, environmental, psycho-social, educational, and sanitation [10].

The results of the current study revealed that the leading symptom in IBS patients who were infected with *Blastocystis* was fatigue (OR=15.15), followed by abdominal pain (OR=4.7), bloating (OR=4.7), joint pain (OR=4.4), constipation (OR=4.1) and the differences between the IBS patients and control subjects regarding the frequency of these symptoms were statistically significant ( $P=0.0486-0.0001$ ). In contrast, no significant differences were observed between the two groups regarding the frequency of diarrhea (OR=1.5;  $P=0.5752$ ), vomiting (OR=1.1;  $P=0.9419$ ) and rash (OR=0.4;  $P=0.2656$ ). In addition, the findings of this study showed a significant differences ( $P=0.0154-0.0001$ ) between the IBS patients who were found infected with *Giardia* and their counterparts asymptomatic healthy individuals regarding the frequency of bloating (OR=14.6), followed by constipation (OR=11.3), fatigue (OR=7.2), abdominal pain (OR=5.7) and joint pain (OR=5.4).

It has been reported that the prevalence of *Blastocystis* infection was higher among the patients with gastrointestinal complaints including IBS than their counterpart's asymptomatic individuals and in most of these studies, the most leading clinical symptoms in IBS patients infected with *Blastocystis* were abdominal pain followed by diarrhea, distention, constipation, vomiting, and flatulence [1,18,21,23,33]. The results of the current study revealed that the leading symptom in IBS patients who were infected with *Blastocystis* was fatigue (OR=15.15), followed by abdominal pain (OR=4.7), bloating (OR=4.7), joint pain (OR=4.4), constipation (OR=4.1) and the differences between the IBS patients and control subjects regarding the frequency of these symptoms were statistically significant ( $P=0.0486-0.0001$ ). In contrast, no significant differences were observed between the two groups regarding the frequency of diarrhea (OR=1.5;  $P=0.5752$ ), vomiting (OR=1.1;  $P=0.9419$ ) and rash (OR=0.4;  $P=0.2656$ ). In addition, the findings of this study showed a significant differences ( $P=0.0154-0.0001$ ) between the IBS patients who were found infected with *Giardia* and their counterparts asymptomatic healthy individuals regarding the frequency of bloating (OR=14.6),

followed by constipation (OR=11.3), fatigue (OR=7.2), abdominal pain (OR=5.7) and joint pain (OR=5.4).

In conclusion, prevalence of the parasites that belong to the genera *Blastocystis* and *Giardia* was significantly higher in IBS patients than in controls and these parasites are likely to have a role in the pathogenesis of IBS. The findings of the study support a possible link between parasitic infections and IBS. Control measures such as education, personal hygiene and sanitation should be considered in order to prevent the infection with the intestinal parasites that play a role in the pathogenesis of IBS.

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