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INTESTINAL PARASITIC INFECTIONS IN PATIENTS WITH IRRITABLE BOWEL SYNDROME IN SOHAG, EGYPT

By
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Abstract

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder of uncertain etiology. Several studies have proposed the possible role of intestinal parasites in the pathogenesis of IBS. The study evaluated the prevalence of intestinal parasitic infections (IPIs) in patients with IBS in comparison with healthy control group in Sohag. Case-control study was conducted on 100 patients with IBS and 100 healthy controls. The IBS patients were selected based on the Rome IV criteria. All participants filled a structured questionnaire that covered demographic and clinical data. Stool samples were collected and examined by direct wet mounts, formalin ethyl acetate concentration technique and modified Kinyoun's acid-fast stain and *in vitro* culture for *Blastocystis* spp. IBS patients were 30% males and 70% females (32.82±12.89) years and controls were 35% male and 65% females (29.4±10.41) years. Prevalence of IPIs in IBS patients (66%) was significantly higher than in controls (30%) with (P<0.0001) and the most common parasites in IBS patients were *Blastocystis hominis* (46%) and *Cryptosporidium* spp. (20%) with significant difference between groups (p<0.001, P<0.0001) respectively. The infection rate was significantly associated with residence (P< 0.027**),** but neither associated with sex (P< 0.4) nor age (P< 0.123) among IBS patients. The study data support a possible link between parasitic infections and IBS.

Keywords: Irritable bowel syndrome, Parasites, *Blastocystis hominis, Cryptosporidium* spp.

Introduction

Irritable bowel syndrome (IBS), a functional disorder affecting the gastrointestinal tract, is defined as recurrent abdominal pain for at least one day per week over the previous three months whose onset was associated with at least two of the following three criteria: improvement upon defecation; change in stool frequency or stool appearance, where no particular organic pathology was found (Lacy *et al,* 2016). IBS prevalence rates among 26 countries according to Internet surveys using the Rome IV criteria ranged from a low of 1.3% (0.8%-1.8%) in Singapore to a high of 7.6% (6.4%-8.7%) in Egypt (Sperber and Drossman, 2012).

The IBS has been divided into four subtypes, Diarrhea (IBS-D), Constipation (IBS-C), Mixed (IBS-M) and Unclassified (IBS-U) types (Lacy and Patel, 2017). IBS causes a significant financial burden in terms of direct costs such as higher use of medical with ages ranged from 15 to 65 years resources and indirect costs due to decreased work productivity (Buono *et al*, 2017).

However, the IBS's etiology and pathogenesis are still poorly understood, and a variety of variables, including genetic, physical, and mental, health problems, and environmental factors have been proposed (Lovell and Ford, 2012). Persistent low-grade inflammation may play a role in IBS and it is one of proposed mechanism of IBS through persistent antigenic exposure as in persistent carriage. It is estimated that 7-31% of patients with infectious gastroenteritis went on to the development of the post-infectious IBS (PI-IBS) (Wang *et al*, 2016).

Studies reported a significant association between intestinal parasitic infections (IPIs), such as *Blastocystis* spp., *Giardia lamblia, Entamoeba histolytica* and *Cryptosporidium* spp. and IBS (Wadi *et al,* 2021, Das *et al,* 2022), but others didn't report (Vasquez-Rios *et al*, 2016, Khademvatan *et al*, 2017).

This study aimed to determine the preval-

ence of IPIs in IBS via a case-control study in Sohag University Hospitals, Upper Egypt.

Materials and Methods

Study area: This study was conducted in Sohag Governorate, Upper Egypt. Sohag is located in south of the country, 467km south of Cairo, and covers the Nile Valley with a total of 1547km^2

Study design: A case-control study was conducted on 100 patients with IBS attending outpatient clinics in Sohag University Hospitals and 100 healthy control subjects from April 2023 to April 2024. IBS patients were selected based on the Rome IV criteria. All subjects had to fulfill the criteria: not taking anti-parasitic drugs 2 weeks before sample collection. They filled a structured questionnaire that covered demographic information and clinical data.

Morning stool samples were collected from patients and controls in clean, disposable plas-tic containers and processed on the same day. Samples were examined macroscopically for consistency, blood, mucus, or adult parasites and microscopically by direct wet mounts, formalin ethyl acetate concentration methods (FECT), modified Kinyoun's acid-fast stain (Garcia, 2016) and *in vitro* culture for *Blastocystis* spp.

Culture preparation: Stool samples (50mg of formed stool or 0.5 ml of diarrheic one) were immediately cultured in 10ml sterile glass tubes contained medium prepared from 500ml ringer solution, 0.5gm yeast extract and 5gm Peptone, mixed well and adjusted to pH 7.2-7.4. After sterilization 20ml of water cooked rice (Cook rice well with water and boil after that taking a part of this water) and streptomycin 50-100mg were added. Cultures were incubated at 37°C and examined at 24, 48, & 72 hours (Sulaiman and Kamal, 2015).

Ethics approval: The study protocol was approved by the Medical Research Ethical Committee, Faculty of Medicine, Sohag University number (Soh-Med-23-04-23MS), as well as registered at clinical trials.gov, number (NCT05830370).

Written informed consents were obtained from all the participants after clarifying the aims and procedures of the study.

Statistical analysis: Data were collected, computerized and analyzed by using SPSS version 26 (IBM Inc., Chicago, IL, USA). Quantitative data were presented as mean ± SD, frequency and percentage (%). The Chi-square test or Fisher's exact test were used for qualitative variables comparison as appropriate. P-value was considered significant at $P< 0.05$.

Results

A total of 200 participants were enrolled included 100 IBS patients and 100 healthy controls. IBS patients were 30% males and 70% females with ages ranged from 15 to 65 years (32.82 ±12.89), 70% live in rural areas and 30% in urban ones. Control cases were 35% males and 65% females, with ages ranged from 15 to 65 years (29.4±10.41), 66% live in rural areas and 34% in urban ones. Of IBS patients, 66% were infected with at least one intestinal parasite compared to 30% of healthy controls group with significantly difference ($P<0.0001$). There was significant difference between IBS patients and controls as to single and mixed infections ($P<0.002$ & $P<0.005$) respectively.

The IBS patients infection rate was significantly associated with residence ($P< 0.027$) but neither associated with sex ($P< 0.4$), nor age ($P< 0.123$). The most common parasites in these IBS patients were *Blastocystis hominis* (46%) and *Cryptosporidium* spp. (20%) with significant differences ($P<0.001$, & $P <0.0001$) respectively.

Details were given in tables (1, 2, 3, 4, 5 & 6) and figure (1).

Table 1: Prevalence of IPIs in IBS patients and control.

Groups	Total number	Positive, no. (%)	P value
IBS patients	100	66 (66%)	<0.0001*
Control group	100	30 (30%)	

*Significant as P value≤0.05.

Table 2: Types of IPIs in IBS patients and control.

Infection	IBS patients	Control	P value
Single	38	18	<0.002*
Mixed	28	12	<0.005*

*Significant as P value≤0.05

Table 3: IPIS in IBS patients and control group according to sexes.

Groups	Sex	Total number	Positive, no. (%)	P value
IBS patients	Male	30	18 (60%)	0.4
	Female	70	48 (68.57%)	
Control	Male	35	11 (31.4%)	0.819
	Female	65	19 (29.2%)	

*Significant as P value≤0.05

Table 4: IPIS in IBS patients and control group according to residences.

Groups	Residence	IBS patients	Positive, no. (%)	P value
IBS patients	Rural	70	51 (72.85%)	0.027*
	Urban	30	15 (50%)	
Control	Rural	66	20 (30.3%)	0.927
	Urban	34	10 (29.4%)	

*Significant as P value≤0.05

Table 5: Distribution of IPIS in IBS patients and control as to age groups

Groups	Age groups	IBS patients	Positive, no. (%)	P value
IBS patient	15-24	31	22 (70.96%)	0.123
	25-34	28	22 (78.57%)	
	35-44	18	9 (50%)	
	45-54	15	10 (66.66%)	
	55-65	8	3 (37.5%)	
Control	15-24	43	11 (25.58%)	0.886
	25-34	23	7 (30.4%)	
	35-44	21	8 (38.09%)	
	45-54	9	3 (33.33%)	
	55-65	4	1 (25%)	

*Significant as P value≤0.05

Table 6: Distribution of intestinal parasites in IBS patients and control group.

Parasites	IBS Patients			Control			P value
	Total	Single	Mixed	Total	Single	Mixed	
Pathogenic protozoa							
Blastocystis hominis	46	21	25	23	11	12	<0.001*
Cryptosporidium spp.	20	6	14	3	1	2	<0.0001*
Entameba histolytica/dispar	13	5	8	8	2	6	0.249
Giardia lamblia	8	3	5	4	1	3	0.234
Cyclospora cayetanensis	2	-	2	1	-	1	0.561
Non-pathogenic protozoa							
Entameba coli	7	3	4	5	3	2	0.552
Endolimax nana	1	-	1	-	-	-	0.316
Helminths					-	-	
H.nana	2	-	2	-			0.155

*Significant as P value≤0.05.

Discussion

The irritable bowel syndrome (IBS) is described as recurrent chronic gastrointestinal functional disturbances. IBS patients are often presented with abdominal discomfort, distension, and alteration in bowel behavior in the form of constipation, diarrhea, or both predominant (Oka *et al,* 2020).

El Sharawy *et al*. (2022) in Egypt reported that even many of the medical students suffered from the IBS, and that some dietary habits, anxiety, and depression of them during final examination time could be risk factors related to development of its syndrome.

In the present study, the prevalence of IPIs in IBS patients (66%) was significantly higher than controls (30%) with P<0.0001. These data were consistent with previous studies reported that the IPIs prevalence in IBS patients was significantly higher than in healthy controls. Brair *et al*. (2016) in the Sudan reported that the prevalence 32% in

IBS patients were more than 16% in controls (P<0.005). Also, Salem *et al.* (2019) in Egypt reported the IBS patients 50% as compared 12.5% in healthy controls (P<0.002). Again, Shafiei *et al.* (2020) in Iran who recognized 30% in IBS patients compared to 16% in healthy controls (P<0.019). Moreover, Wadi *et al.* (2021) in Iraq reported 94% in IBS patients as compared to 44% in healthy controls (P<0.0001). Nevertheless, the findings disagreed with Vasquez-Rios *et al*. (2015) in South America, Krogsgaard *et al.* (2015) in Denmark, Khademvatan *et al*. (2017) in Iran and Kebayer *et al.* (2021) in Khartoum State, they all didn't find significant difference in the incidence of IPIs in IBS patients and healthy controls. These differences could be attributable to other potential confounding factors investigated included water source, sanitation (toilet or latrine use), household construction and neighborhood as well as socioeconomic level.

In the present study, IBS patients (38%, & 28%) were significantly higher than (18%, &12%) controls as to single and mixed parasitic infections (P<0.002, & P<0.005) respectively. This agreed with Shafiei *et al.* (2020), who found that IBS patients with two or more parasites were 5% & 0% in controls with significant difference (P<0.024). Also, Wadi *et al.* (2021) found that multiparasitism in IBS patients were 39% & 8% in controls with significant difference (P< 0.0001). However, this disagreed with Jadallah *et al*. (2017) in Jordan, who found that mixed infection in IBS patients were 1.8% compared to 1% in controls without significant difference (P< 1).

The present study showed that the IPIs prevalence in IBS females (68.57%) was higher than males (60%), but sex was not significantly associated with IPIs in IBS patients (P < 0.4). This agreed with Abdalla *et al.* (2014) in the Sudan, who found that the IBS prevalence among males & females was more or less equal 55% & 57% respectively.

In the present study, the prevalence of IPIs in IBS patients in the rural houses (72.85%) was higher than those in urban ones (50%), with significant associated with IPIs in IBS patients (P< 0.027). This agreed with El-Badry *et al*. (2018) in Egypt, who reported that IBS cases from rural areas were at risk of acquiring *Blastocystis* spp. infection ten times more than those in urban areas, but with a particular focus on correlation of IBS with different Blastocystis sp. subtypes and gut microbiomes.

In the present study, the highest prevalence rates (78.57% & 70.96%) were reported among age group (25-34 &15-24 years) respectively in IBS patients. However, age group of IBS was not significantly associated with IPIs (P< 0.123, & P < 0.886 respectively). This agreed with Wadi *et al.* (2021), who found that the prevalence of IPIs was high among patients aged 14-30 & 31-40 without significant difference.

In the present work, the most common parasitic infections in IBS patients were *Blastocystis* spp. (46%) followed by *Cryptosporidium* spp. (20%) with significantly difference between both groups (P< 0.001, and P< 0.0001) respectively, but other parasites such as *E. histolytica/dispar* (13%), *G. lamblia* (8%), *E. coli* (7%), *C. cayetanensis* (2%), *E. nana* (1%) & *Hymenolepis nana* (2%) didn't show significant differences between both groups.

These data more or less agreed with Brair *et al.* (2016) who found the most common parasites in IBS patients were *Blastocystis* spp. (20%) followed by *E. histolytica/dispar* (10%) and *G. lamblia* (3%). Also, this agreed with Jadallah *et al*. (2017), who found *Blastocystis spp.* (14.7%), *Cryptosporidium* spp. (9.2%) and *G. lamblia* (8.3%), also it agreed with Salem *et al*. (2019) in Egypt, who found *Blastocystis* spp. (45%), *E. histolytica/dispar* (5%) and *D. fragilis* (5%), they concluded that *B. hominis* was independent of demographic characters, IBS subtype, *Helicobacter pylori* infection or treatment, but with a positive association with a history of antibiotic intake with IBS. Besides, this agreed with Shafiei *et al.* (2020), who re-

ported that *Blastocystis* spp. (15%), *G. lamblia* (8%), as well as agreed with Wadi *et al*. (2021) who found *Blastocystis* spp. (57%), *G. lamblia* (43%) and *Cryptosporidium* spp. (13%). Moreover, Maghsood *et al.* (2024) in Iran reported that there was no significant difference in the detection of *B. hominis* infection between the IBS patients and healthy controls.

However, the results disagreed with Kebayer *et al.* (2021), who that found *E. histolytica/dispar* (22%) was the most common parasite followed by *E. coli* (18%) and *G. lamblia* (16%) in IBS patients, as well as Das *et al.* (2022) in India, who found that *G. lamblia* (27.5%) and *E. histolytica/dispar* (13%) among the IBS patients. The differences in the prevalence of *Blastocystis* spp. between the present study and previous studies may be attributed to the differences in the diagnostic tools used as *in vitro* culture was used in current study and differences in the regions and populations studied. There were several limitations to this study. First, the sample size of the study was relatively small. Second, the genotypes of *Blastocystis* have not been assessed to investigate the possible association of the *Blastocystis* subtypes.

Conclusion

The outcome results recommended possible link between parasitic infections among IBS patients. Intestinal parasites, particularly *Blastocystis* spp. and *Cryptosporidium* spp., have a role in the pathogenesis of IBS.

Proper diagnosis of the IBS in endemic areas, will suggest specific treatment that minimize the chance of PI-IBS development.

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Explanation of figure

Fig 1: *In vitro* culture of *Blastocystis* spp. showed binary fission (arrows, x1000).