

Frequency of Intestinal Parasites Among Sudanese Patients with Irritable Bowel Syndrome

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To cite this article:

Sara Lavinia Brair, Linda Bashier Eltayeb, Sara Abdelghani Elgazy, Abdelsalam Mohammad Nail, Alfatih Saifudinn Aljafari. Frequency of Intestinal Parasites Among Sudanese Patients with Irritable Bowel Syndrome. *American Journal of Health Research*. Vol. 4, No. 4, 2016, pp. 82-85. doi: 10.11648/j.ajhr.20160404.12

Received: May 22, 2016; **Accepted:** June 3, 2016; **Published:** June 18, 2016

Abstract: The objective of this case control study is to identify the relationship between intestinal parasitic infection and Irritable Bowel Syndrome (IBS) among Sudanese patients. Method: Two hundred patients diagnosed with irritable bowel syndrome (IBS) and were attended Health Facilities at Khartoum State were enrolled, together with 99 apparently healthy controls. Patients were classified into three groups (IBS-all types, IBS with diarrhea (IBS-D), and IBS with constipation (IBS-C)). Stool specimens were collected from study population and all were subjected to microscopic examination following wet saline preparation, formal-ether concentration technique, Z. N stain, and *in vitro* cultivation in Locke egg medium. Results: Intestinal parasites were detected in 32% (64/200) of IBS-all types; and in 16.2% (16/99) of control group (P. value 0.005). For patients, 20 (10%) were positive for the *Entamoeba* cyst, 3 (1.5%) *Giardia lamblia* cysts, and 41 (20.5%) were positive for *Blastocystis hominis* cyst. For control, *Entamoeba* species were detected in 10 (10.1%), *G. lamblia* one case (1.1%), and *B. hominis* in 5 (5.2%). *Blastocystis hominis* was significantly prevalent among IBS-all types than the control group (P. value 0.02). It is more frequent among patients with IBS- D than those with IBS-C (P. value 0.001). Conclusion: This study concluded that, there is a statistical association between presence of intestinal parasites and IBS on study population. *Blastocystis hominis* was found more frequently detected parasite, and it was found significantly associated with IBS- diarrhea.

Keywords: Irritable Bowel Syndrome (IBS), Intestinal Parasites, *Blastocystis hominis*, *Giardia Lamblia*

1. Introduction

Irritable bowel syndrome (IBS) is a group of functional disorders that is associated with abdominal pain and alterations in bowel habit in the absence of an organic cause. The diagnosis IBS usually based on symptom criteria known as Rome criteria [1- 4]. It is one of the most commonly diagnosed gastrointestinal illnesses with prevalence rates of 10-15% in North America and Europe leading to an estimated cost to the

United States of 1.7 billion dollars in 2000. IBS is negatively affecting the quality of life of patients [5-7]. The pathophysiology of IBS is not well defined [8, 9]. 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 go on to develop IBS (post- infectious IBS) [10, 11].

Intestinal parasitic infection is known to cause IBS-like symptoms that can be continuous, intermittent, and sporadic

or recurrent [12]. Recent studies have described a possible role of protozoan parasites such as *Blastocystis hominis*, *Dientamoeba fragilis*, *Giardia lamblia* and *Entamoeba histolytica* in the etiology of IBS [2, 13].

The objective of this study is to identify the frequency of intestinal parasites among Sudanese patients with IBS.

2. Material and Methods

2.1. Patients

This is a case control study of a qualitative approach in which 200 patients with irritable bowel syndrome (IBS) were enrolled, together with 99 apparently healthy controls. Patients were selected by a professional gastroenterologist according to Rome III diagnostic criteria. Patients with, at least, one of the following conditions were excluded; Crohn's disease, ulcerative colitis, celiac sprue or colon cancer, within 12 months of the IBS diagnosis.

2.2. Method

Natural voided, fresh stool specimen was collected from each one of the study population. Specimens were subjected to; wet saline preparation, formal-ether concentration technique, Z. N stain, and in vitro cultivation.

For wet preparation, specimens were emulsified in normal saline on microscopic slide and then thoroughly examined under the microscope (X10 and X40 objectives). Formed specimens were stained with Lugol's Iodine for cyst identification. Parts of specimens (approximately 5 grams) were preserved in 20 ml of SAF (sodium-acetate formalin) fixative for further permanent staining and formal ether concentration.

One gram (4 ml suspension) of each specimen was concentrated by Ritche formal-ether concentration technique which utilizes di-ethyl acetate instead of ether, sediment was then stained with Lugol's Iodine and was thoroughly examined under the microscope (X10 and X40 objectives) [12].

Part of SAF preserved specimen was smeared and prepared for staining by modified Ziehl- Neelsen stain. Following fixation in methanol for 3 minutes, smears were stained with Carbol fuchsin and decolorized in acid alcohol (1% HCl in methanol) and counter stained with 0.4% methylene blue [14].

Parasite *in vitro* cultivation:

Cultures were done by inoculating approximately 50 mg of feces into Locke egg medium [15]. For cultivating *B. hominis*, starch free Locke egg medium was used. The cultures were incubated at 37°C and examined after 2-3 days. When vacuolar or granular forms of *Blastocystis* were observed, they were sub-cultured in fresh medium for another 3-4 days.

2.3. Statistical Analysis

Data was analyzed using IBM® Statistical Package for Social Science (SPSS) software. Frequencies and percentage were considered as qualitative measures. Significance of

difference was analyzed by Chi-squared test, P value < 0.05 was considered significant.

Ethical consideration:

This work received ethical clearance from the FBC of the College of Graduate, Al-Neelain University. Informed consent was obtained from each participant.

3. Results

The study group was composed of 200 IBS patients (119 females and 81 males) with a mean age of 37.1 (±1.6) years. The demographics of the study population are summarized in Table-1.

Patients were classified into three groups; IBS all types (having IBS regardless the type); the second group contains patients with IBS- diarrhea (IBS-D); and the third group contains patients with constipation predominant IBS (IBS-C).

Intestinal parasites were detected in 32% (64/200) of IBS-all types; and in 16.2% (16/99) of control group. There was statistical difference between IBS-all types and control group in this regard (P. value 0.005). Examination of patients' stool by the different methods collectively revealed that 20 (10%) were positive for the *Entamoeba* cyst, 3 (1.5%) *G. lamblia* cysts, and 41 (20.5%) were positive for *B. hominis* cyst. For control group, *Entamoeba* species were detected in 10 (10.1%), *Giardia lamblia* 1 (1.1%), and *Blastocystis hominis* in 5 (5.2%). *Blastocystis hominis* was more prevalent among IBS-all types than the control group (P. value 0.02). Table-2 illustrates the frequency of intestinal parasites among study population.

Blastocystis hominis was more prevalent among patients with IBS-D than those with IBS-C (P. value 0.000). *B. hominis* was detected in 12.8% of patients with IBS-D, and in 89.1% of patients with IBS-C. 89.1% of *B. hominis* were detected among IBS-all type patients. Table-3 illustrates the detailed frequencies and percentage of intestinal parasites among IBS major subtypes.

Table 1. Frequency of Age among Irritable bowel syndrome and control Group.

Age group	Patients	Control
20-35	55 (27.5%)	46 (46.5%)
36-50	81 (40.5%)	43 (43.4%)
51-75	64 (32.0%)	10 (10.1%)
Total	200	99

Table 2. Frequency and percentage of intestinal parasites among patients (IBS-all types) and control.

	Patients (N%)	Control (N%)
<i>Entamoeba</i> *	20 (10%)	10 (10.1%)
<i>Giardia lamblia</i>	3 (1.5%)	1 (1%)
<i>B. hominis</i>	41 (20.5%)	5 (5.1%)
Total parasites	46 (32%)	16 (16.2%)
Negative	136 (68%)	83 (83.8%)
Total	200	99
P. value 0.005		

* (*E. histolytica*, *E. coli*, *I. buetchelli*, and *E. nana*)

Table 3. Frequency and percentage of intestinal parasites among patients with IBS- D and IBS-C.

Parasites	IBS-D (120)	IBS-C (103)	P. value
<i>Entamoeba</i> species*	15 (12.5%)	5 (4.85%)	0.65
<i>Giardia lamblia</i>	2 (1.66%)	0	0.5
<i>B. hominis</i>	23 (19.16%)	2 (1.94%)	0.000
Total	40 (33.33%)	7 (6.8%)	

* (*E. histolytica*, *E. coli*, *I. buetchelli*, and *E. nana*)

4. Discussion

Association between intestinal parasite infection and IBS is a matter of concern; at least, it is not excluded. Findings of this study go on the stream with many similar works [16]. Several studies have implicated intestinal protozoa in the differential diagnosis of IBS because they cause symptoms resembling IBS or may cause significant flares of IBS with acquisition [17]. Furthermore, they may lead to IBS, secondary to ongoing low grade inflammation through persistent immune activation as a result of antigenic exposure as in persistent carriage/infection that frequently occurs in intestinal parasitic diseases [1].

Intestinal protozoa identified in this study included: *B. hominis*, *G. lamblia*, and *Entamoeba* species. Several studies have implicated these parasites in the etiology of IBS [2, 18-20]. However, fewer studies found no association between exposure to those parasites and predisposition to IBS symptoms [21]. In our case, *B. hominis* was the most prevalent detected protozoa as it was detected in 41 (20%) of IBS patients and in 5 (5.1%) of control. It was lesser than the findings of Yakoob *et. al.*, who reported that fecal carriage of *B. hominis* occurs more frequently in IBS patients (46%) than in the control group (7%) [22]. Symptoms that attributed to infection with *B. hominis* are non-specific, however, they include diarrhea, abdominal pain, cramps or discomfort and nausea, which mimic the IBS-like symptoms [23]. Other augmenting finding of this study is that, *B. hominis* was found significantly associated with IBS-D (diarrhea) subtype. *G. lamblia* infection also may mimic IBS. Evidence for post-infectious IBS secondary to low-grade inflammation was demonstrated by D'Anchino *et. al.*, [19]. In the present study, *G. lamblia* cyst was detected in three (1.5%) of IBS patients; this was similar to the results of Grazioli *et. al.*, [24].

As a long-term infection, a correct diagnosis and treatment should be given for *Giardia lamblia* and *E. histolytic* [12]. However, they may be innocent bystanders, as occurs with asymptomatic carriage or infections by non-pathogenic protozoa. In this study, stool microscopy revealed protozoal cysts in a significant number of IBS patients (32%) compared to controls (16.2%). This result is consistent with Bujanda *et. al.*, who showed that, 49% of the patients with symptoms suggestive of gastrointestinal functional disorders were, or had previously been, diagnosed with parasitic infection [19].

Parasites of Genus *Entameba* including *E. histolytica* was

detected in twenty patient (10%) and 10 (10.1%). Unless there is acute dysentery, the role of *Entamoeba* carriage is not significant for IBS irritation. Early studies implicated amoebic dysentery in the development of IBS among British soldiers returning from Egypt. On the contrary, several other studies from India have suggested that exposure to *E. histolytica* did not predispose patients to IBS symptoms [24, 25].

The findings of this study together with many others prove that, there is a relationship between IBS and intestinal parasites. This relationship differs according to the Genus of parasite and the type of IBS. There is a consensus, that *B. hominis* is associated with IBS-D, and the association of *G. lamblia* is variable and may be depend on cyst shedding. *Entamoeba* is likely associated with post inflammatory-IBS. It still not clear whether the parasites cause IBS, or just provoke some tissue reactions that exacerbate already existing disorder. We can point to inflammatory cytokines, mast cells activity, or neurotransmitter accumulation in the tissue milieu. We are investigating this issue meanwhile.

5. Conclusion and Recommendation

This study concluded that, there is a statistical association between presence of intestinal parasites and IBS on study population. *Blastocystis hominis* was found more frequent detected parasite, and it is significantly associated with IBS-diarrhea.

Acknowledgement

We would like to thank all participants in this study. Our thanks extended to the department of Medical Parasitology at the Faculty of Medical Laboratory Sciences, Al-Neelain University for their kind assistance and help.

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