

Assessment of the Association between *Blastocystis hominis* Infection and Irritable Bowel Syndrome in Phramongkutklao Hospital

Luksana Thamrongwittawatpong, M.D.
Col. Surapon Surangsrirat, M.D.

ABSTRACT

Background: Irritable bowel syndrome (IBS) is a chronic gastrointestinal condition characterized by abdominal discomfort, bloating and disturbed defecation. The etiology of IBS has not been clearly identified. It has been hypothesized that parasitic infection may play a role in IBS patients particularly in developing countries. Previous studies have shown that *Blastocystis hominis* (*B. hominis*), an obligate anaerobic protozoa found in human intestine, was frequently detected in the stool of IBS patients.

Aims: To determine the prevalence of parasitic infection in IBS patients as well as the association between *B. hominis* and IBS patients.

Methods: We enrolled 80 patients. IBS was defined by Rome II criteria. The patients were divided into two groups. 40 patients were enrolled in the IBS group and 40 patients were enrolled in the control group. Medical history, physical examination, and stool specimens for oval and parasites, bacterial culture, and culture for *B. hominis* with Jones' medium were obtained.

Results: Fourteen males and 26 females were identified in the IBS group. The mean age was 51 years. In the IBS group, 23 patients (57.5%) reported diarrhea, 10 patients (25%) reported constipation, and 7 patients (17.5%) reported abdominal pain. Twenty five males and 15 females were identified in the control group. The mean age was 46 years. In the IBS group, microscopic exam of the stool samples identified *B. hominis* in 6 patients (15%) and Microspidia in 1 patient (2.5%). In the control group, *B. hominis* was identified in 5 patients (12.5%), *Ascaris lumbricoides* was identified in 1 patient (2.5%), and *Entamoeba coli* cyst was seen in 1 patient (2.5%). Stool culture was positive for *B. hominis* in 17.5% (7 of 40) of the patients in the IBS group and in 12.5% (5 of 40) of the patients in the control group ($P = 0.75$). Underlying disease, smoking, alcohol, and characteristic of stool were similar in both groups.

Conclusions: *B. hominis* was a predominant parasitic infection in both IBS and control groups. The prevalence of *B. hominis* in both groups was not significantly different suggesting that it may not be associated with the development of IBS.

Key words : *B. hominis*, Irritable bowel syndrome, Parasite infestation

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BACKGROUND

Irritable bowel syndrome (IBS) is a chronic gastrointestinal condition characterized by abdominal discomfort, bloating and disturbed defecation. It is considered a functional gastrointestinal disorder. Rome Committee for the Classification of Functional Gastrointestinal Disorders has defined IBS⁽¹⁾ on the basis of abdominal and bowel symptoms that occur with sufficient frequency in affected patients. It is characterized by the absence of any identifiable physical, radiologic or laboratory abnormalities. IBS is a common condition, with prevalence estimates ranging between 12% and 15% in the western countries.^(2,3) The symptoms of IBS represent common reasons for primary care visits and more than 40% of IBS patients have symptoms that are so frequent and severe that they have to take time off from work, curtail their social life, stop traveling, take medication, and even stay confined to their house for fear of embarrassment.⁽⁴⁾ Danvivat, *et al.* reported that the prevalence of IBS in Thai population is approximately 4.6%.⁽⁵⁾ The cause of IBS is uncertain while the possible hypotheses are 1) altered motility 2) abnormal visceral sensation 3) post infectious 4) psychosocial factors and 5) neurotransmitter interplay to cause symptoms. In developing countries, gastrointestinal infection is frequently found and remains the major problem. Therefore, such infection probably causes and relates to IBS.

Blastocystis hominis (*B. hominis*) is an obligate anaerobic protozoa found in the human intestine. Infection occurs worldwide but is commonly found in the tropics and developing countries. Its significance as a pathogen remains controversial. However, several studies have reported *B. hominis* as a causative agent of gastrointestinal symptoms such as diarrhea and abdominal pain in both immunocompetent and immunocompromised hosts.⁽⁶⁾ It is believed that *B. hominis* is transmitted via the fecal-oral route. In culture, *B. hominis* has ameboid, vacuolated, granular and a cyst form. Generally, detection of *B. hominis* in stool specimen is based on direct microscopic examination of fresh fecal specimens. Culture of *B. hominis*, is although easy to prepare, but it's not done routinely. Although a previous study demonstrated that culture of *B. hominis* was clearly superior to direct microscopy in terms of sensitivity.⁽⁷⁾ *B. hominis* has a prevalence of approximately 10 to 15 percent and 30 to 50 percent in stool samples from healthy asymptomatic

individuals in developed and developing countries respectively. In Thailand, the prevalence of *B. hominis* carriage in various groups is as high as 10-40%.^(8,9) Recently, there have been studies that have examined the link between *B. hominis* and IBS.^(10,11) The purpose of the present study was to determine the prevalence of *B. hominis* and the association between IBS patients and *B. hominis*.

PATIENTS AND METHODS

This cross-sectional controlled prospective study was conducted at Phramongkutklao College of Medicine in Bangkok, Thailand. IBS patients, defined by Rome II criteria, who attended the gastroenterology clinic, were registered. Forty patients were enrolled in the IBS group and 40 patients were enrolled in the control group. The Rome II criteria is at least 12 weeks or more, which need not be consecutive, in the preceding 12 months of abdominal discomfort or pain that has two of three features: relieved with defecation; and/or onset associated with a change in frequency of stool; and/or onset associated with a change in form of stool⁽¹²⁾. The exclusion criteria included 1) alarming symptom such as weight loss, anemia, hematochezia, fever 2) chronic disease: chronic kidney disease, diabetes, coronary artery disease 3) previous antibiotics used especially metronidazole within 1 month. Medical history and physical examination were obtained. Stool specimens were collected for ova and parasites, culture for *B. hominis*, and culture for bacteria such as *Salmonella* spp, *Shigella* spp, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Vibrio cholera* which are common pathogens in the community. The study was reviewed and approved by the ethics review committee of the Department of Medicine at Phramongkutklao College of Medicine. Stool specimens for ova and parasites and culture of *B. hominis* were sent immediately to the Department of microbiology and Department of parasitology. The specimens were examined by the same technicians who did not know the patients' history.

Stool collection and examination.

Stool specimens were examined by expert in parasitology for intestinal parasites immediately after collection by a wet smear preparation in saline and staining with Lugol's iodine solution. All specimens were processed using the formalin/ethyl-acetate concentra-

tion technique. Since the simple smear and concentration technique is rather insensitive for the detection of *B. hominis*,⁽⁹⁾ short-term *in vitro* cultivation was performed for each stool sample in the medium of Jones supplemented with 10% horse serum. The cultures were incubated at 37 °C for 48-72 hours and then examined under a light microscope with 10x and 40x objectives. *B. hominis* positive specimen was defined as identification of any form of *B. hominis*, i.e., vacuolar, granular, multivacuolar, and cystic forms by short-term *in vitro* cultivation. Each stool specimen was also examined for *Cryptosporidium* spp. and Microsporidia using modified acid-fast and gramchromotrope staining, respectively. All samples were also tested for common bacterial pathogens that may cause gastrointestinal symptoms. Stool samples were transferred in transport media and cultured for bacterial pathogens using conventional cultivation and identified by biochemical methods at the Department of Microbiology.

Statistical analysis

Results are expressed as the mean \pm SD for continuous variables (e.g. age) and number (percentage) for categorical data (e.g., sex, stool culture, diarrhea, etc.). Univariate analysis was performed using the independent sample t-test. The Pearson chi-square test and Fisher's exact test were also used whenever appropriate. A P value <0.05 was considered statistically significant. All P values were two sided. Statistical interpretation of data was performed by using the computerized software program SPSS version 10.0 (SPSS Inc., Chicago, IL).

RESULTS

The IBS group consisted of 14 males and 26 females with a mean \pm SD age of 51 years. The bowel habit was described as diarrhea in 57.5% (23 of 40), constipation in 25% (10 of 40) and abdominal pain in 17.5% (7/40) of patients. (Figure 1) Consistency of stool varied from formed 87.5% (35 of 40), soft 10% (4 of 40), and thick watery 2.5% (1 of 40). The control group consisted of 25 males and 15 females with a mean age of 46 years. Consistency of stool varied from formed in 77.5% (31 of 40), soft 22.5% (9 of 40), and no thick watery.

Comparison of the IBS and control groups

There was no significant difference in age between

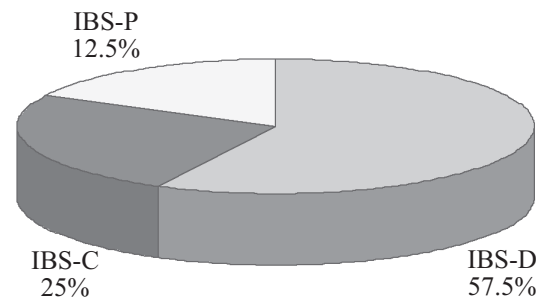


Figure 1 Presentations of IBS
IBS-P = Painful IBS
IBS-C = Constipated IBS
IBS-D = Diarrheal IBS

Table 1 Prevalence of parasitic infestations in Irritable bowel syndrome (IBS) and control patients

Parasite	IBS (N= 40)	Control (N=40)	P-value
<i>B. hominis</i>	7	5	0.75
Microsporidia	1		
<i>Ascaris</i> (egg)		1	
<i>Entamoeba coli</i>		1	

both groups (51 years vs 46 years, P-value = 0.147). There were more women than men in the IBS group (P value = 0.025). All patients in the IBS group and 20% (8 of 40) of the patients in the control group had abdominal pain (P <0.001) while underlying disease, smoking, alcohol, characteristic of stool and mucus were similar in both groups.

Prevalence of parasitic infestations in IBS and control patients were shown in Table 1.

Stool ova and parasites

In the IBS group, microscopic exam of the stool samples identified *B. hominis* in 6 patients (15%) and Microsporidia in 1 patient (2.5%). In the control group, *B. hominis* was identified in 5 patients (12.5%), *Ascaris lumbricoides* was identified in 1 patient (2.5%), and *Entamoeba coli* cyst was seen in 1 patient (2.5%).

Stool culture for *B. hominis* was positive in 17.5% (7 of 40) of the patients in the IBS group and in 12.5% (5 of 40) of the patients in the control group (P = 0.75). Subgroup analysis of diarrhea predominant comparing with other types in IBS patients showed similar results (P = 1.00). (Table 2 and 3)

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Table 2 Prevalence of *B. hominis* in diarrheal type of Irritable bowel syndrome (IBS) and control patients

	<i>B. hominis</i>			P value
	Present	Absent	Total	
IBS-diarrhea	2	21	23	1.00
Control	5	35	40	
Total	7	56	63	

Table 3 Comparison of *B. hominis* infection in each type of Irritable bowel syndrome (IBS)

IBS type	<i>B. hominis</i> positive		
	Diarrhea	Constipation	Abdominal pain
Number of patients	2	3	2

P = 0.233

Table 4 Prevalence of bacteria infections in IBS and control patients

Bacteria	IBS (N = 40)	Control (N = 40)
Salmonella gr B	2	3
Salmonella gr C	1	
<i>Salmonella enteritidis</i>	1	

Prevalence of bacteria infections in IBS and control patients (Table 4)

Culture stool for bacteria revealed Salmonella gr B in 5% (2 of 40), Salmonella gr C in 2.5% (1 of 40) and *Salmonella enteritidis* in 2.5% (1 of 40) of IBS patients. In control group, Salmonella gr B was identified in 3 patients (7.5%).

Comparison of stool microscopy and culture for detection *B. hominis*

Stool microscopy in both groups of patients yielded positive results for *B. hominis* in 11 patients (13.75%) compared with 15% (12 of 80) by stool culture. Stool culture for *B. hominis* was more sensitive than microscopic examination (P < 0.001) (Table 5).

DISCUSSION

Blastocystis hominis is an intestinal protozoan commonly found in human with prevalence between

Table 5 Comparison of *B. hominis* culture and wet preparation

<i>B. hominis</i>	Culture			
	Present	Absent	Total	P value
Wet preparation				
present	9	2	11	<0.001
absent	3	66	69	
Total	12	68	80	

30-50% in the developing countries⁽⁷⁾. The pathogenic potential of *B. hominis* in human intestine remains uncertain because such organism has been demonstrated in both symptomatic and asymptomatic individuals. Controlled studies of the association between *B. hominis* and gastrointestinal symptoms are lacking. However, there is mounting evidence to suggest that *B. hominis* is a cause of intestinal disorders due to its significant correlation with individuals suffering from IBS in recent years.^(10,11,13,14) In addition, levels of IgG antibody to *B. hominis* were increased significantly in the patients with IBS compared with asymptomatic controls.⁽¹⁰⁾

In this study, *B. hominis* was a predominant parasitic infection in both IBS and control groups (17.5% vs 12.5% P = 0.75). Interestingly, the prevalence of *B. hominis* in both groups was not significantly different. This result was similar to the previous studies from Tungtrongchitr A, *et al.*⁽¹⁵⁾ who found that *B. hominis* infection was present in 13.6% of IBS patients and 12% of control patients (P = 0.87). Nevertheless, Giacometti, *et al.*⁽¹⁴⁾ shown that *B. hominis* was recovered from the stool of 38 subjects, 15 of whom belonged to the IBS group (P = 0.006). Yakoob J, *et al.*⁽¹¹⁾ was able to isolate *B. hominis* from the feces in 46% of the IBS patients and this was significantly higher than those seen in the control group. This finding suggests that *B. hominis* is associated with IBS. However, whether such organism causes IBS or not is unclear. The dissimilar results from our study may be explained by the patients coming from different residential areas of the city, diverse walks of life and probably variation of personal hygiene. Also, our study population was small and not sufficient to show the differentiation. The data from subgroup analysis of diarrhea predominant IBS patients was similar to those seen in overall IBS group. This finding is consistent with a recent study,⁽¹¹⁾ suggesting that the presence of

B. hominis may not be causal and responsible for diarrhea type only, but also cause in other types of IBS. We also reported that smoking, alcohol consumption, characteristic of stool and mucus was similar in both groups. In IBS patients, stool culture for bacteria revealed *Salmonella* gr B, *Salmonella* gr C and *Salmonella enteritidis*. This was similar to those detected in the control group. *Salmonella* gr B was identified in 3 patients. Epidemiologic studies examining the incidence of IBS following bacterial gastroenteritis have been observed. However, one important observation is that patients with preexisting IBS may be more likely to present earlier to their physicians with bacterial gastroenteritis compared with those without IBS.⁽¹⁶⁾ Thus, IBS following bacterial gastroenteritis may have been overestimated. Furthermore, this study did not find any significant difference of pathogenic bacteria between both groups.

There are several methods commonly used to detect human infection with *B. hominis*. All are based on the demonstration of parasite in fecal samples. Stool specimen may be examined as fresh, wet smears, by concentration or by *in-vitro* cultivation of the sample prior to the light microscope.⁽¹⁷⁾ Our study showed that microscopic examination of stool is less sensitive than stool culture for *B. hominis*. This finding is similar to the results from previous studies^(7,18).

In conclusions, *B. hominis* was a predominant parasitic infection in both IBS and control groups. The prevalence of *B. hominis* in both groups was not significantly different suggesting that it may not be associated with the development of IBS.

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