

BLASTOCYSTIS HOMINIS INFECTION IN IRRITABLE BOWEL SYNDROME PATIENTS

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Abstract. Irritable bowel syndrome (IBS) is a functional bowel disorder in which abdominal pain is associated with a defect or a change in bowel habits. Subtle inflammation, especially after infectious enteritis, has been sometimes suspected as one mechanism of pathogenesis. This research was performed (1) to evaluate the prevalence of parasitic infections and (2) the possible association of IBS and parasitic infections. Fifty-nine IBS patients were recruited using symptom-based criteria (Rome Criteria II) with an absence of intestinal parasitic infection by direct smear method. Stool samples of individual patients were examined using 7 methods, *ie* examination for stool occult blood, simple saline smear method, formalin-ether technique, culture for *Blastocystis hominis*, modified trichrome stain, modified Ziehl-Neelsen method, and trichrome stain for parasitic and bacterial infections. Of the 59 patients, stool samples of 13 patients (22.1%) were positive for parasites. These were *B. hominis* (13.6%), *Strongyloides stercoralis* larvae (1.7%), *Giardia lamblia* cysts (1.7%), and non-pathogenic protozoa, *ie* *Endolimax nana* cysts (5.1%). The prevalence rate of parasitic infections in the control group (20%) was not statistically different from the patients. There was no statistical difference between *B. hominis* infection in IBS patients and control was found in this study ($p = 0.87$). In the IBS group, *B. hominis* infection predominated (13.6%), while other parasitic infections were found in 8.5%. The culture method for *B. hominis* is more sensitive than the direct (simple) stool smear method, which is the routine diagnostic method in most laboratories. These results were also found in control group.

INTRODUCTION

Irritable bowel syndrome (IBS) is defined as 'a functional bowel disorder in which abdominal pain is associated with defecation or a change in bowel habits with features of disordered defecation and distention' (Drossman *et al*, 1999). The consensus definition and criteria for IBS have been formalized in the 'Rome criteria' which are based on the Manning criteria (Manning *et al*, 1978). The overall prevalence rate is similar (approximately 10%) in most industrialized countries (Camilleri, 2001). The illness has a large

economic impact on health care use, and indirect costs, chiefly through absenteeism. IBS is a biopsychosocial disorder in which three major mechanisms interact: psychosocial factors, altered motility, and/or heightened sensation of intestinal function (Thompson *et al*, 1989, 1999; Camilleri and Prather, 1992; Drossman *et al*, 1997; Camilleri and Choi, 1997). Subtle inflammatory changes suggest a role for inflammation, especially after infectious enteritis. An infectious origin has been suspected but has not been proven. Bacteria, protozoa, and helminths have come under scrutiny, and recent attention has been focused on the alteration of the intestinal ecosystem as a possible pathogenetic cofactor (McKendrick and Read, 1994; Agreus *et al*, 1995; Lembo *et al*, 1996; Barbara *et al*, 1997; Sinha *et al*, 1997). Studies have been in progress for many years to determine whether the so-called 'non-pathogenic'

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intestinal protozoa, such as *Entamoeba* spp (Sinha *et al*, 1997), *Giardia lamblia* (D'Anchino *et al*, 2002), and *Blastocystis hominis* (Giacometti *et al*, 1999) might have a role in some pathologic conditions involving the gastrointestinal tract (Boreham *et al*, 1996; Morgan *et al*, 1996; Hussain *et al*, 1997). The geographic distribution of *Blastocystis hominis* appears to be global, with infections common in tropical, subtropical, and developing countries (Tan *et al*, 2002). In general, studies from developed countries report approximately a 1.5-10% overall prevalence of *B. hominis* (Guignard *et al*, 2000; Jensen *et al*, 2000; Tasova *et al*, 2000; Herwaldt *et al*, 2001; Kaneda *et al*, 2001). Reports of prevalence and the importance of the protozoan *B. hominis*, as an intestinal pathogen in IBS, have been scarce, especially in Thailand. This study was performed to evaluate for the possible association between parasitic infections, especially *B. hominis* infection, and IBS patients, and its prevalence among individuals with gastrointestinal symptoms of IBS.

MATERIALS AND METHODS

The study was performed with a cohort of 59 patients (27 male; 32 female), with 25 normal subjects serving as controls. Thai volunteers who attended the Out-patient Department-General Practice Sections of Siriraj Hospital and Ramathibodi Hospital, Bangkok, for a physical check-up, were investigated. They all visited the clinic voluntarily, and were diagnosed by physical and biochemical laboratory examinations for the inclusion criteria of Rome Criteria II. The exclusion criteria were as follows: IBS patients with red flags, age over 50 years and the presence of chronic diseases, such as diabetic mellitus, coronary heart diseases, etc. Clinical and epidemiological data about each subject were obtained using standardized questionnaires. The questions covered health status, presence of gastrointestinal symptoms, previous parasitic infections, personal hygiene, drug intake, weight loss, and contact with animals. Based on the clinical data, according to the Rome II diagnostic criteria, the patients were diagnosed with IBS. All individuals were asked to provide one stool sample in a disposable stool box for analysis. Samples were sent to the Department of Parasitology, Faculty of Medicine at Siriraj Hos-

pital, Mahidol University for *B. hominis* determination. The sample was then smeared onto a semi-transparent fecal membrane on the surface of a glass slide. The smears were evaluated under a microscope with fresh normal saline solution and iodine solution for the presence of parasites. Trichrome, modified trichrome and acid-fast staining were done after the smears had dried. The stained smears were examined under a microscope. The shape and size of *B. hominis*, including other parasitic infections, were observed. Aliquots of all the stool samples were individually inoculated into monophasic medium for culture of *B. hominis*. If a case was positive for *B. hominis*, both IBS patients and controls were treated with 1,200 mg metronidazole daily for 7 days, and their stools were re-investigated for *B. hominis*.

The study protocol was approved by the Ethics Committee of the Faculty of Medicine at Siriraj Hospital, Mahidol University, Bangkok, No.216/2002, and informed consent was obtained from each participant.

RESULTS

The prevalence of *B. hominis* in both IBS and control subjects is shown in Table 1. Eight out of 59 subjects (13.6%) were found positive for *B. hominis* among the IBS patients while in the control group 12.0% (2 out of 25) were positive. Stool examination of IBS patients found 5.1, 1.7, and 1.7% of the non-pathogenic protozoa *Endolimax nana*, *Strongyloides stercoralis*, and *Giardia lamblia*, respectively. Using different methods to determine *B. hominis* and other parasites, the culture method was found to be more sensitive than the other methods (Table 2).

To examine whether *B. hominis* infections in IBS patients and controls were different, both IBS and control cases positive for *B. hominis* were treated with 1,200 mg of metronidazole for 7 days and the stool was re-investigated for *B. hominis*. The results showed that *B. hominis* infection in IBS patients could not be fully cured using standard treatment (1,200 mg of metronidazole daily for 7 days).

DISCUSSION

The results of this study show that *B. hominis*

infection in IBS patients was high, but not statistically significantly different from *B. hominis* infection in control subjects (Table 1). This result was consistent with the report by Giacometti *et al* (1999), who found that *B. hominis* infection in IBS was 11.1%, compared with 6.1% in non-IBS patients (Giacometti *et al*, 1999). It has been reported that positive rates for *B. hominis* infection in stools taken from patients with diarrhea living in urban and in rural areas were 7.5% (17/226) and 3.95% (7/177), respectively, in contrast to the positive rate for *B. hominis* infection in stools of healthy people, which was only 0.67% (2/300) (Wang *et al*, 2002). These data supported the supposition that *B. hominis* infection was associated with diarrhea. Parasitic infection in IBS patients seemed to be significantly more likely than in control subjects.

Our findings did not show any statistically significant difference between *B. hominis* infec-

tion in IBS and control subjects. One reason might be that the number of IBS patients in our study was too low to show the difference. These results were the same as those of Giacometti *et al* (1999), who reported no significant difference in *B. hominis* prevalence, between IBS patient and controls. Their conclusion suggested that *B. hominis* infection may be an indicator of intestinal dysfunction or resident intestinal flora disorder and an intestinal tract that is abnormal for any reason may provide conditions suitable for the proliferation of *B. hominis* (Udkow and Markell, 1993; Neal *et al*, 1997). Therefore, the interpretation of a prevalence study between the association of IBS and *B. hominis*, requires caution.

The culture method was found to be more sensitive than the other methods, *ie*, direct smear, formalin-ether method and trichrome stain. Although the culture method for *B. hominis* is more costly and time-consuming, it is more reliable than

Table 1
Prevalence of parasitic infections in irritable bowel syndrome (IBS) and control subjects.

Parasite	IBS (N=59)	Controls (N =25)	p-value
<i>B. hominis</i> (vacuolated form)	13.6% (8/59)	12.0% (3/25)	0.87
Non-pathogenic protozoa (<i>Endolimax nana</i> cyst)	5.1% (3/59)	-	-
<i>Strongyloides stercoralis</i>	1.7% (1/59)	-	-
Rhabditiform larva			
Hookworm egg	-	8.0% (2/25)	
<i>Giardia lamblia</i> cyst	1.7% (1/59)	-	-

Table 2
Prevalence of parasitic infections in irritable bowel syndrome (IBS) using different examination methods.

	Hematest	Direct smear	Formalin-ether method	Culture for <i>B. hominis</i>	Trichrome stain	Modified trichrome stain	Modified acid-fast stain	Total
IBS								
<i>B. hominis</i> (vacuolated form)	-	5	3	8	4	-	-	8
Non-pathogenic protozoa, cyst (<i>Endolimax nana</i>)	-		-	-	3	-	-	3
<i>Strongyloides stercoralis</i>	-	1	-	-	-	-	-	1
Rhabditiform larva								
<i>Giardia lamblia</i> cyst	-	1	1	-	-	-	-	1
Controls								
<i>B. hominis</i> (vacuolated form)	-	2	-	3	-	-	-	3
Hookworm egg	-	1	2	-	-	-	-	2

microscopy (Zaman and Khan, 1994).

Individuals whose stool specimens contained *B. hominis* were further investigated and given the standard treatment of metronidazole (1,200 mg/day for 7 days). Up to 10% of IBS cases could not be successfully treated; and *B. hominis* infection remained (Fig 1). It may be that *B. hominis* infection in IBS may develop resistance. It has been reported that IBS may decrease the humoral immune response (Chen *et al*, 1987) and cellular immune function in *B. hominis* infection (Kaneda *et al*, 2000; Long *et al*, 2001; Nasirudeen *et al*, 2001; Tan *et al*, 2001). The level of the CD₃⁺ count, CD₄⁺ count, and CD₄⁺/CD₈⁺ ratio was decreased in *B. hominis*-infected individuals, but the CD₈⁺ count was normal (Wang *et al*, 2002). Compared with the *B. hominis*-negative group, the difference was significant ($p < 0.05$). Recent advances in *B. hominis* research found that, in subjects suffering from immunodepression, *B. hominis* showed a significant association with gastrointestinal symptoms (Germani *et al*, 1998; Ghosh *et al*, 1998; Mathewson *et al*, 1998; Amenta *et al*, 1999; Cimerman *et al*, 1999; Menon *et al*, 1999; Li, 2000; Prasad *et al*, 2000; Wilcox, 2000; Lebbad *et al*, 2001), and *B. hominis* infection was related to host cellular immune function. This may be the reason for the drug resistance of *B. hominis* in IBS. In conclusion, *B. hominis* should be kept in mind by parasitologists and physicians when

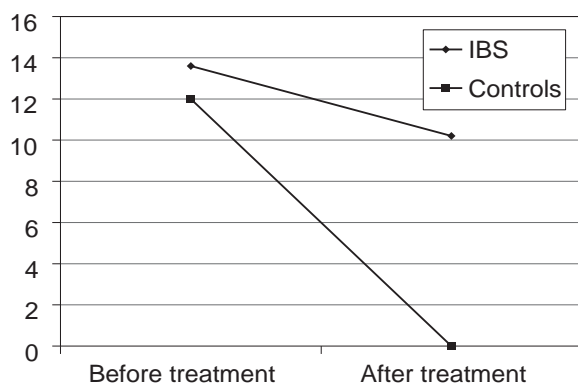


Fig 1—Prevalence of *B. hominis* in IBS and controls before and after metronidazole treatment (1,200 mg/day for 7 day).

dealing with patients with diarrhea. *B. hominis* has long been described as a non-pathogenic protozoan parasite, until recently, when claims have been made that it can result in pathogenic conditions (Cirioni *et al*, 1999; Koutsavlis *et al*, 2001; Waring and Reed, 2001). On the other hand, it is possible that a subgroup of *B. hominis* could be pathogenic in some patients. Recent studies have identified two variants of this organism, on the basis of different polypeptide patterns and DNA nucleotide sequences (Kukoschke and Muller, 1991; Keystone, 1995). Further research into *B. hominis* infection, especially in IBS, should be conducted to elucidate this issue.

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