IRRITABLE BOWEL SYNDROME: IN SEARCH OF AN ETIOLOGY: ROLE OF BLASTOCYSTIS HOMINIS

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Abstract. This study was designed to examine stool specimens of irritable bowel syndrome (IBS) patients for Blastocystis hominis, a common intestinal parasite. One hundred fifty patients were enrolled, 95 IBS cases and 55 controls. These patients provided a medical history, and underwent physical and laboratory evaluations that included stool microscopy and culture for B. hominis and colonoscopy. The 95 cases (51 males and 44 females) had a mean ± SD age of 37.8 ± 13.2 years. Stool microscopy was positive for B. hominis in 32% (30 of 95) of the cases and 7% (4 of 55) of the controls (P = 0.001). Stool culture was positive in 46% (44 of 95) of the cases and 7% (4 of 55) of the controls (P < 0.001). Blastocystis hominis was frequently demonstrated in the stool samples of IBS patients; however, its significance in IBS still needs to be investigated. Stool culture has a higher positive yield for B. hominis than stool microscopy.

INTRODUCTION

Blastocystis hominis (B. hominis) is a unicellular protozoan found in the large intestine of humans. Infection occurs worldwide but is commonly found in the tropics and developing countries. The pathogenic potential of B. hominis in the human intestine is controversial because the organism has been found in both symptomatic and asymptomatic individuals. The morphologic forms observed include vacuolar, granular, and amoeboid. The morphologic form responsible for transmitting the disease has not been identified, and spread is presumed to be via the feco-oral route. The vacuolated form is most commonly found in feces. Infection is usually diagnosed on the basis of direct microscopy of the fecal sample and observing the vacuolated form of the organism using a light microscope. Cultures of B. hominis, although easy to prepare, are not done routinely, although a previous study demonstrated that cultures of B. hominis were clearly superior to direct microscopy in terms of sensitivity. Superficial invasion and mucosal inflammation of the intestine with B. hominis have been observed in studies of gnotobiotic guinea pigs. Although controlled studies of the association between B. hominis and diarrhea are lacking, there have been studies that have examined the link between B. hominis and irritable bowel syndrome (IBS). Levels of IgG antibody to B. hominis were increased significantly in the patients with IBS compared with asymptomatic controls. This is suggestive of a link between B. hominis and IBS. The aim of this study was to determine the prevalence of B. hominis in patients with symptoms suggestive of IBS.

PATIENTS AND METHODS

This prospective study was conducted at the Aga Khan University Hospital in Karachi, Pakistan. Ninety-five patients with symptoms suggestive of IBS, according to the Rome II criteria, who attended the gastroenterology clinic from January 2002 to June 2003 were recruited into the study. 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. Patients in the IBS group presented with abdominal pain or discomfort associated with altered bowel habits. In control group, there was a recent onset of diarrhea, loss of appetite, fever, and abdominal discomfort. These patients provided a thorough medical history and underwent a physical examination, and the following tests were conducted: complete blood count, erythrocyte sedimentation rate, liver function, blood urea nitrogen, creatinine, electrolytes, stool microscopy and culture for B. hominis. Colonoscopy with biopsy was carried out in each of the patients who fulfilled the criteria for IBS. Informed consent was obtained for colonoscopy in all the patients as per Aga Khan University Hospital policy. The study was reviewed and approved by ethics review committee of the Department of Medicine of Aga Khan University. Technologists were unaware of the classification status of the patients. All stool specimens for microscopy and culture of B. hominis were processed by the same technicians and a note was also made of presence of other parasites such as Giardia lamblia, Entamoeba histolytica, etc. A microbiologic investigation was also performed to detect Salmonella spp, Campylobacter jejuni, Clostridium difficile, and Vibrio cholera. A viral screen was not performed on stool specimens obtained in view of cost limitations.

Microscopy of fecal smears. Fecal sample microscopy was done as previously described. Briefly, approximately 2 mg of feces was thoroughly emulsified on a glass slide in one drop of physiologic saline and covered with a cover slip. A similar preparation was made on another slide using Lugol’s iodine. These preparations were examined under both the low power (10×) and high dry (40×) objectives.

Culture of feces. Cultures were done by inoculating approximately 50 mg of feces into Jones’ medium. For culturing B. hominis, Jones medium without starch was used because it supports good growth of the parasite as previously described. The cultures were incubated at 37°C and examined after 24, 48, 72, and 96 hours. If no B. hominis were seen up to the end of this period, the cultures were regarded as negative. The sediment was examined under both the low power (10×) and high dry (40×) objectives.

Statistical analysis. Results are expressed as the mean ± 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 was used.
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**

**Irritable bowel syndrome group.** This group was composed of 51 males and 44 females with a mean ± SD age of 37.7 ± 13 years. Symptoms were equally common in males and females. Abdominal pain was seen in 80% (70 of 95) and was described as cramping in 64% (45 of 70). The bowel habit was described as diarrhea in 73% (69 of 95) and constipation in 13% (12 of 95). Consistency of stool varied from semi-formed in 33% (31 of 95), normal 25% (24 of 95), and loose 42% (40 of 95). Colonoscopy showed patchy erythema in the rectum and sigmoid colon in 11% (10 of 95) and 3% (3 of 95), respectively. This was later confirmed to be nonspecific inflammation.

**Control group.** This group was composed of 19 males and 36 females with a mean ± SD age of 44.6 ± 20.5 years. A vague abdominal pain was present in only 40% (22 of 55). The bowel habit was described as diarrhea in 87.3% (48 of 55) with loose consistency of stools.

**Comparison of the IBS and control groups.** The IBS symptoms were equally common in males and females. Seventy-four percent (70 of 95) of the cases and 40% (22 of 55) of the controls had abdominal pain ($P < 0.001$). Seventy-three percent (69 of 95) of the cases and 87% (48 of 55) of the controls had a bowel habit described as diarrhea ($P = 0.042$). In control group, 33% (18 of 55) presented with fever and 42% (23 of 55) with loss of appetite, while patients in IBS group did not demonstrate these features.

**Stool microscopy.** Stool microscopy was positive for *B. hominis* in 32% (30 of 95) of the IBS patients and in 7.3% (4 of 55) of the control group. Two percent (2 of 95) of the IBS patients were positive for cysts of *Entamoeba coli*.

**Stool culture.** Stool culture was positive for *B. hominis* in 46% (44 of 95) of the IBS patients and in 7.3% (4 of 55) of the control group. The organisms isolated from stool microscopy and culture in the control group is shown in Table 1.

**Comparison of stool microscopy and culture.** Stool microscopy in both groups of patients yielded positive results for *B. hominis* in 23% (34 of 150) compared with 32% (48 of 150) by stool culture. Stool culture for *B. hominis* was more sensitive than microscopy ($P < 0.001$) (Table 2).

**TABLE 1**

<table>
<thead>
<tr>
<th>Frequency of organisms isolated from the stool of patients in the control group ($n = 55$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td><em>Vibrio cholera</em></td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
</tr>
<tr>
<td><strong>Parasites</strong></td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
</tr>
<tr>
<td><em>Blastocystis hominis</em></td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
</tr>
<tr>
<td><em>Giardia lamblia</em></td>
</tr>
<tr>
<td><strong>Idiopathic</strong></td>
</tr>
</tbody>
</table>

**ISOLATION OF Blastocystis hominis BY STOOL MICROSCOPY AND CULTURE IN THE IRRITABLE BOWEL SYNDROME AND CONTROL GROUPS**

<table>
<thead>
<tr>
<th><strong>Stool culture</strong></th>
<th><strong>Negative</strong></th>
<th><strong>Positive</strong></th>
<th><strong>P</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Count</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>16</td>
<td>32</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>Negative</td>
<td>100</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>116</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

*Blastocystis hominis* is one of the most common intestinal protozoa in humans. It appears in both immunocompetent and immunocompromised individuals. Although several reports have suggested that *B. hominis* could cause gastrointestinal disorders, the specific pathogenicity of this organism has not yet been defined. The clinical consequences of *B. hominis* infection are mainly diarrhea or abdominal pain with nonspecific gastrointestinal symptoms such as nausea, anorexia, vomiting, weight loss, lassitude, dizziness, and flatulence. It has been speculated that thick-walled cysts might be responsible for external transmission, while thin-walled cysts might reinfest within a host’s intestinal tract. The transmissible form of this organism has not yet been defined. It has been suggested that *B. hominis* could be transmitted via untreated water. The various mechanisms suggested for *B. hominis*-mediated gastrointestinal symptoms include adherence of *B. hominis* to the gut epithelium, triggering a lysis mechanism as shown for *E. histolytica*, *G. lamblia*, and existence of a diarrheagenic toxin present in culture filtrates and in a *B. hominis* cell-free fraction.

In this study, *B. hominis* was isolated from the feces of 46% of the IBS patients. These patients came from different residential areas of the city and diverse walks of life; however, a high prevalence of *B. hominis* would not reflect their personal hygiene. Although few reports have found that *B. hominis* was common in various age groups, our study failed to show a significant association in any age group. Several reports have suggested that the association of persistent bowel dysfunctions is likely to be associated with deeper penetration of the *B. hominis* and thus more severe mucosal inflammation. However, evidence of ulceration or invasion of *B. hominis* into the tissue could not be found by colonoscopy and biopsies in our patients with IBS. This might be consistent with the results of Phillips and Zierdt, who showed that invasion of *B. hominis* took place only under certain condition in germ-free guinea pigs. Histopathologic examination of rectal and sigmoid colonic tissues with non-specific inflammation failed to demonstrate *B. hominis*. This represents a mild increase in inflammatory cells for which there may not be a necessary and specific etiology, and may be part of the normal variation in our population.

In IBS patients, cysts of *Entamoeba coli* were also demonstrated on stool microscopy, which is associated with asymptomatic carriage. Stool culture appeared to be more sensitive in diagnosing *B. hominis* infection than microscopy (46% versus 32%), which is consistent with our previous study.

This prevalence study shows an association between *B. hominis* and IBS, although the study population was small. In
some IBS patients, the presence of *B. hominis* may not be casual and responsible for diarrhea. It has been previously demonstrated that IBS patients had significantly high-levels of specific IgG antibodies against *B. hominis.* Also, a subgroup of IBS patients have persistently increased concentrations of inflammatory cytokines that include interleukin-1, which by inhibiting absorption of sodium and water, could contribute to persistent diarrhea. In our study, a significant proportion of IBS patients demonstrated *B. hominis* in their fecal samples. However, in view of its pathogenic and non-pathogenic strains, it requires further studies to confirm the pathogenic role of *B. hominis* in IBS in a larger sample size. Stool culture again has a higher yield for *B. hominis* than stool microscopy.

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