Short Communication

Irritable bowel syndrome in Upper Egypt: The role of intestinal parasites and evidence of Th2 response

Soha Saoud Abdelmoneim a,*, Lamia Abdelaziz Galal b, Amani Osama c, Nadia Abdel-Salam a

a Tropical Medicine and Gastroenterology Department, Faculty of Medicine, Assiut University, Assiut, Egypt
b Department of Parasitology, Faculty of Medicine, Assiut University, Assiut, Egypt
c Department of Biochemistry, Faculty of Medicine, Assiut University, Assiut, Egypt

A R T I C L E   I N F O

Article history:
Received 7 November 2009
Accepted 11 April 2010

Keywords:
Irritable bowel syndrome
Parasitic infection
Th2 immune response

A B S T R A C T

Background and study aims: The pathophysiology of irritable bowel syndrome (IBS) remains elusive. In countries where enteric parasitic infection is common, its role in the development of IBS is controversial. Parasites induce the Th2 immune response that elaborates cytokines such as interleukin (IL)-5, which causes eosinophilia. Eosinophilic cationic protein (ECP) is one of the mediators released during the activation of eosinophils. This study aims to determine the relationship between symptoms suggestive of IBS and parasitic infection in IBS patients and to evaluate the serum levels of IL-5, ECP and eosinophilic count as potentially useful serological tests in those patients.

Patients and methods: Thirty-five IBS patients fulfilling Rome II criteria with absence of intestinal helminthic infection by direct smear method and no history of associated allergic conditions were studied. Ten healthy controls were included. Microscopic examination of stools for intestinal parasites, eosinophilic count and erythrocyte sedimentation rate were done. Colonoscopy was performed to rule out inflammatory bowel changes. Serum levels of IL-5 and ECP were measured.

Results: Intestinal parasitic infection was present in 37% (13/35) of IBS patients vs. 20% (2/10) in controls. Of the 35 IBS patients, 13 (37%) had protozoal infection. Mean eosinophilic count, IL-5 level and ECP were significantly high in IBS patients than in controls. Eosinophilic count and ECP serum level were significantly high in IBS patients with parasitic infection.

Conclusion: A significant number of patients with symptoms suggestive of IBS demonstrated evidence of parasitic infection in their stool samples. The IL-5 serum level, eosinophilic count and ECP serum level might be useful tests for detecting parasitic infection aetiology in IBS patients after exclusion of conditions inducing the Th2 response. Larger case-controlled studies are required to clearly define the parasitic pathophysiology in IBS.

Introduction

Irritable bowel syndrome (IBS) is a chronic disorder of the gastrointestinal tract classically manifesting a symptom complex of abdominal pain associated with constipation or diarrhoea, or both [1]. Although IBS has received less attention in non-Western countries, the prevalence of IBS in some developing countries is in the 35–43% range [2,3]. The pathophysiology of IBS remains elusive. More recent studies have identified chronic immune activation in IBS patients [4]. Indeed, in countries where enteric parasitic infection is common, there is a controversy regarding the role of parasites in the development of IBS, as eradication of the parasite may not improve IBS symptoms [5]. Intestinal parasitosis is known to cause IBS-like symptoms that can be continuous, intermittent, sporadic or recurrent. Recent studies have also described a possible role of protozoan parasites such as Blastocystis hominis, Dientamoeba fragilis, Giardia lamblia and Entamoeba histolytica in the aetiology of IBS [6]. It has been shown that strong Th2 cytokine response occurs especially during the chronic phase of helminthes infection with production of high levels of interleukin-4 (IL-4), IL-5 and IL-13, accompanied by eosinophilia and abundant immunoglobulin E (IgE) production [7]. IL-5 is the most important cytokine involved in the transformation and development of eosinophils and acts as an ‘eosinophil activator’. Parasitic diseases are considered as significantly contributing to the increase in the eosinophilic count in the blood [8,9]. Eosinophilic cationic protein (ECP) is one of the mediators released during eosinophilic activation, and it has been shown that its serum level is considered to be valuable in the diagnosis of various eosinophil-mediated tissue inflammatory diseases [10]. This study investigates the prevalence of parasitic infection in patients diagnosed as IBS in our locality and explores
its relationship with IBS symptoms and evaluates the serum levels of IL-5, ECP and eosinophilic count in those patients as potential biological markers for parasitic infection aetiology.

**Patients and methods**

Forty-five patients previously diagnosed as IBS patients according to the Rome II criteria [11] were recruited from the outpatient clinic of Tropical Medicine and Gastroenterology Department at Assiut University Hospital from April 2008 until June 2008. Ten patients were excluded, six refused colonoscopy and in four patients colonoscopy revealed intestinal inflammatory changes. Finally, 35 patients were included. Ten control subjects were recruited among healthy students and volunteers. None of controls included in the study had history of any gastrointestinal symptoms nor any other diseases in the past 2 months. All participants of the study gave informed consent. The ethics committee of the Medical Faculty, Assiut University, approved the study.

Patients provided medical history of IBS fulfilling Rome II criteria (for easier construction of questionnaire compared to Rome III criteria) for at least 12 weeks or more (which did not need to be consecutive) in the preceding 12 months of abdominal discomfort or pain that has two of three features: Relieved with defaecation; and/or the onset associated with change in frequency of stool; and/or the onset associated with the change in the form of stool. Exclusion criteria included IBS patients with red flags, age over 50 years, history of allergy to certain food elements, previous parasitic infection and the presence of chronic diseases such as diabetes mellitus, arterial hypertension and chronic inflammatory bowel disease.

Patients underwent physical examination and laboratory evaluation, which were done in the morning session for all patients and included complete blood count including eosinophilic count and erythrocyte sedimentation rate and stool analysis. Patients were also prepared for routine colonoscopy. Ten millilitres of whole blood were collected from an antecubital vein without stasis and the serum was separated after centrifugation of the sample at 1000–1300 g for 10 min, and stored at −20 °C until the analysis of serum levels of IL-5 and ECP was completed.

**IL-5 assay**

Serum IL-5 assay was done using an enzyme-linked immunosorbent assay (ELISA) kit for quantitative detection of human IL-5 in human serum (Bender Medsystems, a member of Mercure Group GmbH Campus, Vienna Biocenter 2A 1030, Vienna, Austria (cat. No. BMS278)).

**ECP assay**

Serum samples for ECP assay were collected from all patients and controls in the morning and at room temperature. The assay is done using chemiluminescent immunometric method following the manufacturer instructions (Diagnostic Products Corporation, 5210 Pacific Concourse Drive, Los Angles, CA, USA).

**Stool analysis**

One fresh stool sample was collected from each patient in a clean dry container and was referred to the Parasitology Department, Faculty of Medicine, Assiut University. All stool specimens were processed by adding one portion of stool to three portions of sodium acetate/acetic acid formalin (SAF) and mixed thoroughly thereafter. The SAF fixative preserves trophozoites, cysts, eggs and larvae. It is used for direct examination, concentration procedure and permanent staining procedures [12]. One part of the faecal sample was added to three parts of the fixative and mixed thoroughly. Stool samples were subjected to the following tests:

1. **Wet mount examination:**
   - (a) Saline mount.
   - (b) Lacto-phenol cotton blue (LPCB) mount: LPCB wet smear was prepared by mixing a drop of SAF-preserved stool on a glass slide and covered by a cover slip [13,14].

2. **Concentration procedure** [15]: Concentration by formal ethyl acetate technique was conducted using the SAF-preserved stool.

The wet mount preparations and the sediment were examined using low (×10) power, high (×40) power lens and oil immersion lens if needed.

**Statistical analysis**

Statistical analysis was performed using SPSS version 10 for PC. Continuous data were summarised using the mean (±SE). Ordinal and categorical data were summarised using ratios. Group differences were assessed using independent sample Student's t-test. Correlations between clinical and laboratory data were done using Spearman's R test. The p value of <0.05 was considered to be statistically significant.

**Results**

**IBS group**

This group was composed of 35 IBS patients (20 females and 15 males) with a mean age of 37.1 (±1.6) years. The demographics of the study groups are summarised in Table 1. Colonoscopy showed normal colonic mucosa. Presence of parasitic infection was more frequently associated with diarrhoea-predominant IBS (IBS-D) 52.9% (9/17) vs. 22.2% (4/18) in constipation-predominant IBS (IBS-C). The frequency of IBS-related symptoms in infected IBS patients is shown in Table 2.

**Stool microscopy in the IBS group**

Intestinal parasitic infection was present in 37% (13/35) of IBS patients. Microscopic stool examination by different methods re-

### Table 1

Demographics of study groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (±SE), frequency (%) in IBS patients n = 35</th>
<th>Mean (±SE), frequency (%) in controls n = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37.1 (±1.6) 20 (57.1%) 15 (42.9%)</td>
<td>42.1 (±3.4) 7/3 (0%)</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>20/15</td>
<td>7/3</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>35 (100%) 0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>17 (48.6%) 0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>18 (51.4%) 0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Urgency</td>
<td>9 (25.7%) 0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Straining</td>
<td>18 (51.4%) 0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Incomplete evacuation</td>
<td>27 (77.1%) 0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Stool form</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumpy</td>
<td>5 (14.3%)</td>
<td>Normal</td>
</tr>
<tr>
<td>Hard</td>
<td>14 (40%)</td>
<td></td>
</tr>
<tr>
<td>Loose</td>
<td>10 (28.6%)</td>
<td></td>
</tr>
<tr>
<td>Watery</td>
<td>6 (17.1%)</td>
<td></td>
</tr>
<tr>
<td>Protozoal infection</td>
<td>13 (37%)</td>
<td>2 (20%)</td>
</tr>
</tbody>
</table>
revealed that one (2.8%) IBS patients was positive for the Entamoeba coli cyst, *G. lamblia* cysts in three (8.6%), two (5.7%) patients were positive for *E. histolytica* cysts and four (11.4%) were positive for *B. hominis* cyst (Fig. 1).

The control group

The control group included seven females and three males with a mean age 42.1 (±3.4) years. None of the controls included in the study had gastrointestinal symptoms or any other kind of illnesses in the past 2 months. The stool analysis revealed the *E. coli* cyst in two (20%) of them.

**Th2 response mediators in the study groups**

Mean eosinophilic count, IL-5 level and ECP were significantly higher in IBS patients than in controls (Table 3).

**Th2 response mediators in the IBS patients group**

Eosinophilic count and ECP serum level were significantly higher in IBS patients with parasitic intestinal infection than in those without infection (270.2 cells × 10⁹/l [±69.7] vs. 68.76 cells × 10⁹/l [±8.97]; 21.5 ng ml⁻¹ [±3.2] vs. 6.2 ng ml⁻¹ [±0.7], \( p \) values = 0.014, 0.000, respectively). The mean IL-5 level was higher in IBS patients with parasitic infection than in those without, but it did not reach statistical significance (22.9 pg ml⁻¹ [±7.7] vs. 16.8 pg ml⁻¹ [±5.7], \( p \) value = 0.533).

The mean IL-5, ECP serum levels and eosinophilic count were higher in IBS-D patients than in IBS-C patients (23 pg ml⁻¹ [±7.4] vs. 14.9 pg ml⁻¹ [±5.2]; 15.4 ng ml⁻¹ [±3.04] vs. 8.5 ng ml⁻¹ [±1.6]; 153.8 cells × 10⁹/l [±29] vs. 133.9 cells × 10⁹/l [±54]; \( p \) value = 0.382, 0.057, 0.748, respectively). The eosinophilic count and ECP mean serum level were significantly higher in IBS-D patients with parasitic infection than in those without infection (223.9 cells × 10⁹/l [±42.4] vs. 74.9 cells × 10⁹/l [±10.2]; 24.1 ng ml⁻¹ [±3.7] vs. 5.6 ng ml⁻¹ [±1], \( p \) value = 0.008, 0.001, respectively).

**Discussion**

Although enteric parasitic infection is common in the tropics, subtropics and developing countries, the role this infection may play in the pathogenesis of IBS has not been fully investigated. This study evaluated the relationship between the symptoms suggestive of IBS and parasitic infections, and evaluated the eosinophilic count and serum levels of IL-5 and ECP as potentially useful tests for diagnosis of parasitic infection aetiology in IBS patients.

Several studies [16–18] 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. 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 [6]. 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 (37%) compared to controls (20%). This result is consistent with Bujanda et al. [19] who showed that 49% of the patients with symptoms suggestive of gastrointestinal functional disorders were, or had previously been, diagnosed with parasitic infection.

In this study, intestinal protozoa detected in stool samples and associated with IBS-like symptoms included: *B. hominis*, *G. lamblia*, *E. histolytica* and *E. coli*. Several studies have implicated these parasites in the aetiology of IBS [20–23]. However, other studies [24–26] found no association between exposure to these parasites and predisposition to IBS symptoms. In our study group, *B. hominis* cyst was the most frequently detected protozoa as it was detected in six of 35 (17%) of IBS patients but none in controls. Others reported [27] that faecal carriage of *B. hominis* occurs more frequently in IBS patients (46%) than in the control group (7%). The role in IBS is likewise limited by the uncertainty surrounding its pathogenicity. However, symptoms that were attributed to infection with *B. hominis* are non-specific and IBS-like and include diarrhoea, abdominal pain, cramps or discomfort and nausea [28–33]. Furthermore, chronic excretion with persistent symptoms has been reported [31].

*G. lamblia* infection, especially chronic infection, can resemble IBS and must be excluded by diagnosis. Furthermore, evidence for post-infectious IBS secondary to low-grade inflammation was demonstrated by D’Anchino et al. [23]. In this study, *G. lamblia* cyst was detected in four (11.4%) of the 35 IBS patients; this was similar to the results of Grazioli et al. [34] *E. histolytica* cyst was detected in two stool samples (5.7%). Amoebiasis may form part of the differential diagnosis of patients with IBS, especially in those with an acute presentation of IBS symptoms. Early studies implicated amoebic dysentery in the development of IBS among British soldiers returning from Egypt [18,19]. On the contrary, several other studies from India have suggested that exposure to *E. histolytica* did not predispose patients to IBS symptoms [5,25,35]. *E. coli* cyst

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**Table 2**

IBS-related symptoms frequency in IBS patients with intestinal parasitic infection.

<table>
<thead>
<tr>
<th>IBS-related symptoms</th>
<th>Frequency (%)</th>
<th>( n = 13 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>13/13 (100%)</td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>9/13 (69%)</td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>4/13 (30.8%)</td>
<td></td>
</tr>
<tr>
<td>Urgency</td>
<td>5/13 (38.5%)</td>
<td></td>
</tr>
<tr>
<td>Mucous in stools</td>
<td>8/13 (61.5%)</td>
<td></td>
</tr>
<tr>
<td>Incomplete evacuation</td>
<td>4/13 (30.8%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3**

Th2 response mediators in the study group.

<table>
<thead>
<tr>
<th></th>
<th>IBS patients</th>
<th>Controls</th>
<th>( p )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-5 (pg ml⁻¹)</td>
<td>19.1 [±4.5]</td>
<td>2.2 [±0.41]</td>
<td>0.000</td>
</tr>
<tr>
<td>ECP (ng ml⁻¹)</td>
<td>11.9 [±1.8]</td>
<td>2.2 [±0.32]</td>
<td>0.000</td>
</tr>
<tr>
<td>Eosinophilic count</td>
<td>143.6 [±30.8]</td>
<td>6.7 [±2.9]</td>
<td>0.000</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Blastocystis hominis cyst in a stool sample of an IBS patient. The arrow is pointing to Blastocystis hominis cyst (×40) stained by LPCB.
was detected in the stool sample of one IBS patient and two of the healthy controls. Although *E. coli* is not thought to cause gastrointestinal disease, it has been reported to be associated with gastrointestinal symptoms such as abdominal pain and persistent diarrhoea [36,37]. It was also detected in the stool samples of an IBS patient [27].

Intestinal parasitic diseases are commonly accompanied with diarrhoea, abdominal pain, cramps or discomfort. This study revealed that abdominal pain and diarrhoea were the most frequent IBS-related symptoms present in IBS patients with intestinal parasitic infection. The relative prevalence of symptoms of abdominal pain, diarrhoea and constipation in *Blastoceystis* infection and IBS shows remarkable similarities [38,39]. In a study by Armitage et al. [40] patients with gastrointestinal symptoms and harbouring *B. hominis* were found to have high levels of serine protease, which is produced in their gastrointestinal tract and is capable of exciting neurons directly through the protease-activated receptor-2 (PAR2) pathway [41]. This study found that patients with IBS-D exhibited significantly higher protease levels in stool specimens, which were not found in patients diagnosed with diarrhoea from acute infectious causes. Furthermore, colonic biopsies from IBS patients were found to produce elevated levels of serine protease [42]. This may offer an explanation for the comorbidity of patients who experience pain in gastrointestinal illness in the absence of endoscopic findings [43].

Optimal laboratory detection of intestinal protozoa depends on several factors, including the number and type of specimens collected, the processing methods employed and the experience and training of laboratory staff involved in the identification of these organisms. Furthermore, some protozoa (*G. lamblia* and *D. fragilis*) have been shown to have highly variable and intermittent shedding [44]. Currently, there are no available tests for the diagnosis of IBS, and its diagnosis is based on a cluster of clinical symptoms (Rome II criteria). In this study, serum tests detecting Th2 immune response, IL-5, mean eosinophilic count and ECP were significantly higher in IBS patients than in controls. Furthermore, their values were higher in IBS patients with intestinal parasitic infection than in those without. Several studies have reported raised serum IL-5 and eosinophils in serum samples from patients with parasitic intestinal infection [45].

In conclusion, a significant number of patients with symptoms suggestive of IBS demonstrate evidence of parasitic infection and, therefore, meticulous examination of stool for eggs, cyst and parasites is recommended, especially in patients coming from endemic areas, to rule out the presence of parasitic infection as the predisposing agent of IBS when diarrhoea is the major manifestation of IBS. Tests to detect the evidence of Th2 immune response might have an additional value in the diagnosis of parasitic aetiology of IBS. However, more extensive, larger case-controlled studies are required to verify these results and clearly define the potential pathogenicity of intestinal parasites in IBS.

References


