

Intestinal Protozoa Infections among Patients with Ulcerative Colitis: Prevalence and Impact on Clinical Disease Course

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Key Words

Protozoa infections, intestinal · Ulcerative colitis · Enteropathogenic microorganisms

Abstract

Background: Epidemiological and microbiologic studies suggest that enteropathogenic microorganisms play a substantial role in the clinical initiation and relapses of inflammatory bowel disease. **Aim:** To explore the prevalence of intestinal protozoa in patients with ulcerative colitis (UC) and its impact on clinical disease course. **Methods:** A total of 215 patients with definitive diagnosis of UC were studied. Fresh feces samples taken from all UC patients were examined immediately using trichrome-staining methods. **Results:** A total of 103 female and 112 male UC patients were analyzed. The mean age at diagnosis was 30.5 ± 10.8 years. The prevalence of overall parasitic infections was 24% and distributed as follows: *Blastocystis hominis* in 22 patients (10%), *Endolimax nana* in 19 cases (9%), and *Entamoeba histolytica* in 11 cases (5%). A significantly increased frequency of protozoa infection was found in those patients with persistent activity and intermittent activity as compared to active than inactive group ($p = 1 \times 10^{-7}$, OR 13.05, 95% CI 4.28–42.56, and $p = 0.003$, OR 1.42–14.47, respectively). Interestingly, this association remained significant when we compared the persistent activity group versus intermittent activity group ($p = 0.003$, OR 2.97, 95% CI 1.35–6.59). Subgroup analysis showed no as-

sociation between protozoa infection (*E. histolytica*, *B. hominis*, and *E. nana*) and other clinical variables such as gender, extent of disease, extraintestinal complications, medical treatment and grade of disease activity. **Conclusion:** The prevalence of intestinal protozoa infections in Mexican UC patients was 24% and these microorganisms could be a contributing cause of persistent activity despite medical treatment in our population.

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Introduction

Intestinal parasitic infection is one of the major public health problems in developing countries. Approximately 3.5 billion people are infected by intestinal parasites and around 450 million persons are ill due to these infections [1].

In Mexico, the mean annual number of new ulcerative colitis (UC) cases increased from 28.8 in 1987–1996 to 76.1 in 1997–2006 ($p < 0.00008$). The incidence of new cases increased 2.6-fold comparing both time periods in a tertiary referral hospital [2].

The etiology of inflammatory bowel disease (IBD) is unknown. In addition to genetic and environmental factors, microorganisms have been discussed as possibly playing an important role. Recent reports in the literature do not suggest that a specific persistent infection causes

IBD, but indicate that enteric pathogens could cause initial onset of IBD and are associated with reactivation of quiescent disease. Despite their self-limited character, these infections initiate a cascade of inflammatory events leading to chronic, relapsing disease in a genetically susceptible host ('hit-and-run' hypothesis). Epidemiological and microbiologic studies suggest that enteropathogenic microorganisms play a substantial role in the clinical initiation and relapses of IBD. However, similar to traveler's diarrhea, the frequency of infections in the first manifestation and in relapses of IBD is probably understated due to problems in detecting enteric pathogens. Thus, for optimal medical treatment microbiologic screening is helpful in patients with flares of IBD.

Amebiasis, which affects nearly 500 million people in the world, is more prevalent in developing countries in particular [3]. It is difficult to distinguish IBD from colitis associated with ameba according to both the symptomatic and endoscopic appearance of the colon. It is not even possible to establish a differential diagnosis by means of microscopic examination. Sometimes IBD can coexist with amebiasis. This, of course, leads to confusion in the diagnosis and treatment of the disease [4].

Blastocystis hominis is the most common human intestinal protozoa worldwide. It may be identified during a workup for gastrointestinal symptoms, usually in stools. Case reports and series have suggested a pathogenic role of *B. hominis* in causing intestinal inflammation. Also some studies have suggested that IBD and irritable bowel syndrome (IBS) are associated with *B. hominis* infection. The investigators indicate that the stools of all patients presenting with IBD or IBS should be examined, and culture methods for *B. hominis* carried out. Invasion and mucosal inflammation of the intestine with *B. hominis* have been observed in studies of gnotobiotic guinea pigs. The transmission, pathogenicity, culture characteristics, taxonomy, life cycle, biochemistry and molecular biology of *B. hominis* remain unclear. More studies are necessary for this parasite.

The objective of the present study was to determine the prevalence of intestinal protozoa among Mexican UC patients and to evaluate its clinical impact on the clinical course of the disease.

Materials and Methods

At the Inflammatory Bowel Disease Clinic of the Instituto Nacional de Ciencias Médicas y Nutrición Hospital, a total of 215 patients with a definitive diagnosis of UC were included in the study and prospectively followed during the period from January

2007 to January 2009. The diagnosis of UC was made by the presence of the following criteria: a history of diarrhea or blood in stools, and macroscopic appearance by endoscopy and biopsy compatible with UC. Relevant clinical and demographic information in all UC patients was collected from medical records: gender; age at diagnosis; familial aggregation; smoking history; previous appendectomy; disease evolution; extension; extraintestinal manifestations; medical or surgical treatment, and clinical course of disease.

Disease activity was determined by the Truelove and Witts index. The clinical course of the disease was defined as: active, then inactive (first episode with activity and then long-term remission for more than 5 years); intermittent activity (≥ 2 relapses/year), and chronic continual activity (persistent activity despite medical conventional therapy) as previously described [5].

From the Internal Medicine Service a control group of 200 individuals without a diagnosis of IBD was recruited and evaluated, and matched by gender and age to the UC patients.

Collection of Stool Samples

Fresh feces samples taken from all UC patients and the control group were examined immediately using trichrome-staining methods. The trichrome-staining technique was employed for the stools preserved in polyvinyl alcohol (PVA). These PVA preserves were mainly used to detect cyst and trophozoite forms of intestinal protozoa.

Trichrome-Staining Method

The PVA fecal emulsion was strained through a filter into a 15-ml centrifuge tube. The tube was centrifuged at 2,500 rpm for 5 min and the supernatant was discarded. The center of the slide was coated with a thin layer of Mayer's albumin using a fine camel hair brush, a portion of the sediment was lifted and spread evenly on the albumin-coated part of the slide. The smear was stained without letting it dry. The slide was placed in tincture iodine for 1 min. After staining with tincture iodine, the slide was placed in 2 changes of 70% alcohol for 1 min each. The slide was placed in trichrome stain for 8 min and then placed in acid alcohol for 10 s. Then the slide was placed in 2 changes of xylene for 1 min each. The slides were mounted using Depex and examined under a 100 \times objective.

Fecal Examination

Microscopic examination for the occurrence of intestinal parasites was performed using the formalin-ethyl acetate concentration technique. The sediments were examined for intestinal protozoa, eggs and larvae of intestinal helminths under a light microscope.

Statistical Analysis

Descriptive statistics are expressed as mean and standard deviation (SD). Data were analyzed by Student's t test for numerical variables, and χ^2 and Fisher's exact tests for categorical variables. The p value was 2-tailed and <0.05 was considered statistically significant. Data analysis was performed with SSPS Version 15.0 for Windows.

Results

Demographic and Clinical Characteristics

A total of 103 female and 112 male UC patients were analyzed (table 1). The mean age at diagnosis was 30.5 ± 10.8 years. Most of the patients were residing in urban areas (79%), and a total of 146 (68%) patients were non-smokers and 69 (32%) patients were ex-smokers. A prior history of appendectomy was revealed in 13 cases (6%). The predominant clinical features of UC patients were: bloody diarrhea (92%); mucoid diarrhea (48%); abdominal pain (42%); rectal symptoms (35%); weight loss (28%), and fever (9%).

Prevalence of Intestinal Protozoa Infections

In this study, the prevalence of overall protozoa infections was 24.2% and distributed as shown in table 2. Subgroup analysis showed no association between protozoa infection (*Entamoeba histolytica*, *B. hominis*, and *Endolimax nana*) and other clinical variables such as gender, extent of disease, extraintestinal complications, medical treatment, and grade of disease activity. No cases with *Giardia lamblia* and mixed pathogenic intestinal protozoan infection were found in UC patients.

The overall prevalence of protozoa infection in the control group was 10% (20 cases) and distributed as follows: *B. hominis* in 10 (5%); *E. nana* in 5 (2.5%), and *E. histolytica* in 5 individuals (2.5%).

Clinical Course of Disease

Seventy-seven patients (36%) had intermittent activity and 61 (28%) had chronic continual activity. In 52 UC patients, protozoa infection was found and was distributed as follows: 29 cases had persistent activity; 18 patients had intermittent activity, and only 5 cases had inactive UC disease.

Most of the patients (90%) had clinical remission, and the clinical course of the disease (inactive disease) changed after medical treatment (metronidazole or nitazoxanide) against protozoa infections. No infection-specific relation was found.

Association between Protozoa Infection and Clinical Course of Disease

A significantly increased frequency of protozoa infection was found in those patients with persistent and intermittent disease activity as compared to the active, then inactive group ($p = 1 \times 10^{-7}$, OR 13.05, 95% CI 4.28–42.56, and $p = 0.003$, OR 1.42–14.47, respectively) as shown in table 3. Interestingly, this association remained

Table 1. Demographic and clinical characteristics of UC patients

Characteristic	Frequency
Age at diagnosis, years	30.5 ± 10.8
Female/male, n	103/112
Extent of disease, %	
Pancolitis	75
Left-sided colitis	19
Proctitis	6
Clinical course of disease, %	
Active then inactive	36
Intermittent activity	36
Persistent activity	28
Extraintestinal manifestations, %	
Arthropathy	31
Primary sclerosing cholangitis	7.5
Erythema nodosum	3
Sacroiliitis	2.8
Pyoderma gangrenosum	2.2
Anterior uveitis	1.0
Ankylosing spondylitis	0.5

Table 2. Frequency of protozoa infections in patients with UC

Protozoa	n
<i>Blastocystis hominis</i>	22 (10%)
<i>Endolimax nana</i>	19 (9%)
<i>Entamoeba histolytica</i>	11 (5%)

significant when we compared the persistent activity group versus the intermittent activity group ($p = 0.003$, OR 2.97, 95% CI 1.35–6.59).

Extent of Disease

The extent of disease was evaluated using total colonoscopy and biopsies were taken from different segments of the colon in all cases. The Montreal classification was used to define the extent of UC: 75% had pancolitis (E3); 19% had left-sided colitis (E2), and 6% had proctitis (E1).

Extraintestinal Manifestations

One hundred and three UC patients (48%) had extraintestinal manifestations that included: arthropathy (31%), primary sclerosing cholangitis (7.5%), erythema nodosum (3%), sacroiliitis (2.8%), pyoderma gangrenosum (2.2%), anterior uveitis (1.0%) and ankylosing spondylitis (0.5%).

Table 3. Association between clinical course of disease and protozoa infection

Clinical course of disease	Protozoa infection (n = 52)	No protozoa infection (n = 163)	p	OR (95% CI)
Active then inactive	5	72		
Intermittent activity*	18	59	0.003	4.39 (1.42–14.47)
Chronic continual activity*	29	32	1×10^{-7}	13.05 (4.28–42.56)

* Comparison to the active then inactive group was performed.

Medical Treatment

Most of the patients (93%) were taking sulfasalazine or 5-aminosalicylic acid; 39% used oral or systemic glucocorticosteroids; 20% were taking azathioprine, and 3% other treatments. Topical medication based on 5-aminosalicylic acid was used in all patients with proctitis (5%).

Discussion

This study found that the overall prevalence of intestinal protozoa in Mexican UC patients was 24% (*B. hominis* in 10%, *E. nana* in 9% and *E. histolytica* in 5%). To the best of our knowledge, no previous studies have been done in Latin American countries. Few studies have specifically explored the prevalence of amebiasis; however, no study has determined the prevalence of other protozoa infections in UC patients. Bayramicli et al. [6] explored the presence of amebiasis in 19 patients investigated with a preliminary diagnosis of UC and found *E. histolytica* in 69% of the cases. Süleymanlar et al. [7] found *E. histolytica* cysts and trophozoites in 22 (54%) of the patients. These values are higher than those we found. The reason for this is the fact that the incidence of *E. histolytica/Entamoeba dispar* has diminished worldwide, including Mexico. In another study, Prokopowicz et al. [8] reported 5 cases of amebiasis (4.85%) among 103 patients with UC and claimed that this rate was significant in the treatment of chronic UC patients. In our study, we found a low prevalence of amebiasis (5%) contrasting with other studies published 15 years ago in UC patients from Turkey and Singapore [6, 9]. These prevalence differences of amebiasis could be explained by changes in environmental factors such as high temperature and humidity, overuse of anti-parasitic drugs as well as lower immune resistance against the infection, and also to improvements in hygiene.

On the other hand, the prevalence of *B. hominis* and *E. nana* has not been evaluated in patients with IBD. Ac-

ording to data obtained from case reports and series, a pathogenic role of *B. hominis* in causing intestinal inflammation has been suggested [10]. In the present study, the prevalence of *B. hominis* and *E. nana* was 10 and 9%, respectively, in Mexican UC patients. Recently, a study reported that *Blastocystis* sp. subtype 3 was most common in IBD patients from Turkey, followed by *Blastocystis* sp. subtype 2. Identical subtypes of *Blastocystis* are found in patients with IBD and chronic diarrhea. These particular subtypes show low host specificity and are carried by humans and some farm animals [11].

An important finding is that those patients with persistent UC activity showed a statistical increase in protozoa infection compared to the active, then inactive group as well as intermittent activity group. Subgroup analysis showed no specific association with *E. histolytica*, *B. hominis*, and *E. nana*. These data suggest that stools from UC patients should be examined before planning optimization of medical treatment.

Evidence from epidemiological studies indicates an inverse correlation between the incidence of certain immune-mediated diseases, including IBD, and exposure to helminths. Helminth parasites are the classic inducers of Th2 responses. The Th2-polarized T-cell response driven by helminth infection has been linked to the attenuation of some damaging Th1-driven inflammatory responses, preventing some Th1-mediated autoimmune diseases in the host, including experimentally induced colitis. Helminth parasites (the porcine whipworm, *Trichuris suis*) have been tested in the treatment of IBD patients, resulting in clinical amelioration of the disease [12].

It is interesting to note that the predominant intestinal parasites in this cohort were intestinal protozoa while the occurrence of intestinal helminthes was not found. These results confirm the possible role of worm parasites, where helminth carriage has steadily declined, and there is increasing evidence to indicate that helminth carriage

might protect the host from immunological disorders. Most helminths stimulate the production of Th2 cytokines (IL-4, IL-5, IL-9, IL-13). This mechanism would explain the protective role of helminths in Th1-driven disorders such as intestinal inflammation [13].

Infectious gastroenteritis (IGE) is known to exacerbate previously diagnosed IBD. A recent study evaluated the IBD risk in subjects with an antecedent case of IGE. A total of 3,019 incident IBD cases and 11,646 matched controls were evaluated in a conditional logistic regression model. The results showed that after adjusting for potential confounders, an episode of IGE increased the risk of IBD (OR 1.40, 95% CI 1.19–1.66). The risk was slightly higher for Crohn's disease (CD) compared with UC. In addition, there was an approximate 5-fold increase in IBD risk for persons with a previous IBS diagnosis [14].

On the other hand, a multifunctional cellular and secreted barrier separates the microbial flora from host tissues. Altered function of this barrier remains a major largely unexplored pathway to IBD. Although there is evidence of barrier dysfunction in IBD, it remains unclear whether this is a primary contributor to disease or a consequence of mucosal inflammation. Recent evidence from animal models demonstrating that genetic defects restricted to the epithelium can initiate intestinal inflammation in the presence of normal underlying immunity has refocused attention on epithelial dysfunction in IBD [15].

In UC, epithelial leaks appear early due to micro-erosions resulting from upregulated epithelial apoptosis and in addition to a prominent increase in claudin-2. Th1 cytokine effects by IFN- γ in combination with TNF- α are important for epithelial damage in CD, while IL-13 is the key effector cytokine in UC stimulating apoptosis and upregulation of claudin-2 expression. Focal lesions caused by apoptotic epithelial cells contribute to barrier disturbance in IBD by their own conductivity and by confluence toward apoptotic foci or erosions. Another type of intestinal barrier defect can arise from α -hemolysin-harboring *Escherichia coli* strains among the physiological flora, which can gain pathologic relevance in combination with proinflammatory cytokines under inflammatory conditions. On the other hand, intestinal barrier impairment can also result from transcellular antigen translocation via an initial endocytotic uptake into early endosomes, and this is intensified by proinflammatory cytokines as IFN- γ and may thus play a relevant role in the onset of IBD. Taken together, barrier defects contribute to diarrhea by a leak flux mechanism

(e.g. in IBD) and can cause mucosal inflammation by luminal antigen uptake. Immune regulation of epithelial functions by cytokines may cause barrier dysfunction not only by tight junction impairments but also by apoptotic leaks, transcytotic mechanisms, and mucosal gross lesions [16].

The role of infection in the development of IBD is underscored by various clinical observations, such as the delayed age at onset, suggesting that childhood exposure to pathogens is essential, and the clinical improvement that follows decreasing bacterial intestinal load. Over the years, many a pathogen has been linked to the development and exacerbation of IBD, notably *Mycobacterium paratuberculosis*, *E. coli*, *Listeria monocytogenes* and *Chlamydia* as well as viruses such as measles, mumps, rubella, Epstein-Barr virus and cytomegalovirus [17].

Adherent-invasive *E. coli* (AIEC) pathovar has been identified in the intestinal mucosa of patients with CD. AIEC reference strain LF82 is able to adhere to intestinal epithelial cells, to invade epithelial cells via a mechanism involving actin polymerization and microtubules, and to survive and replicate within macrophages.

A study reported in ileal specimens that AIEC strains were found in 21.7% of CD chronic lesions versus in 6.2% of controls. In neo-terminal ileal specimens, AIEC strains were found in 36.4% of CD early lesions ($p = 0.034$ vs. controls) and 22.2% of healthy mucosa of CD patients. In colonic specimens, AIEC strains were found in 3.7% of CD patients, 0% of UC patients, and 1.9% of controls. These findings suggest that AIEC strains are associated specifically with ileal mucosa in CD [18].

In conclusion, the prevalence of protozoa intestinal infections in Mexican UC patients was 24% and these microorganisms could be a contributing cause of persistent activity despite optimal medical treatment in our population.

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