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Short Communication

Is the treatment of *Enterobius vermicularis* co-infection necessary to eradicate *Dientamoeba fragilis* infection?

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SUMMARY

Objectives: *Dientamoeba fragilis* is a pathogenic protozoan of the human gastrointestinal tract with a worldwide distribution, which has emerged as an important and misdiagnosed cause of chronic gastrointestinal illnesses such as diarrhea and 'irritable-bowel-like' gastrointestinal disease. Very little research has been conducted on the use of suitable antimicrobial compounds. Furthermore, higher rates of co-infection with *Enterobius vermicularis* have been described, suggesting that *E. vermicularis* could influence the treatment of *D. fragilis*-infected patients. To study this, the treatment of *E. vermicularis* and *D. fragilis* co-infected patients was evaluated.
Methods: Forty-nine patients with a *D. fragilis* infection, including 25 (51.0%) patients co-infected with *E. vermicularis*, were studied. All of them were treated with metronidazole. Patients with *E. vermicularis* co-infection and/or an *E. vermicularis*-positive case in the family were treated with mebendazole.
Results: Metronidazole treatment failure was significantly more frequent in patients with *E. vermicularis* co-infection and in patients with children in the family.
Conclusions: Co-infection with *E. vermicularis* may act as a factor favoring *D. fragilis* infection by preventing eradication measures. This suggests that both parasites should be treated simultaneously.

1. Introduction

Dientamoeba fragilis is a pathogenic protozoan of the human gastrointestinal tract with a worldwide distribution. It has emerged as an important and misdiagnosed cause of chronic gastrointestinal illnesses such as diarrhea and 'irritable-bowel-like' gastrointestinal disease.[1,2] Higher rates of co-infection with *Enterobius vermicularis* have been described in previous papers.[3,4] Despite the growing importance of this parasite, very little research has been conducted on the use of suitable antimicrobial compounds. Recently, a randomized trial was performed to evaluate the efficacy of metronidazole in children, with negative conclusions.[5] The influence of *E. vermicularis* and its treatment with regard to the usefulness of metronidazole was evaluated in this study.

2. Methods

A standard protocol for the screening of imported diseases is used routinely in all individuals who attend the study unit for the first time, regardless of race, sex, origin, and symptomatology. All individuals who were found to be infected by *D. fragilis* between January 2012 and January 2014, as well as their infected household contacts, were enrolled in this retrospective, descriptive study. This research is part of an overall project entitled "Usefulness of molecular diagnosis techniques in parasitology" validated and approved by the Ethics Committee of Clinical Investigation of Asturias (Spain).

Each patient's clinical history, including diarrhea within the preceding 3 months, nature of the diarrhea, abdominal pain, intensity of fever, nausea and/or vomiting, urticaria, anal pruritus,

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Table 1
Characteristics of patients who were cured and patients who were not cured

Parameter	Cured $n=43$	Not cured $n=6$	p-Value	OR (95% CI)
Demographic characteristics				
Sex (M/F)	22/21	4/2	0.395	NS
Age, years (Standard Deviation)	30 (21)	27 (17)	0.421	NS
Age under 14 years	15/28	3/3	0.656	NS
Children under 14 in family (yes/no)	26/17	6/0	0.065	1.231 (1.042–1.454)
E. vermicularis detection				
Co-infected by *E. vermicularis* (yes/no)	19/24	6/0	0.013	1.316 (1.056–33.29)
Relative with *E. vermicularis* (yes/no)	27/16	6/0	0.079	1.222 (1.041–1.435)
Asymptomatic (yes/no)	23/20	6/0	0.034	1.261 (1.047–1.518)

OR, odds ratio; CI, confidence interval; M, male; F, female; NS, not significant.

anorexia, and weight loss, was collected. Diarrhea was defined as at least three unformed or liquid stools per day for at least 3 days. Treatment history also included anti-parasitic drugs.

Blood tests and biochemical analyses, including liver enzyme levels, were performed for all patients. Eosinophilia was defined as $>0.5 \times 10^9$ eosinophils/l .

Three stool samples per patient were concentrated using a Copropack Extraction Kit C100 (Cromakit S.L., Spain), following the manufacturer's instructions. These were then stained with lugol and screened under a light microscope with a low magnification to detect helminth eggs, protozoa trophozoites, and cysts. An immunofluorescence test (MERIFLUOR *Cryptosporidium/Giardia* kit; Meridian Bioscience, USA) was performed using concentrated stool samples to detect *Cryptosporidium spp* and *Giardia lamblia*. Genome detection of *D. fragilis* as well as *Entamoeba histolytica* and *Entamoeba dispar* was performed in stool samples following previous extraction with a QIAamp DNA Stool Mini Kit (Qiagen, Netherlands), using two methods based on PCR, as described previously.[6,7]

A pinworm test was performed in stool samples of all patients with *D. fragilis*. Thus, pinworm eggs or a few adult worms that had adhered to a piece of transparent cellophane tape applied to the anal region on two consecutive mornings immediately after the infected person woke up and before any bowel movement or cleansing (bath or shower), were identified by examination under a microscope.

Parasitological controls were performed at 4 and 8 weeks after the end of treatment in all patients. Patients who failed to deliver one or more pieces of cellophane tape or stool samples, or who had received anti-parasitic treatment in the previous 6 months, or who were infected by other parasites different to *E. vermicularis* were excluded.

All patients were treated with metronidazole 500 mg/8 h (25–35 mg/kg/day in three doses in children). Patients with *E. vermicularis* co-infection and/or an *E. vermicularis*-positive case in the family were treated with mebendazole 100 mg/12 h for 3 days.

Qualitative variables were compared using the Chi-square test, or Fisher's exact test, when necessary. For quantitative variables, the Student t-test for non-paired variables or the Mann–Whitney U-test was used. Significance was set at $p < 0.05$. Multivariate analysis was performed using logistic regression (enter method) to identify variables that showed an independent association with treatment failure. The variables included in the model were those with a statistically significant association ($p < 0.05$) in the bivariate analysis. All tests were performed using SPSS for Windows version 15 software (SPSS Inc., Chicago, IL, USA).

3. Results

Forty-nine patients were studied and treated. Fifty-three percent of them were male. The mean age was 30 years (range 3–71 years). Eighteen patients were under 14 years of age. Most of them were from Spain (65.3%), followed by Equatorial Guinea (12.2%), Pakistan (10.2%), Colombia (8.2%), and Paraguay (4.1%). In immigrant patients, the average time of permanent residence in Spain prior to the first consultation was 853 ± 540 days and no patient had travelled to their country of origin in the last year. Twenty-nine patients (59.2%) were asymptomatic. For the remaining patients, the most frequent symptoms were abdominal pain (10 patients) and diarrhea (three patients). Only one patient described anal pruritus. Twenty-seven patients (55%) had hypereosinophilia in the blood and 17 of them were asymptomatic. The mean level of eosinophilia was $1.361 \pm 1.676 \times 10^9$ cells/l. Twenty-five (51%) patients had a co-infection with *E. vermicularis*, 64% of them being children under 14 years of age.

All were treated with metronidazole, with a cure rate of 87.8%. Thus, 43 patients had complete resolution of symptoms and a normalization of eosinophil levels. Nevertheless, six patients showed a persistence of *D. fragilis* in control stool samples taken 4 weeks after the treatment. No differences in sex or age between the patients who were cured and not cured were found. No significant differences were found after studying whether age < 14 years was associated with treatment failure (3 vs. 3, $p = 0.656$). Treatment failure was significantly more frequent in patients with *E. vermicularis* co-infection (6 vs. 0, odds ratio (OR) 1.316 (95% confidence interval (CI) 1.056–1.454), $p = 0.013$) and nearly significant in those with a child in the family (6 vs. 0, OR 1.231 (95% CI 1.042–1.454), $p = 0.065$). All patients with treatment failure were asymptomatic (Table 1).

A second treatment with paromomycin (25–35 mg/kg/day, usually divided into three doses, for 5–10 days) was administered to the six patients with no response to metronidazole. A parasitological control test was performed in all of these patients with negative results at 4 and 8 weeks after the end of treatment, indicating that all of them had responded to it.

4. Discussion

Dientamoeba fragilis is a trichomonad parasite, which has been described as a cause of gastrointestinal disease. Although several reports support the efficacy of metronidazole for the treatment of *D. fragilis* infection, others have reported treatment failures and relapses. Preiss et al. administered metronidazole (30 mg/kg/day for 10 days) to children and this was found to be effective in 70% of the cases. The remaining 30% of cases required up to three follow-up treatments for complete resolution of parasites and symptoms.[8] Vandenberg et al. reported parasitological and clinical cure in eight out of 12 patients, although no dosage information was given.[9] Although Stark et al. reported a similar cure rate to that found in the present study (80% of cases), a relatively high rate of treatment failures/relapses (21.4%) was associated with the use of metronidazole. The majority of treatment failures were associated with a 3-day course of metronidazole and these were less likely with a longer duration of therapy.[1]

A recent randomized trial involving children showed that although eradication of *D. fragilis* was significantly greater in a group of metronidazole-treated infected patients than in a group of placebo-treated infected patients, it declined rapidly from 2 weeks to 8 weeks after the end of treatment (62.5% and 24.9%, respectively). These data do not support the routine use of metronidazole for the treatment of *D. fragilis*-positive children with chronic gastrointestinal symptoms.[5] In this trial, a pinworm prevalence of 24% and a relative risk of 1.20 (95% CI 1.01–1.44) for post-treatment *D. fragilis* infection when the subject was pinworm-positive were reported. Higher pinworm prevalence (51%) and a similar relative risk were observed in the present study, where all patients with *E. vermicularis* infection were treated with mebendazole and the parasite was eradicated at 4 and 8 weeks after the end of treatment.

E. vermicularis has been associated with the transmission of *D. fragilis* due to the higher rates of co-infection described in several studies, as well as the isolation of *D. fragilis* DNA on the surface of *E. vermicularis* eggs.[3,4] The present results support the hypothesis that co-infection with *E. vermicularis* may act as a factor favoring *D. fragilis* infection by preventing eradication measures. This suggests that both parasites should be treated simultaneously. The fact that *E. vermicularis* is a common parasite in the child population explains why treatment failures are more common in this age group.

Further research is necessary to evaluate the role of the association of *D. fragilis* and *E. vermicularis* when choosing a treatment and whether the use of mebendazole improves the treatment of *D. fragilis* infection.

Ethical approval: This work was approved by the Ethics Committee of the Hospital Universitario Central de Asturias.

Conflict of interest: All authors state that there are no financial or personal relationships with other people or organizations that could inappropriately have influenced (biased) their work. This study was not funded by any source.

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