Protein-Losing Enteropathy Is Associated with Clostridium difficile Diarrhea but Not with Asymptomatic Colonization: A Prospective, Case-Control Study

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A prospective, case-control study was performed in which enteric protein loss and nutritional status were measured in patients with symptomatic and asymptomatic infections due to Clostridium difficile. Enteric protein loss, measured by elevated levels of fecal α1-antitrypsin, was detected in 14 of 20 cases and controls with diarrhea (9 of 10 cases with C. difficile--associated diarrhea and 5 of 10 age-matched controls with diarrhea not associated with C. difficile) compared with none of 20 asymptomatic cases and controls (10 colonized cases and 10 noncolonized controls without diarrhea who were matched by age and clinical diagnosis) (P < .0001). Cases and controls with diarrhea had higher prognostic nutritional index values (P = 0.005) and lower levels of serum albumin, transferrin, and cholesterol than did the asymptomatic cases and controls. Decreased nutritional status, measured by increased prognostic nutritional index values, was associated with the presence of diarrhea but not with the presence of C. difficile. Protein-losing enteropathy was associated with C. difficile only in the presence of diarrhea, and we did not detect an increased risk of protein-losing enteropathy or malnutrition as a consequence of asymptomatic colonization with C. difficile.

Clostridium difficile is the most commonly identified cause of antibiotic-associated diarrhea and pseudomembranous colitis [1, 2]. In a previous study of protein-losing enteropathy associated with C. difficile [3], elevated levels of fecal α1-antitrypsin were demonstrated in 12 of 12 hospital cases of pseudomembranous colitis and in six of 14 cases of C. difficile--associated diarrhea (CDAD). Levels of α1-antitrypsin were also elevated in the stools from six of 12 nursing home residents known to be asymptptomatically colonized with C. difficile (defined by formed stool that was culture positive and cytotoxin negative). Fifteen of 15 age-matched controls in the Baltimore Longitudinal Study on Aging had normal levels of fecal α1-antitrypsin [3].

Asymptomatic colonization with C. difficile is common in nursing home residents [3, 4] and hospitalized patients [5–7]. The loss of serum proteins into the stool of these individuals could potentially contribute to hypoalbuminemia [8] or hypercoagulability caused by loss of coagulation inhibitors [9], and concern has been raised that occult protein loss into the stool could potentially contribute to malnutrition in some elderly nursing home residents asymptomatically colonized with C. difficile [3, 10]. Therefore, we conducted a prospective, case-control study of fecal α1-antitrypsin levels in and the nutritional status of patients with CDAD and patients asymptomatically colonized with C. difficile.

Patients and Methods

Patient Groups

Four groups each with 10 hospitalized patients at the Minneapolis Veterans Affairs Medical Center were evaluated for protein-losing enteropathy and nutritional status between 1989 and 1992 after written informed consent was obtained. CDAD cases. Ten patients with CDAD were identified as CDAD cases by periodic review of the culture and cytotoxin assay records of the clinical microbiology laboratory. CDAD was defined as six or more unformed stools in a 36-hour period from a patient for whom both culture and cytotoxin assay of stool were positive for C. difficile and for whom no other cause for diarrhea was present. Endoscopy was not routinely performed.

Diarrhea controls. Each CDAD case was matched by age (within 5 years) to a control with diarrhea not associated with C. difficile. These controls were also identified from the records of the clinical microbiology laboratory, but their cultures and cytotoxin assays of stool were negative for C. difficile. Diarrhea was also defined as six or more unformed stools in a 36-hour period.
Asymptomatic cases.  Ten patients without diarrhea but for whom rectal swab cultures were positive for *C. difficile* were identified as asymptomatic cases by periodic surveillance of inpatients [11]. Surveys of rectal swab specimens from patients throughout the hospital were conducted periodically as an infection control measure, and specimens from all patients on one ward were cultured weekly as part of an epidemiologic study. Patients with a history of CDAD were excluded from this study population.

Asymptomatic controls. Each asymptomatic case was matched by age (within 5 years) and by primary clinical diagnosis to a control without diarrhea for whom a stool culture was negative for *C. difficile*. Potential controls for the asymptomatic cases were identified by review of the daily roster of inpatients.

Microbiological Studies

Stool and rectal swab specimens were inoculated onto agar plates containing cycloserine, cefoxitin, and fructose [12], and the plates were incubated anaerobically for 48 hours. *C. difficile* was identified by characteristic morphology and gram staining [13]. Typing by restriction endonuclease analysis (REA) was performed on all *C. difficile* isolates according to a previously described method [7].

A cytotoxin assay was performed on filtered supernatants of stool specimens (final dilution, 1:40 [7]) from the diarrhea cases and controls and on supernatants of cultures of *C. difficile* on chopped meat medium after incubation at 37°C for 96 hours. Supernatants were inoculated onto monolayers of HEp-2 cells, and specific cytopathic effects were confirmed by neutralization with *Clostridium sordellii* antitoxin [14].

Fecal α₁-antitrypsin Levels

A portion of the same stool specimen tested by culture and cytotoxin assay was saved for measurement of α₁-antitrypsin levels. Stools were kept frozen at −20°C until the time of assay. All specimens were assayed in native (wet) and lyophilized (dry) form.

For nonlyophilized specimens, 1.0 mL of liquid stool or 0.50 mL of formed stool plus 0.50 mL of PBS was added to microcentrifuge tubes (Eppendorf Flex-tubes, Brinkmann Instruments, Westbury, NY), mixed for 30 seconds, and centrifuged at 10,000g for 5 minutes. Five microliters of supernatant from each sample was placed in a well of a radial immunodiffusion plate (Hycor Biomedical, Garden Grove, CA). Plates were placed in a humid chamber at room temperature for 24 hours, and the diameters of the precipitin rings were measured (±0.1 mm) by means of a calibrated viewer (Kallestadt Laboratories, Chaska, MN). Ring diameters were converted to α₁-antitrypsin concentrations by interpolation from a standard curve supplied by the manufacturer (Hycor). Control sera supplied by the manufacturer were used to confirm the accuracy of the standard curve.

For lyophilized specimens, 5-mL aliquots of stool were frozen at −20°C until the time of assay and then freeze-dried for 72 hours. Fifty-milligram samples of powdered stool were mixed with 250 μL of PBS for 30 seconds and then were centrifuged at 16,000g for 10 minutes. Five-microliter samples of each supernatant were loaded into plates and processed as above.

The lowest detectable concentration of fecal α₁-antitrypsin was 1.4 mg/g of stool (dry weight) for lyophilized specimens. Other investigators have established normal ranges for fecal α₁-antitrypsin levels, with upper limits of normal (mean ± 2 SD) of 2.2 and 2.4 mg/g (dry weight) for lyophilized stool [15, 16] and 0.55 and 0.65 mg/g (wet weight) for nonlyophilized stool [3, 15]. The upper limits of normal used in this study were 2.4 mg/g for lyophilized stool and 0.65 mg/g for nonlyophilized stool.

Nutritional Status Assessment

Nutritional assessments of the patients were performed as soon as possible following results of the stool culture and cytotoxin assays. The mean time ± SD between submission of the stool specimen and nutritional assessment was 4.1 ± 2.1 days. To assess the nutritional status of patients, the following measurements were obtained: height, weight, absolute lymphocyte count, hemoglobin level, serum albumin level, serum cholesterol level, serum transferrin level, triceps skin fold thickness, and delayed hypersensitivity (DH) skin reaction to mumps virus and *Candida* antigens. One experienced dietitian performed the measurements of skin fold thickness on all patients. Three measurements of triceps skin fold thickness of each patient were obtained and averaged.

A nutritional index was also calculated for each patient from the above data. The prognostic nutritional index (PNI) has been used to predict postoperative complications relating to malnutrition and is a better index of nutrition than any single nutritional parameter [17]. The PNI value is defined by the following relation: PNI (%) = 158 − 16.6 (serum albumin level; g/dL) − 0.78 (triceps skin fold thickness; mm) − 0.2 (serum transferrin level; mg/dL) − 5.8 (DH skin reaction), where PNI is the risk of complications and the DH skin reaction is the maximal reactivity to either of the two antigens that was graded as 0 if nonreactive, 1 if <5 mm of reactivity, and 2 if >5 mm of reactivity. Higher PNI values indicate worse nutritional status.

Statistical Analysis

The proportion of patients with elevated levels of fecal α₁-antitrypsin was compared between groups by the χ² test or the two-tailed Fisher’s exact test if the expected value of any cell was less than five. Fecal α₁-antitrypsin levels were compared between groups with use of the Mann-Whitney U test. Mean values of the nutritional parameters in the four patient groups were compared by the Mann-Whitney U test or the Wilcoxon rank-sum test if the expected value of any cell was less than five.
Results

Characteristics of Patients and C. difficile Isolates

The mean age ± SD of the 40 patients was 68.1 ± 7.5 years, and all patients but one were male, reflecting the demographics of the medical facility. A marked heterogeneity of C. difficile strains isolated from the cases was documented; this heterogeneity reflected the epidemiology and endemic nature of C. difficile infections at this facility during the study period. There were 17 distinct REA types, representing 12 groups of related strains, among the 20 C. difficile isolates. Ten different REA types of C. difficile isolates each were recovered from CDAD cases and asymptomatic cases, respectively. All of the C. difficile strains associated with diarrhea produced cytotoxin in vitro, whereas six of the 10 asymptomatic cases were colonized with cytotoxin-producing strains.

Nine of the 10 CDAD cases and seven of the 10 diarrhea controls had received antibiotic therapy within 14 days of stool culture. Nine of the 10 CDAD episodes and eight of the 10 episodes of diarrhea not associated with C. difficile developed > 48 hours after admission. CDAD was present in one case at the time of admission, but he had been hospitalized 9 days previously, at which time he was also given antibiotic therapy. None of the cases or controls with diarrhea had chronic diarrhea syndromes, and stool specimens cultured and assayed for protein loss were obtained early in the course of diarrhea. The mean time ± SD from onset of diarrhea to specimen collection was 3.6 ± 4.8 days for the seven CDAD cases and 1.9 ± 1.9 days for the eight diarrhea controls for whom these data were known. None of the CDAD episodes were relapses.

Fecal α1-Antitrypsin Levels

None of the 20 asymptomatic cases and controls had elevated levels of fecal α1-antitrypsin regardless of the results of C. difficile cultures (figure 1). In contrast, 14 of the 20 diarrhea cases and controls had elevated levels of α1-antitrypsin in their lyophilized stools (P < .0001, χ² test, diarrhea cases and controls vs. asymptomatic cases and controls). Nine of the 10 lyophilized specimens (and six of the 10 nonlyophilized specimens) from the CDAD cases contained elevated levels of α1-antitrypsin. Five of the 10 lyophilized specimens (and four of the 10 nonlyophilized specimens) from the diarrhea controls had elevated levels of α1-antitrypsin (P = .14, Fisher’s exact test, CDAD cases vs. diarrhea controls). The median levels of fecal α1-antitrypsin for the CDAD cases and the diarrhea controls were 5.4 and 2.7 mg/g (dry weight), respectively (P = .049, Mann-Whitney U test).

Nutritional Assessment

Nutritional status was similar when both case groups were compared with their respective control groups, as reflected by the PNI value or individual nutritional parameters (with the exception of the absolute lymphocyte count) (table 1). The mean PNI value ± SE was also similar for all CDAD and asymptomatic cases compared with all diarrhea and asymptomatic controls (47.6 ± 4.1 vs. 46.9 ± 3.6, respectively, Student’s t-test). However, diarrhea cases and controls, regardless of their C. difficile culture results, had higher PNI values than did the asymptomatic cases and controls (54.4 ± 3.3 vs. 39.7 ± 3.7, P = .005, Student’s t-test). The diarrhea cases and controls also had lower levels of serum albumin, transferrin, and cholesterol than did the asymptomatic cases and controls (31.1 ± 0.1 vs. 35.5 ± 0.1 g/dL, P = .006, Student’s t-test; 193 ± 13 vs. 228 ± 10 mg/dL, P = .04, Student’s t-test; and 134 ± 11 vs. 176 ± 11 mg/dL, P = .012, Student’s t-test, respectively); these lower levels reflect a worse nutritional status in the diarrhea cases and controls.

In a separate analysis of only the patients who had measurable levels of fecal α1-antitrypsin, there was a poor correlation between the PNI value and the fecal α1-antitrypsin level (r = .135, regression analysis).

Discussion

In this study, diarrhea was associated with increased leakage of serum proteins into the gastrointestinal lumen (protein-losing
enteropathy); leakage was measured by fecal α₁-antitrypsin levels. Although elevated levels of fecal α₁-antitrypsin were found in both the diarrhea cases and controls, enteric protein loss may be more extensive in the presence of CDAD than in the presence of other types of nosocomial diarrhea. Most of the diarrhea episodes that were not associated with C. difficile were associated with antibiotics and were nosocomial. The association between acute diarrhea and elevated levels of fecal α₁-antitrypsin has been previously documented [3, 18].

In contrast to the findings of this study, elevated levels of fecal α₁-antitrypsin have been described in nursing home residents asymptptomatically colonized with C. difficile whose stool specimens did not contain detectable levels of cytotoxin [3]. It is possible that a strain of C. difficile causing subclinical mucosal disease in the intestine could have been present in that nursing home; however, no strain typing was performed. In the present study, there was no evidence of protein-losing enteropathy in our asymptomatic cases who were colonized with 10 different REA types of C. difficile strains, six of which were toxigenic in vitro. A wide diversity of C. difficile strains has been noted previously at this and other institutions [7, 19]. Although quantitative cultures and cytotoxin tests of stool specimens from asymptomatic cases in this study were not performed, the diversity of the C. difficile strains, both toxigenic and nontoxigenic, suggests that protein-losing enteropathy was uncommon in our patient population.

Another possible explanation for the different findings is that some asymptomatic nursing home patients in the previous study [3] had recent CDAD and were continuing to have some protein loss into the stool following CDAD, but persistence of organisms is not uncommon [20]. It is also possible that the nursing home patients had other systemic or enteric disease that accounted for the enteropathy. Another study [21] demonstrated a modest correlation between titers of C. difficile antigen (measured by counterimmunoelectrophoresis) and α₁-antitrypsin content in stools from healthy infants. In our study, all 20 asymptomatic cases and controls (who were hospitalized elderly patients with formed stools) had normal levels of fecal α₁-antitrypsin.

Currently, there is no convincing evidence that asymptomatic colonization with C. difficile is harmful to the individual. Colonization is usually transient, and treatment is not recommended [22]. Compared with other hospitalized patients exposed to antimicrobial therapy, asymptomatically colonized patients are not at increased risk of C. difficile disease, although they may be reservoir hosts and serve as sources from which other patients can be infected with C. difficile [7]. Asymptomatic cases were followed prospectively and did not have diarrhea despite subsequent exposure to antimicrobial therapy—even those cases colonized with a strain responsible for epidemic diarrhea in other patients [7]. Treatment with vancomycin can temporarily eliminate C. difficile excretion, but this therapy may promote recolonization with the original or a new strain of C. difficile [22, 23].

In this study, radial immunodiffusion was used to screen for the presence of protein-losing enteropathy. This method has been shown to be a simple, sensitive, specific measure of the α₁-antitrypsin level in stools [16, 24, 25]; it is not significantly affected by freezing and thawing, long-term frozen storage, incubation at room temperature [16], or the presence of hematochezia [26, 27]. However, increased levels of fecal α₁-antitrypsin have been reported in cases of colonic disease with the simultaneous presence of elevated levels of serum α₁-antitrypsin [28]. Watery stools can mask abnormal protein leakage by dilution unless stools are lyophilized [16], hence the greater reliance on the results for lyophilized stools in our study. Two potential difficulties encountered in interpreting fecal α₁-antitrypsin

Table 1. Nutritional parameters for patients with symptomatic and asymptomatic C. difficile infections and for symptomatic and asymptomatic controls.

<table>
<thead>
<tr>
<th>Nutritional parameters</th>
<th>Study group</th>
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<tbody>
<tr>
<td></td>
<td>Patients with C. difficile-associated diarrhea (n = 10)</td>
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<tr>
<td>Prognostic nutritional index*</td>
<td>53.6 ± 5.8</td>
</tr>
<tr>
<td>Triceps skinfold thickness (mm)</td>
<td>10.6 ± 2.1</td>
</tr>
<tr>
<td>Delayed hypersensitivity†</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.2 ± 0.2</td>
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<tr>
<td>Transferrin (mg/dL)</td>
<td>195 ± 18</td>
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<tr>
<td>Absolute lymphocyte count (mm³)†</td>
<td>1,600 ± 200</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.2 ± 0.5</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>122 ± 15</td>
</tr>
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</table>

NOTE. All values are expressed as mean ± SE.
* Values refer to risk of complications and are given as a percentage (see Methods for further details).
† Arbitrary units from 0 to 2 (see Methods for further details).
‡ P = .036 for patients with C. difficile–associated diarrhea vs. controls with diarrhea by Student’s t-test.
trypsin levels are the definition of a normal range and the relative insensitivity of the assay. The previously reported normal ranges are based on rather small sample sizes and can differ depending on the assay used [16]. The assay used in this study was designed to measure α₁-antitrypsin levels in serum, which are ~10 times higher than those in stool. Therefore, it was important to prospectively define the normal and abnormal range for the fecal α₁-antitrypsin level for the assay used. Additional studies with larger sample sizes and a more sensitive assay would be helpful to better delineate the normal levels of fecal α₁-antitrypsin.

A nutritional assessment, as measured by the PNI value, was included in this study to determine if protein loss into the stool was associated with a decreased clinical nutritional status. Nutritional assessment of these patients was important because a previous report [3] suggested that decreased nutritional status in elderly nursing home residents may be due, in part, to fecal protein loss into the stool as a consequence of asymptomatic colonization with C. difficile. Although the optimal method for quantification of nutritional status is controversial, the PNI value was used in this study because it is objective, easy to use, and a better indicator of nutritional status than any single nutritional parameter used alone [17]. Measurements of DH skin reactions, in particular, may be difficult to interpret (and to compare between studies) because antigen preparations have not been standardized.

Nutritional status was significantly worse in diarrhea cases and controls than in asymptomatic cases and controls, but the presence or absence of C. difficile had no association with nutritional status in any of the cases or controls. Therefore, diarrhea was more predictive of decreased nutritional status than infection or colonization with C. difficile. However, even though decreased nutritional status was associated with diarrhea and diarrhea was associated with protein-losing enteropathy in this study, one cannot conclude that decreased nutritional status was related to protein-losing enteropathy. In fact, for those patients with a measurable level of fecal α₁-antitrypsin, there was a poor correlation between the PNI value and the fecal α₁-antitrypsin level, thus suggesting that the patients with diarrhea may have had a decreased nutritional status on the basis of a condition other than protein-losing enteropathy. Factors such as underlying clinical disease (e.g., infection and malignancy) and nutritional intake may have been more important determinants of nutritional status.

In conclusion, this study confirmed the association between protein-losing enteropathy and C. difficile only in the presence of diarrhea and demonstrated that diarrhea is more predictive of protein loss than is the presence of C. difficile; however, it did not document an increased risk of protein-losing enteropathy or malnutrition as a consequence of asymptomatic colonization with C. difficile. Studies in nonhospital settings, such as nursing homes and chronic care facilities, are needed to verify these findings for other populations; this prospective, case-control study of 40 patients in which objective measurements of nutritional status were used suggests that asymptomatic colonization with C. difficile in hospitalized patients is unlikely to be complicated by protein-losing enteropathy.

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References


