ORIGINAL ARTICLE

Toxin A and B frequency in mildly and moderately active inflammatory bowel disease patients

Hafif ve orta aktiviteli inflamatuvar barsak hastalarında toksin A/B sıklığı

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Background/aims: *Inflammatory bowel disease patients are at an increased risk for Clostridium difficile infection because of frequent hospitalizations and usage of immunosuppressive/immunomodulator drugs as well as antibiotics. The frequency of Clostridium difficile infection increases in parallel with the increase in disease activity. We aimed to evaluate the frequency of Clostridium difficile toxin A/B in patients with mildly and moderately active inflammatory bowel disease because a search on PubMed revealed a scarcity of literature knowledge in this regard.* ***Methods:*** *One hundred inflammatory bowel disease patients (48 females, 52 males) with mild and moderate activity were consecutively enrolled in the study; none of the patients had a history of hospitalization and/or antibiotic usage for the last three months. A stool sample was investigated for Clostridium difficile toxin A/B with enzyme immunoassay method by a microbiologist who was blinded to the study. Additionally, samples were evaluated for parasites and culture. Toxin A/B-positive and -negative cases were compared according to age, gender, disease type (Crohn's disease, ulcerative colitis), duration and location, extraintestinal findings, and any drugs used (azathioprine, salazopyrin, methotrexate, infliximab, adalimumab). Results were evaluated statistically.* ***Results:*** *Clostridium difficile toxin A/B positivity was found in only two patients (1 female, 1 male) (2%). In comparisons, we found no significant differences between the two groups. There was no growth in stool cultures and no parasite was found in stool samples.* ***Conclusions:*** *Clostridium difficile toxin A/B positivity in mildly and moderately active inflammatory bowel disease patients is the same as in the normal population and not higher as hypothesized.*

Key words: *Clostridium difficile, toxin A and B, inflammatory bowel disease*

Giriş ve Amaç: *İnflamatuvar barsak hastalığı olan hastalar sık hastaneye yatış ve immünsüpresif/immünmodülatör ilaç ve antibiyotik kullanımı nedeniyle Clostridium difficile enfeksiyonu açısından artmış riske sahiptir. Clostridium difficile enfeksiyonu sıklığı hastalık aktivitesiyle paralel olarak artar. Bu çalışmadaki amacımız hafif ve orta aktiviteli inflamatuvar barsak hastalarında toksin A/B sıklığını araştırmaktır.* ***Gereç ve Yöntem:*** *Son üç ayda hastaneye yatış ve/ya antibiyotik kullanım öyküsü olmayan 100 inflamatuvar barsak hastası (48 kadın, 52 erkek) çalışmaya alındı. Her hastadan bir adet dışkı örneği alınıp EIA metoduyla Clostridium difficile toksin A/B açısından çalışıldı. Ek olarak dışkı örnekleri parazit açısından incelendi ve örneklerin kültürü yapıldı. Toksin A/B açısından pozitif ve negatif olan hastalar yaş, cinsiyet, hastalık tipi (Crohn hastalığı, ülseratif kolit), lokalizasyonu ve süresi, ektsraintestinal bulgular ve kullanılan ilaçlar (azatioprin, salazoprin, metotreksat, infliximab ve adalimumab) açısından karşılaştırıldı.* ***Bulgular:*** *Clostridium difficile toksin A/B pozitifliği sadece iki hastada saptandı (1 erkek, 1 kadın). İki grup arasında karşılaştırılan parametreler açısından anlamlı bir fark saptanmadı. Dışkı örneklerinde parazit saptanmadı ve dışkı kültürlerinde herhangi bir üreme olmadı.* ***Sonuçlar:*** *Hafif ve orta aktiviteli inflamatuvar barsak hastalarında Clostridium difficile toksin A/B pozitifliği normal popülasyonla benzerdir ve sıklığı daha önce düşünüldüğü kadar yüksek değildir.*

Anahtar Kelimeler: *Clostridium difficile, toksin A ve B, inflamatuar barsak hastalığı*

INTRODUCTION

Bacillus difficilis (Clostridium difficile) was first described in the mid 1930s and found to be a part of the normal flora of neonates. Pseudomembranous colitis was first identified in the 1950s, but was initially attributed to either *Staphylococcus aureus or Candida albicans.* In 1974, a prospective study reported the development of diarrhea and pseudomembranous colitis in a series of patients treated with the antibiotic clindamycin. In 1977, a toxin produced by a *Clostridium* species was proposed as the cause of clindamycin-induced ileocecitis in hamsters,

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and finally in 1978, *Clostridium difficile* was identified as the causal agent of antibiotic-associated pseudomembranous colitis in humans (1).

C. difficile is a gram-positive, spore-forming anaerobic bacteria, produces several exotoxins including toxin A and B, and is associated with the development of a spectrum of clinical illnesses ranging from diarrhea to pseudomembranous colitis, toxic megacolon, sepsis, and death. The spores of *C. difficile* can be found in the environment, and the carrier rate of *C. difficile* was reported as 1–3% in adults (2). In addition to severe morbidity and mortality, *C. difficile*-associated disease (CDAD) increases the cost of medical care of patients with this infection.

The incidence of *C. difficile* has increased, doubling in the United States (US) and Canada over the past years, affecting up to 1.2% of hospitalized patients and causing life-threatening disease in 3.2% of those individuals who have been infected (3).

Historically, little overlap was identified between *C. difficile* infection and inflammatory bowel disease (IBD), and studies (4–10) performed two decades ago suggested that specific testing for this pathogen was not warranted in IBD patients experiencing colitis flare (5). More recently, *C. difficile* has been identified to exert a significant negative impact on patients with IBD, and is associated with increasing numbers of patients experiencing disease activity. Studies from single referral institutions as well as national trends in the US identified from the Nationwide Inpatient Sample (NIS) have demonstrated that IBD patients are at increased risk for the development of CDAD, and have increased rates of hospitalization, surgery and mortality as a result of this infection (11–13).

Historically, subgroups of patients are known to be at increased risk for the acquisition of CDAD (14). Patients recently treated with broad-spectrum antibiotics, hospitalized patients (15), oncology patients, and immunocompromised individuals as well as the elderly are believed to be at increased risk for CDAD. Patients with the two major forms of IBD, Crohn's disease and ulcerative colitis, share many of these same clinical risk factors for the development of CDAD. Many IBD patients are maintained on long-term immunosuppression, frequently require antibiotic use for their treatment, and are often hospitalized.

More recent reports have suggested that up to 20% of IBD flare was associated with a positive stool analysis for *C. difficile* (16). Given that the ability to clear infection is dependent on the generation of an immune response against *C. difficile* toxin A, IBD patients are a particularly high-risk subgroup (17).

Because the IBD flares are routinely treated with high-dose corticosteroids, an effective antibody response against *C. difficile* might be compromised, further worsening this infectious complication.

Immunomodulator use is reported as a risk factor for *C. difficile* infection in studies (11). *C. difficile* infection might present in a form that can mimic IBD activation and this might contribute to a delay in diagnosis, thereby increasing morbidity and mortality.

Disease activity is important for IBDs because clinical deterioration parallels the increased presence of *C. difficile* infection. There are many grading scales concerning disease activity in IBDs. The most frequently used grading scale for disease activity of Crohn's patients is Crohn's disease activity index (CDAI). Scores <150 indicate a patient in remission, while a score >450 indicates a severely active stage of the disease. The mostly frequently used grading scale for disease activity of ulcerative colitis patients is Truelove-Witts severity grading scale, which assesses fever (no fever, mean evening temperature >37.5°C or at least 2 days out of 4), diarrhea (<5/d, 5-10/d, >10/d), sedimentation rate (<30 mm/h or >30 mm/h), tachycardia (no tachycardia or >90/minute), and anemia (not severe or Hb <10.5 g/dl). Patients with a stool frequency <5, temperature <37.5°C, pulse <90/per minute, Hb >10 g/dl, and sedimentation rate <30 mm/h are accepted as having mild disease activity.

The association of CDAD and hospitalization, antibiotic and immunosuppressive use, and bowel resection (partial or total) has been studied by several researchers in IBD, but our review of the English and Turkish literature in PubMed did not reveal any clear data about the incidence of *C. difficile* infection in patients with IBD who have no history of hospitalization and/or antibiotic use in the last three months.

In the present study, we aimed to investigate the prevalence of *C. difficile* toxin A/B in patients with mild and moderately active IBD who had no history of hospitalization and/or antibiotic usage for three months, and we also investigated the association of this prevalence with age, gender, disease duration, and immunomodulatory agents.

MATERIALS AND METHODS

This study was designed as a prospective observational

cohort study. The study protocol was approved by the local ethics board. All patients were informed about the study protocol and written consent was obtained from all patients.

Patients with IBD seen in our outpatient clinic between 1 April 2007 and 31 March 2008 were consecutively enrolled in the study if they had no history of hospitalization and/or antibiotic usage in the last three months. The demographic features of patients (age, gender, IBD type [ulcerative colitis, Crohn's disease]), disease duration, anatomic distribution of the disease, (isolated small intestine, any colon involvement), extraintestinal findings, and drugs (mesalazine, immunomodulatory drugs [azathioprine, salazopyrin, methotrexate] and anti-tumor necrosis factor [TNF] agents [infliximab, adalimumab]) were recorded.

There were 38 patients with Crohn's disease and 62 patients with ulcerative colitis. For determining the disease activity, we used CDAI for Crohn's patients and Truelove-Witts severity grading scale for ulcerative colitis patients. All patients with Crohn's disease had CDAI score <250 and patients with ulcerative colitis were determined to have mild or moderate activity.

One stool sample was obtained from each patient enrolled in the study and all samples were immediately sent to the laboratory. These stool samples were stored at -20°C in the laboratory until evaluated for *C. difficile* toxin A/B with enzyme immunoassay (EIA) method (Ridascreen *C difficile* toxin A/B, R-Biopharm; Germany) by a microbiologist who was blinded to the patients' data. Stool culture was carried out for *Salmonella spp, Shigella spp and Aeromonas spp.* in all samples, and samples were also evaluated in terms of parasites. Both toxin A/B-positive and -negative patients were compared regarding age, gender, IBD type (Crohn's disease, ulcerative colitis), disease duration, anatomic involvement of the disease (isolated small intestine, any colon involvement), extraintestinal involvement, and drugs (mesalazine, immunomodulatory drugs [azathioprine, salazopyrin, methotrexate], and anti-TNF agents [infliximab, adalimumab]).

Patients who tested positive for the *C. difficile* toxin A and/or toxin B stool enzyme-linked immunosorbent assay (ELISA) were considered infected if they presented with concomitant symptoms of colitis (i.e., diarrhea, increased stool frequency, rectal bleeding, cramping, and/or tenesmus).

ANOVA was used for continuous variables and the results were presented as mean ± standard deviation (mean±SD). Chi-square test (χ^2) was used for categorical variables and independent t-test was used for comparing two groups. Statistical evaluation of the obtained data was carried out using SPSS 15.0 for Windows (SPSS, Inc, Chicago, IL, USA). A p value <0.05 was approved as statistically significant.

RESULTS

Overall 100 patients (48 female, 52 male) were enrolled in the study. The demographic features of patients and disease characteristics are shown in Table 1.

We found *C. difficile* toxin A/B positivity in only two patients (1 male, 1 female) (2%). The male patient (62 years old) had a diagnosis of ulcerative pancolitis, while the female (50 years old) had colonic Crohn's disease. Disease duration was 6 and 7 years in the male and female patients, respectively. The female patient used mesalazine and azathioprine, while the male patient used only azathioprine. Both patients had steroid history because of IBD attacks. There was no gastrointestinal surgery in either patient with positive *C. difficile* toxin A/B result. No classic pseudomembranous or fibrinous exudates were seen in endoscopic and histologic evaluations, respectively.

Both *C. difficile* toxin A/B-positive and -negative patients were compared for age, gender, IBD type (Crohn's disea-

Tablo 1. Demographic features of patients and disease characteristics

	***C. difficile*-positive (n=2)**	***C. difficile*-negative (n=98)**	**P**
Mean age (year)	43	41	0.9
Gender (Female/Male)	1/1	47/51	0.9
IBD type (Crohn/UC)	1/1	37/61	0.7
Mean disease duration (year)	6	5	0.8
Colonic IBD involvement	2/2	81/98	0.5
Immunomodulatory use	2/2	46/98	0.1
Biologic therapy	0/2	12/98	0.6

IBD: Inflammatory bowel disease. UC: Ulcerative colitis.

se, ulcerative colitis), disease duration, anatomic distribution of the disease (isolated small intestine, any colon involvement), extraintestinal findings, and drugs (mesalazine, immunomodulatory drugs [azathioprine, salazopyrin, methotrexate], anti-TNF agents [infliximab, adalimumab]) (Table 1).

Stool samples in all patients were negative for *Salomella spp, Shigella spp and Aeromonas spp.* There was no parasite in any stool sample.

DISCUSSION

In the present study, we found the incidence of *C. difficile* toxin A/B in mildly and moderately active IBD patients as 2% in our tertiary reference center. Colonic distribution of the disease, use of immunomodulatory drugs in maintenance therapy and above average disease duration were the common features of patients who tested positive for *C. difficile* toxin A and B.

C. difficile is a gram-positive, spore-forming anaerobic bacteria that can colonize the large intestine, producing a range of clinical activity from asymptomatic carriage to severe fulminant colitis in humans (18,19). Studies reported from North America have described a 2–10-fold increase in *C. difficile* incidence in the last two decades (20,21).

Traditional risk factors, including advanced age (>65 years) and use of broad-spectrum antibiotics, must now include use of fluoroquinolone as a major risk factor (21). The immunomodulator use and presence of IBD colitis (either ulcerative or Crohn's colitis) are also shown as significant risk factors for the development of *C. difficile* infection (10). Other important clinical factors include host susceptibility, virulence of the *C. difficile* strain concerned and the nature and extent of antimicrobial exposure (22).

Patients with immunosuppression from chemotherapy or as a desired therapeutic goal in the setting of organ transplantation are known to be at increased risk for CDAD.

In a retrospective cohort study, it was shown that the use of maintenance immunomodulator therapy in IBD patients was significantly associated with *C. difficile* infection (10). Interestingly, in the same study, the authors concluded that the use of anti–TNF–antibody therapy did not correlate with increased risk for acquisition of CDAD in patients with IBD. These findings suggest that specific regimens of immunosuppression might carry differential risk for the acquisition of *C. difficile* infection and associated disease. They also concluded that colonic involvement with IBD was also significantly associated with *C. difficile* infection, and isolated small bowel Crohn's disease was found in a disproportionately lower number of patients. They did not propose any clear reason for IBD colitis predisposing to *C. difficile* infection. They hypothesized that the previously damaged colonic mucosa that was subject to chronic inflammation becomes more susceptible to infection with *C. difficile*.

In a study that evaluated *C. difficile* toxin A/B in patients with diarrhea, the incidence was reported as 34%, while the incidence in the control group was 4.3% (23), but in another study conducted in a tertiary reference center, the incidence of *C. difficile* toxin A/B was reported as 4.3% in patients with antibiotic-associated diarrhea (24).

In another study from Ireland, the incidence of toxigenic *C. difficile* infection was reported as 1% and 8.2% in healthy controls and IBD patients in remission, respectively (25).

In the present study, we determined the incidence of *C. difficile* toxin A/B as 2% in mildly and moderately active IBD patients. Interestingly, it is lower than the rate in healthy controls reported two decades ago. The possible reasons for this low incidence are: first, we studied the IBD patients with mild and moderate activity who had no history of hospitalization and/or antibiotic usage in the last three months, though some of them had used immunomodulatory or immunosuppressive drugs. Second, we used a different *C. difficile* toxin A/B kit from that used in the studies cited above, and in fact, Aygün et al. (24) reported a relatively low incidence of *C. difficile* toxin A/B in patients with diarrhea using the same kit. The third and possibly the most important reason is that we studied only one stool sample from each patient, whereas it was shown in a study that the detection rate of toxin A/B increases in parallel with the number of stool samples (10).

In conclusion, according to our results, the incidence of *C. difficile* toxin A/B in IBD patients with mild and moderate activity and with no history of hospitalization and/or antibiotic usage in the last three months was similar to that of the normal population; however, this need to be confirmed with further comprehensive studies.

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