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Original Article

Clinical and Microbiological Risk Factors for Severe *Clostridioides difficile* Infections

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중증 *Clostridioides difficile* 감염의 임상 및 미생물학적 위험인자

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ABSTRACT

Background: There has been a marked increase in the mortality rate associated with *Clostridioides difficile* infection (CDI) globally since 2003, with the emergence of binary toxin-producing ribotype 027 strains. However, the molecular epidemiology of *C. difficile* shows regional differences and ribotype 027 is not common in Korea. In this study, the risk factors for severe CDI were evaluated, while considering the region-specific molecular epidemiology.

Methods: A retrospective case-control study was performed. Cases (n = 149) included patients with severe CDI or severe complicated CDI. Controls (n = 155) consisted of patients with non-severe CDI.

Results: Advanced age (odds ratio [OR] = 1.017, *P* = 0.0358), a history of chemotherapy (OR = 2.695, *P* = 0.0464), and ribotype 002 (OR = 3.406, *P* = 0.0231) were statistically significant factors associated with severe CDI in a multivariate analysis.

Conclusion: Ribotype 002 was found to be a significant risk factor for severe CDI in this study. Therefore, the surveillance of *C. difficile* ribotypes is recommended to monitor the spread of high-risk clones.

Keywords: *Clostridioides difficile,* Risk factor, Severe infection

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INTRODUCTION

Clostridioides difficile is a gram-positive anaerobe that causes infectious diarrhea that can range in severity from mild to severe [1]. The incidence and mortality rate of *C. difficile* infection (CDI) has also increased dramatically worldwide since 2003, and severe clinical conditions of CDI were reported in association with binary toxin-producing ribotype 027 strains [2,3]. However, molecular epidemiology is different according to regions and the ribotype 027 is not prevalent in Korea [4,5].

Advanced age, antibiotic use, gastric acid suppression, and infection with hypervirulent strain are well-known risk factors for recurrent CDI [6]. According to a recent large national cohort study, treatment with

certain antibiotics, proton pump inhibitors (PPIs), immune suppressants, and underlying disease were also important risk factors for the first CDI recurrence [7].

Although severe CDI can precede recurrence or treatment failure, the contexts were mixed [8]. Early prediction of severe CDI is essential so that adequate management can be applied to high-risk patients. However, the recent data is limited as far as we know. In this study, we evaluated risk factors for severe CDI, considering the region-specific molecular epidemiology.

MATERIALS AND METHODS

Study population and definition

All study populations had visited Ilsan Hospital or Gangnam Severance Hospital in 2019 and they had been diagnosed with CDI based on clinical and laboratory evidence (stool culture for *C. difficle* plus nucleic acid amplification tests for *C. difficile* toxin genes). The first infection case was only included to avoid duplication. A retrospective case-control study was performed. Cases (n = 149) included patients with severe CDI or severe complicated CDI. Controls (n = 155) consisted of patients with non-severe CDI.

The level of severity was classified as follows [9]: The severe CDI case was defined if they have a serum albumin level < 3.0 g/dL plus either a white blood cell (WBC) count > 15,000/mm^3 or abdominal tenderness. The severe complicated CDI case was defined if they were admitted to the intensive care unit with any one of the following attributes (hypotension, body temperature > 38.5 ℃, ileus or significant abdominal distension, mental changes, WBC > 35,000/mm^3 or < 2,000/mm^3, serum lactate levels > 2.2 mmol/L, and development of end-organ failure).

We obtained clinical features by reviewing the electronic medical records. Variables included age, sex, associated disease, history of antimicrobials within the previous 12 weeks, history of chemotherapy within the previous 12 weeks, history of PPIs within the previous 12 weeks, sites of acquisition, CDI treatments, history of CDI within the previous 12 weeks, recurrence after eight weeks, death, toxin types, and *C. difficile ribotype*. Community-associated cases were those cases that had occurred in the community without admission to a healthcare facility during the previous 12 weeks [10]. Others were regarded as hospital-associated cases.

Molecular study

The toxin production and molecular epidemiology were determined with polymerase chain reaction (PCR)-sequencing [4]. For toxin A and B genes, the primer pairs we used were tcdA-F and tcdA-R for tcdA, NK104 and NK105 for tcdB, cdtA-pos and cdtA-rev for cdtA, and cdtB-pos and cdtB-rev for cdtB [4]. PCR ribotyping with CD1-CD1445 primers was performed as previously described [4]. We visually compared PCR ribotyping patterns with known standards (VPI 10463, UK078, 48489ATCC9689, ATCC43598, and ATCC70057). Those ribotype patterns that differed by at least 1 band were assigned to different types. Multilocus sequence typing (MLST) was done, using a previously described scheme with a set of seven housekeeping genes (*adk, atpA, dxr, glyA, recA, soda,* and *tpi*) [11]. PCR of the seven loci and sequenced

amplicons was done with forward and reverse primers. DNA sequences were submitted to the MLST database (https://pubmlst.org/cdifficile/) to obtain the sequence type (ST).

Statistical analysis

Continuous variables were analyzed by the Mann-Whitney U test. The chi-squared test was used for the comparative analysis of categorical variables to determine independent risk factors. The odds ratio (OR) was calculated at 95% confidence interval (CI) values for binomial variables. Variables with *P* values of less than 0.1 in univariate analyses were included in a multivariate logistic regression analysis model to determine the independent risk factors. We defined the statistical significance as being $P < 0.05$. We used the SPSS 23.0 software (SPSS, Chicago, IL, USA) for univariate analyses and multivariate analyses. This study was approved by the Institutional Review Boards as required by the hospital policy (IRB No. NHIMC-2020-05-014).

RESULTS

Clinical features of patients with severe or severe complicated CDI

More frequent factors in severe or severe complicated CDI were advanced age, pneumonia, heart failure, previous use of penicillin, previous use of carbapenem, previous use of teicoplanin, crude mortality, ribotype 002, and ribotype 018. Whereas, previous use of narrow-spectrum cephalosporin and more frequent recovery were observed in non-severe CDI (Table 1).

Table 1. Comparison of severe (or severe complicated) CDI and non-severe CDI (to be continued)

Variable	Severe or severe complicated CDI (n = 149)	Non-severe CDI (n = 155)	*P*-value
Age (yr)	**74.0+15.0**	**65.9+18.3**	**< 0.0001**
Sex, male	70 (47.0)	70 (45.2)	0.7505
Hospital-associated	100 (67.1)	94 (60.7)	0.2411
Charlson comorbidity index	2.4+1.7	2.5+1.8	0.6365
Associated disease			
Biliary tract disease	5 (3.4)	7 (4.5)	0.6047
Cancer	32 (21.5)	47 (30.3)	0.0799
Pneumonia	**33 (22.2)**	**14 (9.0)**	**0.0021**
Heart failure	**10 (6.7)**	**2 (1.3)**	**0.0295**
Chronic respiratory disease	6 (4.0)	2 (1.3)	0.1574
Chronic renal disease	29 (19.5)	27 (17.4)	0.6460
Diabetes mellitus	28 (18.8)	20 (13.0)	0.1613
Cerebrovascular disease	12 (8.1)	17 (11.0)	0.3890
Alcohol disorder	1 (0.7)	1 (0.7)	0.9776
Atherosclerosis	3 (2.0)	4 (2.6)	0.7423
Esophageal disorder	1 (0.7)	0 (0.0)	0.9856
Nutrition deficiency	6 (4.0)	1 (0.7)	0.0859
Inflammatory bowel disease	1 (0.7)	5 (3.2)	0.1474
Gastric ulcer	2 (1.3)	1 (0.7)	0.5477
Liver cirrhosis	1 (0.7)	3 (1.9)	0.3556

Table 1. Comparison of severe (or severe complicated) CDI and non-severe CDI

Variable	Severe or severe complicated CDI (n = 149)	Non-severe CDI (n = 155)	*P*-value
History of antimicrobial use			
Any	137 (92.0)	135 (87.1)	0.1720
Penicillin	**39 (26.2)**	**24 (15.5)**	**0.0228**
Narrow-spectrum cephalosporin	**10 (6.7)**	**23 (14.8)**	**0.0262**
Extended-spectrum cephalosporin	40 (26.9)	48 (31.0)	0.4285
Inhibitor-combination	23 (15.4)	22 (14.2)	0.7604
Carbapenem	**36 (24.2)**	**21 (13.6)**	**0.0191**
Fluoroquinolone	37 (24.8)	27 (17.4)	0.1145
Teicoplanin	**19 (12.8)**	**8 (5.2)**	**0.0243**
History of PPI use	10 (6.7)	16 (10.3)	0.2638
History of chemotherapy	13 (8.7)	13 (8.4)	0.9162
Treatment			
Antimicrobial stop	78 (52.4)	80 (51.6)	0.8978
Metronidazole	26 (17.5)	17 (11.0)	0.1077
Vancomycin	54 (36.2)	44 (28.4)	0.1437
Prognosis			
Recovery	**118 (79.2)**	**145 (93.6)**	**0.0005**
Recurrence	6 (4.0)	10 (6.5)	0.3482
CDI-related mortality	1 (0.7)	1 (0.7)	0.9776
Crude mortality	**26 (17.5)**	**7 (4.5)**	**0.0007**
History of CDI	2 (1.3)	2 (1.3)	0.9683
C. difficile toxin			
A+B+CDT+	5 (3.4)	12 (7.7)	0.1057
B only	11 (7.4)	6 (3.9)	0.1901
A+B+CDT-	133 (89.3)	137 (88.4)	0.8091
C. difficile ribotype			
AB24 (ST129)	5 (3.4)	4 (2.6)	0.6919
AB25 (ST102)	4 (2.7)	7 (4.5)	0.3978
Ribotype 001	4 (2.7)	12 (7.7)	0.0590
Ribotype 002	**16 (10.7)**	**6 (3.9)**	**0.0265**
Ribotype 012	4 (2.7)	10 (6.5)	0.1290
Ribotype 014/020	20 (13.4)	28 (18.1)	0.2687
Ribotype 017	8 (5.4)	4 (2.6)	0.2219
Ribotype 018	**42 (28.2)**	**24 (15.5)**	**0.0080**
Ribotype 046	5 (3.4)	5 (3.2)	0.9494
Ribotype 070	2 (1.3)	5 (3.2)	0.2891
Ribotype 106	10 (6.7)	9 (5.8)	0.7448
Others*	29 (19.5)	41 (26.5)	0.1492

Data in number (%) except for age, Charlson comorbidity index, and laboratory findings, which were in mean + standard deviation.

Bold formatting indicates statistical significance.

*Other included AB11, AB16, AB21, AB27, AB28, AB37, AB43, AB46, AB47, AB48, AB67, AB68, AB76, AB79, AB84, AB89, AB90, AB91, C14, C29, C32, R005, R023, R027, R078, R103, R122, R126, R137, R159, R161, R163, R267, and R369.

Abbreviations: CDI, *C. difficile* infection; PPI, proton pump inhibitor; ST, sequence type.

The risk factors of severe or severe-complicated CDI

In univariate analysis, variables with *P* values of less than 0.1 were advanced age, cancer, pneumonia, heart failure, nutrition deficiency, previous use of antimicrobials (penicillin, narrow-spectrum cephalosporin, carbapenem, and teicoplanin), history of chemotherapy, recovery, crude mortality, specific ribotypes (ribotype 001, ribotype 002 and ribotype 018).

These variables were included in multivariate analysis. Advanced age (odds ratio [OR] = 1.017, *P* = 0.0358), history of chemotherapy (OR = 2.695, *P* = 0.0464), and the ribotype 002 (OR = 3.406, *P* = 0.0231) were statistically significant (Table 2).

Table 2. Risk factors for severe or severe complicated CDI over non-severe CDI: a multivariate analysis

Risk factor	OR (95% CI)	*P*-value*
Advanced age	1.017 (1.001-1.034)	0.0358
History of chemotherapy	2.695 (1.016-7.146)	0.0464
Ribotype 002	3.406 (1.183-9.803)	0.0231

*Statistical significances were maintained after the adjustment for age, associated disease (cancer, pneumonia, heart failure, nutrition deficiency), previous use of antimicrobials (penicillin, narrow-spectrum cephalosporin, carbapenem, teicoplanin), recovery, ribotype 001, ribotype 002, and ribotype 018.
Abbreviations: CDI, *C. difficile* infection; OR, odds ratio; CI, confidence interval.

DISCUSSION

Risk factors such as malignancy, chronic obstructive pulmonary disease, immunosuppression, antiperistaltic medications, renal failure, or clindamycin use were previously reported to be predictive of either intensive care unit admission or death in patients with CDI [12]. Others reported that the mortality was associated with variables such as low serum albumin, an abrupt decrease of serum albumin, use of more than three antibiotics, and persistence of positive cytotoxin in *C. difficile* colitis [13]. These early studies were performed in the late 1990s and focused on predictors of survival.

After the rise of hypervirulent strains, other definitions were used [2]. Briefly, they defined severe cases as being positive *C. difficile* cytotoxicity assay result or endoscopic (histopathological) evidence of pseudomembranous colitis. Complicated cases had one or more of the following: megacolon, perforation, colectomy, shock requiring vasopressor therapy, or death within 30 days following diagnosis. This approach seems to be more practical for clinicians to use in predicting which patients have a higher risk of severe CDI, many of whom do not respond to the recommended anti-CDI antibiotic therapy [14].

The ribotype 027 is a well-known risk factor for the severe or severe complicated CDI and this ribotype produces a binary toxin with a higher toxin level (16 to 23-times greater than do the wild-type strains) [15]. In Korea, ribotype 027 has been known as a minor major type [4,5] and only three *C. difficile* isolates were typed to ribotype 027 also in this study. Therefore, the hypervirulent strain with ribotype 027 can't play a big role in the severe clinical presentation of CDI in Korea.

The ribotype 002 was defined as a significant risk factor for severe CDI in this study. The *C. difficile* ribotype 002 has been reported as a major clone in Hong Kong [16]. Moreover, this clone was associated with a higher virulence of toxin production, sporulation, and germination rates [17]. *C. difficile* ribotype 002 showed increased mortality [18]. The *C. difficile* ribotype has been monitored as part of the South Korean national antimicrobial resistance surveillance system, Kor-GLASS [19].

This is the first report that the risk factors for severe CDI were evaluated, considering the region-specific molecular epidemiology. Considering the clinical importance of *C. difficile* ribotype 002, it is needed to monitor the spread of high-risk clones in Korea. In conclusion, we found advanced age, history of chemotherapy, and ribotype 002 as being significant risk factors for severe CDI.

요약

배경: *Clostridioides difficile* 감염(CDI)의 사망률은 binary toxin을 생성하는 ribotype 027 균주가 출현한 2003년 이후 전세계적으로 급격히 증가했다. 하지만 국내에서는 ribotype 027 *C. difficile*의 분리가 흔하지 않고 분자역학은 지역에 따라 다른 것으로 알려져 있다. 본 연구에서는 이를 고려하여 중증 CDI의 위험 인자를 평가하고자 한다.

방법: 후향적 환자-대조군 연구를 수행하였다. 환자(n = 149)에는 중증 CDI 또는 중증 복합 CDI 환자를 포함하였고, 대조군(n = 155)은 비감염 CDI 환자로 하였다.

결과: 다변량 분석에서 고령(odds ratio [OR] = 1.017, P = 0.0358), 항암화학요법력(OR = 2.695, P = 0.0464), 및 ribotype 002 (OR = 3.406, P = 0.0231)가 통계적으로 의미가 있었다.

결론: 본 연구에서는 ribotype 002가 중증 CDI의 중요한 위험 인자임을 알 수 있었다. 따라서 *C. difficile* ribotype의 감시를 통해 고위험 클론의 확산을 모니터링할 필요가 있겠다.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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