Do statins protect against the development of *Clostridium difficile*-associated diarrhoea?

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Objectives: To assess whether prior statin use protects against the development of *Clostridium difficile*-associated diarrhoea (CDAD) in hospitalized patients.

Patients and methods: A retrospective case–control study conducted in three hospitals included all hospitalized patients diagnosed with CDAD in the Internal Medicine Departments (IMDs) during a 1 year period. Subjects were determined to have CDAD if their stool sample was positive for *C. difficile* toxin in the context of diarrhoea at the time of diagnosis. Patients with CDAD were compared with patients without CDAD, hospitalized during the same period and in the same departments, matched for age, gender, comorbidities (Charlson score), length of hospitalization and antibiotic use during the last 3 months.

Results: Prevalence of CDAD was 2.87% (197/6850 patients hospitalized in the IMDs). The 197 cases with CDAD were compared with 169 hospitalized patient controls. Sixty-four out of 197 (32.5%) patients in the CDAD group were statin users versus 87/169 (51.5%) of the controls (P = 0.02). Multivariate analysis showed that a Charlson score >3 [OR = 2.2 (95% CI 1.8–2.8), P = 0.024], chemotherapy during the last 6 months [OR = 3.09 (95% CI 1.95–3.91), P = 0.002], a history of intra-abdominal surgery [OR = 2.99 (95% CI 2.58–3.24), P = 0.003] and no statin use [OR = 2.2 (95% CI 1.82–2.73), P = 0.034] were associated with CDAD.

Conclusions: Prior statin use may provide protection against CDAD. Further studies are warranted to evaluate this association.

Keywords: CDI, simvastatin, risk factors, predictors

Introduction

*Clostridium difficile*, a Gram-positive anaerobic spore-forming bacterium, is the most common cause of hospital-acquired infectious diarrhoea among adults in developed countries.¹⁻⁴ The incidence of *C. difficile*-associated diarrhoea (CDAD) is increasing in some countries and is associated with significant mortality, morbidity, increased length of hospital stay and healthcare cost.⁶⁻⁹ The clinical manifestation of CDAD ranges from an asymptomatic carrier state to life-threatening conditions (including toxic megacolon and colonic perforation) and death.¹⁰

The most common risk factor for developing CDAD identified in the literature is the use of antimicrobial agents.¹¹⁻¹³ Other risk factors have been described and include advanced age, hospitalization, severe comorbidities, exposure to cytotoxic chemotherapy, immunosuppressive therapy and the use of acid suppressive therapy, especially proton pump inhibitors (PPIs).¹⁴⁻¹⁶

Statins are 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors, which exert anti-inflammatory and other pleiotropic effects independent of their cholesterol-lowering actions.¹⁵⁻¹⁷ The association between statins and CDAD is still unclear. Medical literature investigating the impact of statins on CDAD is very scarce. Three previous studies presented conflicting results regarding the effect of statin therapy on the development of CDAD.¹⁸⁻²⁰ Against this background, we conducted a retrospective study to assess the association between statin use and CDAD.
Methods

Subjects and study design

A retrospective multicentre study was carried out to investigate whether prior statin use can reduce the risk of CDAD in adult hospitalized patients. The study included all consecutive patients with CDAD hospitalized from January 2011 to December 2011 in the Internal Medicine Department (IMD) and Infectious Diseases Unit, Holy Family Hospital, Nazareth, Israel; IMD B Ziv Medical Center, Safed, Israel; and Infectious Diseases Unit, Rabin Medical Center, Petah-Tiqva, Israel. The study was reviewed and approved by the local Ethics Committee of the Holy Family Hospital, Nazareth, the Ziv Medical Center, Safed, and the Rabin Medical Center, Petah-Tiqva, Israel. In these IMDS, general medical patients and elderly, cardiology, gastroenterology, infectious, respiratory and neurology patients are hospitalized. Excluded patients were <18 years old or lacked sufficient data for calculating the Charlson comorbidity index score. Data on demographic characteristics, underlying conditions, previous hospitalizations, abdominal surgery, prescribed chronic drug use and antibiotic therapy during the previous 90 days, and clinical and laboratory findings were collected and reviewed by three of the authors (W. N., W. K. and J. M.). Any covariate in the medical information or physical examination not noted as present was considered absent.

The control group included patients without CDAD who had been hospitalized during the same period and in the same departments. The controls (169 patients) were matched for age (+5 years), gender, comorbidity, length of hospitalization (+2 days) and antibiotic use during the last 3 months.

The majority of patients (>90%) who used statins were treated daily with simvastatin at 20–40 mg/day. The most common PPI was omeprazole at 20 mg/day. The study was reviewed and approved by the local Ethics Committee of the Holy Family Hospital, Nazareth, the Ziv Medical Center, Safed, and the Rabin Medical Center, Petah-Tiqva, Israel.

Definitions

Diarrhoea was defined as the passage of three or more unformed stools per day for at least 2 consecutive days. CDAD was defined as diarrhoea not attributed to any other cause and associated with at least one of the following positive tests: endoscopy revealing pseudomembranes or stool enzyme immunoassay for toxin A or B (TechLab®, Inc., Blacksburg, VA, USA).

Statin users were defined as individuals taking statins before and during hospitalization according to data obtained from the medical charts, in addition to a list of drugs supplied by the patients’ family physicians.

Statistics

Data were statistically analysed using WinSTAT® (Kalamia, Cambridge, MA, USA), the statistics add-in for Microsoft® Excel. Data are shown as means ± SD. For statistical analyses, the χ² test for categorical variables and Student’s t-test for continuous variables were used for testing differences between CDAD cases and controls. Spearman rank correlation and univariate regression analysis were used to determine the strength of the relationship between the different risk factors and CDAD. A risk factor associated with a P value of <0.05 in univariate analysis was then used for feature analysis. A multivariate analysis was performed to determine the association between the risk factors and CDAD. Statistical significance was set at 5%.

Results

Of 6850 hospitalized patients in IMDS, 197 met the CDAD diagnosis criteria. The prevalence of CDAD in IMD patients was 2.87% (197/6850) per year. No significant difference was found between the CDAD cases and controls in terms of age (65 ± 17 versus 62.5 ± 17, respectively; P = 0.98), gender (male 47% versus 51%, respectively; P = 0.51), Charlson score (2.62 ± 2 versus 2.15 ± 1.7, respectively; P = 0.08), antibiotic use during the last 3 months (51% versus 48%, respectively; P = 0.98) and length of hospitalization (8 ± 5.7 days versus 8.6 ± 5.9 days, respectively; P = 0.68) (Table 1). In addition, there were no significant differences between groups regarding types of previous antibiotics (data not shown). In contrast, a significant difference was found between CDAD cases and controls in terms of PPI use (36.5% versus 26.6%, respectively; P = 0.046), statin use (32.5% versus 51.5%, respectively; P = 0.02), nasogastric tube insertion (10% versus 4%, respectively; P = 0.03), history of intra-abdominal surgery (25% versus 10%, respectively; P < 0.001) and chemotherapy during the last 6 months (4.7% versus 0%, respectively; P = 0.004).

Table 2 shows the results of multivariate analysis of risk factors for CDAD after adjusting for the confounders of gender, length of hospitalization and antibiotic use during the last 3 months. The analysis showed that Charlson score >3, OR = 2.9 (95% CI 1.8–2.82), P = 0.024, chemotherapy during the last 6 months, OR = 3.09 (95% CI 1.95–3.91), P = 0.002, history of intra-abdominal surgery, OR = 2.99 (95% CI 2.58–3.24), P = 0.003 and no statin use, OR = 2.2 (95% CI 1.82–2.73), P = 0.034, were all significant and independent predictors of CDAD.

Discussion

In this multicentre, hospital-based cohort study, we found that patients who were on statin therapy had a lower risk of CDAD than patients who did not use statins. In addition, we found that a Charlson score of >3 points, past history of intra-abdominal surgery and post-chemotherapy during the last 6 months were significantly associated with CDAD. These results are in agreement with previous studies. In contrast, PPI use and nasogastric tube insertion in this study were not significantly associated with

Table 1. Demographic and clinical characteristics of patients with CDAD and matched controls

<table>
<thead>
<tr>
<th></th>
<th>CDAD group, n=197</th>
<th>Control group, n=169</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD</td>
<td>65 ± 17</td>
<td>62.5 ± 17</td>
<td>0.98</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>92 (47)</td>
<td>87 (51)</td>
<td>0.51</td>
</tr>
<tr>
<td>Charlson score, mean ± SD</td>
<td>2.62 ± 2</td>
<td>2.15 ± 1.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Antibiotic use during the last 3 months, n (%)</td>
<td>100 (51)</td>
<td>81 (48)</td>
<td>0.98</td>
</tr>
<tr>
<td>Length of hospitalization in days, mean ± SD</td>
<td>8 ± 5.7</td>
<td>8.6 ± 5.9</td>
<td>0.68</td>
</tr>
<tr>
<td>Statin use, n (%)</td>
<td>64 (32.5)</td>
<td>87 (51.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>PPI use, n (%)</td>
<td>72 (36.5)</td>
<td>45 (26.6)</td>
<td>0.046</td>
</tr>
<tr>
<td>Chemotherapy during the last 6 months, n (%)</td>
<td>9 (4.6)</td>
<td>0</td>
<td>0.004</td>
</tr>
<tr>
<td>Nasogastric tube, n (%)</td>
<td>20 (10)</td>
<td>7 (4)</td>
<td>0.03</td>
</tr>
<tr>
<td>History of intra-abdominal surgery, n (%)</td>
<td>49 (25)</td>
<td>17 (10)</td>
<td>&lt;0.001</td>
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</tbody>
</table>
CDAD, probably due to our small sample size, or perhaps due to some unseen confounding variable in our population.

Recently, Motzkus-Feaganus et al.\(^\text{18}\) in a large retrospective study demonstrated that statins may provide protection against *Clostridium difficile* infection. However, in their study, patients who received either metronidazole or oral vancomycin, according to the ICD-9-CM code, were considered incidental cases of *Clostridium difficile* infection.\(^\text{18}\) A similar possible protective effect was recently reported by Naggie et al.\(^\text{20}\) in a smaller study of community-acquired *Clostridium difficile* infection. In their study, however, there could still be more significant differences in health status between the cases and the controls than those observed in a hospital-based study. Because of their design, these studies often suffer from multiple unseen confounding variables, which are hard to adjust for.

In contrast, Lee et al.\(^\text{19}\) speculated that simvastatin may potentiate the *Clostridium difficile* toxin A cytotoxic effect by inhibiting interferon-γ-induced CD40 gene expression by suppressing STAT-1a.

The mechanisms by which statins might act as protective agents against the development of CDAD remain unknown. Statins have also been reported to have pleiotropic effects, such as immune-modulatory, antioxidative and anti-thrombotic actions, as well as antibacterial action.\(^\text{23–25}\) Statins as anti-inflammatory agents inhibit both acute and chronic inflammation by interfering with the endothelial adhesion molecules and trans-endothelial cell migration of polymorphonuclear leucocytes to sites of inflammation.\(^\text{26}\) In addition, statins reduce activation of the monocyte/macrophage system, and this suppression of activation results in many favourable effects on T-lymphocytes, including a reduction in their cytotoxicity.\(^\text{27}\) Statins inhibit Th-helper cell subclasses that promote inflammation (Th-1 subclass), simultaneously stimulating T-helper subclasses that promote anti-inflammatory effects (Th-2 subclass).\(^\text{28}\) Therefore, by these possible anti-inflammatory effects, statins can combat the inflammation processes induced by *Clostridium difficile* toxins.

In contrast, statins lead to apoptosis by inhibiting Rho, which has been characterized as a molecular switch involved in inflammation, cell cycle regulation and cytoskeletal processes.\(^\text{29}\) One of the classic Rho inhibitors is the *Clostridium difficile* toxin.\(^\text{30}\) Therefore, negative effects on the course of CDAD may occur when statins are used in patients exposed to other Rho inhibitors, such as the *Clostridium difficile* toxin.\(^\text{31}\)

Antibacterial action of statins is another mechanism that could explain the beneficial effect of statins in *Clostridium difficile* infections. Animal studies have shown that simvastatin inhibits the inflammatory response to *Staphylococcus aureus* α-toxin in rats.\(^\text{32}\) Liappis et al.\(^\text{33}\) found that the mortality rate of bacteraemia patients who were taking statins was substantially lower than that of those who were not. Recently, Masadeh et al.\(^\text{34}\) studied the antibacterial activity (MICs) of three statins against 18 different bacteria, demonstrating that statins, especially atorvastatin and simvastatin, had good antibacterial activity.

The antibacterial and anti-inflammatory effects of statins were investigated in a retrospective and meta-analysis, which suggested that statin use may be associated with better outcomes in the treatment and prevention of different infections.\(^\text{35–37}\) It is not clear whether statins act directly on *Clostridium difficile* as an antimicrobial agent or induce an indirect effect on the anti-inflammatory response produced by *Clostridium difficile* toxins.\(^\text{38}\) In addition, the development of CDAD is related to perturbations in the intestinal microbiota\(^\text{39}\) and has shown that Bacteroidetes and bifidobacteria play an important role in the mechanism of resistance to colonization by *Clostridium difficile*.\(^\text{40}\)

Previous studies have shown lower concentrations of Bacteroidetes in the intestines of CDAD patients\(^\text{40}\) and higher intensities of Firmicutes and Proteobacteria in patients with CDAD than controls.\(^\text{41}\) Whether statins have an impact on intestinal microbiota remains to be elucidated.

This study has limitations. First, the observed beneficial effects of statins can be further explained by a ‘healthy user effect’ (‘healthy user’ bias). Statin users tend to live at home, and engage in other positive health behaviours. Observational studies are often unable to adjust for these variables. However, the most important risk factors for *Clostridium difficile* infections are previous antibiotic therapy, comorbidities and risk of exogenous exposure to the organism.

In our study, patients from both groups were all hospitalized during the same period and in the same departments. In addition, patients in the statin group were more likely to be treated with antibiotics during the last 3 months and have a higher Charlson score, although they did not reach statistical significance. Second, the use of ELISA Tox A/B assays for case definition, which could underestimate the prevalence of the disease. Third, some of our cases were of community onset; therefore, their exposure to *Clostridium difficile* prior to admission was not controlled for.

In conclusion, in this cohort of hospitalized patients, statin use was associated with a lower risk of CDAD. Further randomized controlled studies are needed to assess whether the lower rates of *Clostridium difficile* infection observed in statin-treated patients are directly related to the drugs or whether statin use is just a marker of a lower-risk group of patients.

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**Transparency declarations**

None to declare.

**Author contributions**

W. N. and J. B.: study design, analysis and interpretation of data, drafting the article and revising the article critically for important intellectual content. J. M., M. M., W. K. and M. T.: acquisition of data, analysis and interpretation of data, and final approval of the article. R. F.: acquisition of data, analysis and interpretation of data, critical review and final approval of the article.

**References**


