PREVENTION OF RELAPSE FOLLOWING CLOSTRIDIUM DIFFICILE INFECTION USING PROBIOTIC LACTOBACILLUS CASEI SHIROTA

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ABSTRACT: Once patients have had Clostridium difficile infection (CDI), recurrence rates are high with many patients experiencing a relapse of their disease. Lactobacillus casei Shirota is a probiotic that reduces rates of antibiotic-associated diarrhoea. There have been no studies analysing the use of this probiotic in patients who have had an episode of CDI to prevent relapse. This study was a single site, cohort-control study of patients with CDI and treated with either antibiotics alone or antibiotics and probiotics (L. casei Shirota). 66 patients were included for analysis in this study, 31 had probiotics and antibiotics and 35 who had no-probiotics. The median age of the patients was 78 years and 33.3% were male. Rates of recurrent CDI were significantly lower in the probiotics cohort, 3.2% vs 20.0% (p=0.007). We conclude, patients who have had CDI have a high rate of early re-admissions to hospital with significant morbidity and mortality. This study suggests that the use of the widely available probiotic strain L. casei Shirota at the time of initial infection may be associated with lower rates of CDI recurrence and lower readmission rates. Further prospective studies are required.

KEY WORDS: Clostridium difficile, Colitis, Diarrhoea, Lactobacillus casei Shirota, Probiotics, Relapse

INTRODUCTION: Clostridium difficile (C. difficile) is an anaerobic Gram-positive spore-forming bacillus that proliferates when the normal microbiota of the gastrointestinal tract is disrupted by broad spectrum antibiotics. The incidence in the United Kingdom remains at 26.7 cases per 100,000, despite significant decreases in rates since 2009 (Public Health England, 2013). Once patients have had C. difficile infection (CDI), recurrence rates are high with up to 35% of patients having a relapse of their disease (Barbut et al., 2000). Patients have similar recurrence rates when treated with short courses of metronidazole (500mg BD) or vancomycin (125mg QDS), however the best strategy to reduce disease recurrence is to use a prolonged tapering dose of vancomycin (Nelson et al., 2011)2010. The costs of retreating patients with recurrence CDI is high, up to £11,000 per episode as these patients require prolonged hospital stays often in intensive care with large impact on quality of life (Ghantoji et al., 2010).

Bacterial therapies have been proposed to reconstitute the gut microbiota and allow competitive antagonism of C. difficile from other bacteria. These ‘healthy’ bacteria limit C. difficile proliferation via mechanisms such as occupation of gut adhesion sites, consumption of nutrient sources and the production of antimicrobial substances (Sekirov et al., 2010).

Probiotics contain a live strain or combinations of strains of bacteria that confer a health benefit to the host. Only three types of probiotics have been demonstrated to reduce the incidence of antibiotic-associated diarrhoea (Saccharomyces boulardii, Lactobacillus rhamnosus GG and a probiotic mixture) (McFarland et al., 2006)abstract;”CONTEXT: Antibiotic-associated diarrhea (AAD, with only the probiotic mixture showing efficacy in the primary prevention of CDI (Hickson et al., 2007). In terms of preventing recurrence of CDI, there is only evidence for the probiotic yeast strain S. boulardii. This probiotic has been shown in two double-blind randomized controlled trials to be an effective treatment, however, it requires a prolonged treatment period of 4 weeks following the initial episode of CDI (McFarland ., 1994).

Lactobacillus casei Shirota is a widely commercially available probiotic, marketed as the fermented milk drink Yakult. There has been one study analysing the use of this probiotic for the primary prevention of CDI. In this open-label study of 678 elderly hospital patients on antibiotics, half were concurrently given L. casei Shirota and half did not receive any probiotic.
The incidence of antibiotic-associated diarrhoea decreased from 18% to 5% and rates of CDI were also significantly decreased. There have been no studies looking at the use of L. casei Shirota in patients who have had CDI with the aim of preventing recurrent episodes of CDI.

MATERIALS AND METHODS

Study Population
All inpatients from June 2010 to September 2012 at a UK district general hospital that had CDI were included in this study. Potential study participants were identified from the microbiology database of stool assays performed for diarrhoea. Criteria for inclusion in the study were adult patients (aged >18 years at time of infection), presence of diarrhoea (defined as ≥ 3 non-formed stool in 24 hours) and positive stool C. difficile toxin A or B. Patients were excluded from the study if they had colonization of non-toxigenic C. difficile (C. difficile antigen-positive, C. difficile toxin negative).

Study Design
The study was a single site, cohort-control study.

Identification of toxin
Stool specimens were stored at 4°C and processed within 24 hours. The assay was an enzyme-immunosorbent assay to C. difficile antigen, toxin A and toxin B (TechLab, C.Diff Quik Chek Complete).

Patient treatment
Patients were treated with either oral metronidazole or oral vancomycin +/- intravenous metronidazole if there was evidence of severe disease. Severe disease was defined as evidence of acute kidney injury, high grade fevers (>38.5°C), radiological evidence of colitis or leukocytosis (white cell>15 x 10⁹/L) or endoscopic evidence of pseudomembranous colitis. The study cohort of patients consisted of patients concurrently treated with the probiotic L. casei Shirota and antibiotics during the hospital admission. The probiotic was generally available for prescription from the hospital dispensaries. The comparison cohort consisted of patients with CDI who did not receive probiotic.

Assessment of recurrence of C. difficile
Recurrence of C. difficile was defined as diarrhoea and C. difficile toxin A/B positivity >28 days after resolution of symptoms of the initial episode of CDI.

Statistical design and analysis
Statistical analysis was performed using SPSS version 20 (SPSS Inc, Chicago, Illinois, USA). The primary outcome measure was the recurrence rate of CDI. A Hazard Function curve was created that demonstrated the cumulative hazard against time. A log-rank test was used to determine statistical significance with a cut off p-value of 0.05.

RESULTS

Patient demographics (Table 1).
Sixty-six hospital inpatients were included for analysis in this study. The median age of the patients was 78 years and 36.4% were male. The median follow up period was 7.9 months.

<table>
<thead>
<tr>
<th></th>
<th>Probiotic cohort (n=31)</th>
<th>Non-probiotic cohort (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age/years (std dev)</td>
<td>80 (9.9)</td>
<td>76 (12.6)</td>
</tr>
<tr>
<td>% &gt;60 years of age</td>
<td>93.5</td>
<td>94.3</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>8 (25.8)</td>
<td>16 (43.2)</td>
</tr>
<tr>
<td>Median follow up period/months (std dev)</td>
<td>10 (8.8)</td>
<td>6 (8.2)</td>
</tr>
<tr>
<td>Underlying Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Respiratory (%)</td>
<td>9 (29.0)</td>
<td>6 (17.1)</td>
</tr>
<tr>
<td>- Cardiac (%)</td>
<td>13 (41.9)</td>
<td>8 (22.9)</td>
</tr>
<tr>
<td>- Neurological (%)</td>
<td>9 (29.0)</td>
<td>5 (17.1)</td>
</tr>
<tr>
<td>- Renal (%)</td>
<td>1 (3.2)</td>
<td>3 (8.6)</td>
</tr>
<tr>
<td>- Malignancy (%)</td>
<td>5 (16.1)</td>
<td>8 (22.9)</td>
</tr>
<tr>
<td>Temp &gt;38.3°C (%)</td>
<td>3 (9.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Mean albumin (g/L)</td>
<td>26.6</td>
<td>25.5</td>
</tr>
<tr>
<td>Mean WBC Count (x10⁹/L)</td>
<td>11.8</td>
<td>11.9</td>
</tr>
<tr>
<td>Hospitalized in the ITU</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Presence of pseudomembranous colitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C.diff treatment administered</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Metronidazole (%)</td>
<td>17 (54.8)</td>
<td>24 (68.6)</td>
</tr>
<tr>
<td>- Vancomycin (%)</td>
<td>8 (25.8)</td>
<td>7 (20.0)</td>
</tr>
<tr>
<td>- Metronidazole + Vancomycin (%)</td>
<td>6 (19.4)</td>
<td>4 (11.4)</td>
</tr>
<tr>
<td>Mean probiotic dose/day</td>
<td>12.8 x 10⁶ CFU</td>
<td>n/a</td>
</tr>
<tr>
<td>Total number of recurrent c.diff episodes</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Number of patients with c.diff recurrences (%)</td>
<td>1 (3.2)</td>
<td>7 (20)</td>
</tr>
</tbody>
</table>

Treatment
In the probiotic cohort, 54.8% were treated with single-agent metronidazole. The dose of the probiotic was on average 12.8 billion colony forming unit per day (1.96 bottles). In the non-probiotic cohort, 68.6% were treated with single-agent metronidazole.
Severity of CDI

In the probiotic cohort, three patients had high-grade temperatures (>38.3°C) and there were no cases of ITU admissions or pseudomembranous colitis. In the non-probiotic cohort, there were no cases of high-grade temperatures, ITU admissions or pseudomembranous colitis.

Recurrence rates of CDI

There was one patient with recurrent laboratory-confirmed CDI in the probiotic cohort equivalent to a recurrence rate of 3.2%. In contrast there were seven patients with recurrent laboratory-confirmed CDI in the non-probiotic cohort, with a total of nine episodes, equivalent to a recurrence rate of 20.0%. A log-rank test showed this was a statistically significant difference (p=0.007). The number of patients needed to treat (NNT) to prevent one case of recurrent CDI was 6. (Table 1) (Figure 1).

FIGURE 1. Hazard-function curve showing rates of recurrence-free survival of CDI patients.

Hospital re-admissions rates

In the probiotic cohort, six patients required re-admissions for diarrhoea within three months (19.4%). This occurred at a mean of 4.5 weeks from initial infection. In the non-probiotics cohort, thirteen patients required re-admissions for diarrhoea within three months (35.1%). This occurred at a mean of 4.3 weeks from the initial infection.

DISCUSSION

In the United Kingdom, rates of C. difficile have been consistently decreasing due to strategies such as improved antibiotic stewardship. However, rates still remain at 26.7 cases per 100,000 population and interventions to reduce cases further is of clinical importance.

Patients who have suffered CDI have a high rate of clinical recurrence of diarrhoea and also of recurrence of CDI. Other third of patients are readmitted within three months (2). These re-admissions have a large healthcare cost and are associated with significant morbidity and mortality. Probiotics have been proposed to prevent recurrence of CDI with evidence for the probiotic strain Saccharomyces Boulardii (McFarland et al., 1994 and Surawicz et al., 2000) container-title: "Clinical infectious diseases: an official publication of the Infectious Diseases Society of America", page: "1012-1017", volume: "31", issue: "4", source: "NCBI PubMed", abstract: "Recurrent Clostridium difficile disease (CDD). A key characteristic of oral probiotics is their ability to survive passage through the stomach and subsequently modulate the profile of the gastrointestinal microbiota (Pirker et al., 2012). The range of benefits shown by probiotics is expanding, including reduction of rates of traveller’s diarrhea, improvement of symptoms of chronic constipation and reduction of plasma ammonia concentrations in hepatic encephalopathy (Aureli et al., 2011)."

Cohort studies of patients with Clostridium Difficile infections are difficult to perform as cases are increasingly rare and occur in a wide variety of clinical specialties. This study of all C. difficile infections within a two year period at a district general hospital provides evidence that the widely available probiotic strain, L. casei Shirota may be associated with lower recurrence rates of CDI in patients who have had a CDI. The mechanisms of how L. casei Shirota is able to exert this effect is not proven, though the authors theorise that alterations in the gut microbiota may play a role.

There are limitations that arise as a result of the retrospective nature of this study. The data collected for this study were dependent on clinical notation by clinicians who were unblinded to the treatments. Furthermore, the baseline characteristics of the cohorts were slightly different in terms of antibiotic use and disease severity, with relatively more patients in the probiotic cohort having a more severe disease requiring vancomycin. However, recurrence rates of C. difficile is not known to be associated with either disease severity or type of antibiotic use, and the recurrence rates of CDI in this retrospective study were very compatible to published literature, providing further support to this study.

In summary, it is evident that patients who have suffered CDI have a high rate of recurrence of diarrhoea, recurrent CDI and hospital admissions. Re-admissions have a large healthcare cost and are associated with significant morbidity and mortality. In this small cohort, the probiotic L. casei Shirota is associated with reduced C. difficile relapse rates in a fashion similar to the probiotic yeast S. boulardii. Prospective studies are required to confirm this association and further research is required to ensure that morbidity and mortality from recurrence of diarrhoea and CDI may be minimized.

CONFLICTS OF INTEREST STATEMENT

None of the authors have any financial or personal relationships with any people or organization that could inappropriately influence their work.
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REFERENCES


