Bifidobacteriaceae Family Diversity in Gut Microbiota of Patients with Renal Failure

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Abstract

Bifidobacteriaceae family are belonged to the gut microbiota that could exhibit probiotic or health promoting effects on the host. Several studies suggested that gut microbiota are quantitatively and qualitatively altered in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD). The present study was aimed to assess the members of Bifidobacteriaceae family in fecal samples of patients with CKD and ESRD in compared to non-CKD/ESRD patients to find any changes of their counts and diversions in these patients. Twenty fresh fecal samples of patients with CKD/ESRD and twenty from non-CKD/ESRD patients were included. The whole DNA of fecal samples was extracted and the gut microbiota composition was analyzed by next generation sequencing (NGS) method. Total 651 strains were identified from 40 fecal samples, which 8 (1.23%) strains were identified as family Bifidobacteriaceae. The most abundance species in both control and disease groups were Bifidobacterium adolescentis and Bifidobacterium longum subsp. longum, and the lowest abundance species in disease group was Bifidobacterium animalis subsp. lactis. There was no significant differentiation in the abundance of various species between disease group and control group (p < 0.05). This study has confirmed that the members of Bifidobacteriaceae family are not alters in patients with CKD/ESRD.

Keywords: Bifidobacteriaceae, Chronic Kidney Disease (CKD), End-Stage Renal Disease (ESRD), Next Generation Sequencing (NGS)
INTRODUCTION

Recent studies have focused on the gut microbiota that exhibit probiotic or health promoting effects on the host. These studies revealed that the gut microbiota are associated with some physiological effects on the host by modulation of the immune system, metabolic and hormonal regulation, competitive exclusion of pathogens, breakdown of nondigestable dietary carbohydrates for provision of nutrients (O’Hara and Shanahan, 2007; Hooper et al., 2002; Leahy et al., 2005; Reinhardt et al., 2009). In addition, alterations in the gut microbiota have been associated with a number of diseases such as colorectal cancer, allergic diseases, fatty liver disease, obesity and diabetes and many other metabolic, non-metabolic and inflammatory diseases (Reinhardt et al., 2009; Bisgaard et al., 2011; Azcárate-Peril et al., 2011; Gholizadeh et al., 2019; Mouzaki et al., 2013). Particular interests of studies have focused on the genus *Bifidobacterium*, which are included as probiotic bacteria (Ventura et al., 2007).

*Bifidobacteriaceae* family is belonged to *Actinobacteria* class and includes nine genera including 55 species of the genus *Bifidobacterium*, and members of the genera *Scardovia*, *Pseudiscardovia*, *Parascardovia*, *Neoscardovia*, *Gardnerella*, *Bombiscardovia*, *Alloscardobia* and *Aeriscardovia* (Parte et al., 2012; Zhang et al., 2016). This family are Gram-positive, anaerobic and facultative anaerobic, non-motile and non-spore forming bacteria, which are isolated from various ecological niches such as the gastrointestinal tract of human and various mammals, the insect gut, the oral cavity, sewage and water kefir (Ventura et al., 2004; Laureys et al., 2016). Several studies suggested those gut microbiotas are quantitatively and qualitatively altered in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) (Hida et al., 1996; Vaziri et al., 2011). In addition, in patients with CKD and ESRD, counts of both anaerobic and aerobic bacteria are greatly increased in the intestinal microbial population compared to healthy persons (Vaziri et al., 2013). Notably, both *Prevotellaceae* and *Lactobacillaceae* families are decreased in patients with CKD (Vaziri et al., 2013). As well as, higher numbers of *Clostridium perfringens* and lower *Bifidobacteriaceae* family are significantly revealed in hemodialysis patients (Hida et al., 1996). The gastrointestinal tract is a major source of chronic inflammation, which could be one of the factors that play a role in cardiovascular pathology of CKD (Kalantar-Zadeh et al., 2003; Lau and
Recent studies demonstrated that probiotics such as *Streptococcus* spp., *Lactobacillus* spp., and *Bifidobacterium* spp. could affect inflammatory state via alterations of gut microbiota (Konstantinov et al., 2008; Van Baarlen et al., 2009). As well as, treatment of hemodialysis patients with *Lactobacillus acidophilus* could decrease serum dimethylamine, as a potential uremic toxins (Simenhoff et al., 1996). Identification and classification of bacteria with the development methods are facilitated by the sequencing of 16S rRNA genes that are amplified DNA extracted from fecal samples, which next generation sequencing (NGS) is one of these developed methods (Vrieze et al., 2010).

As noted above, gut microbiota could affect inflammation, uremic toxicity, cardiovascular and other complications in patients with CKD. Therefore, the present study was aimed to assess the members of *Bifidobacteriaceae* family in fecal samples of patients with CKD and ESRD in compared to non-CKD/ESRD patients to find any changes of their counts in these patients.

**MATERIALS AND METHODS**

**Fecal samples collection.** Twenty fresh fecal samples of patients with CKD or ESRD were directly collected from anus of patients admitted to kidney transplantation ward of Imam-Reza teaching and treatment hospital, Tabriz, Iran. At the same time, twenty fresh fecal samples were collected from patients that were admitted to other wards of this hospital as a control group. The underlying causes of CKD or ESRD in the study population included hypertensive nephrosclerosis, glomerulonephritis, chronic pyelonephritis, post renal and urolithiasis, polycystic kidney disease, chronic kidney disease with unknown etiology. Patients with gastrointestinal individuals, infections, active inflammatory disorders, malignancy, diabetes and individuals who had been treated with antibiotics within 3 months before the enrolment in the study were excluded. The collected fecal samples were immediately stored at -80 °C until DNA extraction.

**DNA extraction, PCR amplification and sequencing.** Each fecal sample was vigorously and aseptically mixed and homogenized with a spoon and 4 gr of each samples was weighted. DNA of all fecal samples were extracted by the QIAamp Stool Mini Kit (Qia gene, Germany), according to the manufacturer’s instruction. Template DNA of each sample was amplified by two sequences of universal bacterial 16S rRNA gene including Illumina V3: 5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3’ and 3’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-5’. 

Ix, 2013).
Illumina V4: 5’- GTCTCGTGGGCTCGGAGATGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3’ (Klindworth et al., 2013). The amplifications were performed in a T100™ thermal (Bio-Rad, USA) and 1 cycle of initial denaturing at 95 °C for 5 min, 35 cycles of denaturing at 95 °C for 1 min, annealing at 55 °C for 45 s and extension at 72 °C for 1 min, and a final extension at 72 °C for 1 min were used. The amplified products were detected using electrophoresis in 1% agarose gel in Tris-boric acid-EDTA buffer, stained with ethidium-bromide and visualized under UV light. The sequencing of amplified products was performed on a MiSeq system (100k 2 x 300 bp paired-end reads) (Illumina, USA) in Omega Bioservices company. Illumina’s BaseSpace in parallel with Illumina’s in-house QIIme 2 pipeline was used for bioinformatics analysis.

**Statistical analysis.** Statistical analysis was performed in GraphPad Prism 8 and Microsoft Excel 2016. The study data was analyzed by using descriptive statistics including mean and standard deviation (STDEV) and unpaired t test with Welch’s correction and Mann-Whitney nonparametric test were applied to compare mean of data.

**RESULTS**

The characteristics of participants between disease group did not differ with control group - and gender (male: 14 and female: 6 vs. male: 10, female: 10) and age (53.20 ± 12.03 vs. 59.3 ± 7.89). Total 651 strains were identified from 40 fecal samples, which 8 (1.23%) strains were identified as family *Bifidobacteriaceae*. The strains were classified into 4 genus and 8 species. The identified genus were included *Bifidobacterium* (5 species), *Scardovia* (1 species), *Parascardovia* (1 species), and *Alloscardovia* (1 species). The most abundance species in both control and disease groups were *Bifidobacterium adolescentis* (2.10% ± 1.05% vs. 1.98% ± 1.53%, respectively) and *Bifidobacterium longum* subsp. *longum* (1.59% ± 1.14% vs. 0.92% ± 0.74%, respectively). In addition, the lowest abundance species in control group was *Alloscardovia omnicolens* (0.001% ± 0.002%) and in disease group was *Bifidobacterium animalis* subsp. *lactis* (0.0007% ± 0.0009%). The abundance of various species is shown in Table 1 and Figure 1. There was no significant differentiation in the abundance of various species between disease group and control group (p < 0.05).
DISCUSSION

This study describes the diversity and abundance of *Bifidobacteriaceae* family in fecal samples of patients with CKD/ESRD in compared to control group that was patients who was not admitted to the hospital for kidney diseases. We assessed the hypothesis concerning the existence of difference in the abundance and diversity of the members of *Bifidobacteriaceae* family and its relationships to patients with CKD or ESRD. Therefore, we demonstrated lack of association between the abundance and diversity of *Bifidobacteriaceae* family and CKD/ESRD. In other words, In CKD/ESRD patients, the abundance of different species of *Bifidobacteriaceae* family identified in fecal samples was not different in compared to control group. Cruz-Mora et al. (2014) assessed the effects of probiotics, especially *Bifidobacteria* in two groups including ESRD patients and control group. They observed a significant increase of *Bifidobacteria* on the test group after treatment with probiotic and there is no significantly differences in the counts of *Bifidobacteria* in control group.

Present study is one of the few researches concerning the diversity and abundance of *Bifidobacteriaceae* family and their effects on patients with CKD/ESRD, specifically comparing of *Bifidobacteriaceae* family between CKD/ESRD patients and non-CKD/ESRD patients. However, the importance of this type of study in patients with CKD/ESRD lies in benefits that probiotics such as strains of *Bifidobacteria* could not promote symptoms in these patients. Some studies such as Vaziri et al. (2013) study reported uremia and CKD could alter the microbial population of the gut. As well as, gut microbiota by the production of uremic toxins could contribute in the uremic syndrome and translocation of bacteria and their LPS to blood from the gut takes place in renal failure. As well as, in the dialysis patients, the gut microbiota contributes to the chronic inflammatory (Kotanko et al., 2006). The gut microbiota such as *Bifidobacteria* could effect on essential fatty acids that causes a beneficial results of anti-inflammatory properties (Chapkin et al., 2008; Simopoulos, 2002). Several studies reported that the decreased of *Bifidobacteria* along with the expression of tight junction proteins such as occludin and ZO-1 (zonula occludens-1) due to high-fat diets are adversely associated with high portal plasma concentration of LPS (Lipopolysaccharide), which could initiates inflammatory responses via TLRs (toll-like receptors), and proinflammatory cytokines (Cani et al., 2008; Brun et al., 2007; Cani et al., 2007). de Goffau et al. (2013) reported that a decreased in the abundance of butyrate-producing species, *B. adolescentis* and *B. pseudocatenulatum* negatively affect inflammation and
the intestinal epithelial barrier function. Recent studies demonstrated that disequilibrium in the gastrointestinal microbial ecosystem and abnormalities in the gut mucosa are associated with uremia (Vaziri et al., 2013; Vaziri et al., 2011). The changes in the gut microbial population in CKD increases transformation of aminoacids into uremic retention solutes including trimethylamine n-oxide (TMAO), p-cresylsulfate (PCS), and indoxyl-sulfate (IS) among others (Evenepoel et al., 2009). Goetze et al. (2008) study suggested that intake *Bifidobacteria* as probiotics could present positive results in prevention of constipation, improvement of blood lipid profile and sugar, absorption of minerals and nutrition and synthesis of vitamins. In addition, Taki et al. (2005) study suggested that *B. longum* could be effective in decreasing the pre-hemodialysis serum levels of IS, triglyceride and hemocystein. As well as, Koppe et al. (2015) suggested that the efficacy of probiotic bacteria to improve renal function and to decrease production of uremic toxin has been confirmed in *in vitro* models and in various CKD patients of human and animals. These studies suggested that inflammation and inflammatory responses could cause kidney disorders followed by kidney failure, as well as, probiotics could promote health of the host. As noted these studies, we was not find significantly alteration in the abundance of members of *Bifidobacteriaceae* family to confirm this hypothesis that changes in the abundance of *Bifidobacteria* could effect on kidney disorders. In addition, the abundance of *Bifidobacteria* as probiotics did not alters in CKD/ESRD patients in compared to control group. A limitation of this study was measurement of the biochemical parameters such as serum creatinine, blood urea nitrogen. Because of the patients did not fast, we could not measure these parameters.

It is clear that gut microbiota via some ways contribute in the pathogenesis of chronic kidney disease. However, in this study, we have confirmed that the members of *Bifidobacteriaceae* family are not alters during chronic kidney diseases in the gut microbiota population but may be play roles in the metabolism of uremic toxin precursors and normalizing gut microbiota population. Use of the members of *Bifidobacteriaceae* family as a probiotic could be health promoting properties in these patients.

**Ethics**

We hereby declare all ethical standards have been respected in preparation of the submitted article.
Conflict of Interest
The authors declare that they have no conflict of interest.

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References


the duodenum of healthy humans correlating with immune tolerance. Proc Natl Acad Sci U S A 106, 2371-2376.


Table 1. The abundance of different species of *Bifidobacteriaceae* family identified in fecal samples of both control and disease groups.

<table>
<thead>
<tr>
<th>Spec</th>
<th>Control</th>
<th>Individuals</th>
<th>Disease</th>
<th>Individuals collected</th>
<th>Min</th>
<th>Max</th>
<th>STDEV</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. adolescentis</em></td>
<td>6273.1</td>
<td>20</td>
<td>121</td>
<td>25</td>
<td>7200.85</td>
<td>5732.35</td>
<td>20</td>
<td>48</td>
</tr>
<tr>
<td><em>B. longum subsp. longum</em></td>
<td>4747.75</td>
<td>20</td>
<td>18</td>
<td>33</td>
<td>7767.72</td>
<td>2654.7</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td><em>B. bifidum</em></td>
<td>235.5</td>
<td>17</td>
<td>0</td>
<td>37</td>
<td>840.84</td>
<td>791.85</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td><em>B. kashiwae</em></td>
<td>374.35</td>
<td>19</td>
<td>0</td>
<td>30</td>
<td>677.94</td>
<td>327.95</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td><em>Scardovia wiggsiae</em></td>
<td>95.35</td>
<td>16</td>
<td>0</td>
<td>67</td>
<td>176.10</td>
<td>66.55</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td><em>B. animalis subsp. lactis</em></td>
<td>63.45</td>
<td>3</td>
<td>0</td>
<td>125</td>
<td>280.23</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><em>Parascardovia denticolens</em></td>
<td>11.2</td>
<td>5</td>
<td>0</td>
<td>127</td>
<td>29.35</td>
<td>1.25</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><em>Alloscardovia omnicolens</em></td>
<td>4.15</td>
<td>4</td>
<td>0</td>
<td>65</td>
<td>14.58</td>
<td>11.85</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>11804.85</td>
<td>20</td>
<td>0</td>
<td>33552</td>
<td>16987.61</td>
<td>9588.5</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 1.** Heatmap graph of the abundance of different species of *Bifidobacteriaceae* family identified in fecal samples of both control and disease groups.