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Original Article

Bifidobacteriaceae Family Diversity in Gut Microbiota of Patients with Renal Failure

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Abstract

Bifidobacteriaceae family are gut microbiota that exhibit probiotic or health promoting effects on the host. Several studies have 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 aimed to assess the members of *Bifidobacteriaceae* family in fecal samples of patients with CKD and ESRD and compare them with non-CKD/ESRD patients to find any changes in their counts and diversions in these patients. Twenty fresh fecal samples from patients with CKD/ESRD and twenty from non-CKD/ESRD patients were examined. Whole DNA was extracted from fecal samples and the gut microbiota composition was analyzed by next generation sequencing (NGS). A total of 651 strains were identified from 40 fecal samples, 8 (1.23%) strains of which were identified as family *Bifidobacteriaceae*. The most abundant species in both control and disease groups were *Bifidobacterium adolescentis* and *Bifidobacterium longum* subsp. *longum*, and the least abundant species in the disease group was *Bifidobacterium animalis* subsp. *lactis*. There was no significant difference in the abundance of various species between the disease and control groups ($p < 0.05$). This study confirms that the members of the *Bifidobacteriaceae* family are not altered in patients with CKD/ESRD.

Keywords: *Bifidobacteriaceae*, chronic kidney disease (CKD), end-stage renal disease (ESRD), next generation sequencing (NGS)

Diversité de la Famille des *Bifidobacteriaceae* dans le Microbiote Intestinal des Patients Atteints d'insuffisance Rénale

Résumé: La famille des *Bifidobacteriaceae* est un microbiote intestinal qui présente des effets probiotiques ou favorisant la santé sur l'hôte. Plusieurs études ont suggéré que le microbiote intestinal est altéré quantitativement et qualitativement chez les patients atteints d'insuffisance rénale chronique (IRC) et d'insuffisance rénale terminale (IRT). La présente étude visait à évaluer les membres de la famille des *Bifidobacteriaceae* dans des échantillons fécaux de patients atteints d'IRC et d'IRT et à les comparer avec des patients non-IRC/IRT pour trouver des changements dans leurs décomptes et détournements chez ces patients. Vingt échantillons fécaux frais de patients atteints d'IRC/IRC et vingt de patients non-IRC/IRT ont été examinés. L'ADN entier a été extrait d'échantillons fécaux et la composition du microbiote intestinal a été analysée par séquençage de nouvelle génération (SNG). Au total, 651 souches ont été identifiées à partir de 40 échantillons fécaux, dont 8 (1.23%) souches ont été identifiées comme appartenant à la famille des *Bifidobacteriaceae*. Les espèces les plus abondantes dans les groupes témoins et malades étaient *Bifidobacterium adolescentis* et *Bifidobacterium longum*

subsp. *longum*, et l'espèce la moins abondante dans le groupe de la maladie était *Bifidobacterium animalis subsp. lactis*. Il n'y avait pas de différence significative dans l'abondance des diverses espèces entre les groupes malades et témoins ($p<0.05$). Cette étude confirme que les membres de la famille des *Bifidobacteriaceae* ne sont pas altérés chez les patients atteints d'IRC/IRT.
Mots-clés: *Bifidobacteriaceae*, insuffisance rénale chronique (IRC), insuffisance rénale terminale (IRT), séquençage de nouvelle génération (SNG)

1. Introduction

Recent studies have focused on gut microbiota that exhibit probiotic or health promoting effects on the host. These studies revealed that gut microbiota are associated with some physiological effects on the host through modulation of the immune system, metabolic and hormonal regulation, competitive exclusion of pathogens, and breakdown of non-digestible dietary carbohydrates for the provision of nutrients (1-4). In addition, alterations in gut microbiota have been associated with a number of diseases such as colorectal cancer, allergic diseases, fatty liver disease, obesity, diabetes, and many other metabolic, non-metabolic, and inflammatory diseases (4-8). Particular studies have focused on the genus *Bifidobacterium*, which is included in the probiotic bacteria (9).

The *Bifidobacteriaceae* family belongs to class *Actinobacteria* 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* (10, 11). This family comprises 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 humans and various other mammals, the insect gut, the oral cavity, sewage, and water kefir (12, 13). Several studies have suggested those gut microbiotas are quantitatively and qualitatively altered in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) (14, 15). 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 (16)., both *Prevotellaceae* and *Lactobacillaceae* families are decreased in patients with CKD (16), and significantly higher numbers of *Clostridium perfringens* and lower numbers of *Bifidobacteriaceae* are observed in hemodialysis patients (14). The gastrointestinal tract is a major source of chronic inflammation, which could be one of the factors that play a role in the cardiovascular pathology of CKD (17, 18). Recent studies have demonstrated that probiotics such as *Streptococcus* spp., *Lactobacillus* spp., and *Bifidobacterium* spp. could affect inflammatory state via alterations in gut microbiota (19, 20). Moreover, the treatment of hemodialysis patients with *Lactobacillus acidophilus* could decrease serum dimethylamine as a potential uremic toxin (21). Identification and classification of bacteria with development methods are facilitated by the sequencing of 16S rRNA genes that are amplified DNA extracted from fecal samples, and next generation sequencing (NGS) is one of these developed methods (22).

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

2. Material and Methods

2.1. Fecal Sample Collection

Twenty fresh fecal samples of patients with CKD or ESRD were collected directly from the anus of patients admitted to the 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, and chronic kidney disease with unknown etiology. Patients with gastrointestinal infections, active inflammatory disorders, malignancy, or diabetes, and individuals who had been treated with antibiotics within 3 months before enrollment in the study were excluded. The collected fecal samples were immediately stored at -80 ºC until DNA extraction.

2.2. DNA Extraction, PCR Amplification and Sequencing

Each fecal sample was vigorously and aseptically mixed and homogenized with a spoon, and 4 gr of each sample was weighed. The DNA of all fecal samples was extracted by QIAamp Stool Mini Kit (Qia Gene, Germany), according to the manufacturer's instructions. Template DNA of each sample was amplified by two sequences of universal bacterial 16S rRNA genes Illumina V3: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and Illumina V4: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3' (23). The amplifications were performed in a T100^{TM} thermal cycler (Bio-Rad, USA), and 1 cycle of initial denaturing at 95 ºC for 5 min, 35 cycles of denaturing at 95 ºC for 1 min, annealing at 55 ºC for 45 s, extension at 72 ºC for 1 min, and a final extension at 72 ºC 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 amplified products were sequenced on a MiSeq system (100k 2 x 300 bp paired-end reads) (Illumina, USA) in the Omega Bioservices company. Illumina's BaseSpace in parallel with Illumina's in-house QIIme 2 pipeline was used for bioinformatics analysis.

2.3. Statistical Analysis

Statistical analyses were performed in GraphPad Prism 8 and Microsoft Excel 2016. The study data was analyzed 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 means.

3. Results

Participant characteristics did not differ between the disease and control groups in gender (male: 14 and female: 6 vs. male: 10, female: 10, respectively) or age (53.20 ± 12.03 and 59.3 ± 7.89, respectively). In total, 651 strains were identified from 40 fecal samples, 8 (1.23%) strains of which were identified as family *Bifidobacteriaceae*. The strains were classified into 4 genera and 8 species. The identified genera were *Bifidobacterium* (5 species), *Scardovia* (1 species), *Parascardovia* (1 species), and *Alloscardovia* (1 species). The most abundant species in both control and disease groups were *Bifidobacterium adolescentis* (2.10% ± 1.05% and 1.98% ± 1.53%, respectively) and *Bifidobacterium longum* subsp. *longum* (1.59% ± 1.14% vs. 0.92% ± 0.74%, respectively). The least abundant species in the control group was *Alloscardovia omnicolens* (0.001% ± 0.002%) and in the 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 difference in the abundance of various species between the disease and control groups ($p < 0.05$).

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

Species	Control group mean	Individuals collected	Min	Max	STDEV	Disease group mean	Individuals collected	Min	Max	STDEV	P-value
B. adolescentis	6273.1	20	121	25238	7200.85	5732.35	20	48	40142	9889.32	0.844
B. longum subsp. longum	4747.75	20	18	33552	7767.72	2654.7	20	10	15990	4810.62	0.313
B. bifidum	235.5	17	0	3796	840.84	791.85	13	0	13071	2934.91	0.420
B. kashiwanohense	374.35	19	0	3046	677.94	327.95	13	0	4288	977.04	0.862
Scardovia wiggsiae	95.35	16	0	675	176.10	66.55	15	0	532	153.17	0.584
B. animalis subsp. lactis	63.45	3	0	1254	280.23	2	4	0	24	5.68	0.339
Parascardovia denticolens	11.2	5	0	127	29.35	1.25	3	0	14	3.53	0.147
Alloscardovia omnicolens	4.15	4	0	65	14.58	11.85	3	0	163	38.84	0.414
Total	11804.85	20	0	33552	16987.61	9588.5	20	0	40142	18813.11	0.812

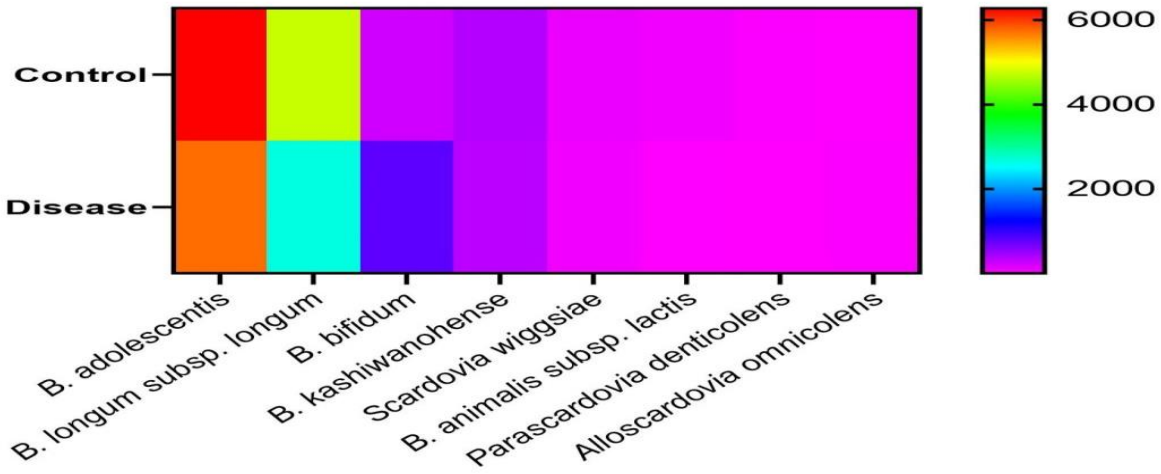

Figure 1. Heatmap graph of the abundance of different species of *Bifidobacteriaceae* family identified in fecal samples of both control and disease groups

4. Discussion

The current study described the diversity and abundance of *Bifidobacteriaceae* family in fecal samples of patients with CKD/ESRD compared with a control group of patients admitted to the hospital for something other than kidney disease. The hypotheses concerning the existence of differences in the abundance and diversity of the members of *Bifidobacteriaceae* family and their relationships to patients with CKD or ESRD were assessed. The results demonstrated the lack of an 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 did not differ with that in the control group. Cruz-Mora, Martinez-Hernandez (24) assessed the effects of probiotics, especially *Bifidobacteria*, in ESRD patients and a control group. They observed a significant increase in *Bifidobacteria* in the test group after treatment with probiotics and no significant differences in the counts of *Bifidobacteria* in the control group.

The present study is one of the few concerning the diversity and abundance of *Bifidobacteriaceae* and their effects on patients with CKD/ESRD, specifically comparing the *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 the benefits that probiotics such as strains of *Bifidobacteria* could not promote symptoms in these patients. Some studies such as one by Vaziri, Wong (16) have reported that uremia and CKD could alter the microbial population of the gut and that gut microbiota, by producing uremic toxins, could contribute to the uremic syndrome and translocation of bacteria and their LPS to blood from the gut that takes place in renal failure. In dialysis patients, gut microbiota contribute to chronic inflammation (25). Gut microbiota such as *Bifidobacteria* could affect essential fatty acids that cause beneficial results of anti-inflammatory properties (26, 27). Several studies have reported that decreases in *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 concentrations of LPS (lipopolysaccharide), which could initiate inflammatory responses via TLRs (toll-like receptors) and proinflammatory cytokines (28-30). de Goffau, Luopajarvi (31) reported that a decrease in the abundance of the butyrate-producing species *B. adolescentis* and *B. pseudocatenulatum* negatively affected inflammation and the intestinal epithelial barrier function. Recent studies have demonstrated that disequilibrium in the gastrointestinal microbial ecosystem and abnormalities in the gut mucosa are associated with uremia (15, 16). Changes in the gut microbial population in CKD increases the transformation of amino acids into uremic retention solutes, including trimethylamine n-oxide (TMAO), p-cresylsulfate (PCS), and indoxyl-sulfate (IS) among others (32). Goetze, Fruehauf (33) suggested that taking *Bifidobacteria* as probiotics could present positive results in the prevention of constipation, improvement of blood lipid profiles and sugar, absorption of minerals and nutrition, and synthesis of vitamins. In addition, Taki, Takayama (34) suggested that *B. longum* could be effective in decreasing the pre-hemodialysis serum levels of IS, triglycerides, and hymocysteine. Moreover, Koppe, Mafra (35) suggested that the efficacy of probiotic bacteria in improving renal function and decreasing the production of uremic toxins has been confirmed in *in vitro* models and in various human and animal CKD patients. These studies suggest that inflammation and inflammatory responses could cause kidney disorders followed by kidney failure, and probiotics could promote the health of the host. As noted in these studies, the current study found no significant alteration in the abundance of members of the *Bifidobacteriaceae* family to confirm the hypothesis that changes in the abundance of *Bifidobacteria* could affect kidney disorders. In

addition, the abundance of *Bifidobacteria* as probiotics was not altered in CKD/ESRD patients compared to the control group. One limitation of the current study was that biochemical parameters such as serum creatinine and blood urea nitrogen could not be measured, because the patients did not fast.

It is clear that gut microbiota contribute to the pathogenesis of chronic kidney disease; however, this study has confirmed that members of the *Bifidobacteriaceae* family are not altered during chronic kidney diseases in the gut microbiota population, but they may play roles in the metabolism of uremic toxin precursors and the normalization of gut microbiota populations. Use of members of the *Bifidobacteriaceae* family as probiotics could be health promoting in these patients.

Authors' Contribution

Study concept and design: H. S. K., M. A. and H. T. K.
Acquisition of data: Gh. R. H.
Analysis and interpretation of data: H. S. K. and R. Sh.
Drafting of the manuscript: Gh. R. H., R. Sh. and M. A.
Critical revision of the manuscript for important intellectual content: Gh. R. H.
Statistical analysis: Gh. R. H.
Administrative, technical, and material support: H. S. K., M. A. and H. T. K.

Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Islamic Azad University of Zanjan, Iran (587968786354).

Conflict of Interest

The authors declare that they have no conflict of interest.

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