Bifidobacteriaceae family diversity in gut microbiota of patients with renal failure

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Research

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Abstract

Background: 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 quantitative and qualitative 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 in these patients.

Methods: Twenty fresh fecal samples of patients with CKD/ESRD and twenty from non-CKD/ESRD patients were included. The whole DNA of fecal samples were extracted and the gut microbiota composition was analyzed by next generation sequencing (NGS) method.

Results: 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 group were Bifidobacterium adolescentis (2.10% ± 1.05% vs. 1.98% ± 1.53%, respectively) and the lowest abundance species in disease group was Bifidobacterium animalis subsp. lactis (0.0007% ± 0.0009%).

Conclusions: 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.

Introduction

Recent studies have focused on the gut microbiota that exhibit probiotic or health promoting effects on the host [1–3]. 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 [4–7]. In addition, alters in the gut microbiota have been associated with number of diseases such as colorectal cancer, allergic diseases, fatty liver disease, obesity and diabetes and many other metabolic, non-metabolic and inflammatory diseases [7–12]. Particular interest of studies have focused on the genus Bifidobacterium, which are included as probiotic bacteria [13].

Bifidobacteriaceae family is belonged to Actinobacteria class and includes nine genera including 55 species of the genus Bifidobacterium [14], and members of the genera Scardovia, Pseudiscardovia, Parascardovia, Neoscardovia, Gardnerella, Bombiscardovia, Alloscardobia and Aeriscardovia [14, 15]. 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 [16, 17]. Several studies suggested that gut microbiota are quantitative and qualitative altered in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) [18, 19]. 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 [19]. Notably, both Prevotellaceae and Lactobacillaceae families are decreased in patients with CKD [19]. As well as, higher numbers of Clostridium perfringens and lower Bifidobacteriaceae family are significantly revealed in hemodialysis patients [18]. 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 [20, 21]. Recent studies demonstrated that probiotics such as Streptococcus spp., Lactobacillus spp., and Bifidobacterium spp. could affect inflammatory state via alterations of gut microbiota [22, 23]. As well as, treatment of hemodialysis patients with Lactobacillus acidophilus could decrease serum dimethylamine, as a potential uremic toxins [24]. Identification and classification of bacteria with the development methods are facilitated by the sequencing of 16srRNA genes that are amplified DNA extracted from fecal samples [25], which next generation sequencing (NGS) is one of these developed methods.

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.

Methods And Materials

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. Sample collection was done during September 2018 till June 2019 after patients volunteer collaboration and for all samples informed consent form filled by patients. Study was conducted after approval of Human subjects local ethic review committee at Tabriz University of Medical Sciences and was conducted according to Declaration of Helsinki and Registered in Iranian Registry of Clinical Trials with reference number:IRCT2016062628644N1.

The underlying of CKD in the study population had inclusion criteria: diagnosis of hypertensive nephrosclerosis, glomerulonephritis, chronic pyelonephritis, post renal and urolithiasis, polycystic kidney disease, chronic kidney disease with unknown etiology according to defined protocols such as Estimated glomerular filtration rate (eGFR), Mean Hb, Transferrin saturation (Tsat), Clinical signs, Serum vitamin B12 and folate levels. ESRD patients were identified as patients who had maintenance dialysis such as haemofiltration, haemodialysis, peritoneal dialysis or haemodiafiltration for a month. Exclusion criteria were patients with gastrointestinal individuals, infections, active inflammatory disorders, malignancy, diabetes and individuals who had been treated with antibiotics within 3 months before the enrolment. 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 g of each sample was weighted. DNA of all fecal samples were extracted by the QIAamp Stool Mini Kit (Qia gene, Germany), according to the manufacturer's instruction [26]. Template DNA of each samples was amplified by two sequences of universal bacterial 16srRNA gene including Illumina V3: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and Illumina V4: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3' [27]. The amplifications were performed in a T100^{TM} thermal (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 and 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 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 in 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 group 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 are shown in table 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. Mora et al. [28] 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 [28].

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. [19] study reported uremia and CKD could alters 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 contribute to the chronic inflammatory [29]. The gut microbiota such as bifidobacteria could effect on essential fatty acids that causes a beneficial results of anti-inflammatory properties [30, 31]. 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 [32–34]. de Goffau et al. [35] reported that a decreased in the abundance of butyrate-producing species, B. adolescentis and Bifidobacterium 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 [19, 36]. 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 [37]. Goetze study [38] 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. study [39] 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. 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 [40]. 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.

Conclusion

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 have health promoting properties in this patients.

Declarations

- **Ethics approval and consent to participate**

Study was conducted after approval of Human subjects local ethic review committee at Tabriz University of Medical Sciences and was conducted according to Declaration of Helsinki and Registered in Iranian Registry of Clinical Trials with reference number:IRCT2016062628644N1. All participants filled consent form and is available by authors and ethic local committee.

- **Consent for publication**

All authors declare agreement and consent for publication.

- **Availability of data and material**

All data and materials (DNA samples) are available by request from Author.

- **Competing interests**

The authors declare that they have no conflict of interest.

- **Funding**

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- **Authors' contributions**

Gholamreza Hanifi: Data collection, Formal analysis, Writing - original draft. **Hamid Tayebi Khosroshahi**: Supervision, Writing - original draft. **Reza Shapouri**: Adviser, Writing - original draft.. **Mohammad**

Asgharzadeh: Formal analysis, Writing - original draft. **Hossein Samadi Kafil**: Supervision, Methodology, Funding acquisition, Formal analysis, Writing - original draft

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Table

Table 1: The 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

Figures

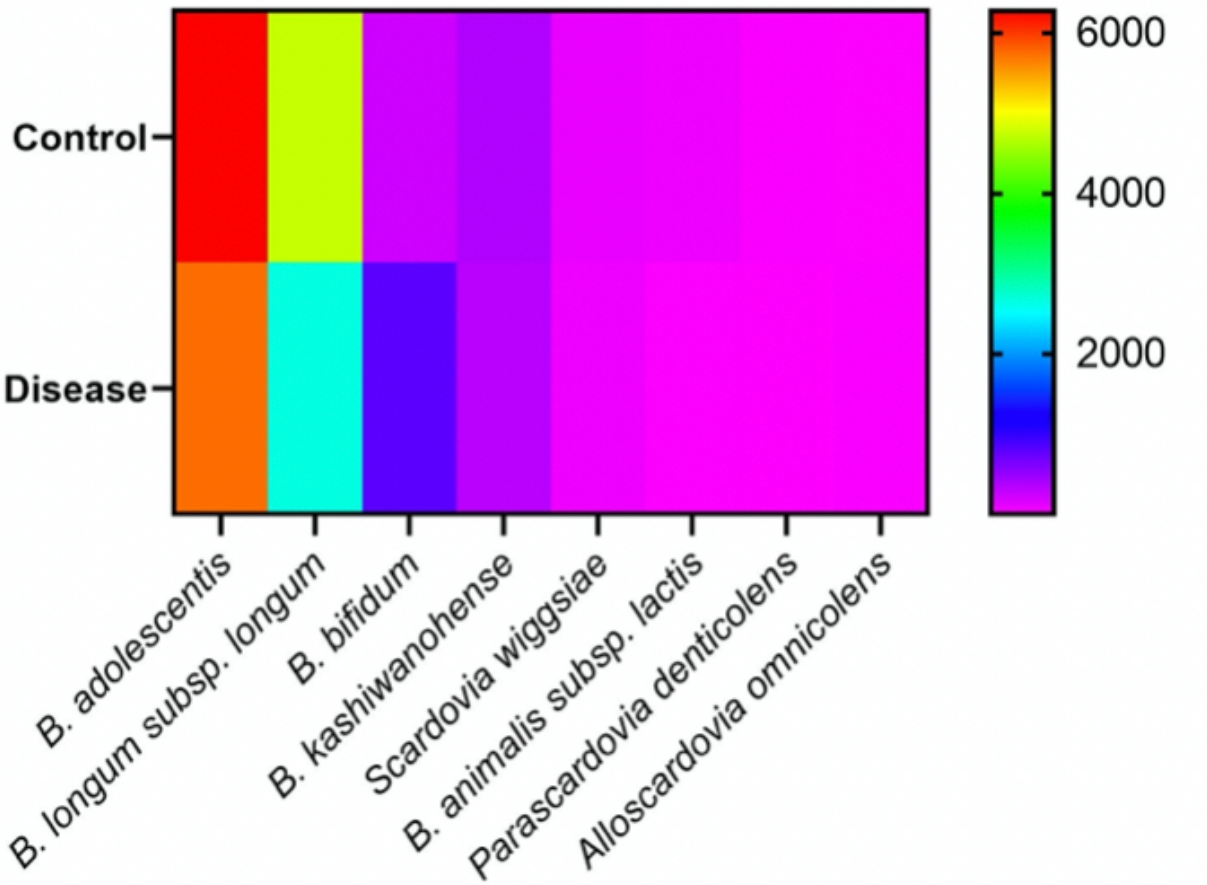

Figure 1

Heatmap graph of the abundance of different species of Bifidobacteriaceae family identified in fecal samples of both Control and Disease groups.