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Prebiotics Improved the Defecation Status via Changes in the Microbiota and Short-chain Fatty Acids in Hemodialysis Patients

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Hemodialysis patients often become constipated. We analyzed the effect of prebiotics on the defecation status due to the intestinal environment in hemodialysis patients. Fifteen patients received prebiotics as partially hydrolyzed guar gum for four weeks. The defecation status was assessed using both the Bristol Stool Form Scale and the Japanese version of the Constipation Assessment Scale. The fecal status, microbiota measured by a terminal restriction fragment length polymorphism analysis, and fecal short-chain fatty acid concentrations by gas chromatography were compared before and after prebiotics ingestion. Prebiotics ingestion improved the individual stool form and decreased the constipation score from 5.1 to 3.0. The ratio of short-chain fatty acid-producing microbiota, such as *Bifidobacterium* and *Bacteroides*, increased after ingestion (2.35- and 3.17-fold, respectively). Furthermore, the concentration of short-chain fatty acids significantly increased (1.58-fold). The individual dendrogram distribution after ingestion was changed in 8 participants (53.3% of the subjects). In 5 participants (33.3% of the subjects), the clusters were even more noticeably different. Prebiotics improved the defecation status in hemodialysis patients due in part to the composition of intestinal microbiota and short-chain fatty acid concentrations.

INTRODUCTION

Hemodialysis (HD) patients are often constipated due to dietary restrictions and side effects of their medications and are therefore treated with laxatives. An average of 53% HD patients were reported to suffer from constipation (2). Severe constipation induces anorexia, and the subsequent decreased dietary intake then causes malnutrition. Healthy defecation habits in HD patients are thus an important factor influencing the quality of life.

A dysbiotic microbiota is a susceptibility factor for kidney disease and vice versa, with the progressive loss of the renal function worsening the intestinal dysbiosis in chronic kidney disease (CKD)/end-stage renal disease (ESRD) patients (14). In HD patients, the proportion of microbiota drastically differs from those in healthy individuals; for example, the proportion of *Enterobacteriaceae* is increased while that of *Bifidobacteria* is decreased. Where the percentage of culturable aerobes in healthy individuals ranges from 0.1% to 0.2%, that in HD patients ranges from 11.2% to 15.6% (3, 11). This increase in the proportion of aerobes causes opportunistic infections due to uremic toxins, such as phenol, indican, and ammonia (3, 11).

Prebiotics are non-digestible food constituents beneficial to the host. Useful information in prebiotics treatment for chronic constipation has been provided in recent reviews (16, 25). In HD patients, however, only a few studies so far have evaluated the effects of prebiotics on constipation, with promising results obtained. For example, the ingestion of prebiotics such as oligofructose-enriched inulin and partially hydrolyzed guar gum (PHGG), which is a soluble dietary fiber, have been reported to decrease serum uremic toxins, to improve the nutritional status, and to relieve constipation in HD patients (8, 10, 21). Indeed, the ingestion of PHGG for six weeks in HD patients improved the presence of constipation and the stool form (21).

In these previous papers, culture-based methods have been utilized to detect microbiota. Recently, a terminal restriction fragment length polymorphism (T-RFLP) analysis was developed to detect microbiota simultaneously (13, 18). Regarding the detection efficiency for microbiota and the simplicity of the procedure, the T-RFLP analysis is assumed to be superior to conventional culture-based methods and to have a high throughput and scientific reproducibility. However, few reports have evaluated the effects of prebiotics on intestinal microbiota and constipation in HD patients by T-RFLP analyses.

Short-chain fatty acids (SCFAs), the end product of anaerobic bacterial fermentation of carbohydrates in the colon, play important roles in the biology of colonocytes and exert effects on various cell types. SCFAs regulate tissue-specific homeostasis, including gastrointestinal motility, colitis, metabolic syndrome, airway disease, and

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even carcinogenesis (7). In addition, many studies have reported that SCFAs improved the renal function in experimental animal models, including those of acute kidney injury and CKD (7).

We therefore hypothesized that a change in the intestinal environment by prebiotics with PHGG could improve the stool form and relieve constipation in HD patients. However, the definition of constipation still lacks objectivity. We aimed to clarify the effects of prebiotics on the stool form and severity of constipation in a broad sense and assessed the composition of the fecal microbiota by high-throughput T-RFLP analyses and fecal SCFA concentrations in HD patients.

MATERIALS AND METHODS

Participants

This study involved 15 outpatient maintenance dialysis (8 males and 7 females, mean age: 63.9 ± 2.1 years old) who had no fever or other infection at Suzuran Hospital (Kobe, Japan). The mean duration of HD was 51.7 ± 9.4 months. Their primary diseases were diabetic nephropathy (8 patients), chronic glomerulonephritis (3 patient), nephrosclerosis (1 patient), lecithin cholesterol acyltransferase deficiency (1 patient), antineutrophil cytoplasmic antibody associated vasculitis (1 patient), and unknown (1 patients). This study was approved by the Ethics Committee of Kobe University School of Health Sciences (permission No: 596). All participants were included in this study after providing their informed consent.

Study design

The study was a non-randomized controlled trial, as there is often considerable individual variability in the gut microbiota, which hampers comparisons among individuals. As the prebiotics, we used Sunfiber® (Taiyo Kagaku Co, Tokyo, Japan) 6 g/packet containing 5.1 g PHGG. Of note, 100 g of Sunfiber® contains 207 kcal, 8.9 g carbohydrate, 0-1 g protein, 0 g lipid, 84.7 g fiber, 50-150 mg sodium. The participants ingested two packets of prebiotics daily for four weeks. During the examination period, participants' concomitant medications and diet contents were not changed, but *Lactobacillus* preparations and foods containing *Lactobacillus* were discontinued. Blood samples for the biochemical analysis were collected every two weeks from two weeks before ingestion until the end of ingestion and then again two weeks after the end of ingestion. Feces samples and a questionnaire on the defecation status were collected before and after ingestion.

Biochemical analyses

The laboratory data collection was outsourced to a clinical laboratory testing facility (Fukuyama Medical Laboratory, Hiroshima, Japan).

Defecation status and satisfaction of ingestion

The stool form was evaluated using the Bristol Stool Form Scale, which classifies samples into seven types based on the shape and hardness of the stool: Type 1, separate hard lumps, like nuts (hard to pass); Type 2, sausage-shaped but lumpy; Type 3, like a sausage but with cracks on its surface; Type 4, like a sausage or snake, smooth and soft; Type 5, soft blobs with clear-cut edges (passed easily); Type 6, fluffy pieces with ragged edges, a mushy stool; Type 7, watery, no solid pieces, entirely liquid (Table I).

The severity of constipation was evaluated using the Japanese version of the Constipation Assessment Scale (CAS-J), which was derived from a partial modification applied by Fukai et al. (2) to the scale developed by McMillan et al. (9) (Table II). The scale consists of eight descriptors for constipation, with responses on a three-point rating scale, ranging from "no problems" to "severe problems". "No problems" is scored as 0, "some problems" as 1, and "severe problems" as 2. The subjective item ratings are summed to obtain a total score for each subject ranging from 0 to 16.

Satisfaction with prebiotics ingestion was evaluated using a visual analog scale (VAS) (1).

Fecal bacteriological examinations and determination of SCFA concentrations

Feces samples were collected in a test tube and maintained at -30 °C until assayed. Analyses of the intestinal microbiota and SCFA concentrations were outsourced to TechnoSuruga Laboratory Co., LTD. (Shizuoka, Japan). The intestinal microbiota were analyzed by a T-RFLP analysis (13) and compared by a hierarchical cluster analysis of the ratio of the peak area to the total area of each operational taxonomic unit (OTU), after which a dendrogram was constructed.

The concentrations of SCFAs, namely acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and caproic acid, were analyzed by gas chromatography with a flame ionization detector. These results were expressed as micromoles per gram of feces.

Statistical analyses

Statistical analyses were conducted using the Statcel2 software program (OMS Publishing Inc., Saitama, Japan). Data are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using a repeated measure analysis of variance, Pearson's product-moment correlation, Wilcoxon's signed-ranks test, a paired *t*-test, Student's *t*-test, or Mann-Whitney's U test. $P < 0.05$ was considered statistically significant.

Table I. The Bristol Stool Form Scale.

Type	Form of human faeces	
1		separate hard lumps, like nuts (hard to pass)
2		sausage-shaped but lumpy
3		like a sausage but with cracks on its surface
4		like a sausage or snake, smooth and soft
5		soft blobs with clear-cut edges (passed easily)
6		fluffy pieces with ragged edges, a mushy stool
7		watery, no solid pieces, entirely liquid

Table II. The Japanese Version of the Constipation Assessment Scale (CAS-J).

Question	Options		
Abdomen appears distended or swollen	0. Never	1. Sometimes	2. Always
Amount of flatus	0. Normal or large	1. Sometimes small	2. Always small
Frequency of stool passag	0. Normal or high	1. Low	2. Very low
Rectum appears filled with feces	0. Never	1. Sometimes	2. Always
Pain at the anus during defecation	0. Never	1. Sometimes	2. Always
Amount of stool	0. Normal or large	1. Small	2. Very small
Easiness of defecation	0. Easy	1. Sometimes difficult	2. Always difficult
Diarrhea or watery stools	0. Never	1. Sometimes	2. Always

RESULTS

Participant characteristics

There were no marked differences in sex in our population (Table III). The average age was 64.6 ± 3.6 years old for male and 63.0 ± 1.8 years old for female, and the median HD period was 48.9 ± 14.2 months for male and 54.9 ± 12.9 months for female.

Table III. Characteristics of the study participants.

	Male	Female	Total
n	8	7	15
age (years)	64.6 ± 3.6	63.0 ± 1.8	63.9 ± 2.1
duration of hemodialysis	48.9 ± 14.2	54.9 ± 12.9	51.7 ± 9.4
primary diseases			
diabetic nephropathy	4	4	8
chronic glomerulonephritis	2	1	3
nephrosclerosis	1	0	1
lecithin cholesterol acyltransferase deficiency	0	1	1
antineutrophil cytoplasmic antibody associated vasculitis	0	1	1
unknown	1	0	1

Mean ± SEM

Blood biochemical tests

There were no significant differences regarding blood biochemistry after prebiotics ingestion compared with before (Table IV). The renal function values, such as the creatinine and BUN, and electrolyte levels also remained relatively unchanged. Total cholesterol and triglyceride were measured only before ingestion, and those were within the normal level, 4.64± 0.84 and 1.48 ± 0.22 mmol/L, respectively.

Defecation status

Based on the Bristol Stool Form Scale, some subjects showed extremely disordered properties, such as type 1, type 2, type 6, or type 7 stool, before ingestion (Figure 1A). Prebiotics improved the stool form in most subjects to type 3, type 4, and type 5, from 60.0% to 66.7%, indicating significant improvement in constipation.

The severity of constipation was measured using the CAS-J, and the score decreased from 5.1 to 3.0 ($p < 0.05$, Figure 1B). Table V shows the results for each of the eight descriptors, with “Abdomen appears distended or swollen” and “Rectum appears filled with feces” showing improvement ($p < 0.01$-0.05). These results were more pronounced in male than in female.

The satisfaction with prebiotics ingestion was evaluated by a VAS (Figure 1C). The score increased from 5.77 ± 0.62 cm to 7.31 ± 0.56 cm ($p < 0.05$) after ingestion.

Table IV. Effects of prebiotics ingestion on laboratory test findings in hemodialysis patients.

	2 weeks before ingestion	2 weeks after ingestion	4 weeks after ingestion	2 weeks after the end of ingestion
Glucose (mmol/L)	7.82 ± 0.96	7.56 ± 0.85		6.91 ± 0.44
Total Protein (g/L)	66.21 ± 1.73			66.57 ± 2.02
Albumin (g/L)	36.53 ± 0.97	36.40 ± 0.61	36.64 ± 0.69	37.29 ± 0.75
Total Bilirubin (μmol/L)	5.93 ± 0.97			6.11 ± 1.28
ALP (U/L)	289.00 ± 45.65	321.53 ± 52.99		285.71 ± 43.52
AST (U/L)	13.20 ± 1.57	14.73 ± 2.26		13.00 ± 2.63
ALT (U/L)	12.67 ± 2.52	12.87 ± 2.46		11.57 ± 2.31
γ-GT (U/L)	30.00 ± 8.65	38.47 ± 12.20		35.21 ± 12.63
Creatine kinase (U/L)	76.87 ± 8.09	79.80 ± 8.71		73.64 ± 7.06
BUN (mmol/L)	20.16 ± 1.42	19.59 ± 1.44	23.22 ± 1.55	21.63 ± 1.44
Creatinine (μmol/L)	816.70 ± 50.10	844.10 ± 50.27	853.50 ± 51.97	856.47 ± 57.51
Uric acid (μmol/L)	378.12 ± 30.75			364.95 ± 28.67
Na (mmol/L)	140.13 ± 0.87	139.67 ± 1.01	139.43 ± 0.88	139.57 ± 1.13
K (mmol/L)	4.55 ± 0.25	4.43 ± 0.19	4.78 ± 0.20	4.69 ± 0.17
Cl (mmol/L)	102.93 ± 1.10	102.00 ± 1.08	102.14 ± 1.20	101.93 ± 1.17
Ca (mmol/L)	2.18 ± 0.05	2.14 ± 0.05	2.14 ± 0.05	2.13 ± 0.04
P (mmol/L)	1.54 ± 0.09	1.59 ± 0.14	1.67 ± 0.11	1.64 ± 0.09

Mean ± SEM, repeated measure analysis of variance

ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, γ-glutamyl transferase; BUN, blood urea nitrogen

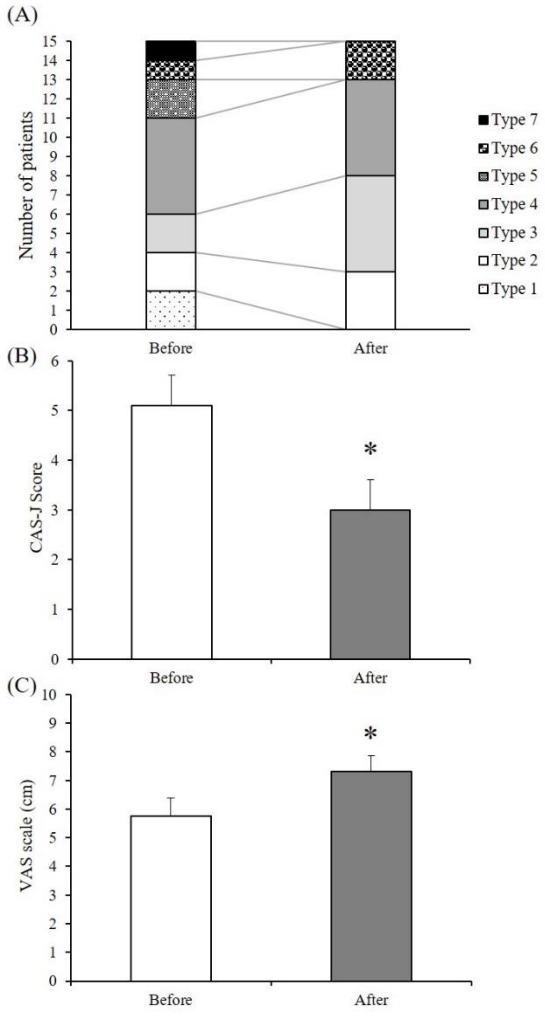

Figure 1. Defecation status before and after the ingestion of prebiotics in hemodialysis patients.
(A) The Bristol Stool Form Scale. Type 1, separate hard lumps, like nuts (hard to pass); Type 2, sausage-shaped but lumpy; Type 3, like a sausage but with cracks on its surface; Type 4, like a sausage or snake, smooth and soft; Type 5, soft blobs with clear-cut edges (passed easily); Type 6, fluffy pieces with ragged edges, a mushy stool; Type 7, watery, no solid pieces, entirely liquid. (B) The Japanese version of the Constipation Assessment Scale (CAS-J) score. The scale consists of eight descriptors for constipation, and "no problems", "some problems", and "severe problems" are scored as 0, 1, and 2, respectively. The subject items are summed to obtain a total score, ranging from 0 to 16, with 16 representing severe constipation. (C) Satisfaction of ingestion by a visual analog scale (VAS) scale.

Table V. Change of percentage in hemodialysis patients who answered 'zero' before and after prebiotic ingestion by The Japanese Version of the Constipation Assessment Scale.

	Male		Female		Total	
	Before	After	Before	After	Before	After
Abdomen appears distended or swollen	42.9	100.0*	33.3	66.7	40.0	90.0 *
Amount of flatus	42.9	42.9	33.3	33.3	40.0	40.0
Frequency of stool passag	57.1	42.9	66.7	66.7	60.0	50.0
Rectum appears filled with feces	42.9	100.0*	33.3	100.0	40.0	100.0**
Pain at the anus during defecation	28.6	57.1	33.3	100.0	30.0	70.0
Amount of stool	57.1	71.4	66.7	66.7	60.0	70.0
Easiness of defecation	14.3	14.3	33.3	66.7	20.0	30.0
Diarrhea or watery stools	57.1	57.1	33.3	66.7	50.0	60.0

* $p < 0.05$, ** $p < 0.01$ vs. before by the paired *t*-test

Fecal microbiota

The composition of the intestinal microbiota changed over the course of the study (Table VI). Since the intestinal microbiota differed markedly among individuals, we calculated the "after ingestion to before ingestion" ratio. Prebiotics increased this ratio for *Bifidobacterium*, *Bacteroides*, and *Lactobacillales* to 2.35 ± 0.59, 3.17 ± 1.49, and 1.88 ± 0.48, respectively ($p < 0.05$). In contrast, prebiotics significantly decreased the ratio of *Clostridium* clusters XVIII to 0.54 ± 0.19 ($p < 0.05$). Furthermore, the ratio of SCFA-producing *Clostridium* clusters IV, IX, and XI showed an increasing trend after prebiotics ingestion. There were no marked gender differences in our population.

Based on the results of the T-RFLP analysis, a dendrogram was constructed (Figure 2). In 8 participants (53.3% subjects), the clusters of intestinal microbiota drastically changed after ingestion, although no marked changes were seen in the other 7 participants. Interestingly, the distribution pattern in 5 participants (33.3%; subjects 3, 8, 9, 12, and 15) significantly varied from one cluster to another one after prebiotics ingestion.

Table VI. Composition of fecal microbiota using T-RFLP after/before ratio of prebiotics in hemodialysis patients.

	Male	Female	Total
Bifidobacterium	2.74 ± 0.98	1.89 ± 0.61	2.35 ± 0.59 *
Bacteroides	1.85 ± 0.60	4.69 ± 3.15	3.17 ± 1.49 *
Clostridium cluster IV	1.08 ± 0.43	1.27 ± 0.37	1.17 ± 0.28
Clostridium subcluster XIVa	0.80 ± 0.12	1.17 ± 0.18	0.97 ± 0.11
Clostridium cluster IX	0.91 ± 0.62	1.66 ± 0.86	1.24 ± 0.50
Clostridium cluster XI	1.26 ± 0.87	1.02 ± 0.42	1.14 ± 0.43
Clostridium cluster XVIII	0.83 ± 0.32	0.25 ± 0.14	0.54 ± 0.19 *
Lactobacillales	2.61 ± 0.74	0.91 ± 0.16	1.88 ± 0.48 *
Enterobacteriales	0.84 ± 0.18	1.01 ± 0.26	0.92 ± 0.16
Others	1.27 ± 0.07	1.30 ± 0.39	1.28 ± 0.18

Mean ± SEM (after/before ratio), * $p < 0.05$ vs. before by the paired *t*-test
The bacterial family abundance was calculated as the percent of the peak area to the total area of each OTU.

Fecal SCFA concentrations

The total SCFA concentrations significantly increased 1.58-fold ($p < 0.05$, Table VII). The major SCFAs of acetic acid, propionic acid, and butyric acid increased more than 1.5-fold, especially in male. Caproic acid was not detected.

Table VII. Fecal short-chain fatty acid concentrations after/before ratio of prebiotics in hemodialysis patients.

	Male	Female	Total
Total short-chain fatty acids	1.89 ± 0.35	1.23 ± 0.07	1.58 ± 0.21*
Acetic acid	1.74 ± 0.25	1.35 ± 0.14	1.56 ± 0.15
Propionic acid	2.11 ± 0.51	1.05 ± 0.13	1.62 ± 0.30
Butyric acid	2.55 ± 0.60	1.22 ± 0.17	1.93 ± 0.37
Isobutyric acid	2.50 ± 1.40	1.35 ± 0.26	2.00 ± 0.80
Valeric acid	2.32 ± 0.89	1.26 ± 0.27	2.02 ± 0.60
Isovaleric acid	3.16 ± 2.12	1.34 ± 0.31	2.38 ± 1.21

Mean ± SEM (μmol/g of feces), * $p < 0.05$ vs. before by the paired *t*-test

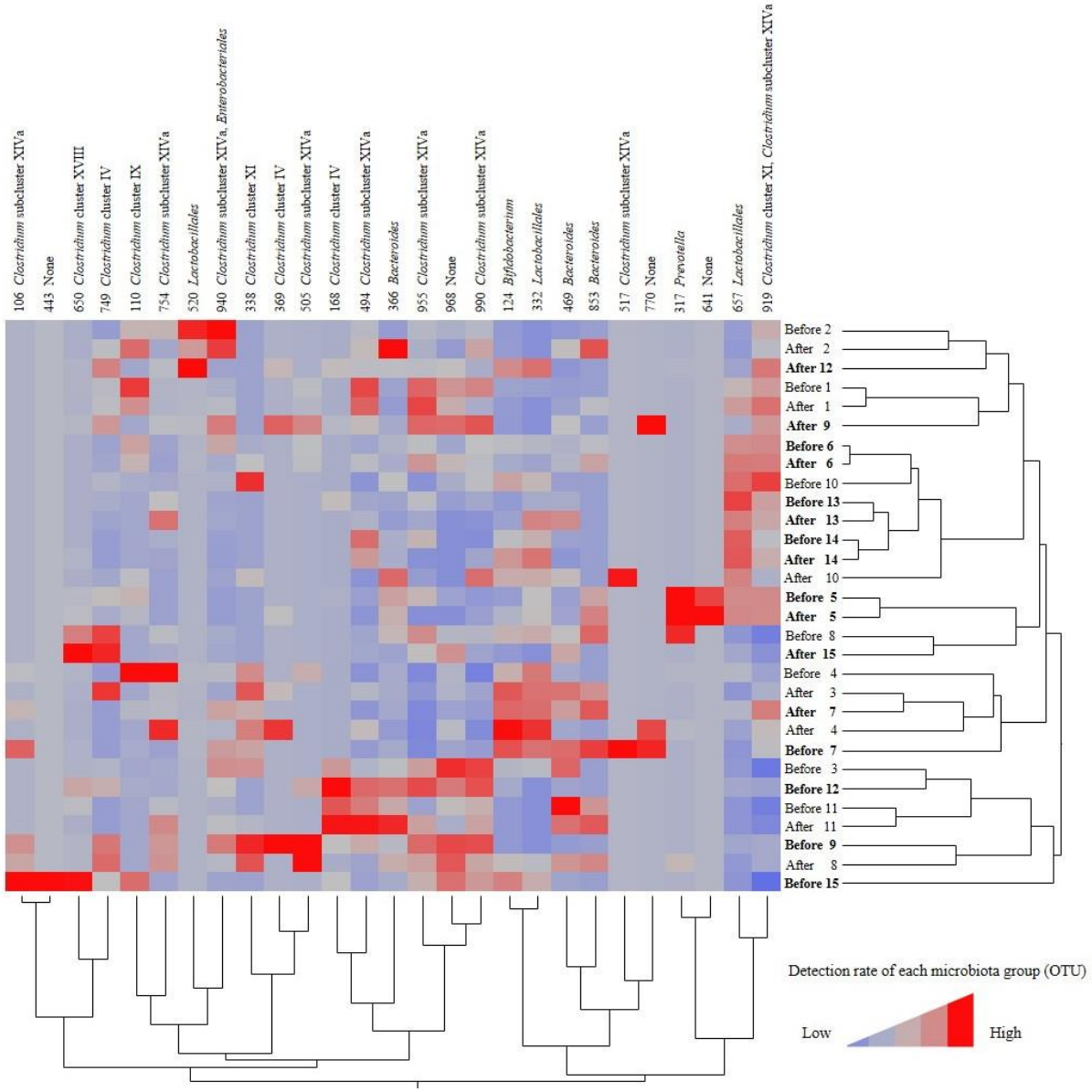

Figure 2. A dendrogram with a heatmap of the intestinal microbiota distribution before and after the ingestion of prebiotics in hemodialysis patients.
The red part shows a high proportion of operational taxonomic units (OTUs). Bold, Male.

We next evaluated the correlation coefficient between fecal microbiota and SCFAs. After ingestion, total SCFAs as well as acetic acid and propionic acid showed a positive correlation with *Bifidobacterium* ($p < 0.01$, Table VIII). Acetic acid was negatively correlated with *Clostridium* cluster XIVa ($p < 0.01$).

Table VIII. Correlations between fecal microbiota and fecal short-change fatty organic acids in hemodialysis patients.

	correlation coefficient (r)	*p* -value
Bifidobacterium – total short-chain fatty acid	0.727	0.005
Bifidobacterium – acetic acid	0.747	0.003
Bifidobacterium – propionic acid	0.724	0.005
Clostridium subcluster XIVa – acetic acid	-0.645	0.009

Pearson's correlation coefficient test using After/Before Ratio

DISCUSSION

The primary aim of the present study was to evaluate the effects of prebiotics on constipation in HD patients. Ingestion of prebiotics for four weeks considerably improved constipation based on the stool form and CAS-J score and altered the composition of the intestinal microbiota in HD patients. The total amount of fecal SCFAs produced by intestinal microbiota also increased. Prebiotics influenced the intestinal environment and changed it into different clusters on the dendrogram by T-RFLP. Only a few studies have used the T-RFLP method for analyses in HD patients, although this approach is a high-throughput analysis and superior to culture-based methods. To our knowledge, this is the first report of the simultaneous evaluation of constipation, the intestinal microbiota by a T-RFLP analysis, and the fecal SCFA concentration after the administration of prebiotics to HD patients.

Regarding the baseline microbiota in healthy individuals as assessed by a T-RFLP analysis, previous studies have reported that the amounts of *Bifidobacterium* and *Enterobacteriales* were 8.0% and 10.1%, respectively (5). In our HD patients, the amounts of *Bifidobacterium* and *Enterobacteriales* according to a T-RFLP analysis were 9.3% and 10.3%, respectively. Compared with the results obtained using the T-RFLP method, the levels of *Bifidobacterium* and *Enterobacteriales* as well as aerobic bacteria were not markedly changed in HD patients. This also differed from the results of a previous analysis in HD patients and functional constipation patients using the conventional culture method of microbiota, which found decreased *Bifidobacterium* and increased *Enterobacteriales* (3, 11, 14). The difference in the distribution of microbiota in HD patients from other patients might be influenced by the method of assessing the microbiota, as a T-RFLP analysis is superior to conventional culture-based methods and has a high throughput and scientific reproducibility.

Soluble dietary fiber increases microbiota, such as *Bifidobacterium* and *Lactobacillus*, which contribute to improved human health (21). In the present study, prebiotics with PHGG increased *Bifidobacterium*, *Bacteroides*, and *Lactobacillales*, and the distribution patterns changed after ingestion. Since the flora changes with age, constipation flora results are inconsistent. However, in adults with functional constipation, including chronic constipation, the *Bifidobacterium* and *Bacteroides* levels are reduced (14). Taken together, the present findings suggest that prebiotics might improve the stool form and subsequent constipation due to compositional changes in the intestinal microbiota.

Bifidobacterium; *Clostridium* clusters IV, IX, and XI; and the *Bacteroides fragilis* group produce SCFAs, and dietary fiber stimulates their growth (20). In the present study, the ingestion of prebiotics also increased the ratio of those microbiota and the fecal concentrations of total SCFAs and showed a positive correlation between *Bifidobacterium* and SCFAs. Acetic acid has been shown to be positively correlated with moisture (24). Gargari et al. reported that the fecal *Clostridiales* distribution and SCFAs reflect bowel habits in irritable bowel syndrome (IBS), and fecal SCFA concentrations were found to be negatively correlated with the colon transit time. In brief, IBS of constipation type is characterized by decreased fecal levels of SCFAs (4). Taken together, these present and previous findings suggest that an increase in SCFAs followed by an increase in SCFA-producing microbiota can improve the stool form and constipation in HD patients.

Wang et al. reported that *Bifidobacterium adolescentis* or oligosaccharides relieved constipation by increasing the fecal SCFA concentration in an experimental mouse model (22, 23). Increased SCFAs in the intestine leads to acidification in the intestinal tract, suppression of the growth of aerobes, and decreased production of intestinal putrefactive products (11). SCFAs act on not only the intestinal mucus but also the liver, nerves, and immune cells and are considered to play an important role in cancer, inflammation, diabetes, obesity, and other ailments (6). Therefore, the ingestion of prebiotics in HD patients may exert beneficial effects on the whole body.

Prebiotics improved the severity of constipation, changed composition of microbiota, and increased concentration of SCFA. In the present study, these results were more pronounced in male than in female but not significant. Since sexual maturation and sexual hormones are considered as major determinants of the differences in the intestinal microbiota structure between the sexes (19), gender difference might influence on the effect of prebiotics.

Our study is associated with several major limitations. Prebiotics improved both the defecation status and intestinal environment, such as the composition of microbiota and SCFA concentration, but the mechanism underlying how microbiota change the stool form is still unknown. Dietary fiber decrease blood cholesterol directly or through modification of intestinal microbiota, but several reports have been shown that prebiotics had insignificant effects on lipid profiles (15). Since we did not measure the concentration of cholesterol after ingestion of prebiotics in this study, effects of PHGG on lipid profile is still unknown. Probiotics, beneficial bacteria, have been reported to increase *Bifidobacterium* in HD patients (11). However, there are few reports that simultaneously analyzed both composition of intestinal microbiota, particularly by T-RFLP analysis and stool form in HD patients. Probiotics, beneficial bacteria, have been reported to increase *Bifidobacterium* in HD patients (11). However, there are few reports that simultaneously analyzed both composition of intestinal microbiota, particularly by T-RFLP analysis and stool form in HD patients. Since synbiotics is a combination of probiotics and prebiotics, it is possible

that synbiotics may change the intestinal microbiota and cause the improvement of the defecation status compared with prebiotics alone. Further studies will be necessary to clarify those point.

We concluded that prebiotics with PHGG improved constipation by increasing the proportions of *Lactobacillales* and SCFA-producing *Bifidobacterium*, *Bacteroides*, and *Clostridium* (IV, IX, XI), which subsequently increased the SCFA levels in HD patients. Improvement of the stool form by prebiotics will help relieve constipation, thus improving the quality of life in HD patients.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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