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Decrease in *Escherichia-Shigella* in the gut microbiota of ESKD patients undergoing maintenance hemodialysis

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Abstract

Background Gut dysbiosis is thought to be involved in the pathogenesis and progression of chronic kidney disease and end-stage kidney disease (ESKD). However, differences in the composition and function of gut microbiota in hemodialysis patients are not consistently concluded.

Methods A total of 20 patients receiving maintenance hemodialysis (MHD) treatment at the Blood Purification Center of Bethune International Peace Hospital from March 2021 to December 2022 were included based on the inclusion criteria. Additionally, 20 healthy volunteers matched for age, gender, and body mass index were recruited from the Health Examination Center as the healthy control (HC) group. The structure of the gut microbiota community in the study subjects was analyzed using second-generation high-throughput sequencing technology based on 16S rRNA and amplicon sequence variants (ASV) analysis.

Results There were significant differences in gut microbial communities between the two groups. At the genus level, significant differences were found in 19 genera. Among them, *Escherichia-Shigella*, *Lachnospira*, *Parasutterella*, *[Ruminococcus]-torques-group*, *Butyricicoccus*, and *Streptococcus* were significantly decreased, while *Phascolarctobacterium*, *Ruminococcaceae-UBA1819*, *Erysipelotrichaceae-UCG-003*, *Flavonifractor*, and *Erysipelatoclostridium* were significantly increased in MHD patients. In particular, the abnormal decrease in the abundance of *p-Proteobacteria.c-Gammaproteobacteria.o-Enterobacterales.f-Enterobacteriaceae.g-Escherichia-Shigella* might be a significant characteristic of gut microbiota in MHD patients.

Conclusion The decreased abundance of *Escherichia-Shigella* is a signature gut microbiota alteration in patients with ESKD undergoing MHD, and *Escherichia-Shigella* may represent a key bacterial group warranting exploration in the field of hemodialysis. The dysbiosis of gut microbiota holds promise as a therapeutic target and biomarker for the diagnosis and treatment of MHD.

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Keywords Maintenance hemodialysis, End-stage kidney disease, Chronic kidney disease, Gut microbiota, Escherichia-Shigella

Introduction

Chronic kidney disease (CKD) has become a prominent global public health issue. With the increasing number of patients with end-stage kidney disease (ESKD) and the rapid aging progress, as well as the quality and rate of dialysis treatment have been continuously improving, the number of dialysis patients sustained increased at a rate of 10–13% per year [1, 2]. The high mortality rate among maintenance hemodialysis (MHD) patients is mainly associated with factors such as advanced age, cardiovascular disease, diabetes, malnutrition, inflammation, and uremic solute accumulation [3]. Therefore, it is urgent to understand the effects of various types of dialysis on the body and improve treatment methods to reduce complications and mortality rates.

The human gut microbiota is composed of various bacteria, fungi, viruses, and other microorganisms, which exhibit individual variations and maintain relative stability under steady-state conditions. These gut microbiotas play important roles in various aspects of health, including digestive system function, immune system regulation, and nutrient metabolism [4]. Gut dysbiosis and changes in its metabolites may lead to an imbalance between immune response and immune tolerance, producing autoantibodies and inflammatory factors, which lead to the occurrence and development of various types of CKD [5]. Meanwhile, dyspepsia and gastrointestinal dysfunction are common complications in patients with ESKD and various renal replacement therapy [6, 7], and changes in the gut microbiota of hemodialysis patients are related to inflammation and renal function changes [8, 9]. Furthermore, studies have demonstrated that dialysis can effectively eliminate excess water from the body and maintain electrolyte balance as well as acid-base homeostasis, thereby contributing to the improvement of intestinal ecological disorders [10]. Longitudinal studies have also shown that Succinivibrio and Anaerostipes were associated with an increased risk of death in hemodialysis patients [11]. Therefore, gut microbiota may be a key factor affecting the symptoms and prognosis of MHD patients. Recent research has focused on the differences in the composition and function of the gut microbiota between hemodialysis patients and healthy groups, revealing alterations in *Enterobacteriaceae* and *Bifidobacteriaceae*, as well as changes in functions related to carbohydrate metabolism, amino acid metabolism, energy metabolism, translation, and membrane transport [8, 12–14]. However, these results are inconsistent. It is still unclear about the specific changes in the gut microbiota of MHD patients and whether these changes are associated with adverse outcomes.

In this study, we explored the characteristics of changes in gut microbiota in MHD patients using 16S rRNA sequencing technology. We compared the changes in fecal microbiota between the MHD patient group and the healthy group, and analyzed the correlation between changes in gut microbiota and clinical parameters, to depict the characteristics of gut microbiota in MHD patients and investigate the impact of gut microbiota changes on the disease condition of patients.

Materials and methods

The study design was carried out according to the PRoBE concept for prospective specimen collection and retrospective blinded evaluation [15], and approved by the Ethics Committee of Bethune International Peace Hospital (2022-KY-05). Informed consent for this study was obtained from all participants, who provided written informed consent that met the study requirements.

Study subjects

Patients undergoing MHD at the Renal Disease Department Blood Purification Center of the Bethune International Peace Hospital from March 2021 to December 2022 were selected as the study subjects. All patients had vascular access established via a forearm arteriovenous fistula. Inclusion criteria were as follows: (1) adults aged ≥ 18 years; (2) receiving regular hemodialysis for at least 6 months (3 times/week, 4 h/session), with no adjustments in the hemodialysis mode, dialysate, or dialyzer model in the past month; (3) no intake of prebiotics, probiotics, or other interventions for modulating gut microbiota within the past 3 months. The exclusion criteria were: (1) the presence of gastrointestinal diseases, urinary tract stones, acute infections, etc., in the clinical setting; (2) a recent history of antibiotic use, immunosuppressants, proton pump inhibitors, or chemotherapy drugs (< 3 months); (3) inability to eat normally or requiring enteral or parenteral nutrition intervention; (4) other situations deemed unsuitable for inclusion by the researchers.

Healthy subjects in the physical examination center of Xijing Hospital were selected as the control group. The inclusion criteria were: (1) age, gender, and body mass index matched to the patient group; (2) willingness to participate in the study and signed informed consent. Exclusion criteria were: (1) a current or past history of physical or mental illnesses; (2) a recent history of

antibiotic use, probiotics, or proton pump inhibitors for treatment (< 3 months).

The general demographic data and clinical characteristics of the enrolled population were recorded, including gender, age, height, weight, blood pressure, medical history, biochemical indicators, and treatment. The above indicators were extracted from the patient's electronic medical record system.

Sample collection

Following the concept that fecal microbiota represents the gut microbiota, the study subjects were instructed to collect their first bowel movement of the day after emptying their urine. The stool was collected in a clean bedpan, and a disposable sterile plastic spoon was used to obtain a sample from the middle of the stool (approximately 0.5–2 g per tube). Upon completion of the collection, the patient immediately placed the fecal sample in an insulated container with ice packs. Thereafter, it was transported by staff to a freezer maintained at -80 °C.

16S rRNA sequencing

High-throughput sequencing technology was used for DNA extraction and genomic sequencing of the fecal samples. The V3-V4 region of the 16S rRNA gene was amplified for sequencing (sequencing platform: Illumina Miseq). The upstream primer was 341 F (5'-CCTACGGGNGGCWGCAG-3'), and the downstream primer was 805R (5'-GACTACHVGGGTATCTAATCC-3') [16]. The specific process included sample freezing and aliquoting, DNA extraction and quality control, PCR amplification and product purification, library preparation and quality control, Illumina Miseq sequencing, and raw data quality control and statistics. After DNA extraction from the samples, the concentration of DNA was measured using a Nanodrop instrument. The quality of the DNA was assessed through the ratios of A260/A280 and A260/A230. The integrity of the DNA was observed via agarose gel electrophoresis. Additionally, negative controls were included during both the DNA extraction and PCR amplification stages to assess whether the sample DNA and PCR products were contaminated by environmental bacteria during the procedural steps, thereby guaranteeing that the sequencing data obtained were indeed derived from the original bacterial genome present in the samples.

Bioinformatics analysis

The DADA2 algorithm was used to identify amplicon sequence variants (ASVs). The representative sequences of each ASV were annotated using the SILVA reference database. QIIME feature classifier was used for species annotation. Bray-Curtis, weighted UniFrac, unweighted UniFrac, and Jaccard-binary dissimilarity were calculated in QIIME. Principal coordinate analysis (PCoA) plots and permutational multivariate analysis of variance (PERMANOVA) were generated using the vegan 2.5-7 package in R (version 3.6.0) to test for statistical significance between groups, using 10,000 permutations. Linear discriminant analysis effect size (LEfSe) was used to detect taxonomic groups with differential abundance between groups (LEfSe 1.1, https://github.com/SegataLab/lefse). The 'heatmap' package in R was used to generate heatmaps of key ASVs identified by the random forest model. Based on the 16S rRNA gene sequences, PICRUSt2 v2.4.1 (https://github.com/picrust/picrust2/wiki) was used to predict functional abundances in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database [17]. Spearman's rank correlation analysis was used to assess the correlation between ASVs and clinical characteristics of MHD patients. The explanations of relevant terminology are provided in Table S1.

Statistical methods

Count data were described using composition ratios or rates, and measurement data were described using means, standard deviations, maximum values, minimum values, or quartiles. Non-parametric Mann-Whitney U test was used for comparisons between the two groups. Non-parametric Kruskal-Wallis test was used for comparisons among multiple groups. Non-normally distributed data were described using maximum values, minimum values, medians, and quartiles. A P-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 25.0 (IBM) and R (version 3.6.0).

Results

Baseline clinical characteristics of patients with MHD and healthy control (HC)

A total of 20 MHD patients and 20 HCs were enrolled in this study. The baseline clinical characteristics of the two groups are shown in Table 1. The duration of dialysis was 47.7 (31.0, 128.2) months. Among MHD patients, we observed 5 cases with renal anemia, 5 cases with diabetes, 7 cases with hypertension, and 17 cases with hyperphosphatemia. Among them, constipation symptoms were present in 5 patients, while alternating constipation and diarrhea were observed in 2 patients. MHD patients had significantly higher systolic blood pressure (SBP) compared to HC, while significantly lower levels of hemoglobin (HB), platelets, and serum albumin (ALB). Serum creatinine (Scr) levels in MHD patients reached 1142.5 (911.3, 1218.8) umol/L, parathyroid hormone levels were 337 (158, 543) pg/ml, and blood phosphorus was 2.2 ± 0.5 mmol/L. There were no statistically significant differences in age, gender, body mass index, or white blood cell count between the two groups.

Table 1 Demographic and clinical characteristics of the participants

Characteristic	MHD patients ($n=20$)	Healthy control ($n=20$)	*P* value
Age, year	49.3 ± 10.3	50.1 ± 10.4	0.797
Dialysis months	47.7 (31.0, 128.2)	-	
Hypertension, *n* (%)	7(35)	0(0)	<0.001
Diabetes, *n* (%)	5 (25)	0 (0)	<0.001
Renal anemia, *n* (%)	15(75)	0(0)	<0.001
Hyperphosphatemia, *n* (%)	17(85)	0(0)	<0.001
Male, *n* (%)	17 (85)	17 (85)	1.0
BMI, kg/m^2	22.1 ± 2.3	23.4 ± 1.7	0.056
SBP, mmHg	134(125, 143)	121 (118.125)	0.002
WBC, $10^9/L$	5.78 ± 1.60	6.19 ± 1.39	0.390
Hb, g/L	112.7 ± 14.1	144.6 ± 14.1	<0.001
PLT, $10^9/L$	188.5 ± 69.6	253.4 ± 65.4	0.004
TP, g/L	70.7 (65.9, 74.2)	73.3 (72.3, 76)	0.044
Albumin, g/L	40.9 ± 2.5	46.4 ± 3.5	<0.001
Serum Creatinine, umol/L	1142.5 (911.3,1218.8)	64.0 (58.0,81.5)	<0.001
UA, umol/L	437.5 ± 86.2	364.3 ± 66.2	0.005
BUN, mmol/L	26.9 (20.4, 32.2)	5.3 (4.0, 6.2)	<0.001
CO_2CP, mmol/L	20.8 ± 3.6	-	
PTH, pg/ml	337(158, 543)	-	
P, mmol/L	2.2 ± 0.5	-	
Ca, mmol/L	2.3 ± 0.2	-	

Abbreviations: Values are presented as the mean ± standard deviation, median (interquartile range) or *n* (%). MHD, maintenance hemodialysis; BMI, body mass index; SBP, systolic blood pressure; WBC, white blood cell; Hb, hemoglobin; PLT, platelet; TP, total albumin; UA, uric acid; BUN, blood urea nitrogen; CO_2CP, binding force of carbon dioxide; PTH, parathyroid hormone; P, serum phosphorus; Ca, serum calcium

The quality of sequencing data and changes in gut microbiota diversity

The rarefaction curve shows the number of ASVs in each sample at different sequencing depths(Fig. 1A). When the curve approaches a plateau, it indicates that the sequencing data is sufficiently large to reflect the majority of microbial information in the sample. According to the Venn diagram, there were a total of 491 ASVs shared between the two groups, with 87 ASVs unique to MHD and 107 ASVs unique to HC (Fig. 1B). Community richness was measured by Chao1 and ACE indices, and diversity was measured by Shannon and Simpson indices. There was no significant difference in community richness between the two groups, but the Shannon index was significantly higher in MHD than in HC (Fig. 1C, D). The gut microbiota communities of MHD patients and HCwere clustered separately (Fig. 1E), and the difference between the two groups was significantly greater than the difference within each group based on Analysis of Similarities (ANOSIM) analysis using Jaccard binary distance (Fig. 1F, $R=0.111$, $P=0.0007$).

Differently abundant taxon of gut microbiota at the phylum and genus levels in MHD patients

Based on ASVs classification, the relative abundance of gut microbiota at different taxonomic levels (phylum, class, order, family, genus) was identified in the 40 samples. The composition of gut microbiota at the phylum and genus levels in each group is shown in Fig. 2.

At the phylum level, *Firmicutes*, *Bacteroidota*, and *Proteobacteria* were the three dominant populations in both groups, accounting for an average proportion of 90% of the sequences (Fig. 2A). The F/B ratio was significantly decreased in MHD patients. Compared to the HC group, MHD patients showed a significant decrease in the abundance of *Proteobacteria* and *Patescibacteria* (Fig. 2B).

At the genus level, *Bacteroides*, *Faecalibacterium*, and *Subdoligranulum* were the three dominant populations in MHD patients, while *Bacteroides*, *Faecalibacterium*, and *Prevotella* were the three dominant populations in HC, with each population accounting for more than 5% of the sequences on average (Fig. 2C). Wilcoxon rank test revealed significant differences in the relative abundance of 19 genera between the two groups, as shown in Fig. 2D. Compared to the HCs, MHD patients had significantly lower abundance of *Escherichia-Shigella*, *Lachnospira*, *Parasutterella*, *[Ruminococcus]-torques-group*, *Butyricicoccus*, and *Streptococcus*, while significantly enriched abundance of *Phascolarctobacterium*, *Ruminococcaceae-UBA1819*, *Erysipelotrichaceae-UCG-003*, *Flavonifractor*, and *Erysipelatoclostridium* (Fig. 2D). Among them, *Escherichia-Shigella* was the genus with the highest abundance and significant changes.

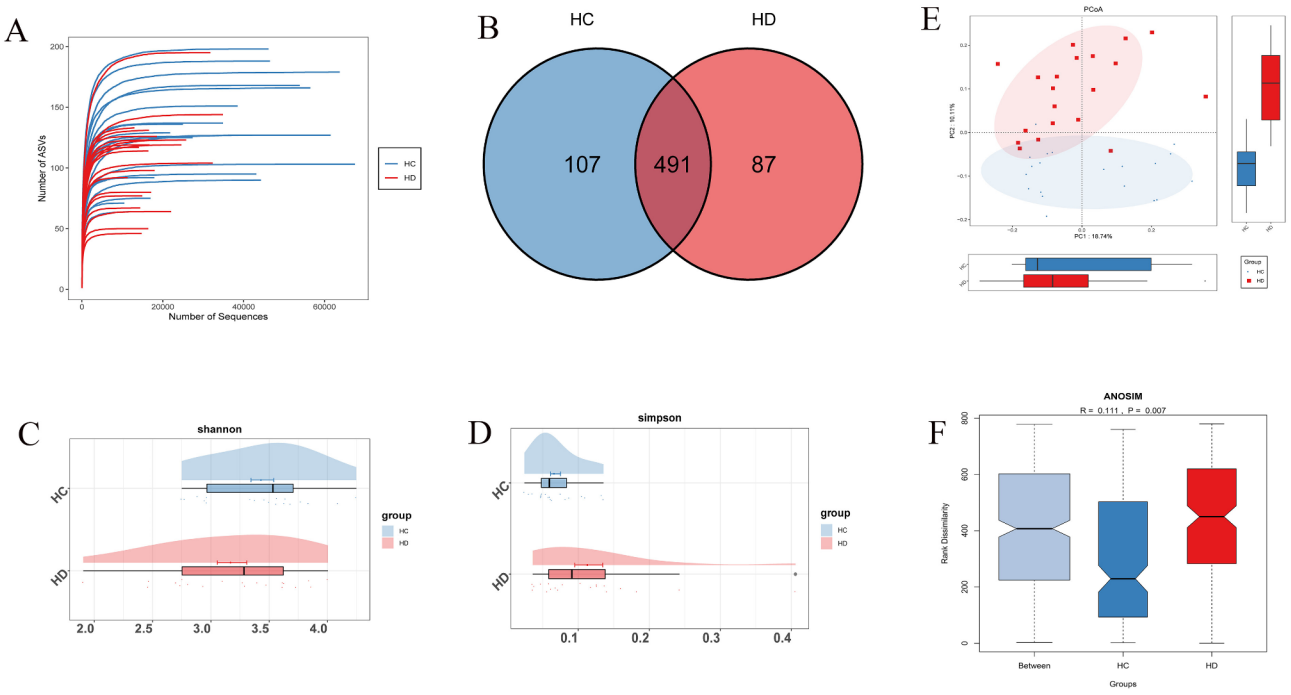

Fig. 1 Quality of sequencing data and diversity of gut microbiota in MHD patients. **(A)** Raresome curve showed the number of ASVs in each sample under different sequencing volumes. **(B)** Venn diagram showing overlapping ASVs between the two groups, 87 of which were specific to MHD. **(C)** Comparison of Shannon index between the two groups. **(D)** Comparison of ACE index of intestinal flora between the two groups. **(E)** Adonis analysis based on Jaccard binary distance. **(F)** ANOSIM analysis based on Jaccard binary distance

Phylogenetic characteristics of gut microbiota in MHD patients

In addition to differences in gut microbiota abundance at different taxonomic levels, LEfSe was used to analyze the gut microbiota to identify specific taxa associated with patients after MHD. The histogram of LDA scores distribution (Fig. 3A) showed that 19 microbial biomarkers distinguished MHD patients from HC (LDA > 3, all $P < 0.05$). Moreover, the evolutionary branching diagram of fecal microbial structure and major bacteria (Fig. 3B) revealed significant changes in the classification of gut microbiota between MHD patients and HC. Specifically, the abnormal decrease in the abundance of *Escherichia-Shigella* within *p-Proteobacteria.c-Gammaproteobacteria.o-Enterobacterales.f-Enterobacteriaceae.g-Escherichia-Shigella*, *Streptococcus* within *p-Firmicutes.c-Bacilli.o-Lactobacillales.f-Streptococcaceae.g-Streptococcus*, and *Haemophilus* within *p-Proteobacteria.c-Gammaproteobacteria.o-Pasteurellales.f-Pasteurellaceae.g-Haemophilus* were the significant characteristics of MHD patients.

Prediction function of gut microbiota in MHD patients

To elucidate the functional and metabolic changes in gut microbiota between MHD patients and HC, PICRUSt2 analysis was used to predict functional changes based on 16S rRNA gene sequences (Fig. 4). The results showed that compared to HC, MHD patients exhibited functional enrichment in multiple KEGG pathways, including inositol phosphate metabolism, TCA cycle, amino sugar and nucleotide sugar metabolism, N-glycan biosynthesis, glycosaminoglycan degradation, flavonoid biosynthesis, pentose and glucuronate interconversions, valine leucine and isoleucine degradation, pyruvate metabolism, steroid hormone biosynthesis, lipoic acid metabolism, and other glycan degradation. On the other hand, Shigellosis, tryptophan metabolism, purine metabolism, bacterial invasion of epithelial cells, and RNA transport were significantly decreased in MHD patients compared to HC.

Correlation between differently abundant ASVs and clinical features of MHD

The Spearman correlation analysis was used to investigate the correlation between ASVs and clinical features of MHD patients. The diagonal heatmap displayed the correlation results between gut microbiota and clinical parameters. A total of 21 solid lines showed strong correlations (all $P < 0.01$), which may be the focus of further research (Fig. 5). For example, ASV1 (*Escherichia-Shigella*) was positively correlated with patient ALB and HB, while negatively correlated with Scr. SBP was significantly positively correlated with ASV18 (*Phascolarctobacterium*), while significantly negatively correlated with ASV44 (*Bifidobacterium*). ASV31 (*Bifidobacterium*) was positively correlated with HB and ALB, while negatively correlated with Scr, blood urea nitrogen (BUN), and SBP.

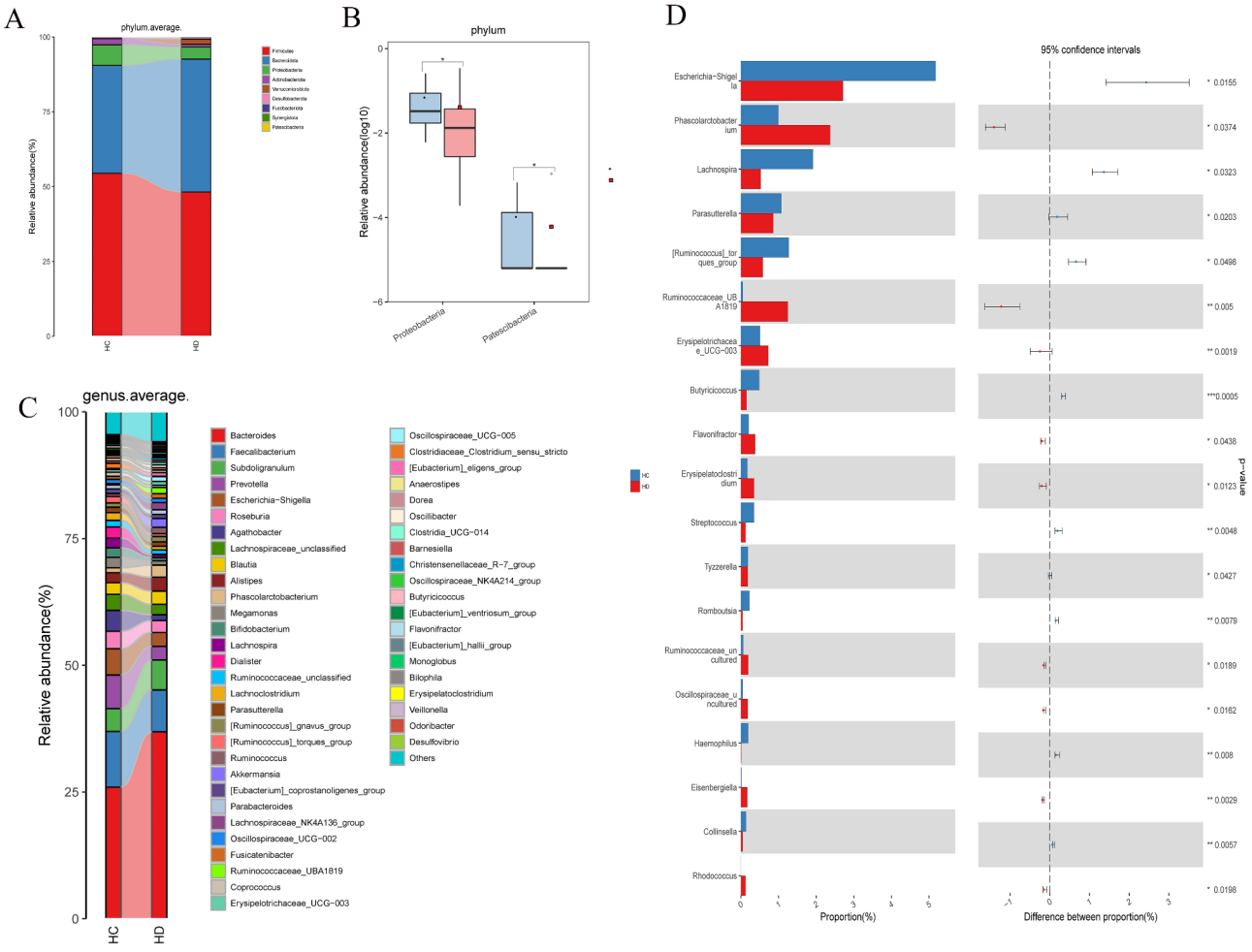

Fig. 2 Composition and comparison of gut microbiota between MHD and HCs. Gut microbiota composition of MHD and HC at phylum (**A** and **B**) and genus **(C)** levels. **(D)** There were significant differences in microbial communities between the two groups at the genus level. *P* values were calculated by the Wilcoxon rank sum test, * $P < 0.05$, ** $P < 0.01$

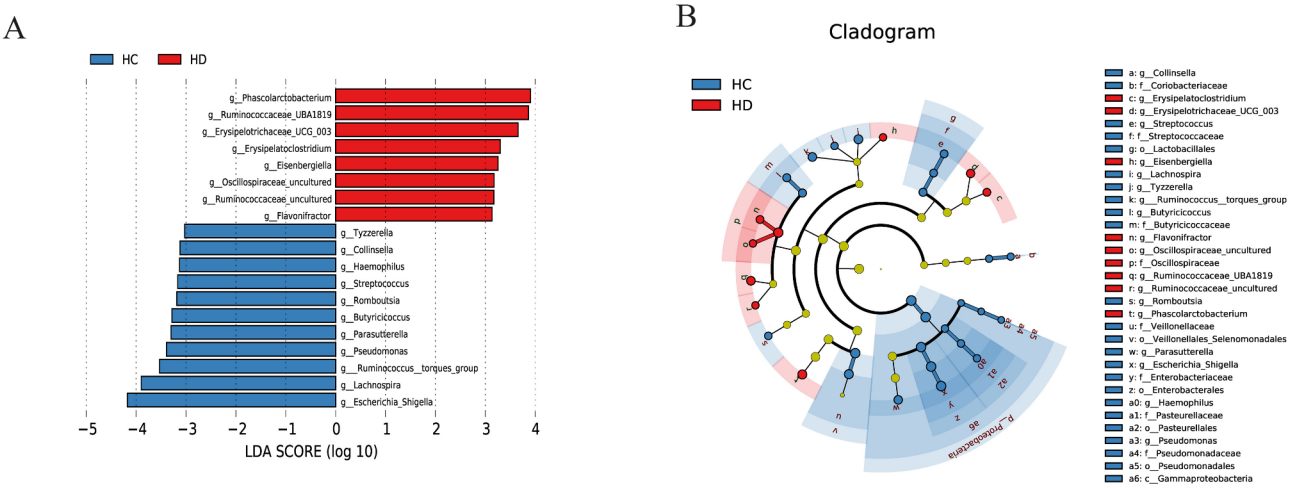

Fig. 3 LEfSe analysis based on ASVs characteristics of gut microbiota. **(A)** Histograms of the LDA scores calculated for the selected taxa show significant differences in microbial types and abundance between MHD(red) and HC(blue). **(B)** LEfSe dendrogram showing the phylogenetic distribution and developmental characteristics of gut microbiota. Red nodes indicate taxa enriched in MHD compared to HC, and blue nodes indicate taxa enriched in HC compared to MHD. p, phylum; c, class; o, order; f, family; g, genus

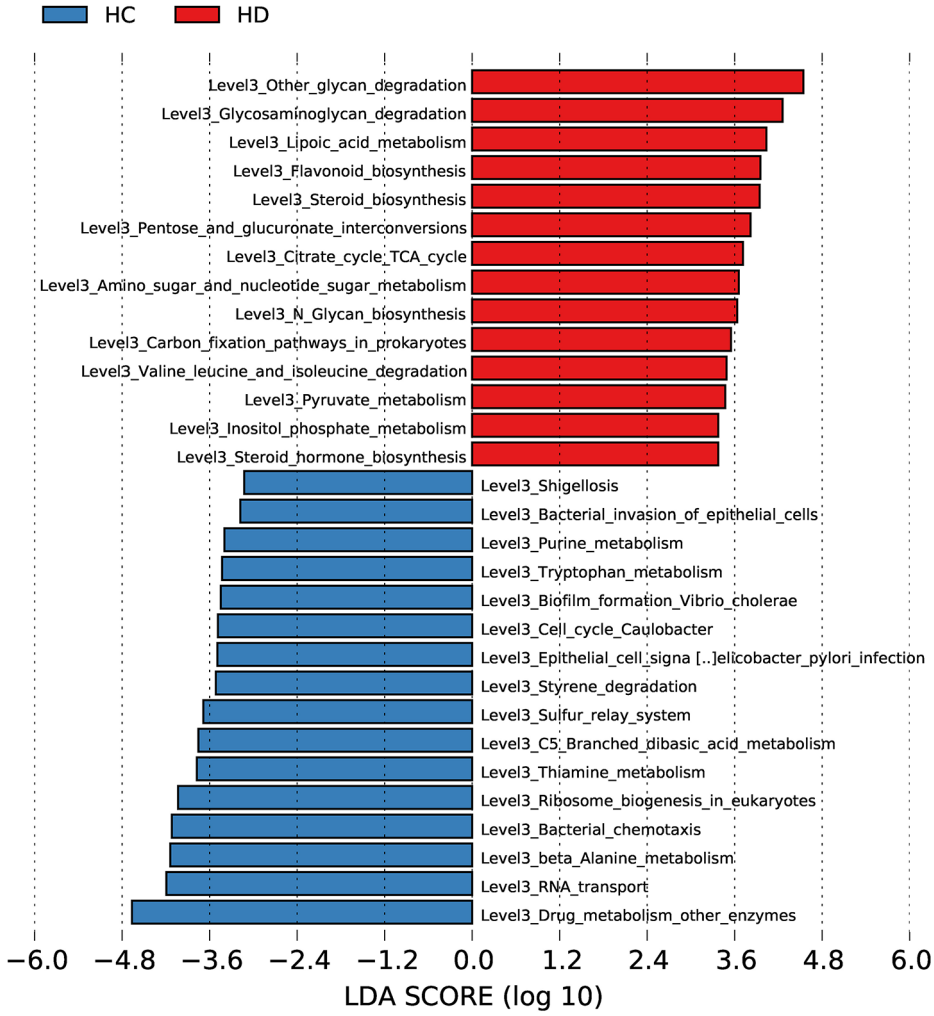

Fig. 4 Prediction of differential functional and metabolic alterations of gut microbiota in MHD patients by PICRUSt2 analysis. Selected KEGG pathways were calculated using LDA score histograms displayed

ASV42 (*Erysipelotrichaceae-UCG-003*) was positively correlated with HB, ALB, and total protein.

Relationship between gut microbiota and complications in MHD

We conducted further subgroup analyses to elucidate the relationships between the characteristics of the gut microbiota in MHD patients and various complications, including renal anemia, hypertension, diabetes, and hyperphosphatemia. Compared with 15 cases of with anemia, *Ruminococcaceae_UBA1819* and *Lachnospiraceae-FCS020-group* were significantly enriched in 5 cases without renal anemia (Fig. 6A). While one bacterial taxon, *p-Bacteroidota.c-Bacteroidia.o-Bacteroidales* was increased in anemia patients (Fig. 6B). In addition, compared with 13 patients without hypertension, 7 patients with hypertensive MHD showed increased abundance of *Roseburia* and *Faecalibacterium*, and decreased abundance of *Romboutsia* and *Phocea* (Fig. 6C). The decrease of *p-Firmicutes.c-Clostridia.o-Peptostreptococcales-Tissierellales.f-Peptostreptococcaceae.g-Romboutsia* in hypertensive patients was noteworthy note (Fig. 6D). Unfortunately, no meaningful bacterial changes were found in the complications of diabetes and hyperphosphatemia.

Discussion

A large number of studies have attempted to determine the specific changes in gut microbial parameters in patients with CKD. However, it is still not possible to obtain the full spectrum of intestinal microbiota from the data. In this study, we observed significant differences in gut microbial community dysbiosis, taxonomic changes, and functional changes between MHD and HCs, and investigated the role of gut microbiota to provide novel insights into the incorporation of gut microbiota in the management of chronic diseases among MHD patients and enhancement of their clinical interventions.

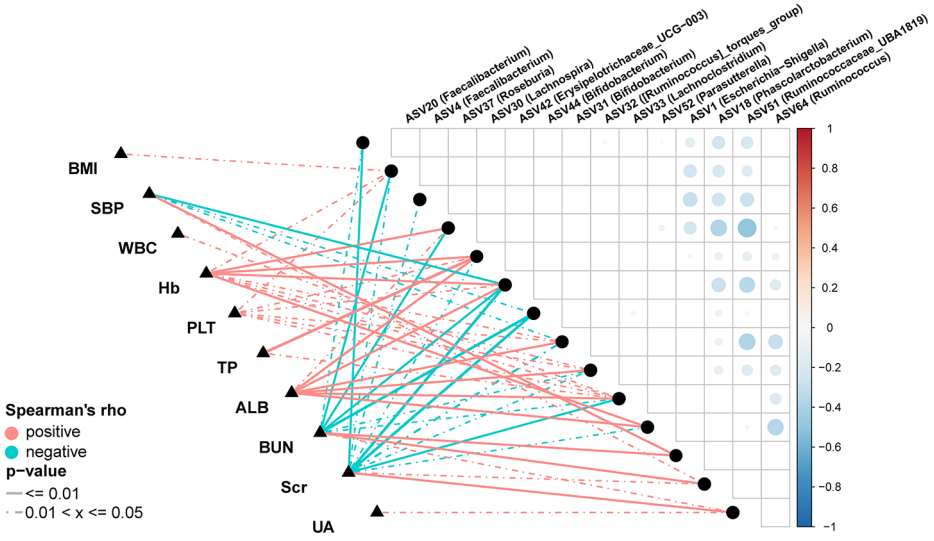

Fig. 5 Spearman rank correlation test was used to analyze the correlation between different ASVs and clinical characteristics in MHD patients. Positive values (red) indicate a positive correlation and negative values (blue) indicate a negative correlation. Solid lines indicate $P \leq 0.01$, and dashed lines indicate $0.01 \leq P \leq 0.05$

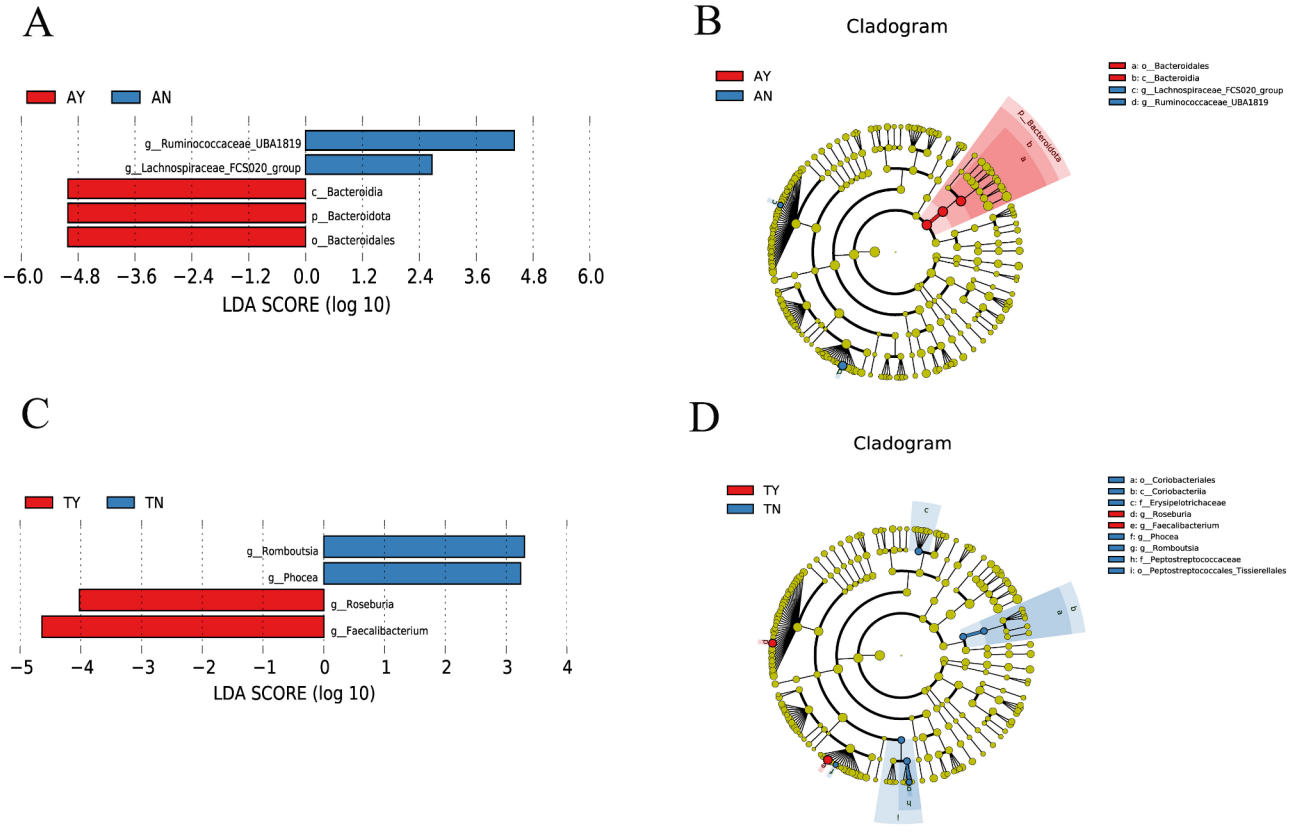

Fig. 6 Association between gut microbiota and complications in MHD. **(A)** Histogram of LDA scores calculated for selected taxa showed significant differences in microbe type and abundance between AY(red) and AN(blue). LDA scores on a log10 scale are indicated at the bottom. The default criteria was LDA > 2.5 and $p < 0.05$. **(B)** LEfSe dendrogram showing the phylogenetic distribution and developmental characteristics of gut microbiota. **(C)** Histogram of LDA scores calculated for selected taxa showed significant differences in microbe type and abundance between TY (red) and TN (blue). LDA scores on a log10 scale are indicated at the bottom. The default criteria was LDA > 3.0 and $P < 0.05$. **(B, D)** LEfSe dendrogram showing the phylogenetic distribution and developmental characteristics of gut microbiota. AY, patients with anemia. AN, patient without anemic. TY, patients with hypertension. TN, patient without hypertension. p, phylum; c, class; o, order; f, family; g, genus

Furthermore, our study provides new evidence of microbiome alterations in CKD.

In our study, the abundance index was increased in MHD patients, but its diversity index was preserved. This is consistent with the results of previous studies [8]. However, peritoneal dialysis may lead to a reduction in gut microbial diversity [18]. In addition, β diversity analysis showed that the differences between the two groups were significantly greater than the within-group differences, further confirming the value of the between-group comparisons. The factors contributing to Intestinal flora disorder in patients with uremia are delineated as follows [19]: Dietary restrictions and eating habit modifications; Antibiotic overutilization; Metabolic acidosis; Impairment of the Intestinal Mucosal Barrier, this alteration in the bacterial habitat facilitates bacterial translocation; Additional factors: such as oral iron therapy. Following hemodialysis, there may be a removal of somatic toxins or shifts in the internal milieu, potentially contributing to a further changes in the gut microbiota. Among the changes in the abundance of gut bacteria in MHD patients, *Escherichia-Shigella* is the most noticeable one. *Escherichia-Shigella* is known to be closely associated with various afflictions such as hypertension, hemorrhagic colitis, septicemia, inflammatory bowel disease, and thrombocytopenia [20], and has recently been found to be abnormally increased in patients with CKD [5]. In IgAN, gut dysbiosis is characterized by significant expansion of the *Escherichia-Shigella* and the imbalance is reversed by successful immunosuppressive therapy [16]. An abnormal increase in abundance was also found in primary and secondary kidney diseases such as membranous nephropathy and diabetic nephropathy [21, 22]. In HD patients, *Escherichia-Shigella* was positively correlated with vascular calcification and was the most contributing bacterial genus [13]. The PhyloChip analysis revealed that the abundance of seven *E. coli* species was abnormally enriched in the gut microbiota of patients with CKD and ESKD [23]. Interestingly, we observed an abnormal decrease in the abundance chain of *p-Proteobacteria.c-Gammaproteobacteria.o-Enterobacterales.f-Enterobacteriaceae.g-Escherichia-Shigella* in MHD patients. This implies that MHD may have reduced *Escherichia-Shigella*. Whether this is related to uremic toxin clearance in the body after hemodialysis needs to be further confirmed, but *Escherichia-Shigella* may be considered to be a key bacterium in the renal disease state.

Escherichia-Shigella, belonging to the *Enterobacteriaceae* family of the *Proteobacteria* phylum, is an extremely diverse single intestinal flora with a wide range of strain utilization niches from beneficial intestinal symbiotic bacteria to obligate extraintestinal pathogens [24]. The results of feature frequency profiles (FFPs) showed that *Escherichia-Shigella* belonged to group B2, and the pathogens in this group may be facultative or opportunistic. Under some environmental conditions, these strains are harmless, while under other conditions they are pathogenic [25]. The expansion of *Escherichia-Shigella* in gut microbiota may be a direct cause of the occurrence and development of CKD. However, there is a lack of literature to verify the causal relationship between *Escherichia-Shigella* and CKD. It is currently believed that the exposure of *Escherichia-Shigella* is associated with the activated nod-like receptors protein 3 (NLRP3) inflammasome that excessively activates mucosal immunity, and the autophosphorylation of double-stranded RNA-dependent protein kinase [26]. The link between *Escherichia-Shigella* overgrowth and activation of the intestinal NLRP3 inflammasome was thought to contribute to the pathogenesis of acute pancreatitis and lung tissue inflammation [26, 27]. Meanwhile, in the acetate-treated bronchopulmonary dysplasia model, the reduction of *Escherichia-Shigella* in the gut was followed by a reduction of NLRP3 [28]. In tuberculous meningitis, a high proportion of intestinal Escherichia coli is associated with increased plasma levels of TNF-a and down-regulates the brain-tight junction protein claudin-5, exacerbating the inflammatory response [29].

Gut microbiota is thought to be the driving force affecting tryptophan metabolism in the gut [30]. In PICRUSt2 analysis, tryptophan metabolism was significantly decreased in MHD patients, which may be directly related to the reduced abundance of *Escherichia-Shigella*. Patients with CKD often show a disorder of tryptophan metabolism, which induces up-regulation of IDO activity to interfere with metabolism and activate AhR, leading to renal fibrosis and aggravation of CKD [31]. Tryptophan metabolism produces various bioactive substances involved in the pathophysiology of CKD, among which indoxyl sulfate (IS) is one of the most important. Studies have shown that the expansion of *Enterobacteriaceae* induced by gut dysbiosis will produce excessive accumulation of IS, leading to the deterioration of renal function and the inability to extilate it from the body [32]. Whereas in *Enterobacteriaceae*, some *Escherichia coli* alleles are highly homologous to tryptophanase (TnaA) and can encode TnaA homologs, which directly affect the expression of IS [23]. Therefore, changes in *Enterobacteriaceae* caused by genus *Escherichia-Shigella* in MHD patients may be the direct cause of altered tryptophan metabolism and affect the overall metabolic environment of patients.

In the correlation analysis between gut microbiota and clinical indicators, *Bifidobacterium* was closely related to not only blood pressure but also uremic toxin, hemoglobin, and other key indicators. Previous evidence proved that a supplement of *Bifidobacterium* was associated with

a lower level of uremic toxins in CKD models [33] and a lower prevalence of obesity [34]. Disturbance of *Bifidobacterium* homeostasis may reduce plasma SCFAs levels and increase nephrotoxic toxins levels [35]. Randomized controlled clinical trials have also shown that *Bifidobacterium* supplementation improves blood pressure control and reduces uremic toxins, symptoms, inflammation, and cardiovascular risk in patients with advanced CKD [36, 37]. Thus, *Bifidobacterium* may help alleviate some clinical indicators in MHD patients.

There are several limitations in our study. Firstly, this is a cross-sectional study with a small sample size, and further studies with a larger sample size are needed to generalize this study to other MHD patients. Secondly, although the study was conducted in the same area, some confounding factors, such as dietary habits and lifestyle habits, need to be considered in further studies. Thirdly, the current research predominantly relies on 16S rRNA sequencing, which possesses insufficient specificity to discern alterations at the strain-specific level. The *Escherichia-Shigella* decrease may be specific to certain pathogenic strains, while commensal strains may be unaffected or even increased. Lastly, the prognosis data of patients were not collected in this study, and the relationship between gut microbiota and disease progression needs to be further analyzed. Although the specific assessment of gut microbiota is not widely used in clinical practice, we believe that this study provides new evidence for further exploring the changes in gut microbiota in MHD and CKD patients and developing targeted interventions.

Conclusion

In conclusion, for the first time, we analyzed microbiota differences using the ASVs method in this research field. The decreased abundance of *Escherichia-Shigella* is a signature gut microbiota alteration in patients with ESKD undergoing MHD, and *Escherichia-Shigella* may represent a key bacterial group warranting exploration in the field of hemodialysis. The change of tryptophan metabolism in the gut microbiota of MHD deserves further investigation. In addition, the intervention of some key genera such as *Bifidobacterium* may help to improve the clinical indicators of MHD patients, which warrants further investigation and validation. The dysbiosis of gut microbiota holds promise as a therapeutic target and biomarker for the diagnosis and treatment of MHD.

Abbreviations

CKD	Chronic kidney disease
ESKD	End-stage kidney disease
MHD	Maintenance hemodialysis
ASVs	Amplicon sequence variants
PCoA	Principal coordinate analysis
PERMANOVA	Permutational multivariate analysis of variance
LEfSe	Linear discriminant analysis effect size
KEGG	Kyoto Encyclopedia of Genes and Genomes
HC	Healthy control
SBP	Systolic blood pressure
HB	Hemoglobin
ALB	Albumin
Scr	Serum creatinine
ANOSIM	Analysis of similarities
BUN	Blood urea nitrogen
FFPs	Feature frequency profiles
NLRP3	Nod-like receptors protein 3
IS	Indoxyl sulfate

Supplementary Information

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Supplementary Material 1

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Author contributions

Conceptualization, Y. Qin., and K. Luo.; Methodology, J. Zhao., and Y. Qin.; Software, Y. Qin., and K. Luo.; Data Collection, J. Guo., F. Wang., and S. Li.; Writing-original draft, Y. Qin., L.Wang. and J. Zhao., Writing-review and editing, K. Luo., X. Yang., J. Wang., and Y. Chen. Each of the authors contributed an important role in drafting the manuscript and ensuring the accuracy or completeness of the overall work is properly investigated and resolved.

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Data availability

The raw data for 16S rRNA gene sequences are available at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) with the accession numbers PRJNA1031320, PRJNA574226, and PRJNA801894. Other data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Bethune International Peace Hospital and conducted following the Helsinki Declaration (ethical number: 2022-KY-05). Informed consent for this study was obtained from all participants, who provided written informed consent that met the study requirements.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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