DOI: https://doi.org/10.17816/brmma624008

Changes in the composition of the gut microbiota and content of microbial-derived uremic toxins in patients undergoing hemodialysis

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ABSTRACT

Recent data highlight a significant role of the gut microbiota in the pathogenesis of chronic kidney disease, particularly in its terminal stage. However, little is known about the features of intestinal dysbiosis in people undergoing programed hemodialysis. Changes in the intestinal microbiota and blood levels of uremic toxins of microbial origin in patients with terminal renal insufficiency receiving hemodialysis were analyzed. This cross-sectional study included 80 patients receiving hemodialysis and 20 individuals with normal kidney function. The state of the microbiocenosis of the colon was studied using a polymerase chain reaction with a commercial set Colonoflor 16 (premium) manufactured by Alfalab (Russia). Serum levels of trimethylamine and its metabolite trimethylamine-N-oxide were determined by liquid chromatography/mass spectrometry. The concentrations of indoxyl sulfate and p-cresyl sulfate were evaluated by enzyme immunoassay according to the instructions of a commercial kit. In patients undergoing programed hemodialysis, increased colonization of enterococci was combined with the reduction of lacto and bifidophlora, *E. coli*, ruminococci, bacteria producing short-chain fatty acids (*Faecalibacterium prausnitzii, Eubacterium rectale, Roseburia inulinivorans*, and *Blautia spp*.) and microorganisms involved in maintaining the integrity of the intestinal barrier (*Bacteroides thetaomicron* and *Akkermansia muciniphila*). In addition, high titer levels of representatives of opportunistic and even pathogenic flora were often found in this group. Intestinal dysbiosis in patients undergoing programed hemodialysis was accompanied by a significant increase in the concentration of uremic toxins in the blood. Compared with individuals with normal renal function, the trimethylamine level in patients undergoing programed hemodialysis was increased 22 times; trimethylamine-N-oxide, 23 times; indoxyl sulfate, 21 times; and p-cresyl sulfate, 5 times. Thus, patients receiving hemodialysis exhibited pronounced pathological changes in intestinal microbiocenosis, accompanied by a significant increase in serum levels of uremic toxins of microbial origin..

Keywords: intestinal microbiota; intestinal dysbiosis; the intestinal barrier; microbiocenosis; uremic toxins; chronic kidney disease; tubulointerstitial nephritis; programmatic hemodialysis.

To cite this article

Pyatchenkov MO, Shcherbakov EV Trandina AE, Glushakov RI, Leonov KA, Kazey VI. Changes in the composition of the gut microbiota and content of microbial-derived uremic toxins in patients undergoing hemodialysis. *Bulletin of the Russian Military Medical Academy.* 2024;26(1):51–60. DOI: https://doi.org/10.17816/brmma624008

Received: 29.01.2024 **Accepted:** 28.02.2024 **Published:** 30.03.2024

УДК 616.61-036.12:612.336.3

DOI: https://doi.org/10.17816/brmma624008

Изменения состава кишечной микробиоты и содержания уремических токсинов микробного происхождения у больных, находящихся на программном гемодиализе

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АННОТАЦИЯ

Последние данные свидетельствуют о значительной роли кишечной микробиоты в патогенезе хронической болезни почек, особенно в ее терминальной стадии. Между тем мало что известно об особенностях кишечного дисбактериоза у лиц, находящихся на программном гемодиализе. Анализируются характер изменений кишечной микробиоты и содержание в крови уремических токсинов микробного происхождения у больных, страдающих терминальной почечной недостаточностью, получающих лечение гемодиализом. В исследование включено 80 пациентов, находящихся на лечении программным гемодиализом, и 20 сопоставимых по полу, возрасту, индексу массы тела и статусу курения лиц без нарушения функции почек. Состояние микробиоценоза толстой кишки исследовали с помощью полимеразной цепной реакции, используя коммерческий набор «Колонофлор 16 (премиум)» производства «Альфалаб» (Россия). Определение уровня триметиламина и его метаболита триметиламин-N-оксида в сыворотке крови проводили путем жидкостной хроматографии/масс-спектрометрии. Концентрацию индоксил сульфата и п-крезил сульфата оценивали способом иммуноферментного анализа по инструкции коммерческого набора. У пациентов, находящихся на программном гемодиализе, повышенная колонизация энтерококков, сочеталась с редукцией лакто- и бифидофлоры, кишечной палочки, руминококков, бактерий, продуцирующих короткоцепочечные жирные кислоты (*Faecalibacterium prausnitzii, Eubacterium rectale, Roseburia inulinivorans, Blautia spp.*), а также микроорганизмов, участвующих в поддержании целостности кишечного барьера (*Bacteroides thetaomicron, Akkermansia muciniphila*). Кроме того, в этой группе нередко обнаруживались повышенные титры представителей условно-патогенной и даже патогенной флоры. Кишечный дисбактериоз у больных, находящихся на программном гемодиализе, сопровождался значительным повышением концентрации в крови уремических токсинов. По сравнению с лицами с нормальной функцией почек уровень триметиламина у больных, находящихся на программном гемодиализе, был повышен в 22 раза, триметиламин-N-оксида — в 23 раза, индоксил сульфата — в 21 раз, п-крезил сульфата — в 5 раз. Таким образом, у лиц, получающих лечение гемодиализом, наблюдаются выраженные патологические изменения микробиоценоза кишечника, сопровождающиеся значительным повышением сывороточного уровня уремических токсинов микробного происхождения.

Ключевые слова: кишечная микробиота; кишечный дисбактериоз; кишечный барьер; микробиоценоз; уремические токсины; хроническая болезнь почек; тубулоинтерстициальный нефрит; программный гемодиализ.

Как цитировать

Пятченков М.О., Щербаков Е.В., Трандина А.Е., Глушаков Р.И., Леонов К.А., Казей В.И. Изменения состава кишечной микробиоты и содержания уремических токсинов микробного происхождения у больных, находящихся на программном гемодиализе // Вестник Российской военно-медицинской академии. 2024. Т. 26, № 1. С. 51–60. DOI: https://doi.org/10.17816/brmma624008

Рукопись получена: 29.01.2024 **Рукопись одобрена:** 28.02.2024 **Опубликована:** 30.03.2024

ЭКО • ВЕКТОР

DOI: https://doi.org/10.17816/brmma624008

方案血液透析患者肠道微生物群组成和微生物源尿毒症毒素含量的变化

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摘要

最近的证据表明，肠道微生物群在慢性肾脏病的发病机制中起着重要作用，尤其是在其晚期阶段。然而，人们对血液透析患者肠道菌群失调的特殊性知之甚少。该研究分析了接受血液透析治疗的终末期肾衰竭患者肠道微生物群变化的特征以及血液中微生物源尿毒症毒素的含量。研究对象包括 80 名接受血液透析方案治疗的患者和 20 名在性别、年龄、体重指数和吸烟状况方面无肾功能障碍的可比人员。使用 Alphalab（俄罗斯）公司生产的商业试剂盒 "Colonoflor 16（高级）"，通过聚合酶链式反应对结肠微生物增生状况进行了研究。血清中三甲胺及其代谢物三甲胺-N-氧化物的含量是通过液相色谱法/质谱法进行测定。采用商用试剂盒指令的酶联免疫吸附法评估硫酸吲哚基和硫酸对甲苯基的浓度。在血液透析患者中，肠球菌定植率增加的同时，乳酸菌和双歧杆菌、大肠埃希氏菌、反刍球菌和产生短链脂肪酸的细菌（Faecalibacterium prausnitzii、Eubacterium rectale、Roseburia inulinivorans、Blautia spp.）减少， 并且，还观察到参与维持肠道屏障完整性的微生物（Bacteroides thetaomicron、Akkermansia muciniphila）减少。此外，在这组患者中，还经常发现机会性甚至是致病性菌群代表的滴度增加。方案血液透析患者肠道菌群失调的同时，血液中的尿毒症毒素浓度也显著增加。与肾功能正常的人相比，方案血液透析患者的三甲胺水平升高了22倍，三甲胺-N-氧化物升高了23倍，硫酸吲哚酯升高了21倍，硫酸对甲酚酯升高了5倍。因此，在接受血液透析治疗的人中，肠道微生物生态会发生明显的病理变化，同时血清中微生物源尿毒症毒素的水平也会显著升高。

关键词：肠道微生物群；肠道菌群失调；肠道屏障；微生物群落；尿毒症毒素；慢性肾脏病；肾小管间质性肾炎；方案血液透析。

To cite this article

Pyatchenkov MO, Shcherbakov EV Trandina AE, Glushakov RI, Leonov KA, Kazey VI. 方案血液透析患者肠道微生物群组成和微生物源尿毒症毒素含量的变化. *Bulletin of the Russian Military Medical Academy.* 2024;26(1):51–60. DOI: https://doi.org/10.17816/brmma624008

收到： 29.01.2024 **接受：** 28.02.2024 **发布日期：** 30.03.2024

INTRODUCTION

Interest in the potential role of intestinal dysbiosis in the development and progression of chronic kidney disease (CKD) has significantly increased over the last decade [1, 2]. Several experimental and clinical studies have demonstrated that CKD is associated with distinct qualitative and quantitative alterations in the intestinal microflora. These changes are accompanied by increased production and accumulation of uremic toxins, such as p-cresyl sulfate (PCS), indoxyl sulfate (IS), and trimethylamine-N-oxide (TMAO) [3, 4]. In contrast, patients with renal failure exhibit a reduction in short-chain fatty acids (SCFAs), which serve as a source of energy for enterocytes, maintain the integrity of the intestinal barrier, and have anti-inflammatory and anticarcinogenic effects [5]. The nature of changes in the intestinal microbial community can vary significantly depending on the etiology of renal failure and the degree of renal dysfunction. Individuals in the terminal stage of CKD receiving hemodialysis treatment exhibit the most pronounced changes [6].

Uremia can cause damage to the intestinal epithelial barrier, which can lead to the translocation of immunogenic bacterial products into the bloodstream. This can contribute to the development of chronic subclinical inflammation, CKD progression, and associated cardiovascular, metabolic, and other complications [7]. Therefore, intestinal dysbiosis should be considered an independent risk factor for adverse outcomes in individuals with severe renal dysfunction.

Studies on the relative composition and abundance of intestinal bacteria and their potential relationship with the levels of uremic toxins in the blood are insufficient. The data accumulated to date on the features of intestinal dysbacteriosis in CKD are highly heterogeneous [8]. Studies on the intestinal microbiota in our country are significantly limited because of the high cost and low availability of modern metagenomic and proteomic techniques [9].

This study aimed to investigate changes in the composition of the intestinal microbiota and the content of uremic toxins of microbial origin in a Russian cohort of patients on program hemodialysis.

MATERIALS AND METHODS

The study included 80 patients (40 men and 40 women) with a median age of 62.5 (range, 51.3–69.8) years who underwent hemodialysis for a median of 52 (range, 21.5–120) months. The median urea clearance rate was 1.46 (range, 1.39–1.57). In the hemodialysis group, the causes of terminal renal failure (TRF) were type 2 diabetes mellitus (33.7%), chronic glomerulonephritis (28.8%), polycystic kidney disease (10%), tubulointerstitial nephritis (5%), type 1 diabetes mellitus (3.8%), and other diseases (18.7%). The patients were primarily from the Northwest region and did not adhere to any specific diets, except for limiting fluid intake and avoiding foods high in potassium and phosphorus, which is recommended for severe renal failure.

The control group consisted of 20 healthy individuals (10 men and 10 women) with a median age of 55 (range, 49.3–66.8) years, and they had no renal dysfunction. The groups were comparable in terms of sex, age, body mass index, and smoking status.

The exclusion criteria were as follows: presence of acute inflammatory and uncompensated chronic diseases, viral hepatitis, and enteropathies such as Crohn's disease, nonspecific ulcerative colitis, and celiac disease; intake of antibiotics, laxatives, and pre- and probiotics; or surgeries on gastrointestinal tract (GIT) organs in the preceding 12 months.

To determine the state of colonic microbiocenosis, 1 g of fecal samples was examined. Deoxyribonucleic acid (DNA) was extracted from the bacterial fraction of feces using the DNA-sorbB kit (Next-Bio, Russia), amplification was performed by reverse-transcription polymerase chain reaction using the Colonoflor 16 Premium kit produced by Alfalab (Russia) with the help of the DT-prime detection amplifier produced by DNA-Technology (Russia). The manufacturer's software was used to interpret the results.

A comparative analysis was conducted on the composition of the intestinal microbiota using both qualitative (frequency of deviation from reference values) and quantitative criteria (magnitude of change in the trait). The degree of change in the investigated indicator was considered in the statistical analysis. For example, if the abundance of *Escherichia coli* was 3×10^5 colony-forming units (CFU)/mL, 5 were considered for analysis. The total microbial content was compared between groups without considering cases with unknown microorganism counts, mainly due to a decrease in bacterial abundance below the sensitivity threshold of the technique.

Liquid chromatography/mass spectrometry was used to determine trimethylamine (TMA) and its metabolite TMAO in the serum. The Shimadzu-8060 system in combination with a Shimadzu LC-30AD liquid chromatograph (Japan) was employed for this purpose.

The serum concentrations of PCS and IS were determined using an enzyme immunoassay following the instructions of a commercial kit from Cloud-Clone Corp. (USA). The analysis was performed on a Victor X5 tablet analyzer by PerkinElmer Inc. (USA).

Data were statistically processed using the SPSS Statistics 26 program. To account for the small number of observations in the control group, all parameter numerical values are presented as median (*Me*) and interquartile range (*Q25–Q75*). Qualitative characteristics are presented as the absolute number and proportion of the total number (in %). The Mann – Whitney criterion was used to compare groups based on quantitative indicators. Qualitative variables were compared using Pearson's chi-square test and Fisher's exact test. A value of $p < 0.05$ was considered statistically significant.

DOI: https://doi.org/10.17816/brmma624008

The study was approved by the local ethical committee of the Kirov Military Medical Academy (Protocol No. 262 of April 26, 2022).

RESULTS AND DISCUSSION

The results of the comparative analysis of the composition of intestinal microbiota in both groups are presented in Tables 1 and 2. The analysis revealed that the total number of intestinal bacteria increased in 5 (6.3%) patients on hemodialysis, and the number of microbial cells decreased in 2 (2.5%). No deviations in this indicator were observed in the control group. No significant differences in the total number of bacteria were found between the groups. The abundance of *Lactobacillus spp*. and *Bifidobacterium spp*. decreased in 35 (43.8%) and 24 (30%) patients on hemodialysis, respectively. A combined violation was found in 19 (23.8%) patients. In contrast, only 1 (5%) case of similar abnormalities was recorded in the control group. A significant reduction in the absolute number of intestinal bifidobacteria and lactobacilli was noted in the hemodialysis group.

In patients undergoing hemodialysis, the total population of *E. coli* decreased, and in 8 (10%) patients, its content in fecal samples was $<10^5$ CFU/mL. In addition, enteropathogenic strains of *E. coli* were detected in 6 (7.5%) patients with TRF, of which in 4 (5%), its titer exceeded the clinically significant threshold of 10^4 CFU/mL. The percentage of patients with TRF and elevated *Bacteroides spp*. titers (15%) was slightly higher than that of relatively healthy volunteers (5%). However, no significant differences in the total number of *Bacteroides spp*. were found between the two groups.

In the hemodialysis group, the total number of microorganisms that produce butyrate and other SCFAs, including *Faecalibacterium prausnitzii, Eubacterium rectale*, and *Roseburia inulinivorans,* significantly decreased. The content of *Faecalibacterium prausnitzii* was reduced in 18 (22.5%) patients on hemodialysis, *Eubacterium rectale* in 30 (37.5%), and *Roseburia inulinivorans* in 21 (26.3%), relative to normal values. Patients undergoing hemodialysis exhibited higher *Bacteroides spp./Faecalibacterium prausnitzii* ratio (9.95 [3.63–54.24]) than those not receiving treatment (4.25 [2.54–9.5], $p = 0.011$). An increase in this index may indicate an anaerobic imbalance in the intestinal microflora, with values >100 indicating clinically significant disorders. Such abnormalities were observed in 13 (16.3%) patients in the hemodialysis group.

Table 1. Total number of representatives of the intestinal microbiota in both groups
Таблица 1. Общая численность некоторых представителей кишечной микробиоты у пациентов обеих групп

Indicator	Reference values	Hemodialysis group	Control group	*p*
Total bacterial mass	10^{11}–10^{13}	11 (10–12)	10.5 (10–11)	= 0.073
Lactobacillus spp.	10^7–10^8	6 5–7)	8 (7–8)	< 0.001
Bifidobacterium spp.	10^9–10^{10}	8 (7–9)	9 (8–10)	= 0.014
Escherichia coli	10^6–10^8	6 (5–6)	7 (6–8)	< 0.001
Bacteroides spp.	10^9–10^{12}	10 (10–11)	10 (9–11)	= 0.213
Faecalibacterium prausnitzii	10^8–10^{11}	7 (9–10)	10 (9–10.75)	= 0.046
Eubacterium rectale	10^8–10^{11}	6 (5–8)	10 (9–11)	< 0.001
Roseburia inulinivorans	10^8–10^{10}	7 (6–9)	9 (8.25–10)	< 0.001
Bacteroides thetaomicron	–[†]	7.5 (7–9)	10 (9–10.75)	< 0.001
Akkermansia muciniphila	⩽ 10^{11}	8 (6.25–10)	10 (9–10)	< 0.001
Enterococcus spp.	⩽ 10^8	7 (6–9)	6.5 (5.75–7.25)	= 0.037[§]
Blautia spp.	10^8–10^{11}	8 (7–9)	8 (7–9)	= 0.536[¥]
Prevotella spp.	⩽ 10^{11}	7 (6–9)	9 (8–10)	= 0.005
Methanobrevibacter smithii	⩽ 10^{10}	7.5 (6–9)	9 (7.25–10)	= 0.036
Ruminococcus spp.	⩽ 10^{11}	7 (6–8)	9 (8–10)	= 0.001[€]
Relationship between *Bacteroides spp.* and *Faecalibacterium prausnitzii* (*Bfr/Fprau*)	0.01–100	9.95 (3.63–54.25)	4.25 (2.54–9.5)	= 0.011

Note: [†] — any amount is acceptable; [§] — value calculated for 54 patients in the study group and 10 in the control group wherein the exact content of *Enterococcus spp*. was determined; [¥] — value calculated for 44 patients in the study group and 18 in the control group wherein the exact content of *Blautia spp.* was determined; [€] — value calculated for 54 patients in the study group and 17 in the control group wherein the exact content of *Ruminococcus spp.* was determined.

Примечание: [†] — допустимо любое количество; [§] — значение рассчитано для 54 пациентов ОГ и 10 лиц КГ, у которых определено точное содержание *Enterococcus spp*.; [¥] — значение рассчитано для 44 пациентов ОГ и 18 лиц КГ, у которых определено точное содержание *Blautia spp*.; [€] — значение рассчитано для 54 пациентов ОГ и 17 лиц КГ, у которых определено точное содержание *Ruminococcus spp.*

Patients undergoing hemodialysis exhibited a significant reduction in the abundance of *Bacteroides thetaomicron*, a bacterium that possesses anti-inflammatory properties and contributes to the barrier function of the intestinal mucosa. The population of *Akkermansia muciniphila*, which breaks down mucin to produce beneficial products such as SCFAs, was also reduced. These metabolites regulate the abundance of other beneficial bacterial species and play a role in the immune modulation of the intestinal wall. The intestinal microbiota profile of patients on hemodialysis was characterized by an increased colonization of enterococci. Excessive content of enterococci (>10^8 CFU/mL) was detected in 15 (18.8%) patients in the hemodialysis group and 1 (5%) in the control group. These patients also showed a predominance of *Enterococcus spp*. over *Escherichia coli*, which is considered a sign of dysbiosis and can often have pathological consequences. Representatives of opportunistic flora were detected in fecal samples of patients undergoing

Table 2. Frequency of occurrence of clinically significant changes in the composition of the intestinal microbiota in both groups, abs. (%)
Таблица 2. Частота встречаемости клинически значимых изменений состава кишечной микробиоты у пациентов обеих групп, абс. (%)

Indicator	Hemodialysis group	Control group	*p*
Total bacterial mass, > 10^{13}	5 (6.3)	0	= 0.58
Total bacterial mass, < 10^{11}	2 (2.5)	0	= 1.0
Lactobacillus spp. < 10^6	35 (43.8)	1 (5)	= 0.001
Bifidobacterium spp. < 10^8	24 (30)	1 (5)	= 0.021
Escherichia coli < 10^5	8 (10)	0	= 0.352
Bacteroides spp. > 10^{12}	12 (15)	1 (5)	= 0.456
Faecalibacterium prausnitzii < 10^7	18 (22.5)	0	= 0.02
Eubacterium rectale < 10^6	30 (37.5)	2 (10)	= 0.018
Roseburia inulinivorans < 10^8	21 (26.3)	3 (15)	= 0.387
Escherichia coli enteropathogenic > 10^4	4 (5)	0	= 0.581
Enterococcus spp. > 10^8	15 (18.8)	1 (5)	= 0.183
Klebsiella pneumoniae > 10^4	6 (7.5)	0	= 0.597
Klebsiella oxytoca > 10^4	1 (1.3)	0	= 1.0
Candida spp. > 10^4	2 (2.5)	0	= 1.0
Staphylococcus aureus > 10^4	1 (1.3)	0	= 1.0
Acinetobacter spp. > 10^6	2 (2.5)	0	= 1.0
Clostridium difficile	4 (5)	0	= 0.581
Clostridium perfringens	4 (5)	0	= 0.581
Proteus vulgaris/mirabilis > 10^4	2 (2.5)	0	= 1.0
Citrobacter spp. > 10^4	3 (3.8)	0	= 1.0
Enterobacter spp. > 10^4	6 (7.5)	0	= 0.597
Fusobacterium nucleatum	11 (13.8)	2 (10)	= 1.0
Streptococcus spp. > 10^8	0	0	–
Parvimonas micra > 10^4	4 (5)	1 (5)	= 1.0
Blautia spp. < 10^7	46 (57.5)	2 (10)	< 0.001
Methanobrevibacter smithii < 10^5	19 (23.8)	3 (15)	= 0.551
Methanobrevibacter smithii > 10^{10}	7 (8.8)	0	= 0.339
Methanosphaera stadmanae > 10^6	18 (22.5)	0	= 0.02
Ruminococcus spp. < 10^5	26 (32.5)	3 (15)	= 0.17
Salmonella spp.	0	0	–
Shigella spp.	0	0	–
Relationship between *Bacteroides spp.* and *Faecalibacterium prausnitzii* (*Bfr/Fprau*) > 100	13 (16.3)	0	= 0.065

hemodialysis and in some cases in clinically significant titers. Thus, *Fusobacterium nucleatum* was detected in 11 (13.8 %) patients, *Klebsiella pneumoniae* in 6 (7.5 %), *Enterobacter spp.* in 6 (7.5 %), *Clostridium perfringens* in 4 (5 %), *Clostridium difficile* in 4 (5 %), *Citrobacter spp.* in 3 (3.8%), *Candida spp.* in 2 (2.5%), *Proteus vulgaris/mirabilis* in 2 (2.5%), *Klebsiella oxytoca* in 1 (1.3%), *Staphylococcus aureus* in 1 (1.3%), and *Acinetobacter spp.* in 2 (2.5%) of the hemodialysis group. This was in contrast to the control group, where bacterial infections were practically not observed among healthy individuals.

Streptococcus spp. microorganisms were detected in fecal samples from 45 (56.3%) patients in the hemodialysis group and 5 (25%) in the control group. However, none of the patients had titers exceeding the clinically significant threshold of 10^8 CFU/mL. In both groups, the frequency of *Parvimonas micra* detection in fecal samples with clinically significant titers was 5%. Detection of this obligate anaerobic bacterium in amounts $>10^4$ CFU/mL is considered an early marker of large-intestine carcinogenesis. In the hemodialysis group, 57.5% of the patients had a decreased abundance of *Blautia spp*., a resident anaerobic bacterium that synthesizes acetate and exerts a protective effect on pathogen introduction. Two (10%) similar cases were noted in the control group.

The dietary pattern of most patients on dialysis, which includes a reduced intake of plant foods (particularly fiber), may be responsible for the significant reduction in the abundance of *Prevotella spp*. Lower abundance levels of this genus may also be associated with atrophic gastritis and gastric cancer. In addition, the total counts of *Methanobrevibacter smithii* decreased in the hemodialysis group, and the content of this methane-forming anaerobic microorganism in the hemodialysis group had a multidirectional characteristics. In 7 (8.8%) patients with TRF, *M. smithii* was detected in a titer > 10^{10} CFU/mL, a finding previously observed in individuals with obesity. However, 23 (28.8%) patients showed a decrease in *M. smithii* < 10^5 CFU/mL, which may contribute to the activation of fermentation and putrefaction in the intestines. In addition, 18 (22.5%) patients with TRF showed an increase in the titer of *Methanosphaera stadmanae,* another representative of the archaea domain. No abnormalities were observed in the control group. *M. stadmanae* is thought to stimulate local immune reactions and the synthesis of proinflammatory cytokines, contributing to inflammation development.

In the hemodialysis group, 32.5% of the patients had decreased abundance of *Ruminococcus spp*. compared with 15% in the control group. The total content of *Ruminococcus spp*. in the hemodialysis group was significantly lower than that in the control group, even when excluding individuals with titers < 10^5 CFU/mL. These changes may result from a deficiency of dietary proteins, essential amino acids, and microelements. However, some bacteria in this genus, such as *R. torques*, produce butyrate. Pathogenic strains of *Salmonella spp*. and *Shigella spp*. were not found in either group.

Changes in the composition of intestinal microbiota in the hemodialysis group led to a significant increase in the blood concentration levels of uremic toxins of microbial origin. Compared with individuals with normal renal function, patients on hemodialysis had a 22-fold increase in TMA levels, 23-fold increase in TMAO levels, 21-fold increase in IS levels, and 5-fold increase in PCS levels (Table 3).

For the first time in Russian patients on hemodialysis, we have studied the peculiarities of changes in intestinal microbiocenosis. This includes both the composition of the intestinal microbiota and uremic toxins of microbial origin, such as TMA, TMAO, IS, and PCS, in the blood. In patients with TRF, multidirectional changes in the content of obligate representatives of intestinal microflora, with signs of anaerobic imbalance, were noted. Specifically, increased colonization of enterococci is combined with a decrease in the abundance of *E. coli*, ruminococci, and various SCFA-producing bacteria. The total number of microbial cells remains unchanged. Furthermore, patients undergoing hemodialysis often exhibit increased levels of opportunistic and pathogenic microorganisms and an altered microbiome associated with systemic metabolic disorders, inflammation, and gastrointestinal carcinogenesis. Intestinal dysbiosis, in one form or another, was diagnosed in all patients undergoing hemodialysis.

The data generally agree with earlier studies, revealing that stool samples from patients on hemodialysis contain significantly lower counts of *Lactobacillaceae* and *Prevotellaceae* families and 100 times higher counts of *Enterobacteriaceae* and *Enterococci* than in age-, sex-, and ethnicity-matched healthy volunteers [10]. Individuals with advanced CKD stages have also been observed to have

Table 3. Serum levels of uremic toxins of microbial origin in both groups
Таблица 3. Сывороточный уровень уремических токсинов микробного происхождения у пациентов обеих групп

Uremic toxin	Hemodialysis group	Control group	*p* <
Trimethylamine, ng/mL	153.8 (95.7–283.1)	7.1 (4.3–13.1)	0.001
Trimethylamine-N-oxide, ng/mL	5223.3 (3389.3–9445.7)	227.1 (140.4–34)	0.001
Indoxyl sulfate, μmol/L	2.1 (1.4–3)	0.1 (0–0.3)	0.001
P-cresyl sulfate, ng/mL	33.6 (19.1–50.6)	6.4 (4.0–9.2)	0.001

increased counts of *Eggerthella* lenta from the *Actinobacteria* genus, *Fusobacterium nucleatum* from the *Fusobacteriota* genus, and *Alistipes shahii* from the *Bacteroidetes* genus [11]. According to J. Zhao, X. Ning, B. Liu, et al. [12], patients with TRF have higher counts of *Proteobacteria* and *Streptococcus* and *Fusobacterium* genera and lower counts of *Prevotella, Coprococcus, Megamonas*, and *Faecalibacterium*.

The intestinal bacterium *Akkermansia*, belonging to the *Verrucomicrobia* genus, plays a crucial role in maintaining the intestinal barrier function and mucus density. It also participates in the utilization of hydrogen sulfide and supports the growth of SCFA-producing bacteria by providing them with carbon, nitrogen, and energy from mucus decomposition [13]. A study of fecal microbial communities revealed a reduction in the abundance of the probiotic bacteria *Akkermansia* in patients with CKD compared with healthy controls [14].

Several studies have shown that *Roseburia* levels decrease in patients with late CKD stages, including patients on hemodialysis [6]. *Roseburia* is one of the main producers of butyric acid (butyrate) in the colon. Its content is also reduced in various inflammatory and metabolic diseases [15].

Q. Hu, K. Wu, W. Pan, et al. [16] found that the predominance of *Ruminococcus gnavus* at the delivery level could be distinguished between patients with early-stage CKD and healthy controls. In patients with Crohn's disease, *R. gnavus* promotes intestinal wall inflammation by producing inflammatory polysaccharides such as glucoramnan. These polysaccharides induce the secretion of the inflammatory cytokine tumor necrosis factor-alpha by dendritic cells [17]. In contrast, the abundance of *Ruminococcus spp*. significantly in the hemodialysis group compared with the control group. A possible explanation for these contradictions is the peculiarities of the methodology used to assess the composition of the intestinal microbiota, which allows for determining the total number of all ruminococci, rather than individual representatives of this genus, as they have antagonistic properties.

Data on the peculiarities of intestinal dysbacteriosis in Russian patients undergoing hemodialysis are limited. Only one paper in the available literature, authored by I.V. Belova, A.E. Khrulev, A.G. Tochilina et al., addressed this topic. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry revealed dysbiotic changes in the intestinal microbiocenosis of 62 patients on maintenance hemodialysis, which were characterized by a complete absence or suppression of lacto- and bifidoflora and a higher count and species diversity of *Bacteroides, Clostridium, Collinsella, Eggerthella*, and some other microorganisms.

Intestinal dysbacteriosis in the hemodialysis group was found to be associated with a significant increase in serum levels of IS, PCS, and TMA (TMAO). The accumulation of these compounds in the body is attributed to a decrease in their renal excretion and the involvement of dysbiotic intestinal microflora [3]. J. Wong, Y.M. Piceno, T.Z. DeSantis, et al. [5] confirmed that microorganisms with enzymes involved in the synthesis of uremic toxins (such as urease, uricase, and tryptophanase) were prevalent among the bacteria in individuals with TRF. Furthermore, in CKD, the bacterial composition shifts toward proteolytic bacterial species that produce protease. This shift is associated with inflammation and increased permeability of the intestinal wall. In addition, the abundance of saccharolytic bacteria, which break down sugars and produce fermentation products necessary for the synthesis of SCFAs, decreases [12, 19].

In the systemic circulation, uremic toxins of microbial origin can induce inflammation through various molecular mechanisms and signaling pathways and have direct pathogenic effects on various cell types. This contributes to the development and progression of cardiovascular, neurological, mineral-bone, alimentary, and other complications in patients with CKD [20].

This study has limitations. The number of participants may have been insufficient to extrapolate the identified features of intestinal dysbiosis to the entire population of patients receiving hemodialysis because of the huge interindividual variability of the intestinal microbiome. The influence of individual dietary habits, medications, and other factors that have previously shown a significant effect on the composition of the intestinal microbiota cannot be excluded.

Moreover, the primary pathology that caused TRF may have a greater effect on intestinal dysbiosis than severe renal dysfunction and the need for dialysis. Furthermore, this study had limited ability to analyze the full spectrum of intestinal microorganisms because we were only able to assess the contents of certain microorganisms. Although full-genome sequencing is the preferred method for such studies, our findings can serve as a foundation for future research in this field.

CONCLUSIONS

1. Patients receiving hemodialysis have pronounced changes in gut microflora accompanied by a significant increase in the blood concentrations of uremic toxins of microbial origin, such as IS, PCS, and TMA (TMAO).

2. The results suggest the need for further research into the characteristics of intestinal dysbacteriosis in patients with kidney disease.

3. Improved understanding of the metabolic interactions between the intestinal microbiota and the body may facilitate the development of new personalized treatment approaches focused on correcting dysbiosis and reducing the concentration of uremic toxins of microbial origin. This could have a significant effect on improving the outcomes of patients with CKD, including those receiving hemodialysis.

ADDITIONAL INFORMATION

Authors' contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

The contribution of each author. M.O. Pyatchenkov — general concept development, research design, article writing; E.V. Shcherbakov — collection and statistical processing of material; A.E. Trandina — enzyme immunoassay; R.I. Glushakov — literature review, article editing; K.A. Leonov — chromatographic analysis; V.I. Kazey — data analysis, content analysis.

Competing interests. The authors declare that they have no competing interests.

Funding source. This study was not supported by any external sources of funding.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Вклад каждого автора. М.О. Пятченков — разработка общей концепции, дизайн исследования, написание статьи; Е.В. Щербаков — сбор и статистическая обработка материала; А.Е. Трандина — иммуноферментный анализ; Р.И. Глушаков — обзор литературы, редактирование статьи; К.А. Леонов — хроматографический анализ; В.И. Казей — анализ данных, контент-анализ.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

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