Chapter

Specific Cerebrovascular Risk Factors, Colon Microbiocenosis and Its Correction in Patients Receiving Long-Term Programmed Hemodialysis

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Abstract

Introduction: The problem of acute and chronic cerebrovascular disorders in dialysis patients remains the most urgent. Risk factors for cerebrovascular diseases in CKD and dialysis patients can be conditionally divided into "*traditional*" (arterial hypertension, diabetes mellitus, hypercholesterolemia) and "*specific*" (associated with renal pathology and dialysis procedures). The spectrum of specific factors of cerebrovascular risk in patients with dialysis stage of the CKD includes specific dialysis factors that form during programmed HD, as well as impaired phosphorus-calcium metabolism and calcification of the arterial microvasculature, increased blood levels of β2-microglobulin, homocysteine, malondialdehyde and superoxide dismutase, a decrease in the level of nitric oxide (II) metabolites, development of nephrogenic anemia and dysfunction of blood cells, malnutrition and dietary features of patients with renal pathology, accumulation of uremic toxins and toxins of intestinal bacteria, etc. Opportunistic gut microorganisms can produce uremic toxins, which are associated with an increased risk of inflammation, increased oxidative stress, and a higher risk of cardiovascular disease (CVD). Description of the spectrum of risk factors for cerebrovascular pathology in dialysis patients and effective control over them seems to be an effective strategy aimed at increasing the duration and quality of life in patients receiving renal replacement therapy. The aim of the investigation was to study the species composition of colon microbiocenosis in patients with CKD receiving programmed HD treatment and to evaluate the effectiveness of its correction using a new immobilized synbiotic. Materials and methods: Samples of colon microbiota from 62 patients undergoing programmed hemodialysis were studied before and after a course of diet therapy that included probiotic components, in particular, the immobilized synbiotic LB-complex L. Isolation of microorganisms was carried out according to our original method; for bacteria identification, a MALDI-TOF Autoflex speed mass spectrometer (Bruker Daltonik, Germany) was used in the Biotyper program mode. The results were assessed using the criteria proposed by the authors and based on the OST 91500.11.0004-2003. The efficacy of the immobilized synbiotic was determined based on the clinical data, questionnaires,

and bacteriological tests. Results: In patients receiving programmed hemodialysis (before the start of the diet therapy), chronic moderate inflammation and azotemia were found. Dysbiotic changes in microbiocenosis were revealed in all the examined patients; in the absence or suppression of lacto- and bifidoflora, the number and diversity of *Bacteroides* spp., *Clostridium* spp., *Collinsella* spp., *Eggerthella* spp. and other bacteria increased, which was consistent with the theory of functional redundancy of gut microbiota. From the answers to the questionnaires, a decrease in the quality of life was found (up to 70 points out of 100) according to six of the eight scales used. After the combined therapy using the synbiotic LB-complex L in the study group, 56% of the examined patients showed their microbiocenosis restored to normal; no grade III dysbiosis was detected in any patient. There was a significant decrease in CRP and ESR in these patients and an improvement in the quality of life by criteria reflecting physical health. Conclusion: Acute/chronic CVD in patients with CKD of the pre-dialysis and dialysis periods are the most frequent and formidable complications. The spectrum of "traditional" and "specific" CV risk factors in dialysis patients will be described in the chapter. Special attention will be paid to the intestinal microbiota and opportunistic intestinal microorganisms. *The aim* was to study the species composition of colon microbiocenosis in HD patients, and to evaluate the effectiveness of its correction using a new immobilized synbiotic. *Materials and Methods*. Samples of colon microbiota from 62 HD patients were studied before/after a course of diet therapy that included probiotic components, the immobilized synbiotic LB-complex L. MALDI-TOF Autoflex speed mass spectrometer was used in the Biotyper program mode. The efficacy of the immobilized synbiotic was determined based on the clinical data, questionnaires, and bacteriological tests. *Results*. Dysbiotic changes in microbiocenosis were revealed in all patients; in the absence/suppression of lacto-and bifidoflora, the number and diversity of *Bacteroides spp., Clostridium spp., Collinsella spp., Eggerthella spp.* and other bacteria increased. After the combined therapy using the synbiotic LB-complex L in the study group, 56% of the examined patients showed their microbiocenosis restored to normal; no grade III dysbiosis was detected in any patient.

Keywords: cerebrovascular disorders, specific risk factors, dialysis factors, calcification of the arterial microvasculature, anemia, uremic toxins, intestinal bacterial toxins, microbiocenosis, colon microbiota, chronic kidney disease, programmed hemodialysis, probiotics, synbiotics

1. Introduction

The prevalence of chronic kidney disease (CKD) is comparable to such socially significant diseases as hypertension, coronary artery disease, diabetes mellitus, obesity, metabolic syndrome throughout the world. The incidence is according to various sources 10–20% [1, 2]. The number of patients with CKD is progressively increasing and reaching the nature of a pandemic according to the opinion of some scientists [3, 4]. As a result, the number of patients receiving renal replacement therapy is growing (currently, more than 2 million people all over the world). In this connection, the share of the annual increase of dialysis programs is estimated at 6–12% [5].

1.1 Cerebrovascular disorders in patients undergoing long-term programmed hemodialysis (HD)

Cerebrovascular disorders in patients with CKD of the pre-dialysis and dialysis periods are usually classified as acute (transient ischemic attacks, ischemic and

hemorrhagic strokes, subarachnoid hemorrhages) and chronic cerebrovascular diseases (chronic cerebral ischemia due to various variants of vascular cerebral microangiopathy (CMA), including cerebral amyloid angiopathy (CAA) and/or calcifying uremic arteriolopathy).

The remodeling of the micro- and macrocirculatory cerebral vessels continues in patients with dialysis stage of CKD, new vascular risk factors directly related to the dialysis procedure are aggravated and added, and cerebrovascular disorders progress [6]. It has been established that CKD and dialysis are also the causes of the development of CMA, which underlies the development of vascular cognitive impairments and acute cerebrovascular pathology. CMA is characterized by the damaged cerebral microvasculature (perforating cerebral arterioles, capillaries and venules) and damage of the white matter and brain nuclei [7–9]. In our studies, neuroimaging signs of CMA of varying severity were found in 100% of the examined patients who had been receiving renal replacement therapy with the long-term programmed HD. Expansion of perivascular spaces (100%) and white matter hyperintensity (81.4%) prevailed in the structure of MR signs of CMA. Cortical atrophy (67%), cerebral microbleeds (47%), asymptomatic lacunas (35.7%) and small subcortical infarctions (2.9%) were somewhat less common.

In dialysis patients, the risk of stroke and chronic cerebrovascular accidents increases multiple times, the risk of developing cognitive impairment is aggravated, and the quality of life of dialysis patients decreases [10, 11]. According to a meta-analysis conducted by Etgen in 2012, CKD is a statistically significant independent somatic risk factor for the development of cognitive impairment [12]. Epidemiological studies have also revealed a clear relationship between a decrease in glomerular filtration rate and high cardiovascular mortality in dialysis patients [13, 14], the proportion of which is extremely high among patients on renal replacement therapy [15]. According to our data, in patients undergoing programmed HD for more than 1-year, cognitive impairments were found much more often (75.5–81.1% of cases, $p = 0.05$) compared with people without renal pathology. The presence of ESRD and the presence of a patient on programmed HD, regardless of gender and educational level, can directly affect the development of cognitive impairment. For the screening assessment of the neuropsychological status of dialysis patients, the timely use of various neuropsychological scales is necessary: in particular, the use of the SLUMS and MoCA scales is possible. The risk factors for the development of cognitive impairment in persons receiving programmed HD can be considered an increase in the dialysis experience and the age of patients, as well as a low calculated dialysis adequacy index for urea (Kt/V less than 1.4).

In general, the problem of acute and chronic cerebrovascular disorders in dialysis patients remains the most urgent. Description of the spectrum of risk factors for cerebrovascular pathology in dialysis patients and effective control over them seems to be an effective strategy aimed at increasing the duration and quality of life in patients receiving renal replacement therapy.

1.2 Specific risk factors for the development of cerebrovascular pathology in patients with chronic kidney disease

Risk factors for cerebrovascular diseases in CKD and dialysis patients can be conditionally divided into "traditional" (arterial hypertension, diabetes mellitus, hypercholesterolemia) and "specific" (associated with renal pathology and dialysis procedures). Specific risk factors remain poorly understood and less well known to medical practitioners. The spectrum of specific factors of cerebrovascular risk in patients with dialysis stage of the CKD includes specific dialysis factors that form during programmed HD, as well as impaired phosphorus-calcium metabolism,

increased blood levels of β2-microglobulin, homocysteine, malondialdehyde and superoxide dismutase, a decrease in the level of nitric oxide (II) metabolites, accumulation of uremic toxins and toxins of intestinal bacteria, development of nephrogenic anemia, dietary features of patients with renal pathology, etc.) [16–18]. Some of them are presented in more detail below.

1. Specific cerebrovascular risk factors that form during the HD procedure (dialysis factors) include disruptions of autoregulation of microcirculatory cerebral blood flow during regular programmed HD procedures, impaired drainage function of the brain against the background of stasis of interstitial fluid, aggravated by osmotic and electrolyte disorders of dialysis functioning of the glymphatic system. The high probability of developing hemodynamic instability and disruption of autoregulation of microcirculatory cerebral blood flow in dialysis patients, in addition to the traditional mechanisms common to patients with cardiovascular risk, may be due to the possibility of several specific complications during the HD procedure itself. In particular, during the programmed HD procedure, several patients may develop intradialysis hypotension, which is formed due to various pathogenetic mechanisms: the effect of ultrafiltration, hypovolemia and a decrease in the volume of extracellular fluid, an increase in body weight in the interdialysis period, electrolyte and osmolar disorders, and frequent autonomic dysfunction of dialysis patients. Also, in patients receiving programmed HD, intradialysis hypertension may develop due to an overestimation of the patient's "dry weight", intradialysis potassium drop, fluctuations in calcium levels, as well as the use of beta-blockers and other specific factors during the programmed HD procedure.

2. Violation of phosphorus-calcium metabolism and calcification of the arterial microvasculature: Already in the early stages of the development of CKD, there is a progressive impairment of phosphorus-calcium metabolism, which reaches its maximum during the dialysis period of CKD. These disorders include an excess of factors that contribute to the calcification of the arterial microvasculature, as well as a lack of factors that inhibit the calcification of the arterial wall. In the pathological circle of disorders of phosphorus-calcium metabolism and calcification of the arterial wall, several stages are distinguished. At the first stage, due to the presence of renal pathology and the development of CKD, there is a decrease in the excretion of phosphates in the urine and the formation of hyperphosphatemia. In response to an increase in serum phosphorus concentration, intestinal absorption of phosphorus decreases (it is discussed that this process is mediated by an increase in the level of phosphatonin (FGF23, fibroblast growth factor-23).

Long-term elevated levels of FGF23 in combination with destruction of the proximal renal tubules in CKD lead to a decrease in calcitriol levels, which means a decrease in calcium absorption in the intestine and suppression of its reabsorption in the kidneys, followed by the formation of hypocalcemia. At the next stage of the vicious circle of disorders of phosphorus-calcium metabolism, the loss of calcium by the body is compensated mainly due to the development of secondary hyperparathyroidism and increased resorption of bone tissue. When calcium is released from the bone, the level of serum phosphorus increases compensatory, thus, the vicious circle of phosphorus-calcium metabolism is closed [19]. Excess serum calcium is subsequently deposited in ectopic soft tissues and in the in the vascular system (in other words, vascular calcification is observed) [20]. It is believed that the process of calcification and increased stiffness of the vascular

wall are associated with an increased risk of cardiovascular events, but there is no separate data on the cerebrovascular risk associated with arterial calcification and calcifying uremic arteriolopathy development [21]. The literature provides data on another possible mechanism by which an excess of phosphates leads to calcification of arteries, namely, a change in the phenotype of vascular smooth muscle cells (SMC) according to the "osteogenic type". Vascular wall SMCs stop producing SM22α-actin and instead synthesize bone formation factors involved in vascular calcification (alkaline phosphatase, osteocalcin) [22]. There is evidence that the mineralization of the vascular wall is also aggravated by the degradation of elastin against the background of the occurrence of osteogenically modified SMCs [23]. The degraded elastin increases the affinity of calcium and promotes the growth of hydroxyapatites along the elastic fibers. The gradual reduction of vascular smooth muscle leads to additional fibrosis of the median membrane of small and medium arteries, a decrease in cerebral blood flow and possible cerebrovascular risks. It is known that in CKD in the pre-dialysis period, there is also a lack of endogenous factors that inhibit the calcification of the arterial wall: FGF-23 klotho coreceptor, MGP protein (matrix glutamate protein), pyrophosphate, etc. reabsorption in the renal tubules [24]. The role of klotho as a protective factor of the vascular wall, which prevents osteogenic differentiation of SMCs, is discussed [25]. It is believed that as CKD progresses, the level of klotho gradually decreases, however, there is insufficient data on the effect of reduced concentrations of klotho on the development of cerebrovascular diseases [26]. Pyrophosphate and MGP protein (vitamin K-dependent glutamate-containing protein) are normally synthesized by healthy SMCs and inhibit vascular mineralization [27].

3. Anemia and dysfunction of blood cells: It is necessary to note another important specific risk factor for the development of cerebral vascular disorders in patients with CKD—the presence of anemia and dysfunction of blood cells, mainly platelets. The development of anemia in patients with CKD is associated with prolonged proteinuria, which is accompanied by losses of erythropoietin, transferrin and ionized iron, leading to a persistent decrease in hemoglobin levels [28]. As renal failure progresses, the anatomical structures that produce erythropoietin are gradually replaced by fibrous tissue, which is accompanied by the loss of hormone-producing properties. Observations by Chang et al. (2013), show that the presence of anemia in CKD increases the prevalence and severity of cerebrovascular disorders in patients with CKD [29]. The results of epidemiological studies indicate that the likelihood of developing an ischemic stroke is significantly higher in patients with anemia associated with CKD, and when the target values of hemoglobin and erythrocytes are reached, the risk of stroke is significantly reduced [30]. Platelet dysfunction in CKD is the result of a combination of intrinsic platelet abnormalities and disorders of platelet–vascular wall interaction [31]. This leads to a deterioration in platelet aggregation and impaired binding between the surface glycoprotein complex GPIIb/IIIa and fibrinogen on the subendothelial surface, thereby contributing to hypoaggregation and possible hemorrhagic events. The anemia that accompanies CKD exacerbates platelet dysfunction. This is due to a deficiency of erythropoietin, which normally improves platelet function by increasing the density of surface GPIIb/IIIa receptors [32]. There are works in the literature on the role of the transmembrane receptor RAGE in the formation of chronic vascular inflammation by inducing proinflammatory cytokines and chemokines. An increase in the concentration of end products of glycation observed in CKD patients due to impaired excretory function leads to an increase in the expression of RAGE in the cells of the vascular wall. This leads

to an increase in the concentration of sRAGE, the serum form of this receptor, which is a marker of inflammation and, in contrast to RAGE itself, can neutralize some of the inflammatory effects through competitive binding to circulating ligands [33]. In patients with CKD, the sRAGE level is 2.4 times higher than in the general population, and the concentration of proinflammatory ligands is 4 times higher than in the control group without CKD [34]. It is assumed that this may affect the formation of microangiopathy in the deep regions of the brain by activating the inflammatory response, impaired permeability of the blood–brain barrier and the occurrence of microbleeds [35], however, there is no convincing data on this yet.

4. Hyperhomocysteinemia and hyperβ2-microglobulinemia: Another specific risk factor for cerebrovascular disorders in CKD is hyperhomocysteinemia, which plays an important role in the formation of malignant atherosclerosis in CKD and thrombovascular disorders. Pathological accumulation of homocysteine occurs due to impaired reabsorption and metabolism of renal tubular cells in CKD [36]. β2-microglobulin is normally eliminated by the kidneys. Impaired elimination of β2-microglobulin from the body by the natural nephrogenic way leads to further deposition of amyloid in the walls of the microvasculature, the formation of secondary amyloidosis and CAA with the risk of cerebral microbleeds [17]. The high incidence of cerebral microbleeds (according to our data, 47% of the examined patients, mainly in the subcortical nuclei and supratentorial localization), can be explained by the development in this category of patients of both sporadic non-amyloid CMA and CAA, including against the background of persistent β2-microglobulinemia.

5. Accumulation of uremic toxins as a specific risk factor for cerebrovascular diseases in the pre-dialysis and dialysis periods of CKD. Uremic toxins can have both direct neurotoxic and vascular effects, and indirect, mediated through the aggravation of the negative effects of the above-described specific risk factors. It has been proven that the influence of such uremic toxins as uric acid, guanidine compounds, indoxyl sulfate, as well as proinflammatory cytokines–interleukins (Il) 1β, Il-6, tumor necrosis factor α, negatively affect cognitive functions and the functioning of the central nervous system under conditions of uremia [37]. The neurotoxic effects of these compounds can be mediated through ligand- and voltage-dependent calcium channels. Other studies have noted a direct effect of uremic toxins on the rate of cerebral blood flow [38]. It is believed that uremic toxins can enhance oxidative stress, chronic inflammation, endothelial dysfunction, and vascular calcification [39]. Studies in mice with CKD have shown that an increase in the level of a number of uremic toxins increases the content of intercellular adhesion molecules: VCAM-1 (CD106) and ICAM-1 (CD31) [40]. Cell adhesion molecules perform important functions of recognition, adhesion, and migration of immune-competent cells. In cases of violations of adhesive function of the endothelium and balance in the ratio of intercellular adhesion molecules, according to several authors, progression of angiopathy and malignant atherosclerosis is possible. In particular, it was found that uremic toxins induce the formation of angiopathy in patients with CKD, provoke calcification of the SMC of the aorta [41]. It is not yet known to what extent these mechanisms affect the development of cerebrovascular diseases, which requires further study of this issue.

6. Intestinal bacterial toxins: Another specific risk factor for the development of cerebral vascular disorders in patients with CKD, which will be presented

below in the form of an original study—intestinal bacterial toxins is distinguished. One of the negative consequences of the use of extracorporeal detoxification methods can be a violation of intestinal microbiocenosis. Recent studies have shown the presence of changes in intestinal microbiocenosis in patients with end-stage CKD. At the same time, in comparison with the pre-dialysis stage patients, the patients receiving programmed HD had more pronounced disorders in the composition of the intestinal microbiota [42, 43]. At present time, the greatest attention is paid to two possible mechanisms for the development of changes in the composition of the intestinal microbiota in the literature: the characteristics of the diet and drugs taken in dialysis patients, as well as the regimen of the selected method of extracorporeal detoxification (HD, PD, or a functioning kidney transplant). Opportunistic gut microorganisms can produce uremic toxins, in particular, indoxyl sulfate and paracresol sulfate, which are associated with an increased risk of inflammation, increased oxidative stress, progression of CKD, and a higher risk of cardiovascular disease (CVD), which appears to predispose to disorders of intestinal microbiocenosis and mediated vascular risks.

Restrictions on the consumption of fruits and vegetables (sources of potassium), cheese, milk and dairy products (sources of phosphorus) contribute to the predominance of bacteria that produce toxic metabolites [44], which negatively affects the integrity of colonocytes and impairs the protective barrier of the colon mucosa [45]. Insufficient protein intake and loss of albumin during dialysis, especially when using high-flux membranes, also lead to a change in the species structure of the intestinal microbiome and increase the risk of bacterial translocation (penetration of microorganisms from the lumen of the gastrointestinal tract through the mucous barrier into the blood and lymph flow) and endotoxemia [42, 46]. On the contrary, if a high-fiber diet is properly followed in patients on PD, circulating concentrations of uremic toxins (in particular, paracresol sulfate) and some other markers of inflammation are reduced [47–49]. The dialysis procedure itself is associated with inevitable dietary restrictions for the dialysis patient, which may partly explain the differences between patients on PH and PD [50]. Thus, patients receiving PD are less prone to hyperkalemia than patients receiving PD [51]. Dietary restrictions in this category of patients are considered milder, and the diet is more varied.

Patients receiving renal replacement therapy are forced to take several medications regularly, which, as well as dietary habits, can negatively affect the composition of the intestinal microbiocenosis. However, the data on this issue are inconsistent. In particular, Khoury et al. (2016), postulate that the frequent use of antibiotics and phosphate binders in patients with end-stage CKD can alter the composition of the intestinal microbiota and, therefore, jeopardize the intestinal barrier [52]. Researchers consider phosphate binders, immunosuppressants, antibiotics, and proton pump inhibitors as drugs that negatively affect the intestinal microbiocenosis of dialysis patients [53].

The aim of our investigation was to study the species composition of colon microbiocenosis in patients with CKD receiving programmed HD treatment and to evaluate the effectiveness of its correction using a new immobilized synbiotic.

2. Materials and methods

The examined patients were on planned outpatient treatment in the department of gravitational surgery of blood and hemodialysis in 2018–2020. Patients were enrolled in this parallel-group randomized controlled clinical trial using a

continuous sample method. The study involved 62 patients, including 36 women (58.1%) and 26 men (41.9%). Inclusion criteria were: age from 18 to 85 years; the presence of the dialysis stage of chronic kidney disease, the experience of program hemodialysis for more than 1 year; the adequacy of the programmed hemodialysis (at least 3 sessions per week, at least 4 hours/session and 720 minutes per week, the calculated dialysis adequacy index (purification coefficient Kt/V for urea) is at least 1.4, calculated based on the proportion of urea reduction weight loss during dialysis, dialysis time and patient weight); no antibiotic intake for 2 months and more, signed informed consent of the patient.

The patients were divided into the main group and the comparison group matched by sex and age. Basic therapy for patients with dialysis stage CKD of both groups included a high-protein diet and, if necessary, the appointment of drug therapy: antihypertensive—β-blockers (bisoprolol), blockers of Ca-channel (amlodipine), blockers of imidazoline receptors (moxonidine) and hypolipidemic—atorvastatin or rosuvastatin; as well as the treatment of anemia: erythropoietin α (or β) or methoxypolyethylene-glycol-epoetin-β; iron preparations (iron (III) hydroxide sucrose complex); correction of mineral-bone disorders: active metabolites of vitamin D (calcitriol, paricalcitol), calcimimetics (cinacalcet), phosphate-binding agents (β-iron (III) oxyhydroxide complex); correction of protein-energy deficiency (keto analogs of amino acids) [43].

The main group consisted of 32 patients with dialysis stage of CKD, including 19 women (59%) and 13 men (41%) aged 38–65 years (mean age 57.1 ± 7.9 years). Dialysis experience—from 12 to 123 months (40.6 ± 29.8 months). They received basic therapy and, as a probiotic, a new immobilized synbiotic "LB-complex L". The comparison group included 30 patients, including 17 women (57%) and 13 men (43%) aged 34–65 years (54.7 ± 8.4 years) with comparable dialysis experience. They received basic therapy and a placebo. All patients underwent an assessment of the nature of complaints, clinical and laboratory data (general blood test with determination of the number of leukocytes, ESR; biochemical blood test with determination of creatinine, blood urea, C-reactive protein); the dialysis adequacy index (purification coefficient Kt/V for urea) was calculated for each patient. The quality of life was assessed using the SF-36 (Short form medical outcomes study) questionnaire [11]. The study of the species composition of the intestinal microbiota and the assessment of the state of the microbiocenosis was carried out using the unified methodology developed by us and OST 91500.11.0004-2003 "Patient Management Protocol. Intestinal dysbiosis" [54, 55]. Microorganisms were identified on an autoflex speed time-of-flight MALDI-TOF mass spectrometer (Bruker Daltonik, Germany) using the Biotyper 4.1.80 RTC program. The author's immobilized multistrain synbiotic LB-complex L (SGR RU.77.99.88.003.E.002522.06.18) [56], recommended as a source of probiotic microorganisms (bifidobacteria and lactobacilli) and zeolites (enterosorbent), which increase the body's nonspecific resistance and have a detoxifying effect. Six strains that make up the synbiotic under study belong to species with a documented history of safe use and are approved for the production of medical immunobiological preparations. They do not have genetically modified analogues, meet the requirements for probiotic strains [36, 57], in particular, they have high antagonistic activity against a wide range of pathogenic and opportunistic microorganisms, antibiotic resistance undetermined by plasmids, and are sufficiently resistant to the action of gastric juice and bile. Zeolites of the Kholinsky deposit, selected as a matrix for the immobilization of probiotic strains, are approved for use in medical practice (SGR KZ.16.01.78.003.E.004706.08.15 from 18.08.2015). A unique property of clinoptilolites is the property of selective ion exchange: they are ultra-elements, if they are not enough, and they remove substances that are in excess from the body. Zeolites

have pronounced sorption properties, since the openwork of the crystal lattice creates a large adsorption volume, they do not break down and do not undergo any changes in the human body [19]. Statistical processing was performed using standard software packages Statistica 6.1 and Microsoft Excel 2007. Data were presented as arithmetic mean (M) and standard error of the mean (m). If the distribution of data in the samples was not characterized as normal, nonparametric methods of analysis were used. The significance of the differences was assessed using the Mann–Whitney test. Differences between independent groups were considered statistically significant with a probability of error $p < 0.05$.

3. Results and discussion

At the beginning of this study, a comprehensive examination of patients on programmed HD was carried out, including the determination of clinical and laboratory parameters, an assessment of the quality of life by a questionnaire method and a bacteriological analysis of the colon microbiocenosis.

When analyzing laboratory data before using the synbiotic, comparable indicators were found in both groups: a moderate increase in ESR (43.7 ± 21.4 and 42.4 ± 18.9 mm/h) and CRP (6.8 ± 3.1 and 6.5 ± 2.9 g/L), normal leukocyte count (6.7–$6.8*10^9$/L), increased levels of blood urea (19.6–19.4 mmol/L) and blood creatinine (697.1–688.5 μmol/L), indicating the presence of chronic moderate inflammation and azotemia in patients receiving PG. The Kt/V index for urea was 1.43 ± 0.16 and 1.41 ± 0.18, which indicated the adequacy of the dialysis dose (**Table 1**).

ESR, erythrocyte sedimentation rate; C-RP, C-reactive protein; Le, leukocyte count; Kt/V for urea index, the dialysis adequacy index (for urea).

An in-depth study of the microbiota in patients with CKD undergoing hemodialysis showed that among representatives of the phylum Actinobacteria in the microbiocenosis of the colon, representatives of the genus *Bifidobacterium* are found in 75% of the examined, in 43.7% of them the detected number of bifidobacteria is significantly lower than normal. 1–2 species of bifidobacteria were isolated from each patient, and *Bifidobacterium longum* prevailed in the species structure −43.75% (**Figures 1–4**).

Using our technique, in this study, such rarely identified species of the Enterobacteriaceae family as *Enterobacter asburiae*, *Enterobacter kobei*, *Citrobacter youngae*, *Serratia liquefaciens*, and *Raoultella planticola* were isolated.

Dysbiotic changes in colon microbiocenosis of varying degrees were detected in 100% of the examined patients (**Figure 5**).

During the analysis of the composition of the intestinal microbiota, it was noted that in the absence or suppression of lacto- and bifidoflora, the number and species diversity of microorganisms of the genera *Bacteroides*, *Clostridium*, *Collinsella*, *Eggerthella*, etc. literature [58]. The concept of functional redundancy has been validated in metagenomic studies. For example, in experimental models

	ESR, mm/hour	C-RP, g/L	Le, cell/L	Urea (blood), mMol/L	Creatinine (blood), μmol/L	Kt/V for urea index
Study group	43.7 ± 21.4	6.8 ± 3.1	$6.7·10^9$/L	19.6	697.1	1.43 ± 0.16
Control group	42.4 ± 18.9	6.5 ± 2.9	$6.8·10^9$/L	19.4	688.5	1.41 ± 0.18

Table 1.
Values of some clinical laboratory test hematology and blood chemistry indicators in study and control groups.
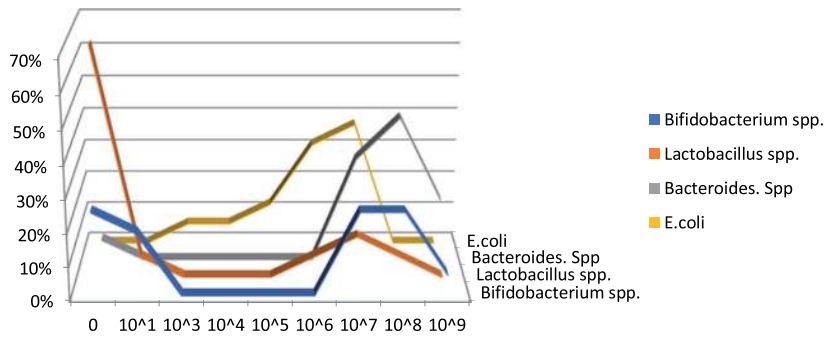
Figure 1.
Quantitative characteristics of major components of the obligate intestinal microbiota in patients with chronic kidney disease receiving programmed hemodialysis.
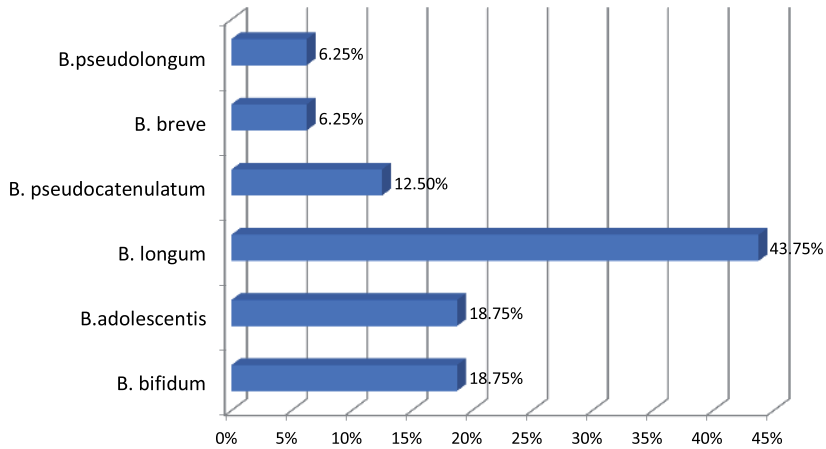

Figure 2.
Occurrence rates for various species of the genus Bifidobacterium in patients with chronic kidney disease receiving programmed hemodialysis.
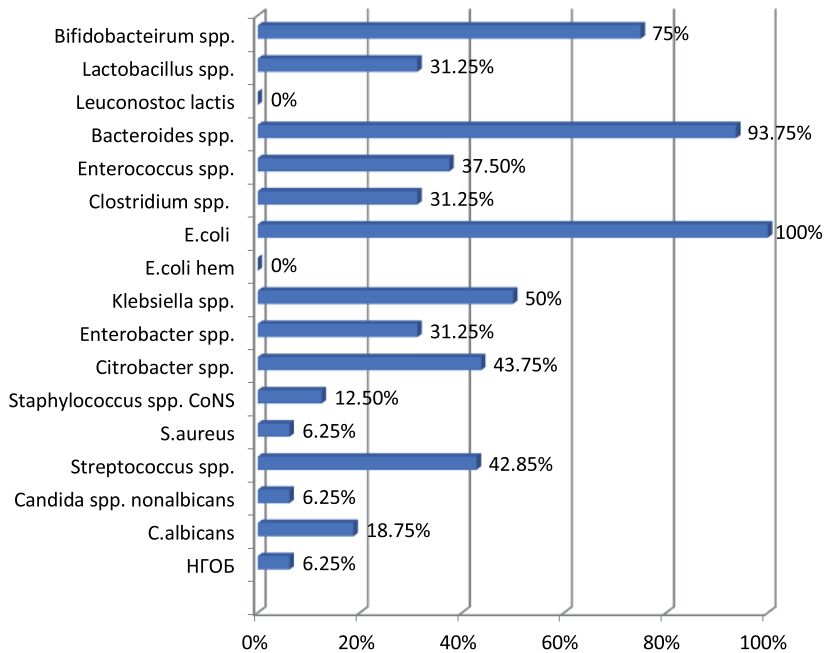

Figure 3.
Occurrence rates for various species of the normal human colon microbiota in patients with chronic kidney disease receiving programmed hemodialysis.
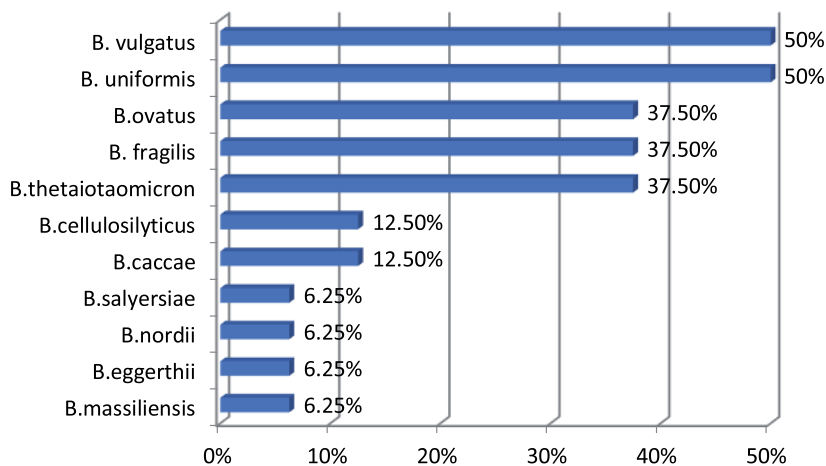
Figure 4.
Occurrence rates for various species of the genus Bacteroides spp. in patients with chronic kidney disease receiving programmed hemodialysis.
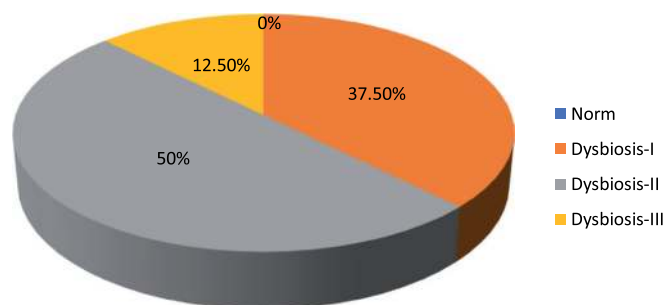

Figure 5.
Dysbiotic disorders (grades I–III) of the colon microbiocenosis in patients with chronic kidney disease receiving programmed hemodialysis before starting treatment with symbiotic.

[59], it was clearly shown how, on the one hand, functional redundancy and, on the other hand, metabolic "specialization" (production of lactate and acetate) of representatives of the two main bacterial phyla—Firmicutes and Bacteroides—provide stability the gut ecosystem as a whole. However, in CKD, due to the progression of renal failure, the concentration of uremic toxins in the intra- and extracellular spaces increases, which leads to their influx into the gastrointestinal tract. With the help of bacterial urease, urea is quickly converted into ammonium hydroxide, irritating of the mucous membrane of the large intestine and, in the future, the development of inflammation. Approximately 68% of creatinine is transformed by bacteria into creatine, and the remainder is converted into 1-methylhydantoin, sarcosine, methylguanidine, etc., by the quantitatively prevailing proteolytic microorganisms (*B. fragilis*, *Bacteroides thetaiotaomicron*, *Enterobacter* spp., *Citrobacter* spp.) recognized as uremic toxins, which contribute to the aggravation of the manifestations of renal failure [21, 60].

Thus, in pathological conditions in humans, the mechanism of functional redundancy of the microbiota can lead to a worsening of the course of the disease [20]. As a result, the restoration of microbiocenosis with the help of undoubtedly probiotic microorganisms (lactobacilli and bifidobacteria) is of particular importance: proteolytic bacteria are excluded from the pathogenesis of the underlying disease formate and succinate) remains unchanged.

After complex therapy, both in the control group and in the group in which the synbiotic "LB-complex L" was used [9], bifidobacteria were detected in 100% of cases.

However, in the main group, they were isolated mainly in amounts of 109–1010 CFU/g, while in the comparison group, their number was 107–108 CFU/g ($p < 0.05$). Lactobacilli were isolated in amounts of 107–108 CFU/g in 100% of patients in the main group and 56.25% of those examined from the comparison group. In 43.75% of patients in the comparison group, lactobacilli were absent in the microbiocenosis. Bacteroids were detected in 100% of the examined in the amount of 108–109 CFU/g in the main group and 106–107 CFU/g in the comparison group ($p < 0.05$).

In the main group, opportunistic microorganisms after treatment were detected with a lower frequency and in a smaller amount than in the comparison group. Thus, *Klebsiella* spp. was isolated in 6.25% versus 50.0% in the comparison group, *Citrobacter* spp.—in 12.5 and 56.25%, respectively. A similar trend was observed about other opportunistic microorganisms and fungi (*Raoultella* spp., *Enterococcus* spp., *Streptococcus* spp., *Acinetobacter* spp., *Corynebacterium* spp., *Microbacterium* spp., *Bacillus* spp., *Candida* spp., etc.). Thus, in the main group, 56% of the examined microbiocenosis recovered, grade III dysbiosis was not detected in any patient. In the comparison group, the microbiological indicators worsened: the number of cases of detection of pronounced microbiocenosis disorders of II and III degrees increased. In clinical and biochemical blood parameters of patients of the main group, attention is drawn to the decrease after treatment in the level of inflammation indicators—CRP (5.3 g/L) and ESR (36.2 mm/h).

The modern concept of providing medical care requires not only the restoration of the biological function of the body but also the normalization of its functioning. When assessing the quality of life using the SF-36 questionnaire, our study revealed an improvement in these indicators on the scales reflecting the physical component of health in the main group. The most positive dynamics after the treatment in the main group was noted on such scales of quality of life as RP—the scale of role activity due to a physical condition (before treatment: 39.2, after: 45.1); P, pain intensity scale (65.2 and 71.3, respectively) and GH, general health scale (52.6 and 58.8, respectively) (**Figure 6**) [60].
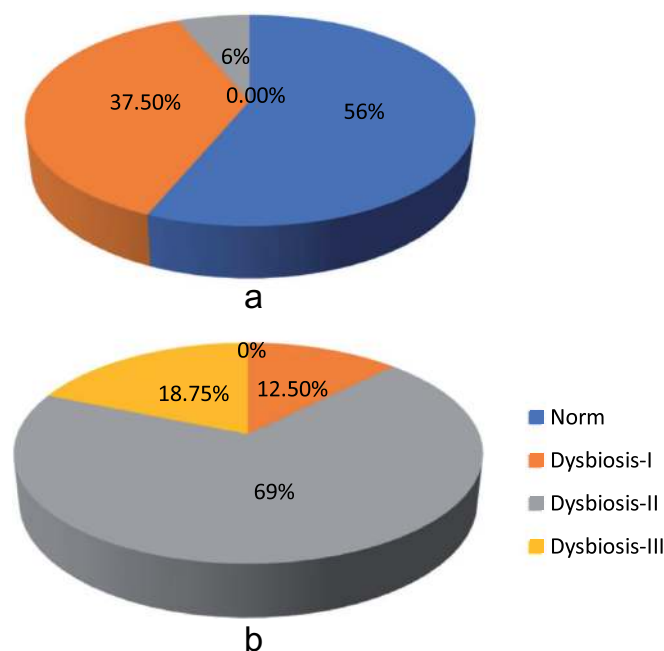

Figure 6.
Dysbiotic disorders of colon microbiocenosis in patients with chronic kidney disease receiving programmed hemodialysis after treatment (a) main group; (b) comparison group.

4. Conclusion

Thus, acute and chronic cerebrovascular disorders in patients with CKD of the pre-dialysis and dialysis periods are the most frequent and formidable complications that develop against the background of "traditional" and "specific" cerebrovascular risk factors. Because of the studies, new knowledge was obtained about the species diversity and species representation of the microbiocenosis of the colon lumen in patients with chronic kidney disease receiving program dialysis. The inclusion of the author's immobilized multistrain synbiotic "LB-complex L" as a dietary component in the basic therapy allows not only to restore the evolutionarily determined microbiocenosis, but also improves the quality of life of patients, and also helps to reduce the risk of cardiovascular events.

Active control of the described specific indicators (impaired phosphorus-calcium metabolism and calcification of the arterial bed, correction of anemia and dysfunction of blood corpuscles, hyperhomocysteinemia and hyperβ2-microglobulinemia, accumulation of uremic toxins and toxins of intestinal bacteria, correction of pathologically altered intestinal microbiocenosis) and conditions of dialysis their timely prevention, as well as the correction of intradialysis hypo-/hypertension, are necessary for the timely prevention of cerebrovascular disorders in dialysis patients.

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