ORIGINAL RESEARCH

Probiotic Supplementation in Chronic Kidney Disease: A Double-blind, Randomized, Placebo-controlled Trial

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Objective: The objective of the study was to evaluate the effects of probiotic supplementation on the gut microbiota profile and inflammatory markers in chronic kidney disease patients undergoing maintenance hemodialysis (HD).

Design and Methods: This was a randomized, double-blind, placebo-controlled study. Forty-six HD patients were assigned to receive 1 of 2 treatments: probiotic (n = 23; *Streptococcus thermophilus*, *Lactobacillus acidophilus e Bifidobacterialongum*, 90 billion colony-forming units per day) or placebo (n = 23) daily for 3 months. Blood and feces were collected at baseline and after intervention. The inflammatory markers (C-reactive protein and interleukin-6) were analyzed by immunoenzymatic assay (enzyme-linked immunosorbent assay). Uremic toxins plasma levels (indoxyl sulfate, *p*-cresyl sulfate, and indole-3-acetic acid) were obtained by Reversed-Phase High-Performance Liquid Chromatography. Routine laboratory parameters were measured by standard techniques. Fecal pH was measured by the colorimetric method, and the gut microbiota profile was assessed by Denaturing Gradient Gel Electrophoresis analysis.

Results: Sixteen patients remained in the probiotic group (11 men, 53.6 ± 11.0 year old, 25.3 ± 4.6 kg/m^2) and 17 in the placebo group (10 men, 50.3 ± 8.5 year old, 25.2 ± 5.7 kg/m^2). After probiotic supplementation there was a significant increase in serum urea (from 149.6 ± 34.2 mg/dL to 172.6 ± 45.0 mg/dL, $P = .02$), potassium (from 4.4 ± 0.4 mmol/L to 4.8 ± 0.4 mmol/L, $P = .02$), and indoxyl sulfate (from 31.2 ± 15.9 to 36.5 ± 15.0 mg/dL, $P = .02$). The fecal pH was reduced from 7.2 ± 0.8 to 6.5 ± 0.5 ($P = .01$). These parameters did not change significantly in placebo group. Changes in the percentage delta (Δ) between groups were exhibited with no statistical differences observed. The inflammatory markers and gut profile were not altered by supplementation.

Conclusions: Aprobiotic supplementation failed to reduce uremic toxins and inflammatory markers. Therefore, probiotic therapy should be chosen with caution in HD patients. Further studies addressing probiotic therapy in chronic kidney disease patients are needed.

Introduction

RESEARCH HAS SHOWN that the imbalance of the microorganisms living in the gut community and the impairment of the colonic epithelium are related to inflammation and oxidative stress in chronic kidney disease (CKD). In fact, CKD patients are constantly exposed to various factors such as malnutrition, edema, stress (physical, psychological, or pharmacological), constipation, dietary restriction, and uremia among others, which compromise the intestinal homeostasis.[1–4]

Alterations in the microbiota composition of CKD patients have been associated with the growth of bacterial species involved in the generation of harmful compounds called uremic toxins such as indole-3 acetic acid (IAA), indoxyl sulfate (IS), and *p*-cresyl sulfate (p-CS).[5] CKD patients present a progressive retention of these uremic toxins,

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with a negative impact on many body functions and increase in cardiovascular mortality.[1,6] The bacterial fermentation of the amino acid tryptophan in the colon leads to production of indoles that are metabolized to IAA in the intestine and IS in the liver.[7] On the other hand, the fermentation of tyrosine and phenylalanine leads to synthesis of *p*-cresol and finally p-CS.[8]

Probiotic supplementation has been suggested as an adjuvant therapy to improve the balance of the gut microbiota contributing to intestinal barrier integrity and metabolic control of these patients.[9-15] Probiotics are "natural or genetically modified microorganisms, expressing specific exogenous enzymes, which are able to survive stomach acid and bile, to increase the colon concentration of symbionts, and confer a health benefit."[16] The mechanisms by which probiotics exert their effects involve changes in intestinal pH, antagonism of pathogens by production of antibacterial components, competition for available nutrients, conjunction with mutagens and carcinogens preventing their actions, and improved intestinal barrier functions.[17,18]

Studies evaluating the effectiveness of probiotics on CKD have reported discrepant results. Thus, the present study evaluated the effects of a probiotic formulation on the biochemical and inflammatory parameters, uremic toxins levels, and gut microbiota profile in CKD patients on hemodialysis (HD).

Methods

Recruitment of Participants

Forty-six HD patients were included in this randomized, double blind, placebo-controlled study (23 received probiotic supplement and 23 received placebo). Patients aged 18 years, undergoing HD for at least 6 months, were included. Patients with inflammatory diseases, cancer, AIDS, autoimmune disease, smokers, use of a central catheter for hemodialysis access, amputated limbs, pregnancy, and patients who had used catabolic drugs, antioxidant vitamin supplements pre, pro, and symbiotic and antibiotics in the last 3 months before starting this study were excluded.

Dialysis duration was 3 to 4.5 hours per session, 3 times per week, with a blood flow greater than 250 mL/minute and a dialysate flow of 500 mL/minute. The study protocol was reviewed and approved by the Ethics Committee of the School of Medicine–Universidade Federal Fluminense (083/11), and all the patients were asked to sign the informed consent.

The random sequence of treatment (probiotic and placebo) was manually generated for a simple randomization. None of the subjects involved in the study had access to the allocation sequence until the end of the statistical analyses. The participants and the researchers who interviewed and visited the subjects were blinded to the contents of the bottles, which contained probiotic or placebo capsules. All laboratory measurements were centralized and performed in a blinded manner.

Intervention

Twenty-three patients were randomly allocated in the probiotic group and 23 patients were allocated in the placebo group. The dosage was 3 capsules of probiotic or placebo per day for 3 months. Each capsule of probiotic contained 30 billion live bacteria, totalizing 90 billion colony-forming units (CFU) per day and included the following strains: *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacteria longum*. Patients were contacted weekly, by phone calls, during the trial by study staff to encourage adherence to supplementation and also to monitor side effects. Adherence was measured by the number of capsules returned to researchers subtracted from the number of capsules dispensed at the previous visit. This number was divided by the number of capsules the patient should have taken during 3 months and then multiplied by 100 to obtain the adherence percentage.

Analytical Procedures and Sample Processing

Blood samples were taken from each subject in the morning, at the start of a dialysis session (after overnight fasting), into a syringe containing ethylenediaminetetraacetic acid (1.0 mg/mL) as anticoagulant. Plasma was separated (15 minutes, 3,000×*g*, 4°C), and stored at −80°C until analysis.

Total concentrations of IS, IAA, and p-CS were quantified by Reversed-Phase High-Performance Liquid Chromatography with fluorescent detection as previously described.[19] Briefly, plasma samples were processed as described[20] and injected into a high-performance liquid chromatography system (Shimadzu Prominence) consisting of a Rheodyne injector (model 7125), a quaternary pump (Shimadzu LC-20AD), and a fluorescence detector (Shimadzu RF-20A) all controlled by LC Solution software.

High-sensitivity C-reactive protein and interleukin-6 were analyzed by immunoenzymatic assay (enzyme-linked immunosorbent assay). Routine laboratory parameters were measured by standard techniques.

The fecal samples were collected in sterile containers and provided to the laboratory on the day of collection before and after the follow-up. The fecal pH was measured by the colorimetric method with homogenized stool samples in distilled water and DNAs were extracted to Denaturing Gradient Gel Electrophoresis (DGGE) analysis.

DNA Extraction and Polymerase Chain Reaction of the 16S Ribosomal RNA

DNA extraction and polymerase chain reaction were analyzed as previously described.[21] Briefly, the DNAs were extracted using the Xpedition Soil/Fecal Miniprep DNA (Zymo, Irvine, CA). The integrity and quality of extracted DNA were analyzed by gel electrophoresis in 0.8%