ORIGINAL RESEARCH

Does Low-Protein Diet Influence the Uremic Toxin Serum Levels From the Gut Microbiota in Nondialysis Chronic Kidney Disease Patients?

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Objectives: To evaluate the effects of low-protein diet (LPD) on uremic toxins and the gut microbiota profile in nondialysis chronic kidney disease (CKD) patients.

Design and Methods: Longitudinal study with 30 nondialysis CKD patients (stage 3-4) undergoing LPD for 6 months. Adherence to the diet was evaluated based on the calculation of protein equivalent of nitrogen appearance from the 24-hour urine analysis. Good adherence to LPD was considered when protein intake was from 90% to 110% of the prescribed amount (0.6 g/kg/day). Food intake was analyzed by the 24-hour recall method. The anthropometric, biochemical and lipid profile parameters were measured according to standard methods. Uremic toxin serum levels (indoxyl sulfate, p-cresyl sulfate, indole-3-acetic acid) were obtained by reversed-phase high-performance liquid chromatography (RP-HPLC). Fecal samples were collected to evaluate the gut microbiota profile through polymerase chain reaction and denaturing gradient gel electrophoresis. Statistical analysis was performed by the SPSS 23.0 program software.

Results: Patients who adhered to the diet (n = 14) (0.7 ± 0.2 g/kg/day) presented an improvement in renal function (nonsignificant) and reduction in total and low-density lipoprotein cholesterol (183.9 ± 48.5-155.7 ± 37.2 mg/dL, $P = .01$; 99.4 ± 41.3-76.4 ± 33.2 mg/dL, $P = .01$, respectively). After 6 months of nutricional intervention, p-cresyl sulfate serum levels were reduced significantly in patients who adhered to the LPD (19.3 [9.6-24.7] to 15.5 [9.8-24.1] mg/L, $P = .03$), and in contrast, the levels were increased in patients who did not adhere (13.9 [8.0-24.8] to 24.3 [8.1-39.2] mg/L, $P = .004$). In addition, using the denaturing gradient gel electrophoresis technique, it was observed change in the intestinal microbiota profile after LPD intervention in both groups, and the number of bands was positively associated with protein intake ($r = 0.44$, $P = .04$).

Conclusion: LPD seems be a good strategy to reduce the uremic toxins production by the gut microbiota in nondialysis CKD patients.

Introduction

CHRONIC KIDNEY DISEASE (CKD) affects the intestinal homeostasis because it influences the microbial metabolism of the colon. This change is due to decreased absorption of intestinal proteins, which increases intraluminal pH due to high ammonia concentration and prolongs intestinal transit time.[1] Other factors can influence microbial metabolism in CKD such as (1) malnutrition, which increases the permeability of the intestinal barrier leading to endotoxin translocation; (2) heart failure, edemas, and oxidative stress that can reduce intestinal blood flow; (3) constipation and uremia *per se*, which may trigger intestinal barrier atrophy; and (4) increased inflammatory processes.[2,3] In fact, studies have shown a strong association between intestinal barrier dysfunction, uremic toxicity, and inflammation in CKD patients.[4–7]

All these complications can provoke a higher influx into plasma of uremic toxins produced by the fermentation of amino acids by gut microbiota, such as indoxyl sulfate (IS), p-cresyl sulfate (p-CS), and indole-3-acetic acid (IAA).[8–12] When tryptophan is metabolized by tryptophanase in the large intestine by intestinal bacteria such as *Escherichia coli*, this produces indole that is absorbed and metabolized in the liver forming IS.[13] Another toxin that is also produced from tryptophan is IAA, which is metabolized to indole directly in the intestine or in the tissue via tryptamine.[14,15] When tyrosine and phenylalanine from dietary protein reach the large intestine, they can be metabolized by putrefactive bacteria of the intestinal microflora including

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Bacteroides, Lactobacillus, Enterobacter, Bifidobacterium, and especially *Clostridium* and produce p-cresol,[16,17] which is converted to p-CS via sulfotransferase in the liver.[18]

These uremic toxins cause many adverse effects in CKD patients, including inflammation, oxidative stress, and increased mortality.[16,19,20] Many strategies have been suggested to reduce the production of uremic toxins by gut microbiota such as pre, pro, or symbiotic supplementation, use of oral adsorbent AST-120, and physical exercise.[21,22] In addition, as these toxins are produced by amino acids from the diet, our hypothesis proposes that a low protein intake may reduce the uremic toxin generation.[23] It is important to emphasize that CKD patients at stages before dialysis treatment (stage 3-4) should receive a low-protein diet (LPD) prescription, which is nutritionally safe and useful to protect residual renal function.[23] Therefore, the aim of this study was to evaluate the effects of LPD on the serum levels of uremic toxins and the gut microbiota profile in nondialysis CKD patients.

Methods

Patients and Study Design

This longitudinal study consisted of 30 nondialysis CKD patients (aged 55.5 ± 14.5 years, 16 males, body mass index [BMI] of 29.1 ± 5.9 kg/m^2), (estimated glomerular filtration rate: 35.6 ± 12.2 mL/min/1.73 m^2) from Renal Nutrition Out-Patient Clinic–Federal University Fluminense-UFF–Niterói, Brazil. Patients were included if they were 18 years or older, CKD categories 3-4, and without any previous nutritional counseling. Patients with inflammatory diseases, cancer, AIDS, autoimmune disease, liver disease or were smokers, or pregnant, 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.

The etiologies of CKD in these patients were hypertensive nephrosclerosis (27), diabetic nephropathy (1), chronic glomerulonephritis (1), and polycystic kidney disease (1). The medications used during the investigation period were angiotensin converting enzyme inhibitor or angiotensin II receptor antagonists (n = 24; 80%), diuretics (n = 14; 46.7%), statin (n = 12; 40%), alpha- and beta-receptor blockers (n = 11; 36.7%), adrenergic blocking agents (n = 10; 33.3%), calcium channel blockers (n = 8; 26.7%), vasodilators (n = 2; 6.7%), sodium bicarbonate (n=11; 36.7%), ferrous sulfate (n=6; 20.0%), and erythropoietin (n=2; 6.7%). These medications were not changed during the study period.

The study protocol was reviewed and approved by the Ethics Committee of the School of Medicine–Federal University Fluminense (565.857/2014), and all the patients were asked to sign an informed consent form.

Dietary Prescription

Patients received an LPD prescription (0.6 g protein/kg/day) and calories according to nutritional assessment of between 30 and 35 kcal/kg/day. Nutritional monitoring consultations were held every 2 months for 6 months. A survey of demographic, clinical, and the assessment of food intake by 24-h food recall data were carried out at the first nutritional appointment. Tubes containing different amounts of salt and food replicas were used to improve the accuracy of the patient's record as described in Mafra and Leal (2016).[24] The intake of energy, macronutrients, and micronutrients were estimated using Excel software (2010), and nutrient composition was obtained through the Brazilian Food Composition Table (TACO).[25] To improve the adhesion to the diet, 2 culinary classes were performed with the patients, and low protein, fat, sodium, potassium, and phosphorus recipes were used. In these classes, nutrition information was provided, and counseling was offered.

Adherence to the diet was evaluated based on the calculation of Protein Equivalent of Nitrogen Appearance (nPNA) from the 24-h urine analysis, which were determined by the colorimetric method. The formula used to calculate the nPNA was nPNA = (urinary urea nitrogen + [0.031 × weight]) × 6.25, where urinary urea nitrogen is (urea/2.14 × urinary volume).[26] Good adherence to LPD was considered when protein intake was from 90% to 110% of the prescribed amount (0.6 g/kg/day).

Nutritional Assessment

The following anthropometric parameters were measured: body weight (kg), height (m), and waist circumference (WC) (cm). BMI (kg/m^2) was calculated as body weight divided by squared stature and used to assess the nutritional status according to World Health Organization guidelines (2000).[27] The abdominal obesity was ranked high when the WC values were above 80.0 cm for women and 94.0 cm for men.[27]

Body composition was assessed by dual X-ray absorptiometry Lunar Prodigy Advanc Plus (Corp/General Electric Madison, Wisconsin, USA). Before body composition measurements, dual X-ray absorptiometry was calibrated according to the standard procedure recommended by the manufacturer.[28]

Analytic Procedures and Sample Processing

Blood samples were drawn from each subject in the morning, after overnight fasting into a Vacutainer® tube containing EDTA as anticoagulant (1.0 mg/mL) and without anticoagulant. Blood was centrifuged (3500 rpm for 15 minutes at 4°C), to obtain a plasma and serum sample, respectively, and was then placed in 1.5 mL polypropylene eppendorf tubes and stored at −80°C until required for analysis. Biochemical parameters were measured in all patients according to standard methods in the routine clinical laboratory. The Glomerular filtration rate was estimated by CKD-Epi equation.[29]

Total uremic toxin levels (IS, p-CS, and IAA) were quantified by high-performance liquid chromatography (RP-HPLC) with fluorescent detection. Briefly, serum samples