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Effects of probiotic supplementation on inflammatory biomarkers and uremic toxins in non-dialysis chronic kidney patients: A double-blind, randomized, placebo-controlled trial

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ABSTRACT

Probiotics may mitigate the generation of uremic toxins and inflammatory biomarkers. The aim of this study was to evaluate the effects of probiotics on uremic toxins and inflammatory biomarkers in CKD. In this randomized, double-blind, placebo-controlled trial, 30 patients (63.8 ± 7.5 years, 14 men, mean BMI of 27.2 ± 3.8 kg/m^2) were assigned to receive one of two treatments: probiotics (n = 15; *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacteria longum*-90 billion CFU per day) or placebo (n = 15) daily for three months. Plasma uremic toxins were measured using reversed-phase liquid-chromatography (RP-HPLC); choline, betaine and trimethylamine-N-oxide (TMAO) were measured using liquid chromatography–mass spectrometry (LC-MS/MS); and inflammatory biomarkers were measured using ELISA. Uremic toxins were not influenced by the probiotics; however, IL-6 levels increased significantly from 15.6 (14.8–20.8) pg/mL to 23.0 (17.6–29.6) pg/mL, p = 0.01. There was a positive correlation between the levels of p-cresyl sulfate and urea (r = 0.55; p = 0.02) and between TMAO and CRP (r = 0.46; p = 0.05) at baseline. These data suggest that probiotic supplementation did not result in expected benefits for non-dialysis CKD patients.

1. Introduction

Gut microbiota imbalance has recently emerged as an important player in the progression and complications of chronic kidney disease (CKD) (Guldris, González, & Amenós, 2017; Mafra et al., 2014). Such an imbalance may be related to the greater influx of urea from the bloodstream into the intestinal lumen, altering the intestinal biochemical environment and gut microbiota composition (Lau & Vaziri, 2017). Consequently, families of bacteria expressing urease and uricase and bacteria expressing indole-forming enzymes and p-cresol become dominant. Thus, the gut microbiota of these patients generates greater amounts of uremic toxins through amino acid metabolism, such as indoxyl-sulfate (IS), p-cresyl sulfate (p-CS) and indole-3-acetic acid (IAA). These uremic toxins have been associated with inflammation and cardiovascular disease (CVD) (Cho et al., 2017). In fact, Stockler-Pinto et al. (2016) demonstrated that the incubation of adipose cells with IS led to elevated levels of TNF-alpha and IL-6, as well as increased production of reactive oxygen species (ROS), mainly through the activation of the enzyme NADPH oxidase.

Another bacterial metabolite in the bloodstream that is associated with CVD is trimethylamine N-oxide (TMAO) (Harris, Kassi, Major, & Chou, 2012; Sallee et al., 2014), which is formed from dietary trimethylamine-containing nutrients, such as phosphatidylcholine (PC), L-carnitine, choline and betaine (Cho et al., 2017). Elevated TMAO levels predict cardiovascular events, even after adjusting for traditional risk factors and renal function (Wang et al., 2014).

In addition to being associated with CVD, the microbiota imbalance is involved with other health hazards, such as increased susceptibility to infections, immune disorders, insulin resistance, oxidative stress and inflammation (Cani et al., 2008; Harris et al., 2012; Manco, Putignani,

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& Bottazzo, 2010). Although the origin of inflammation in CKD is multifactorial, changes in the microbiota profile and leaky intestinal barrier have been recognized as important causes of systemic inflammation in these patients (Vaziri, Zhao, & Pahl, 2015).

Considering that the imbalance of the intestinal microbiota may promote chronic inflammation and CVD, therapeutic interventions, such as the use of probiotics, may be a valid strategy to restore and maintain the balance of the intestinal microbiota in CKD patients, as well as to improve the intestinal barrier and reduce the formation of uremic toxins and inflammation (Borges et al., 2017; Di Cerbo et al., 2013). The effectiveness of probiotics in CKD patients, especially in non-dialysis patients, has not yet been thoroughly investigated, and available results are controversial. Recently, our group supplemented 46 hemodialysis (HD) patients with probiotics for three months and observed that supplementation was not able to reduce the levels of uremic toxins (Borges et al., 2017). Thus, the aim of this study was to evaluate the effects of probiotic supplementation on inflammatory biomarkers and uremic toxins in CKD (stages 3–5) patients.

2. Materials and methods

2.1. Recruitment of participants

Non-dialysis CKD patients (stages 3–5) between 18 and 65 years old receiving a prescribed low protein diet for more than one year were included in this study. Patients with inflammatory diseases, cancer, AIDS, or autoimmune disease; smokers; those who were pregnant; and patients who had used catabolic drugs, antioxidant vitamin supplements, pre-, pro- and symbiotics, or antibiotics in the 3 months before the start of the study were excluded. Based on these criteria, 30 CKD patients on conservative treatment (63.8 ± 7.5 years old, 14 men, BMI of 27.2 ± 3.8 kg/m^2, eGFR 33.8 ± 9.3 mL/min per 1.73 m^2) from one nephrology outpatient department were eligible for inclusion in this randomized, double blind, placebo-controlled study. Fig. 1 shows the flow chart of subjects through the study phases. The study protocol was reviewed and approved by the Ethics Committee of the School of Medicine – UFF (Number 083/11), and all the patients signed the informed consent prior to enrollment (see Fig. 2).

2.2. Intervention

The allocation of patients to active treatment or placebo was randomly performed by a statistician who was not directly involved in the research and not informed of the objectives prior to data analysis.

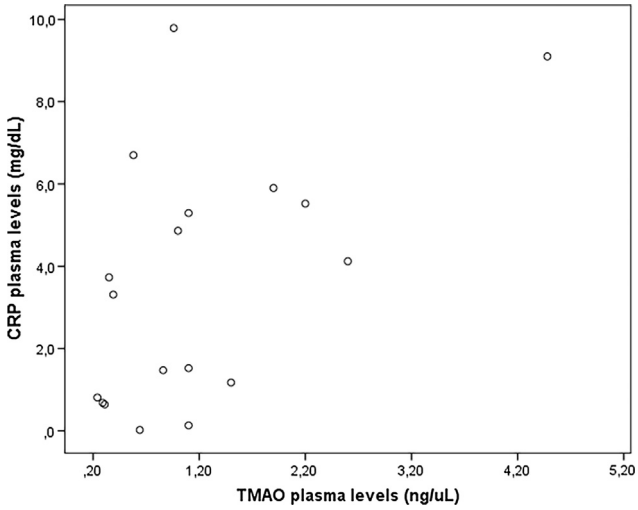

Fig. 2. Correlation between Trimethylamine-N-oxide (TMAO) and C-reactive protein (CRP) plasma levels (r = 0.53, p = 0.023).

Patients involved in the study did not have access to sequential allocation. The supplement used in the treatment group contained a combination of three strains of encapsulated probiotic gram-positive bacteria: *Streptococcus thermophilus*, *Lactobacillus acidophilus and Bifidobacteria longum*. Each capsule had 30 billion colony forming units (CFU). The dosage was 3 capsules a day: one after breakfast, one after lunch and the other after dinner (a total of 90 billion CFU/day) for 3 months. The placebo consisting of capsules of similar appearance in color, size and shape, was packed in plastic bottles identical to the probiotics. Blood samples were taken from the patients at the beginning and at the end of the three (3) month trial period and anthropometric measures were also recorded at the same time. During the intervention period, all patients were followed by a nutritionist involved in the study to certify adherence to the study and report possible symptoms. Compliance was assessed by monitoring the number of capsules returned.

2.3. Inflammatory markers and uremic toxins

Blood samples were drawn from each subject in the morning after overnight fasting into syringes containing EDTA (1.0 mg/mL). Plasma was separated (15 min, 3000*g*, 4 °C) and stored at −80 °C until analysis.

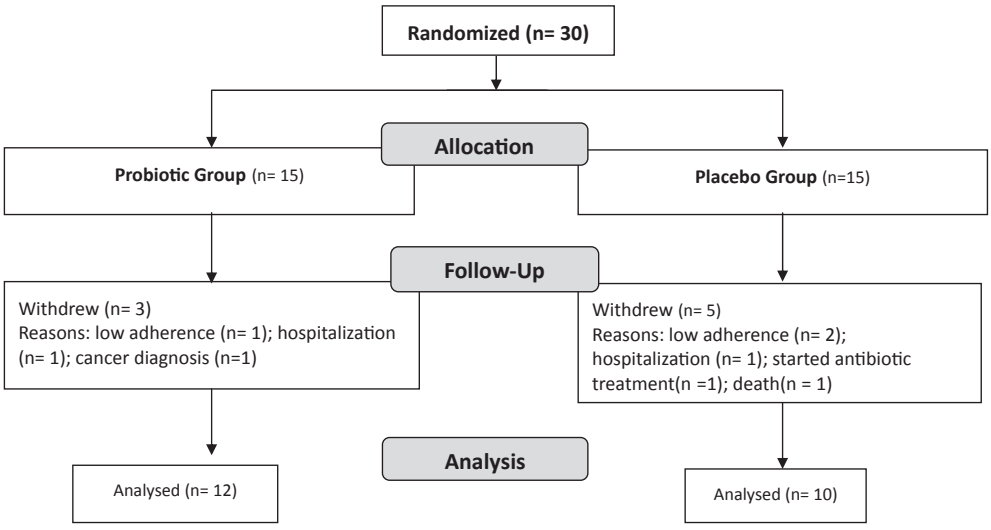

Fig. 1. Flow chart of the study subjects.