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Effect of short-term synbiotic treatment on plasma p-cresol levels in patients with chronic renal failure: A randomized clinical trial

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Abstract *Background and aims:* In patients with chronic kidney disease (CKD), alterations in gut microbiome are posited to be responsible for gastrointestinal symptoms and generation of p-cresol, a uremic toxin that has been associated with CKD progression and cardiovascular mortality. This pilot study investigated whether *Probinul-neutro*®, a synbiotic that normalizes intestinal microflora, may lower plasma p-cresol concentrations and reduce gastrointestinal symptoms in non-dialyzed CKD patients.
Methods and results: This was a double-blind, randomized placebo-controlled trial. Thirty patients on 3–4 CKD stages were randomized to receive either *Probinul neutro*® or placebo for 4 weeks. Total plasma p-cresol concentration was assessed at baseline, and 15 and 30 days after treatment start. At the same study times, ease and frequency of defecation, upper and lower abdominal pain, stool shape, borborygmi, and flatus were quantified by subjective assessment questionnaires. Compared to baseline total plasma p-cresol median concentrations on 15th and 30th day were significantly lower in patients receiving *Probinul-neutro*® (2.31 and 0.78 vs. 3.05 μg/ml, $p < 0.05$; $n = 18$); no changes of plasma p-cresol concentrations were recorded in placebo-treated patients. No significant changes in gastrointestinal symptoms were observed during the study both in *Probinul-neutro*®-treated and placebo-treated patients.
Conclusion: Probinul-neutro® lowered total plasma p-cresol concentrations but did not ameliorate gastrointestinal symptoms in non-dialyzed CKD patients. Because high plasma concentrations of p-cresol in early phases of CKD are predictive of progression to end-stage renal disease, the results of our study suggest that synbiotics deserve attention as possible tools to delay CKD progression towards end-stage renal disease (ESRD).
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Introduction

In patients with chronic kidney disease (CKD), a substantial decrease in gastrointestinal motility occurs because myoelectrical activity is depressed [1]. This causes marked alterations in the gut microbiome with the overgrowth of aerobic bacteria such as *Enterobacteriaceae*,

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Halomonadaceae, *Moraxellaceae*, and *Pseudomonadaceae* and a decrease of anaerobic bacteria such as *Lactobacillaceae* and *Prevotellaceae* [2]. In CKD, gastrointestinal (GI) dysfunction not only causes symptoms that worsen patient quality of life and contribute to anorexia and malnutrition [3] but it also contributes to the pathogenesis of the systemic complications. Indeed, the dysbiotic microflora produces toxic compounds such as phenols, indoles, and amines that are absorbed through the intestinal mucosa and cause systemic toxicity [4]. Compelling evidence showed the association of one of these compounds, p-cresol and of its main metabolite p-cresylsulfate, to cardiovascular risk and mortality in CKD [5,7].

The evidence that alterations in gut microbiome are linked both to GI symptoms and to systemic complications in CKD patients suggests that therapeutic intervention aiming to normalizing intestinal microflora could be beneficial in these subjects. This could be obtained through the administration of probiotics and/or prebiotics. Probiotics are living microorganisms such as *Bifidobacterium* and *Lactobacillus* species that are administered to repopulate the gut with a "normal" microflora [8]. Prebiotics are non-digestible food adjuncts such as inulin or soybeans that can be selectively fermented by probiotics or by bacteria belonging to normal microflora and, therefore, confer a specific selective advantage for these microorganisms respect to other components of pathological gut microflora [9]. Because prebiotics synergize with probiotics they are often administered together in associations known as synbiotics. Previous studies showed that probiotics and synbiotics are effective in restoring the normal gut microbiome in several conditions including post-antibiotic diarrhea and in patients with irritable bowel syndrome [10,11]. Therefore, in the present paper we investigated the effect of a symbiotic on plasma p-cresol concentrations and GI symptoms in CKD patients not yet on dialysis.

Methods

Subjects

Thirty CKD patients (26 male and 4 female) with mean age 59.5 $\pm$ 13.1 years were referred to the Nutrition Unit of the Federico II University of Naples, and enrolled in the study. Patients gave their written informed consent to take part to the study after being informed on study design and aims. The experimental protocol was approved by the Ethics Committee of the School of Medicine of the Federico II University of Naples; procedures were performed according to the WMA Helsinki declaration as revised in 1996.

Inclusion and exclusion criteria

Inclusion criteria were: age >18 years and estimated glomerular filtration rate (eGFR) between 15 and 60 ml/min (stage 3–4 CKD, according to the K/DOQI CKD classification). Patients with kidney transplant or with severe infections, diabetes, malignancy, history of food intolerance, autoimmune disorders, severe malnutrition, or clinical conditions requiring artificial feeding were excluded.

Primary and secondary endpoints

The primary endpoint of the study was the decrease in total plasma p-cresol concentration. Secondary endpoint was the improvement of any of the following GI symptoms: defecation frequency or ease, stool shape, upper or lower abdominal pain, and frequency of borborygmi or flatus.

Study design

This was a double-blind randomized placebo study. Patients were randomly assigned to two different groups. One group received placebo (placebo group, $n = 12$), the other group (synbiotic group, $n = 18$) a commercial synbiotic (*Probinul neutro*®; CadiGroup, Rome, Italy). Patients were allocated to the two study groups by simple randomization using a computer-generated random binary list. The medical doctor performing the first visit was responsible for enrollment and randomized group assignment. Neither the patients nor the medical doctors performing further patient evaluation were aware of group assignment. In all patients, baseline clinical evaluation included anthropometric examination, body composition, and blood chemistry assessment with plasma p-cresol measurement. Patients were asked to fill in questionnaires designed to assess their dietary intakes and bowel habits and symptoms.

Anthropometric evaluation and body composition assessment

Body mass index (BMI) was calculated as the ratio of body weight to height (kg/m^2) [2]. Waist circumference was measured with an inelastic tape at the level of the umbilicus keeping the subject in standing position. Body composition was assessed by bioelectrical impedance analysis. A tetrapolar 50-kHz bioelectrical impedance analyzer (BIA 101 RJL, Akern Bioresearch, Firenze, Italy) was used to measure total body water (TBW), fat mass (FM), and fat free mass (FFM) [12].

Nutritional queries

Dietary intake was quantified with nutritional questionnaires that were collected by experienced dietitians during patient interviews. The questionnaires inquired about amount and frequency of foods and beverages consumed everyday by patients. Data on nutrients composition of the different foods were obtained using the tables of the Italian National Institute of Nutrition, Souci's Food Composition and Nutrition Tables and the European Institute of Oncology as reported elsewhere [13].