ORIGINAL RESEARCH

The Impact of Probiotic Soy Milk Consumption on Oxidative Stress Among Type 2 Diabetic Kidney Disease Patients: A Randomized Controlled Clinical Trial

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Objective: Diabetic kidney disease (DKD) is one the most important complications of diabetes leading to end-stage renal disease. This study aimed to determine the effects of probiotic soy milk consumption on oxidative stress biomarkers in type 2 DKD patients.

Methods: Forty-eight patients were randomly assigned to consume a diet containing 200 mL/day probiotic soy milk in the intervention group or soy milk in the control condition. As determinants of oxidative stress, malondialdehyde, 8-iso-prostaglandin F2α, oxidized glutathione, total antioxidant capacity, reduced glutathione (GSH), glutathione peroxidase, and glutathione reductase were measured after 8 weeks of intervention according to the standard protocol.

Results: Between groups analysis showed that DKD patients in the probiotic soy milk group had a higher mean value of GSH compared with those in the soy milk group. In the final adjusted model, this difference remained significant. Consistently, oxidized glutathione concentration was significantly reduced among patients in the probiotic soy milk group. Also, for activity levels of antioxidant enzymes including glutathione peroxidase and glutathione reductase, significant increased levels were observed between 2 intervention groups in the final adjusted model. However, no significant reduction of the serum 8-iso-prostaglandin F2α or malondialdehyde and no induction of TAC concentrations within and between the 2 groups in the crude and adjusted models were detected.

Conclusion: Overall, the results demonstrate that probiotic soy milk consumption could improve some oxidative stress factors among DKD patients. Further longitudinal studies with consideration of individual variation should be conducted.

Introduction

DIABETIC KIDNEY DISEASE (DKD) is one of the most important complications of diabetes and is the leading cause of end-stage renal failure, disability, and low quality of life worldwide. Hence, DKD patients require a comprehensive care and monitoring plan for controlling the previously mentioned dysfunction.[1–3]

The pathogenesis and mechanisms underlying DKD development are complex and involve both genetic and environmental factors.[4,5] Current evidence suggests that, in the diabetic patients, the balance between free radicals generation and antioxidant defense is impaired, which ultimately contributes to oxidative stress and kidney damage.[6,7]

In the previous studies, regarding the relationship between imbalance of gut bacteria and oxidative stress, probiotics supplementation was recommended for diabetic patients.[8–10]

However, given the difficulty of supplementation adherence among patients, food fortification could be an effective strategy for increasing probiotic intake.[11] As an efficient food matrix, most dairy products have been developed for adding prebiotics.[12,13] However, restricting dairy foods and dietary phosphorus are the major recommendations among DKD patients. Thus, implications of the low phosphorus content of soy milk compared with dairy products, as a probiotic context might be a better choice for DKD patients.[14] Moreover, probiotics are thought to promote the function of soy milk by converting its isoflavones into other active metabolites and increase their favorable effects. Furthermore, soymilk can act as a good substrate for probiotics.[15]

Most previous studies have reported the favorable effects of probiotics supplementation in diabetic patients. To the best of the present authors' knowledge, there are no studies regarding the effects of probiotic soy milk intake on

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oxidative stress profile in type 2 DKD patients. Therefore, this study was conducted to fortify soy milk with *Lactobacillus plantarum A7* and to determine the consumption effects of this product on the oxidative stress biomarkers including malondialdehyde (MDA), 8-iso-prostaglandin F2α (8-iso-PGF2α), oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and total antioxidant capacity (TAC) among type 2 DKD patients.

Methods

Design and Participants

This parallel randomized controlled trial was conducted at Endocrine and Metabolism Research Center. Patients were eligible if they were diagnosed with stages 1 and 2 of nephropathy defined as the meeting criteria including fasting blood glucose >126 mg/dL, hypoglycemic agents or insulin intake, proteinuria >300 mg/day, and glomerular filtration rate >90 mL/minute are eligible for enrollment in this study.[14] The sample size was calculated based on the formula $N = 2[(Z1\text{-}\alpha/2 + Z1\text{-}\beta)^2 \times S2]/d^2$, where α (type 1 error) is 0.05, β (type 2 error) is 0.2, S is the variance of blood urea nitrogen (BUN; S = 3), and d represents the minimal detectable difference of BUN (d = 2.3 mg/dL).[16] Therefore, the power for detecting differences between the treatment conditions for various outcomes in the present study was 80%. Hence, according to this formula, 24 patients were required for the study in each group and 60 diabetic patients were recruited to compensate for any possible exclusion. Finally, 48 adults patients (22 males and 26 females) aging between 32 and 68 years with type 2 DKD were randomized in probiotic soy milk (n = 24, i.e., 12 males and 12 females) and soy milk (n = 24, i.e., 10 males and 14 females) intervention arms using a simple random allocation sequence (www.randomization.com). Concealed envelopes with consecutive numbers were locked up in a drawer and withdrawn in numerical order by the main author. Major exclusion criteria were changing the dosage of medications, allergy or intolerance to soy milk or avoidance of soy milk consumption, smoking, alcoholism, recent antibiotic therapy, use of supplements containing vitamins and minerals, and any medical condition such as inflammatory bowel disease, infection, liver disease, and rheumatoid arthritis.

Study Procedure

This study was approved by the research council (research project number: 394733) and ethics committee (research ethics number: IR.MUI.REC .1394.3733). In addition, this trial is registered at www.clinicaltrials.gov with ID number NCT02704884. The research procedure was explained to the participants, and informed written consent was taken from all the patients. In addition, the Consolidated Standards of Reporting Trials statement guideline[17] was used to design the present study.

Intervention Conditions

After 2-week, single-masked, run-in period, eligible participants were randomly assigned to receive 200 mL/day probiotic soy milk in the intervention group or soy milk in the control group for 8-week. Furthermore, all participants received individualized dietary counseling aimed at achieving a daily energy and restricting dietary protein, sodium, and potassium intake.[17] Discussions during telephone calls and check-in visits focused exclusively on microbial soy milk and soy milk consumption. Adherence to diet containing probiotic soy milk and soy milk was assessed by 24-hour diet recall interview, which was completed by all subjects every 2 weeks of study and during their attendance in the follow-up visits. Both types of milk, probiotic soy milk and soy milk, were produced by Isfahan Soy Milk Company and were provided for each patient. Each patient received a 3-day supply of their probiotic or conventional soy milk every 3 days. Isfahan Soy Milk is registered with the health ministry (No:15/10013).

Measurements

The primary outcomes were the changes in MDA, 8-iso-PGF2α, GSSG, TAC, GSH, GPx, and GR levels after 8 weeks among the 2 groups. Patients were studied and data collected before and after each intervention group. Anthropometric measurements including height, body weight, and waist-to-hip ratio were gathered every 2 weeks during the study. Height was measured in standing position, without shoes. Body weight was measured while patients were minimally clothed without shoes, using the digital scales recording to the nearest 0.1 kg. Body mass index was calculated as body weight (kilogram)/height^2 (meter). Dietary intake data were collected via 24-hour diet recall interviews every 2 weeks. The interviewer also asked each participant to recall the activity performed every 2 weeks by international physical activity questionnaires. A daily physical activity factor was calculated using metabolic equivalent (MET) levels for each reported activity. In addition, medical and medication history, duration of disease, and demographic data were recorded before and after the interventions.

Biochemical Analysis

Blood samples were taken from fasting participants and analyzed at the beginning and end of the intervention period. These samples were collected into tubes containing 0.1% ethylenediaminetetraacetic acid and were centrifuged at 4°C and 500×g for 10 minutes to separate the plasma. Afterward, the plasma samples were collected and frozen at −80°C to be used for their respective analyses. Blood samples also were collected in another tube for Hb measurement. Cell membranes were removed by