Study Protocol

Effects of Synbiotics Supplementation in Lean Patients with Nonalcoholic Fatty Liver Disease: Study Protocol of a Pilot Randomized Double-blind Clinical Trial

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Abstract

Introduction: Currently, Nonalcoholic Fatty Liver Disease (NAFLD) is the most common liver disease in the world. The only approved treatment for it is lifestyle modification and weight loss; however, there is no treatment option for patients with normal or low body mass index (BMI).

The aim of this study is to evaluate the efficacy of synbiotics supplementation in NAFLD patients with normal or low BMI.

Methods and analysis: In our randomized, double-blind, placebo-controlled clinical trial protocol, forty-two patients will be assigned to take either a synbiotic or a placebo capsule for 28 weeks. Both groups will undergo the standard treatment.

Ethics and dissemination: The study protocol has been approved by ethics committee of Shahid Beheshti University of Medical Sciences. At the beginning of the study, a written informed consent form will be signed and dated by subjects and investigators. The results will be published in due time.

Keywords: Body weight, fatty liver, NAFLD (Nonalcoholic Fatty Liver Disease), probiotics, synbiotics

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Introduction

Nonalcoholic Fatty Liver (NAFLD) is the leading etiology of liver disorders worldwide.[1] No proven treatment has been found for it yet; however, lifestyle modification and weight loss have been shown to be effective in disease treatment.[2] All of these studies have been conducted on overweight and obese patients.[2] To our knowledge, there is no clinical trial on lean patients with NAFLD.[3]

Probiotics are live microbes, which have shown many beneficial effects on body health through modulating gut microbiota,[4,5] leading to reduction of pathogenic bacteria, enhancing the gut barrier integrity, modulating the immune system,[4,6] and finally reducing inflammation, especially in the liver due to its anatomical link to the gut.[4,6,7]

Prebiotics are indigestible carbohydrates that provide the optimal environment for probiotics growth and activity.[8] Synbiotics are the combination of probiotics and prebiotics, which are more effective than probiotics or prebiotics alone in modulating the gut microbiota.[9]

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In our previous study on overweight and obese patients with NAFLD, we found that synbiotics supplementation plus lifestyle modification is more effective than lifestyle modification alone in the treatment of NAFLD.[10] On the other hand, previous studies on cholin-methionin deficient model of NAFLD, which is a non-obese model of the disease, have shown the therapeutic effects of probiotics in NAFLD.[11] Thus, we hypothesized that synbiotics supplementation might be effective in management of lean patients with NAFLD.

Aim of the protocol

To evaluate the effects of synbiotics supplementation in lean and normal weight patients with NAFLD

Methods and Design

Study design

Randomized, double-blind, placebo-controlled clinical trial pilot study

Recruitment and eligibility screening

Patients will be diagnosed by a gastroenterologist. A diagnosis of NAFLD will be assigned on the basis of the presence of steatosis and fibrosis on Fibroscan test, associated with a persistently elevated ALT concentration of 60 U/L for 6 months before the study and at the time of randomization, patients should meet the inclusion and exclusion criteria outlined below.

Inclusion criteria

- Age 18 or older - Concentrations of liver enzymes ALT and

AST greater than 1.5 times more than the upper limit of normal range - evidence of hepatic steatosis on Fibroscan test with CAP score of more than 260

- Lack of a history of alcohol consumption
- BMI less than 25
-No evidence of any other acute or chronic disorders of the liver (hepatitis B, C, etc.), biliary disease, autoimmune diseases, cancer and inherited disorders affecting the liver

Exclusion criteria

- Unwillingness to continue the study - Pregnancy or breast-feeding in women,
- Taking antibiotics, and/or hepatotoxic medications during the study
- Loss of more than 10% of body weight during the study period

Sample size

The sample size calculations were performed according to the previous study,[12] so that the 25 U/L difference in ALT indicates a significance difference, with power = 80. The sample size was calculated as 21 patients in each group. We will include at least 10% more samples to compensate for the loss to follow up in this study.

Data collection

General information

General information, medical history, and some confounders will be obtained using a general questionnaire.

Study design

All participants should sign an informed consent form after a full review of the inclusion and exclusion criteria and an explanation of the risks and benefits of the study. The study protocol and consent forms were approved by the ethics committee of the National Nutrition and Food Technology Research Institute and the Digestive Diseases Research Institute of Tehran University of Medical Sciences. Included patients will undergo the standard treatment. Moreover, they will be randomly allocated to receive either synbiotics supplementation or the identical-appearing placebo capsules (maltodexterin) twice daily for 28 weeks, and baseline data will be gathered. Follow-up assessments will be performed every seven weeks to assess dietary intakes and compliance. Randomization lists will be computer-generated by a statistician and given to the interviewer using blocked stratified randomization for age and sex. The age groups include 18–40, 41–65, and 65<. Subjects, investigators, and staff will be blind to the treatment assignment until the end of the study.

On the first visit (week 0), baseline data will be gathered and patients will be provided with a 7-week supply of capsules. On each follow-up visit, patients will be given another set of capsules. Each synbiotics capsule (Protexin) contains 200 million of 7 strains of friendly bacteria *(Lactobacillus casei*, *Lactobacillus rhamnosus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Lactobacillus bulgaricus*) and prebiotic (fructooligosaccharide) and probiotic cultures [magnesium stearate (***source:*** mineral and vegetable) and a vegetable capsule (hydroxypropylmethyl cellulose)]. Adherence will be assessed by capsule counts confirmed on each visit. Both groups will be advised to follow an energy-balanced diet and physical activity recommendations according to the Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults from the NIH and the North American Association for the Study of Obesity.[13] The lifestyle modification recommendation was explained previously.[10]

Anthropometric measurements

Anthropometric measurements, including height, weight and waist circumference, will be performed for all participants. Height will be measured in the standing position without shoes using a height gauge, and weight will be measured using weighing scales with minimal wear. Waist circumference will be measured in the standing position, intermediate between the lower margin of the ribs and the iliac crest, while breathing normally without clothes. Each individual's BMI will be calculated using the following formula: BMI = weight (kg)/height (m^2). Waist-to-hip ratio (WHR) will be measured according to the WHO recommendation.[14]

Paraclinical assessments

Biochemical testing will be performed for each patient at the beginning and the end of the study, after they have fasted for 12 hours. All biochemical assessments will be performed in the same laboratory using standard laboratory methods. Gamma-glutamyl transferase (GGT) will be measured by enzymatic colorimetric assay (Parsazmoun). ALT and AST concentrations will be measured by photometric assay (Reckon). Fasting hs-CRP concentrations will be measured by ELISA (Diagnostics Biochem Canada Inc.). Plasma TNF-a concentrations will be measured using a commercial ELISA kit (KOMA BIOTECH Inc.) with a lower sensitivity limit of 10 pg/mL. Nuclear factor k-B (NF-kB) p65 will be measured in PBMC nuclear extracts using an ELISA kit (Cell Signaling) according to the manufacturer's protocol. Fasting glucose concentrations will be measured using the GOD/POD method. Fasting insulin concentrations will be measured by ELISA (Mercodia AB). HOMA-IR will be used to determine the insulin resistance by using the following formula (22): HOMA-IR = [fasting insulin (mU/L) 3 fasting blood glucose (mg/dL)]/405. Hepatic steatosis and fibrosis will be assessed by transient elastography at baseline and at the end of the study. Transient elastography will be performed by the same equipment and by the same operator who will be also blind to the study randomization and will be unaware of the clinical and laboratory results.

Physical activity questionnaire

As physical activity can be related to NAFLD, it is necessary to estimate physical activity as another confounder. A classified physical activity questionnaire, based on the metabolic equivalent (MET), will be used, which consists of nine levels of activity, from rest and sleep (MET = 0.9) to vigorous activity (MET $\geq$ 6). The questionnaire has been prepared in previous studies in Europe and was validated with the daily physical activity questionnaire and the CSA Accelerometry (Model 7164 Ambulatory Monitor).[15] Reliability and validity of the questionnaire have been confirmed in the study of Kelishadi *et al.* in Iran.[16]

Statistical analysis

Data will be analyzed using STATA software (version 11; StataCorp). For all analyses, a P value < 0.05 will be considered statistically significant. The results will be adjusted for cofounders such

as smoking status, BMI, physical activity, primary liver enzymes, and other paraclinical measurements.

Ethical issues and informed consent process

The study protocol has been approved by the ethics committee of Shahid Beheshti University of Medical Sciences (94-04-563). The investigators will provide subjects with all related information in the preferred language of the subject and at a level of complexity that is understandable to subjects. Prior to the subject's participation in the study, a written informed consent form will be signed and dated by the subject and investigator. The study protocol was registered on Clinicaltrial.gov with registration Identifier: NCT02530138

Discussion

To our knowledge, this is the first randomized, double-blind clinical trial on lean NAFLD patients. The efficacy of synbiotics supplementation in NAFLD has been shown previously.[10] In our previous study, we found some evidence that synbiotics supplementation in addition to lifestyle modification is superior to lifestyle modification alone for the treatment of NAFLD in overweight and obese patients; however, there was no lean patient in that study.

Although obesity is one of the main risk factors for fatty liver, it can also occur in lean subjects. Studies on liver donors and automobile crash victims have roughly found hepatic steatosis in 15% of non-obese subjects,[17,18] which indicates that lean NAFLD might be a frequent cause of cryptogenic liver disorders.[3] Margariti *et al.* found that one in every eight patients with NAFLD has normal body mass index.[19] To our knowledge, there is no proven treatment for lean NAFLD. Since probiotics have shown promising beneficial effects on non obese experimental models of NAFLD,[20] we hypothesized that they may be effective in human lean NAFLD, too. If our results confirm this hypothesis, it will be a novel therapeutic option in lean NAFLD management, which decreases a large socio-economic burden of disease.

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Competing interests: None.

Ethics approval

The ethics committee of Shahid Beheshti University of Medical Sciences has approved the study.

Author Contribution

Fatemeh Mofidi, Hossein Poustchi, and Azita Hekmatdoost designed the study.

Fatemeh Mofidi, Zahra Yari, Hossein Poustchi, Shahin Merat, Babak Nourinayyer, Reza Malekzadeh, Azita Hekmatdoost conduct the research.

Fatemeh Mofidi, and Azita Hekmatdoost wrote the manuscript.

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