Iran Red Crescent Med J. 2017 April; 19(4):e46017. doi: 10.5812/ircmj.46017.

Published online 2017 March 12. **Research Article**

The Effect of Probiotic and/or Prebiotic on Liver Function Tests in Patients with Nonalcoholic Fatty Liver Disease: A Double Blind Randomized Clinical Trial

Leila Javadi,[1] Mostafa Ghavami,[2] Manouchehr Khoshbaten,[3] Abdolrasoul Safaiyan,[4] Abolfazl Barzegari,[5] and Bahram Pourghassem Gargari[6,*]

[1]Nutrition Research Center, Student Research Committee, Tabriz University of Medical Sciences, Tabriz, IR Iran
[2]Faculty of Paramedical, Tabriz University of Medical Sciences, Tabriz, IR Iran
[3]Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, Tabriz, IR Iran
[4]Road Traffic Injury Research Center, Department of Biostatistics and Epidemiology, Tabriz, IR Iran
[5]Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, IR Iran
[6]Nutrition Research Center, Department of Biochemistry and Diet Therapy, School of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, IR Iran

[*]*Corresponding author*: Bahram Pourghassem Gargari, PhD, Nutrition Research Center, Department of Biochemistry and Diet Therapy, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, IR Iran. E-mail: bahrampg@yahoo.com, pourghassemb@tbzmed.ac.ir

Received 2017 January 28; **Revised** 2017 February 16; **Accepted** 2017 February 26.

Abstract

Background: Oral administration of pro- and prebiotics has recently been considered as an effective way for nonalcoholic fatty liver disease (NAFLD) recovery.
Objectives: The current study aimed at evaluating the effect of supplementation with probiotics and/or prebiotics on liver function tests in patients with NAFLD.
Methods: In this double blind, placebo-control clinical trial, 75 subjects with NAFLD were voluntarily recruited from May 2013 to March 2014, in Iran. Participants were randomly assigned to 1 of 4 groups using a block randomization procedure. Group 1 received probiotic capsules (Bifidobacterium longum (BL) and Lactobacillus acidophilus (LA): 2×10^7 CFU/day), group 2 received prebiotic inulin high performance (HP): 10 g/day, group 3 received probiotic and the prebiotic, and group 4 received a placebo for 3 months. The sample size was determined on the basis of a primary outcome of a change in aspartate aminotransferase (AST) level.
Results: An intergroup comparison indicated that the AST (P = 0.006) and alanine aminotransferase (ALT) (P = 0.04) levels decreased at the end of the study. Aspartate Aminotransferase (mean difference of group1 versus placebo with P value of 0.001, group2 versus placebo with P value of 0.045, group3 versus placebo with P value of 0.015) and ALT (mean difference of group 1 versus placebo with P value of 0.009, group 2 versus placebo with P value of 0.041, and group 3 versus placebo with P value of 0.046) serum levels decreased significantly in all of the intervention groups compared to the placebo. The grade of fatty liver in group 1 (P of 0.027, and number needed to treat (NNT) = 3) and group 3 (P = 0.019 and NNT = 3) decreased compared to the placebo group with no significant changes in group 2.
Conclusions: Supplementation with probiotics and/or prebiotics improved aminotransferase enzymes, and supplementation with probiotics or pro- and prebiotics recovered the grade of fatty liver in NAFLD patients.

Keywords: Probiotic, Prebiotic, Liver Function, Steatosis, NAFLD

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease that can lead to nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (1). No effective medication has been reported for NAFLD. However, management strategies, such as lifestyle modifications, have proven to be efficient (2). The gut microbiota is associated with the host's metabolism and seems to have a major role in the pathogenesis of NAFLD (3, 4) via multiple mechanisms, including, regulation of energy homeostasis by increasing the fermentation of carbohydrates to short chain fatty acids (SCFAs) (5), activation of the de novo synthesis of triglycerides in the liver (6, 7), and bacteria-derived toxins (e.g. lipopolysaccharides) (8).

Probiotics are live microorganisms that, in adequate amounts, provide positive health effects on the host (9).

Prebiotics are defined as a group of non-digestible carbohydrates that can alter the composition and activity of the gut microbiota, thus, they have beneficial effects on the host health (10). According to a few studies, adding prebiotics, such as inulin, to a diet, leads to an increased proliferation of Bifidobacterium and Lactobacillus in the intestine (11-13) and the recovery of liver function in diseases such

as NAFLD (14). High Performance inulin (HP) is a prebiotic with specific colonic fermentation characteristics. Inulin HP can change the composition of gut microbiota toward bifidobacteria. Consumption of 5 to 8 g/day inulin can be sufficient to result a positive effect on health (15).

Based on a Cochrane systematic review (16), probiotics may be well accepted in ameliorating liver function tests in the case of NAFLD. Considering the lack of complete pharmaceutical formulations, probiotics may be seen as a complementary therapeutic approach for NAFLD (17). The present study aimed at evaluating the effect of probiotics and prebiotics alone and in combination on liver function tests in patients with NAFLD.

2. Methods

2.1. Patients

In this trial, participants were randomly assigned to 1 of 4 groups, using a block randomization procedure. Participants were recruited from May 2013 to March 2014 and followed-up until April 2015. Eligible subjects were provided with a description of the study and informed written consent was obtained from participants. The ethics committee of Tabriz University of Medical Sciences approved the experimental protocol (university ethical code: 5/4/7041, 1392/9/2). The study was registered at clinical trials, Iranian registry of clinical trials number: IRCT201301223140N6.

Inclusion criteria were as follows, volunteer subjects with nonalcoholic fatty liver, both genders aged 20 to 60 years old, and having serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) higher than the normal range. According to the study of Hyeon, the best cut-off values for the prediction of liver disease were 31 IU/L for aspartate aminotransferase and 30 IU/L for alanine aminotransferase (18). The detection method for fatty liver disease was an ultrasound of the liver and bile ducts and liver aminotransferases enzymes tests. Exclusion criteria were as follows, having cardiovascular, thyroid, kidney, autoimmune diseases, hepatitis A, B, and C, hemochromatosis and Wilson's disease, and taking vitamin supplements (A, E, C, etc.) and alcohol, and being pregnant and lactating.

The primary outcome of the study was the AST level. Values of the remaining variables (i.e., ALT, alkaline phosphatase (ALP), albumin (ALB), bilirubin (BIL), gamma-glutamyl transferase (GGT), weight, body maas index (BMI), and waist-to-hip ratio (WHR) changes) were considered to represent secondary outcomes.

2.2. Sample Size

Sampling for this study was performed by the convenience method. The participants were divided between the study groups (probiotics, prebiotics, probiotics and prebiotics, and the placebo group) by random allocation. The sample size was determined on the basis of a primary outcome of a change in AST, according to Liu et al. (19). Based on the following formula with a power of 80% and confidence interval (CI) of 95%, at least 18 patients per group were required for an adequate sample size. To allow for a dropout rate of 20%, the sample size was increased to 22 in each group. $N=(Z1\text{-}\alpha/2+Z1\text{-}\beta)^2\ (SD1^2+SD2^2)/\Delta^2$.

2.3. Study Design and Interventions

In this double blind placebo-control clinical trial, 75 subjects with NAFLD were voluntarily recruited from Iran Azadi clinic of Tabriz University of Medical Sciences. After matching eligible subjects for age and gender, they were divided to 4 groups using a block randomization procedure, including 3 intervention groups and 1 control group (block sizes of 4 and 8) and an allocation ratio of 1:1:1:1. Group 1 received probiotic capsules (BL and LA: 2×10^7 CFU) and placebo of prebiotics (maltodextrin powder). Group 2 received prebiotic powder (inulin HP: 10 g/day) and a placebo of probiotics (fat and lactose free milk capsules). Group 3 received the probiotic and the prebiotic (BL and LA: 2×10^7 CFU + inulin HP: 10 g) daily. Group 4 received placebos of prebiotics and probiotics. Dosage of supplements was: 2 capsules at 250 mg/day probiotics, 5 g sachet of prebiotic twice/day during morning and evening for 3 months. Both the inulin and the maltodextrin had a similar taste and appearance, and they were given to the participants in similar opaque packages. Capsules of probiotic and placebo had a similar appearance. To ensure blinding, the allocation was performed by an investigator with no clinical involvement in the study, and the main investigator and statistical data analyst, is the same one, remained blinded to the participant group until the end of the analysis. Supplements were divided between volunteers in accordance with their allocation code after randomization.

2.4. Preparation of Probiotic

Bifidobacterium longum and Lactobacillus acidophilus were isolated from traditional homemade dairy products. These strains were screened for conjugated linoleic acid (CLA) isomerase gene with a cholesterol-lowering function. The CLA isomerase gene was detected using a polymerase chain reaction (PCR) technique, and their cholesterol-lowering effect was detected by the digestion of cholesterol in a culture medium, producing a transparent environment. Then the selected microbial

samples were cultured and the probiotic was produced. Capsules that contained 10^7 bacteria were mixed in fat and lactose-free milk powder with water and became a lyophilized homogenous solution. Lyophilized powder was filled in 250 mg capsules by a machine.

2.5. Measurements

2.5.1. Demographic and Anthropometric Assessments

Demographic characteristics including age, gender, education level, and anthropometric indices were collected using a questionnaire. All participants underwent measurements of height, weight, waist, and hip circumferences using standard anthropometric techniques (20). Body weight was measured without shoes and light clothing to the nearest 0.5 kg, using a Seca scale (Seca, Hamburg, Germany). Height was recorded to the nearest 0.5 cm using Seca stadiometers without shoes. The body mass index (BMI) and waist-to-hip ratio (WHR) were calculated using the following formula, respectively: BMI = weight (kg)/height (m^2) and WHR = waste circumference (cm)/hip circumference (cm).

2.6. Dietary Intake

All participants underwent dietary assessments for 3 days (2 workdays and 1 holiday), using a 24-hour food dietary recall at the beginning and at the end of the trial. Dietary data were analyzed using the Nutritionist IV software program (first Databank Inc, Hearst crop, San Bruno, CA, USA).

2.7. Biochemical Parameters

At the beginning and the end of the trial, venous blood was collected after an overnight fast in laboratory of Tabriz University of Medical Sciences. At the beginning and the end of the study, 10 mL of venous blood samples were collected after 12 hours of fasting and place in a Vacationer tube. The serum samples were separated from whole blood by centrifugation at 2500 rpm for 10 minutes (Beckman Avanti J-25; Beckman Coulter, Brea, CA, USA) at room temperature. The serum samples were stored at -70°C until analysis. The AST, ALT, Gamma-Glutamyl Transferase (GGT), Albumin (ALB), Bilirubin (BIL), and Alkaline Phosphatase (ALP) were measured via the enzymatic method by PARS AZMUN (Tehran, Iran) kits using an auto analyzer machine (Alcyon 300, abbott USA), which was calibrated before beginning the tests.

2.8. Ultrasonography

An experienced radiologist, at the Ultrasonic center of Tabriz University Medical Sciences, performed the liver ultrasound. The liver was evaluated for size, echogenicity, structure, and penetration of the ultrasound beam (Medison Sonoace x6). Based on echogenicity, beam penetration, and portal vessel wall distinction, nonalcoholic fatty livers were classified to 3 subscale grades (grade I, II, and III) (21).

2.9. Statistical Analysis

Statistical analyses were performed using SPSS 22.0 software (IBM, Armonk, NY). Before conducting the multivariate analysis, assumptions, including normality of the residuals, homogeneity of residual variance, multicollinearity of independent variables, and independence of the residuals, were studied. First, the normal distribution of all variables was checked with the Kolmogorov-Smirnov test. One-way analysis of variance was performed on all baseline data among the groups. Differences in variables before and after treatment were evaluated with the paired t test. Analysis of covariance in the adjusted models for gender, age, energy intake, and BMI was used for evaluating the differences between groups at the end of the study. A chi-square test was used for categorical variables of continuous data. Statistical significance was set to a P value of < 0.05.

3. Results

In the present study, 88 patients with NAFLD (male = 60, female = 15) were randomly divided to 4 groups. Of these, 13 subjects withdrew from the study that 4 were lost before the intervention and 9 dropped out during flow up because of migration and personal reasons (Figure 1). The mean age of participants was 42.0 $\pm$ 8.9 years and the mean BMI was 30.8 $\pm$ 4.1 kg/m^2 (23.9 - 43.2 kg/m^2), respectively. As shown in Table 1, at the end of the trial, there was a significant reduction in BMI in the treatment groups, and WHR in group 1 compared to the baseline. The completed and detailed results of the study on the anthropometric indices are presented in other articles (under review).

After the intervention, serum levels of AST and ALT in all intervention groups, ALP and GGT in group 3, and BIL in group 1 decreased significantly compared to the beginning of the trial ($P < 0.05$ for all variables). Intergroup comparisons indicated that AST ($P = 0.006$) and ALT ($P = 0.04$) levels decreased at the end of the study (Table 2). The serum levels of AST (change of group 1 versus placebo with P value = 0.001, group 2 versus placebo with P value = 0.045, group 3 versus placebo with P value = 0.015) and ALT (change of group1 versus placebo with P value = 0.009, group 2 versus placebo with P value = 0.041, group 3 versus placebo with P value = 0.046) decreased significantly in all of the intervention groups. Mean score of ALT and AST serum levels in the 4 groups is shown in Figures 2 and 3, respectively. The

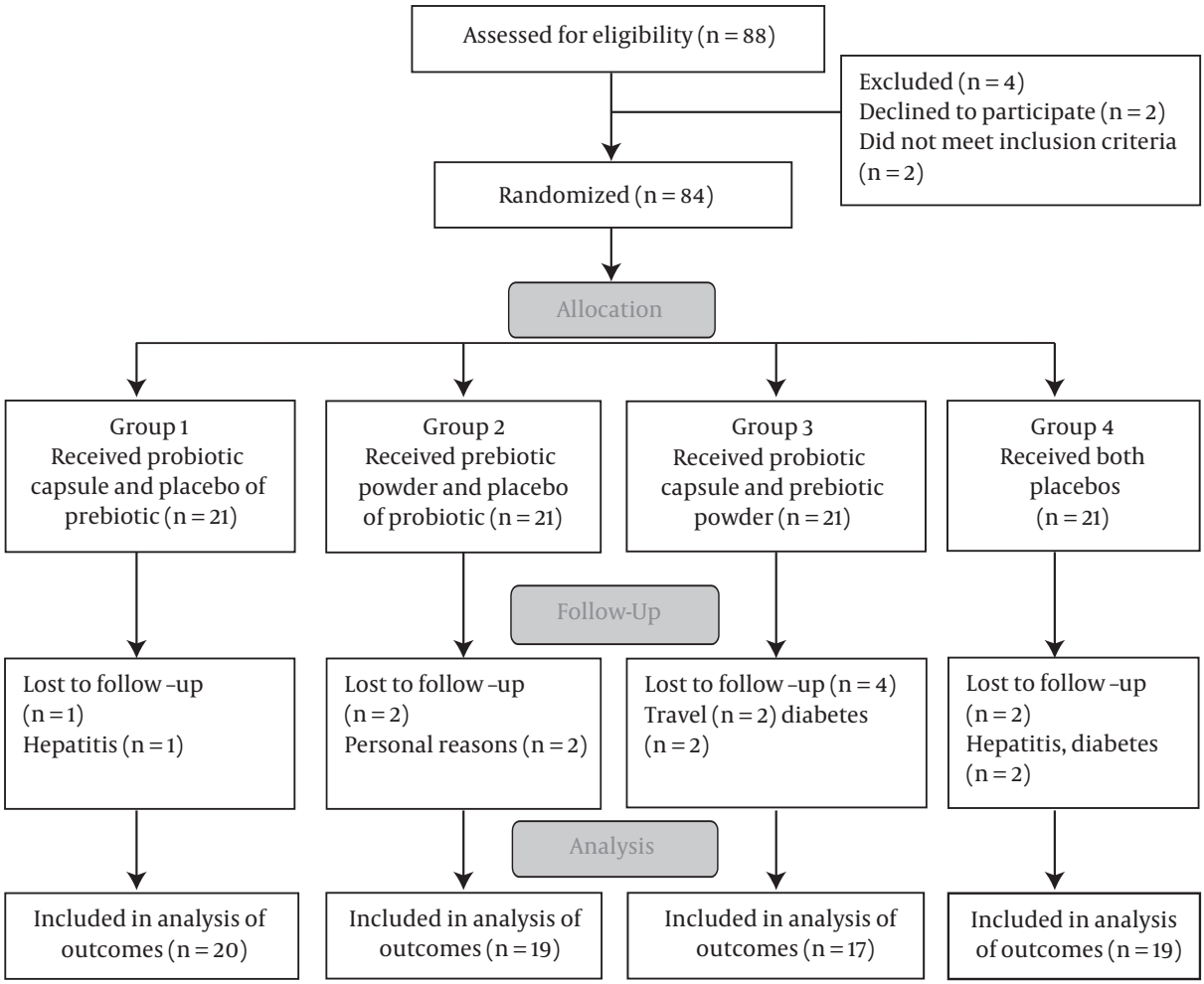

Figure 1. Flowchart of the Study

grade of fatty liver in group 1 and 3 recovered compared to the placebo group with no change in ALP, GGT, ALB, and BIL after the intervention.

Based on the results (Table 3) 3 (15.7% of the total) patients with NAFLD in the placebo group were given a recovery NAFLD grade, yet, there were 11 (55% of total) in group 1 that were given a recovery NAFLD grade; in other words, 3 patients with NAFLD should receive the probiotic supplement to every 1 NAFLD recovery patient (number needed to treat (NNT): 3, absolute risk reduction (ARR): 0.392; P = 0.027). In group 2, 10 (26.3% of total) patients with NAFLD should receive the prebiotic supplement to every 1 NAFLD recovery patient (number needed to treat (NNT): 10, absolute risk reduction (ARR): 0.105; P = 0.158). In group 3, 3 (58.8%) patients with NAFLD should receive the pro- and prebiotic supplement to every 1 NAFLD recovery patient (number needed to treat (NNT): 3, absolute risk reduction (ARR): 0.43; P = 0.019). Mean score of fatty liver grade in the 4 groups is shown in Figure 4.

4. Discussion

It has been proposed that probiotics and prebiotics can be used as a treatment strategy for many metabolic disorders including obesity, hyperlipidemia, and liver diseases (22). There is enough evidence linking the gut microbiota to warrant an intervention aimed for prebiotic-mediated manipulation of the gut microbiota in NAFLD (23). In the present study, supplementation with probiotic (B.L and L.A: 2×10^7 CFU/day) or prebiotic (inulin HP: 10 g/day) for 3 months decreased BMI compared to the placebo group. The completed and detailed results of the study on the an-

Table 1. Demographic and Anthropometric Data of the Study Subjects[a]

Variables	Probiotic (n = 20)	Prebiotic (n = 19)	Probiotic + Prebiotic (n = 17)	Placebo (n = 19)
Gender, %				
Male	17 (85.0)	16 (84.2)	14 (82.4)	13 (68.4)
Female	3 (15)	3 (15.8)	3 (17.6)	6 (31.6)
Age, y	43.90 ± 9.02	38.68 ± 10	43.24 ± 6.95	42.21 ± 9.11
BMI, kg/m^2				
Before	29.91 ± 3.88	30.96 ± 4.39	32.30 ± 4.78	30.38 ± 2.88
After	29.26 ± 3.59	30.38 ± 4.63	31.47 ± 4.58	30.56 ± 2.88
Weight, kg				
Before	86.92 ± 12.37	88.45 ± 10.36	89.88 ± 11.92	86.00 ± 11.98
After	85.08 ± 12.25	86.45 ± 10.54a	87.91 ± 12.08	86.51 ± 12.05
WHR				
Before	0.93 ± 0.06	0.94 ± 0.03	0.95 ± 0.06	0.92 ± 0.05
After	0.92 ± 0.06	0.93 ± 0.04	0.94 ± 0.06	0.92 ± 0.05
Energy, kcal/day				
Before	2369.70 ± 515.66	2296.26 ± 282.16	2153.18 ± 459.56	2158.21 ± 463.99
After	2305.75 ± 616.80	2244.74 ± 174.53	2102.12 ± 254.26	2080.16 ± 408.72

Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio.
[a]Values are expressed as mean ± SD for before and after intervention.

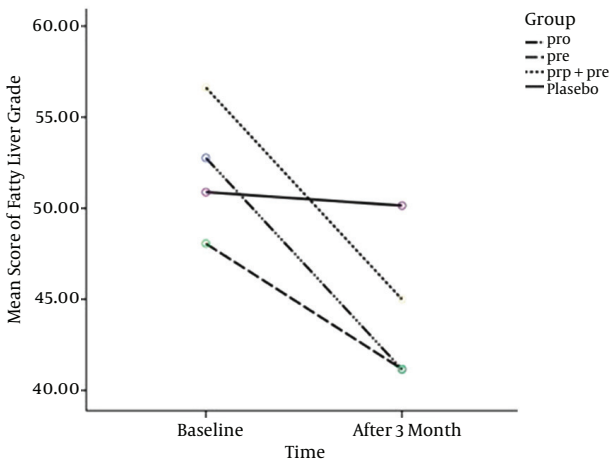

Figure 2. Mean Score of ALT Serum Levels in the Four Groups

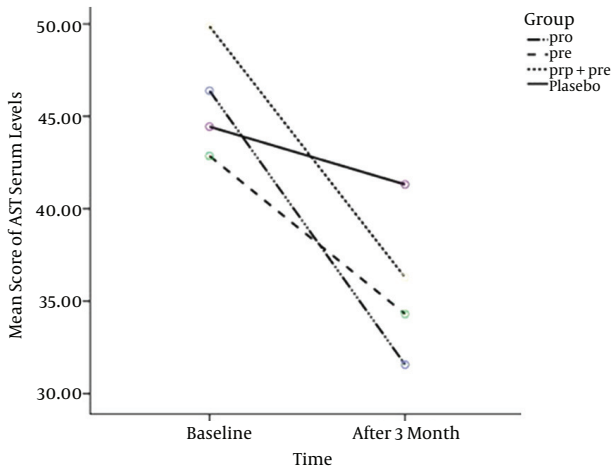

Figure 3. Mean Score of AST Serum Levels in the Four Groups

thropometric indices are presented in other articles (under review). Also, supplementation of the probiotic or/and prebiotic for 3 months decreased AST and ALT serum levels in the patients with NAFLD. Moreover, supplementation with probiotic, with or without prebiotic, significantly recovered the grade of fatty liver in NAFLD patients.

Limited studies with controversial results evaluated the effects of pre/probiotics on anthropometric indices in patients with NAFLD (22), which have been discussed regarding anthropometric and dietary intake in the article under review.

The present study indicated that probiotics and pre-

Table 2. Comparison of Serum Levels of Liver Function Factors at Baseline and at the End of the Trial Among the Study Groups[a,b]

Variables	Probiotic (n = 20)	Prebiotic (n = 19)	Probiotic + Prebiotic (n = 17)	Placebo (n = 19)		P Value$_1$
AST, U/L	Before	45.85 ± 14.54	42.73 ± 10.01	51.58 ± 10.89	43.57 ± 13.20	0.149
	After	31.15 ± 9.08a	33.68 ± 8.87a	37.11 ± 13.65a	41.63 ± 12.46b	0.006
	P Value$_2$	0.001	0.002	0.001	0.269	
	MD (95%CI)	-14.7 (-21.00 to -8.39)	-9.05 (-14.16 to -3.94)	-14.47 (-20.16 to -8.77)	-1.94 (-5.53 to 1.63)	
ALT, U/L	Before	51.15 ± 13.55	49.10 ± 11.80	58.11 ± 13.84	50.21 ± 11.03	0.150
	After	40.30 ± 12.74a	41.05 ± 10.18a	45.82 ± 11.22a	50.42 ± 14.12b	0.046
	P Value$_2$	0.005	0.009	0.002	0.929	
	MD (95%CI)	-10.85 (-18.07 to -3.62)	-8.05 (-13.79 to -2.31)	-12.29 (-19.21 to -5.27)	0.21 (-4.65 to 5.07)	
ALP, U/L	Before	262.30 ± 75.82	266.42 ± 57.89	273.70 ± 88.71	281.84 ± 90.91	0.887
	After	236.75 ± 51.92	262.89 ± 48.82	255.52 ± 88.83	278.47 ± 90.58	0.328
	P Value$_2$	0.47	0.672	0.048	0.809	
	MD (95%CI)	-25.55 (-50.71 to 0.38)	-3.52 (-20.72 to 13.67)	-18.17 (-36.21 to -0.14)	-3.36 (-32.24 to 25.50)	
GGT, U/L	Before	39.75 ± 18.95	31.36 ± 10.82	43.70 ± 10.24	34.78 ± 16.85	0.074
	After	35.75 ± 16.35	34.63 ± 12.74	36.35 ± 12.45	35.89 ± 21.77	0.338
	P Value$_2$	0.071	0.193	0.010	0.776	
	MD (95%CI)	-4.00 (-8.38 to 0.38)	-3.26 (-1.80 to 8.32)	-7.35 (-12.65 to -2.04)	1.10 (-6.91 to 9.13)	
ALB, g/dL	Before	5.42 ± 0.38	5.62 ± 0.34	5.41 ± 0.41	5.36 ± 0.29	0.168
	After	5.39 ± 0.41	5.60 ± 0.33	5.42 ± 0.42	5.57 ± 0.36	0.124
	P Value$_2$	0.724	0.832	0.906	0.060	
	MD (95%CI)	-0.03 (-0.20 to 0.14)	-0.02 (-0.22 to 0.18)	0.01 (-0.19 to 0.21)	0.20 (-0.009 to 0.42)	
BIL, mg/dL	Before	0.60±0.24	0.52 ± 0.15	0.46 ± 0.16	0.50 ± 0.17	0.155
	After	0.46 ± 0.34	0.40 ± 0.18	0.41 ± 0.21	0.48 ± 0.21	0.632
	P Value$_2$	0.038	0.057	0.329	0.595	
	MD (95%CI)	-0.14 (-0.27 to -0.008)	-0.12 (-0.25 to 0.003)	-0.05 (-0.18 to 0.06)	-0.02 (-0.11 to 0.06)	

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase.
[a]Values are expressed as mean ± SD.
[b]PV1 for before of the study resulted from one-way ANOVA test and for after the study resulted from analysis of covariance in the adjusted models sex, age, energy intake, body mass index; PV2 resulted from paired sample t tests; MD, mean difference of within groups (pair sample t test); Data with different superscript letters are significantly different (P < 0.05) to the ANCOVA statistical analysis.

Table 3. Effect of Probiotic or/and Prebiotic on Nonalcoholic Fatty Liver Disease (NAFLD) Grade in Patients with NAFLD[a]

Groups	Recovery, %	NNT (95%CI)	ARR (95%CI)	P Value
Probiotic (n = 20)	11 (55)	3 (2 to 11)	0.392 (0.091 to 0.61)	0.027[b]
Prebiotic (n = 19)	5 (26.3)	10 (-6 to 3)	0.105 (-0.156 to 0.352)	0.691
Probiotic + Prebiotic (n = 17)	10 (58.8)	3 (2 to 9)	0.430 (0.115 to 0.651)	0.019[b]
Placebo (n = 19)	3 (15.7)	-	-	-

Abbreviations: ARR, absolute risk reduction; NAFLD, nonalcoholic fatty liver disease; NNT, number needed to treat.
[a]PV resulted from chi-squared test.
[b]P < 0.05 is significant.

biotics alone or in combination decreased the serum levels of AST and ALT after the 3-month intervention. Limited

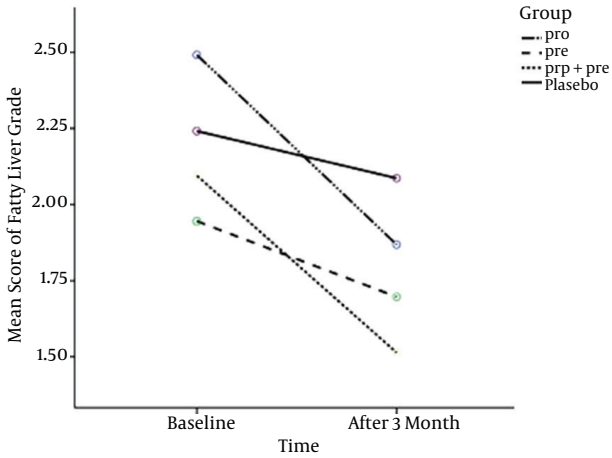

Figure 4. Mean Score of Fatty Liver Grade in the Four Groups

studies evaluated the effects of pre/probiotics on liver function tests in patients with NAFLD. Aller et al. reported that supplementation with Lactobacillus bulgaricus decreased serum levels of AST, ALT, and GGT after 3 months of intervention, yet, after supplementation with Streptococcus thermophilus, no changes were observed in any factor of liver function (24). Some researches demonstrated that probiotics decreased serum levels of AST and ALT in patients with nonalcoholic steatohepatitis (19-25). Our results with regards to reductions in liver aminotransferase are similar to those of Nabavi et al. (26), who showed that probiotic yogurt for 8 weeks in patients with NAFLD caused a decrease in ALT and AST. In another study, probiotic supplementation in pediatric obesity-related liver disease for 8 weeks decreased ALT serum level (27).

Also, a meta analysis study in 2016 reviewed efficacy of probiotics in nonalcoholic fatty liver disease in adult and children, in which a total of 9 clinical trial studies were included and reported significant differences in ALT, AST, and BMI between the 2 childhood groups (28).

Evaluating the true effect of probiotics on NAFLD prevention or treatment is difficult because experiments have used different animal models and different bacterial strains in experimental trials and have not reached general results (29).

Some studies presented different results because prebiotic and/or probiotic supplementations for liver aminotransferase levels showed improvement in patients with NAFLD. The studies with the highest doses and combined treatments showed the amelioration of aminotransferase in the intervention group (19-25, 29, 30). However, the doses of pro- and prebiotics and their combination differed in each study and thus hindered sufficient comparisons (30).

Gut microbiota is an important environmental factor in the pathogenesis of NAFLD (31, 32). Since gut microbiota effects the integrity of the gut barrier, changes in gut integrity effect increase of intestinal permeability and endotoxin translocation (lipopolysaccharide due of gram-negative bacteria) (8, 31-33). In patients with NAFLD, levels of endotoxins increased (34, 35) and have been related to the overgrowth of small intestinal bacteria (31, 36, 37). In addition, gut microbiota can produce endogenous ethanol (30) and ~300 other volatile organic compounds (VOC) (38), some of which may relate to liver pathogenicity. Prebiotics change the gut barrier integrity and endotoxin translocation in favor of host health (39). For example, prebiotics stimulate Bifidobacterium grown, which is related to lower serum endotoxin levels (40). Prebiotics also increased the gut trophic hormone, glucagon-like, peptide-2, which can adjust endotoxin translocation via alterations on epithelial tight junctions. Steatosis itself increases the liver vulnerability to injury from endotoxins (33).

In our study, supplementation with probiotic or pro- and prebiotics recovered the grade of fatty liver in NAFLD patients. A double blind, placebo-controlled pilot study, on obese children showed that supplementation with probiotics showed no alteration in ultrasound liver parameters (27). In the current study, Ahmad Shavakhi et al. reported that probiotics combined with Metformin decreased ultrasound grading of NASH significantly better than metformin alone in patients with NASH (41). Also, in 2012, it was reported that a symbiotic treatment with pre- and probiotics improved the NASH activity index in patients with NASH (12). The intestinal microbiota synthetizes LPS that stimulates the release of cytokines TNF-α and IL-6 through hepatic macrophages that damage the disrupted normal hepatocyte function and reduce the clearance of toxins by the hepatocytes (12); therefore, colonization of the gastrointestinal tract by probiotics and prebiotics is followed by the adjustment of the gut microbiota via a reduction in pro-inflammatory cytokines and an improvement of liver damage.

The most important strength of the current study was that the probiotics were prepared and assessed for their probiotic characteristics in the laboratory. The study can introduce new fields of nutritional interventions for patients with NAFLD. The present trial had some limitations, including a small sample size and a fairly short duration for the intervention. Additionally, gut and fecal microbial compositions were not evaluated in the present study. Since a noninvasive method was preferred for detecting NAFLD, a liver biopsy was not used to derive the pathology score of the disease. Also, generalization to the population is another limitation of the study.

For further clinical investigations, it is necessary to perform clinical trials with larger sample sizes and long-term follow-ups to evaluate all of the possible effects and side effects of probiotics. Expanding the use of probiotics as an intervention for NAFLD is promising.

4.1. Conclusions

Supplementation with probiotics or/and prebiotics improved liver aminotransferase enzymes, and supplementation with probiotics or pro- and prebiotics recovered the grade of fatty liver in NAFLD patients. Although findings of the present study demonstrated positive effects of supplementation with prebiotics and probiotics, more studies are needed to confirm the efficacy of pre/probiotics as an adjunct therapy in NAFLD.

Acknowledgments

The authors would like to acknowledge the patients, who participated in the study.

Footnotes

Authors' Contribution: The authors' contributions were as follows: Bahram Pourghassem Gargari and Leila Javadi designed the study; Bahram Pourghassem Gargari, Leila Javadi, Manouchehr Khoshbaten, Abolfazl Barzegari and Mostafa Ghavami conducted the trial and collected the data; Leila Javadi and Abdolrasoul Safaiyan analyzed the data; Bahram Pourghassem Gargari and Leila Javadi wrote and revised the final manuscript. The article was based on data from a PhD thesis in the field of nutrition.

Conflict of Interest: The authors declare no conflict of interest.

Funding/Support: The study was granted by nutrition research center, Tabriz University of Medical Sciences, Iran. grant number: 5/71/1323, 92/11/1. The authors declare that there were no conflicts of interest.

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