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Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial

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Abstract. – *Objective:* **The present pilot trial was carried out to evaluate the effects of an acute treatment with a mixture containing 500 million of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* per day in patients with non alcoholic fatty liver disease (NAFLD).**

Research Methods: **A sample of 30 patients with NAFLD (diagnosed by liver biopsy) was enrolled and 28 patients were analyzed in a double blind randomized clinical trial. Patients were randomized to one of the following treatments during 3 months: group I, treated with one tablet per day with 500 million of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and group II, treated with one placebo tablet (120 mg of starch).**

Results: **In group I, alanine amino transferase (ALT: 67.7±25.1 vs. 60.4±30.4 UI/L; $p<0.05$), aspartate aminotransferase activity (AST: 41.3±15.5 vs. 35.6±10.4 UI/L; $p<0.05$) and gammaglutamine transferase levels (γGT: 118.2±63.1 vs. 107.7±60.8 UI/L; $p<0.05$) decreased. In group II, all liver function parameters remained unchanged (ALT: 60.7±32.1 vs. 64.8±35.5 UI/L; $p<0.05$), aspartate aminotransferase activity (AST: 31.7±13.1 vs. 36.4±13.8 UI/L; ns) and gammaglutamine transferase levels (γGT: 82.1±55.1 vs. 83.6±65.3 UI/L; ns). Anthropometric parameters and cardiovascular risk factors remained unchanged after treatment in both groups.**

Conclusion: **A tablet of 500 million of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, with a randomized clinical design, improved liver aminotransferases levels in patients with NAFLD.**

Key Words:

Lactobacillus bulgaricus, Non-alcoholic fatty liver disease, Randomized clinical trial, Streptococcus thermophilus.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a common liver disease characterized by elevated serum aminotransferase levels and accumulation of fat in liver accompanied by inflammation and necrosis resembling alcoholic hepatitis in the absence of heavy alcohol consumption[1].

Obesity is considered the most important risk factor. In different series, abdominal fat was correlated with degree of steatosis on liver biopsy[2]. Insulin resistance has been associated with fat liver and NAFLD, too[3]. No proven treatment for patients with NAFLD is currently available. Weight reduction with diet changes are usually recommended as the first step in the treatment of patients with this condition. Achieving and maintaining weight reduction may improve NAFLD, but the results of several reports are inconsistent[4-6].

Numerous pharmaceutical preparations, using various strains of bacteria, are currently available. Few data have been reported on the use of these products in patients with chronic liver disease[7-9].The liver continuously receives blood from the gut through the portal system. Therefore, there is a close relationship between gut and liver.

Intestinal bacteria produce ethanol and acetaldehyde. Thus, gut-derived endotoxins and active metabolites may both contribute to the evolution of alcohol- or obesity-related liver steatosis to steatohepatitis and fibrosis.

The present pilot trial was carried out to evaluate the effects of acute treatment (3 months) with 500 million of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* per day in patients with NAFLD.

Subjects and Methods

Subjects

A sample of 30 patients was randomized (Consort flow-chart, Figure 1) and 28 patients were analyzed in a double blind randomized clinical trial(RCT). The exclusion criteria were

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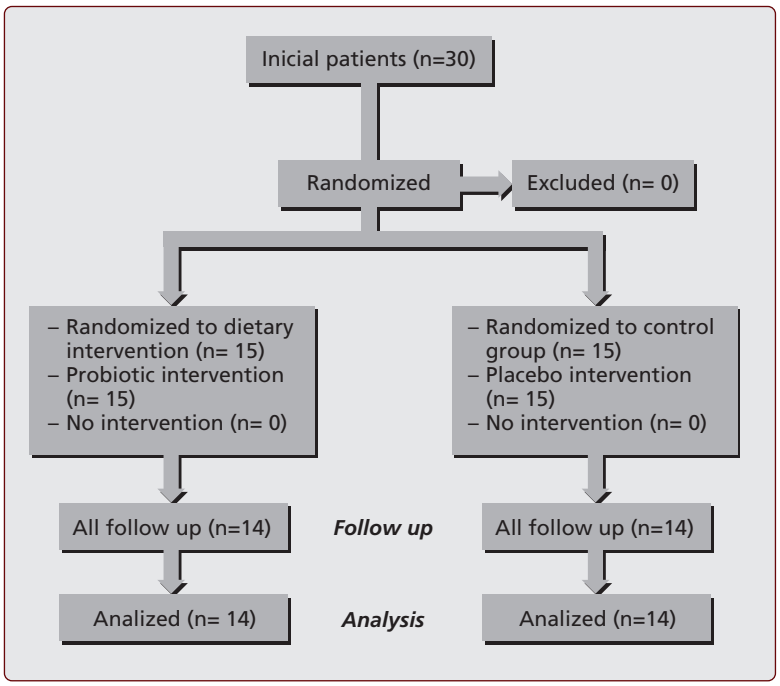

Figure 1. Consort flow-chart of trial.

hepatitis B, C, cytomegalovirus, Epstein Barr infections, non organ-specific autoantibodies, alcohol consumption, diabetes mellitus, impaired glucose tolerance (IGT), medication (blood-pressure lowering medication and statins) and hereditary defects (iron and copper storage diseases and alpha 1-antitrypsin deficiency). The study was approved by the institutional Ethics Committee and the patients signed an informed consent. The diagnosis of NAFLD was confirmed by percutaneous liver biopsy performed in all subjects with a 1.6 mm Menghini-type biopsy needle. Liver samples were routinely processed, sectioned, and stained with hematoxilin-eosin and Manson's trichome.

Procedure

All patients were randomized (table of numbers) to one of the following treatments: group I, treated with one tablet per day with 500 million of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Nutricion Medica, SL, Spain) and group II, treated with one placebo tablet (120 mg of starch). Each patient received a total of 90 tablets and should intake 1 per day, completing 3 months of treatment. It monitored the number of tablets eaten by a daily schedule and getting the final protocol unused tablets. The working methodology was double-blind, neither the patient nor the investigator who followed the patient knew the type of tablet that took the patient. Subject food intakes were recorded at baseline and after dietary advice after 3 months with 3 days written food records.

Weight, blood pressure, basal glucose, transaminases, insulin, HOMA, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, Il6 and TNF alpha blood levels were measured at baseline time and after 3 months of treatment. Anthropometric parameters (weight, BMI, waist to hip circumference, fat mass) and dietary intakes were controlled at basal time and after one month of treatment.

Assays

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA). Insulin was measured by enzymatic colorimetric (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin sensitivity (HOMA) was calculated using these values[11].

Alanine amino transferase, aspartate aminotransferase activity, bilirubin and gammaglutamine transferase were determined by enzymatic colorimetric assay Hitachi 917 (Roche Diagnostics, Geneve, Switzerland).

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant

after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula.

Interleukin 6 and TNF alpha were measured by ELISA (R&D systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal values of IL6 was (1.12-12.5 pg/ml) and TNF-alpha (0.5-15.6 pg/ml).

Anthropometric Measurements

Body weight was measured to an accuracy of 0.1 kg and body mass index (BMI) computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to hip ratio (WHR) were measured, too. Bipolar body electrical bioimpedance was used to determine body composition[12].

Dietary Intake

Patients received prospective serial assessment of nutritional intake with 3 days written food records. All enrolled subjects received instructions to record their daily dietary intake for three days including a weekend day. Handling of the dietary data was by means of a personal computer (Dietsource, Novartis, Geneve, Switzerland), incorporating use of food scales and models to enhance portion size accuracy. National composition food tables were used as reference[13]. Physical activity remained unchanged during the follow up period.

Statistical Analysis

Sample size was calculated to detect differences over 5 UI/L on transaminases levels with 90% power and 5% significance (n=14, in each group). The results were expressed as means ± standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, paired Student's-*t* test. Non-parametric variables were analyzed with the W-Wilcoxon test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. A *p*-value under 0.05 was considered statistically significant.

Results

Thirty patients were included in the protocol (Figure 1 Consort diagram), ending the study a total of 28 patients. The 2 patients excluded from the analysis had taken less than 90% of the tablets set. There were no differences in gender and age distribution of patients. In group 1 (probiotic tablet), patients (10 men and 4 women) had a mean age of 49.4±10.9 years and placebo group 2 (10 men and 4 women) had a mean age of 44.3±15.1 years.

Table I shows the differences in anthropometric variables. In group I after treatment with probiotic, anthropometric parameters remained unchanged. In group 2 (placebo), anthropometric parameters remained unchanged, too. No statistical differences were observed in basal data between both groups.

Table II shows the differences in classic cardiovascular risk factors. No statistical differences were observed in basal data between both groups. After probiotic or placebo tablets, cardiovascular parameters remained unchanged.

In group I, alanine amino transferase (ALT: 67.7±25.1 vs. 60.4±30.4 UI/L; $p<0.05$), aspartate aminotransferase (AST:41.3±15.5 vs. 35.6±10.4 UI/L; $p<0.05$)and gammaglutamine transferase levels (γGT: 118.2±63.1 vs. 107.7±60.8 UI/L; $p<0.05$) decreased. After treatment in group II, all liver function parameters remained unchanged; alanine amino transferase (ALT: 60.7±32.1 vs. 64.8±35.5 UI/L; $p<0.05$), aspartate aminotransferase activity (AST:31.7±13.1 vs. 36.4±13.8 UI/L; ns)and gammaglutamine transferase levels (γGT:82.1±55.1 vs. 83.6±65.3 UI/L; ns).

Table I. Changes in anthropometric parameters.

	Parameters probiotic tablet placebo			
	Basal	3 months	Basal	3 months
BMI	30.2 ± 4.5	31.1 ± 4.8	29.5 ± 5.5	30.1 ± 6.1
Weight (kg)	85.3 ± 15.9	86.2 ± 15.8	88.8 ± 14.1	88.9 ± 14.3
FM (kg)	40.2 ± 8.9	40.5 ± 9.2	37.7 ± 8.2	38.2 ± 8.7
WHR	0.94 ± 0,08	0.95 ± 0,08	0.92 ± 0.04	0.93 ± 0.04

BMI: body mass index. FM: fat mass. WHR: waist to hip ratio. No statistical differences.

Table II. Changes in cardiovascular risk factors and liver function.

	Parameters probiotic tablet placebo			
	Basal	3 months	Basal	3 months
Glucose (mg/dl)	116.0 ± 25.4	114.6 ± 28.1	110.0 ± 28.5	107.7 ± 29.6
Total-chol (mg/dl)	194.8 ± 49.1	200.9 ± 33.1	192.7 ± 38.6	204.7 ± 54.1
LDL-chol. (mg/dl)	110.3 ± 39.9	121.6 ± 53.4	125.6 ± 32.3	136.7 ± 38.9
HDL-chol. (mg/dl)	43.0 ± 11.9	43.0 ± 11.6	40.1 ± 6.7	43.3 ± 8.9
TG (mg/dl)	171.1 ± 95.4	150.9 ± 61.1	134.8 ± 51.8	147.2 ± 48-6
Insulin (mUI/L)	14.5 ± 6.7	14.3± 6.9	13.4 ± 7.1	14.6 ± 6.4
HOMA	4.5 ± 2.6	4.2 ± 2.4	4.2 ± 3 .2	4.3 ± 3.4
IL6 (ng/ml)	1.07 ± 1.24	1.21 ± 1.65	1.47 ± 1.27	1.03 ± 1.11
TNF alpha (ng/ml)	9.18 ± 3.22	9.80 ± 2.13	8.94 ± 2.6	8.80 ± 1.19

Chol: total cholesterol. TG: Triglycerides. HOMA: Homeostasis model assessment. No statistical differences

Table III showed dietary intakes. Before and after treatment, dietary intakes did not show statistical differences.

Discussion

In this study, we found that a tablet containing 500 million of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in patients with NAFLD was associated with alanine amino transferase, aspartate aminotransferase and gammaglutamine transferase improvement.

Animal studies have shown that translocation of bacterial products from the intestinal lumen to the mesenteric lymphatic circulation and activates the Kupffer cells in the liver, induces regional and systemic production of proinflammatory cytokines, and enhances production of free radical species in the splachnic area. Intestinal bacteria produce ethanol, too. Thus, gut-derived endotoxins and active metabolites may both contribute to the evolution of alcohol- or obesity-related liver steatosis or steatohepatitis[14].

Treatment of steatohepatitis is difficult. Based on some data[6], a 4-5% reduction in body weight could be recommended as an initial therapeutic target in patients with NAFLD to improve liver function. ALT and AST levels improved with low fat diet[15]. Marchesini et al[16] have treated these patients with metformin: this drug reduced mean transaminase concentrations, insulin resistance and liver volume. In other study, acarbose attenuated NAFLD progression in an experimental model of NAFLD in rats[17]. Two drugs used in obese patients, orlistat and sibutramine[18-20], have shown that drug-induced weight losses result in reduction of insulin resistance and improvements in biochemical markers of NAFLD. Other treatment is antibiotics. Treatment with oral antibiotics that are poorly absorbed inhibits the shift of steatosis to steatohepatitis in animals with obesity[2,3]. Some studies have shown[21,22] that the combination of two mixtures of bacteria strains (both containing various bacteria strains such as *Streptococcus salivarius, Lactobacillus bifidus, Lactobacillus acidophilus, Lactobacillus Plantarum, Lactobacillus casei, Lactobacillus Bulgaricus*) led to an improvement in liver function in patients with NAFLD, as our study shows. Indeed,

Table III. Dietary intake.

	Parameters probiotic tablet placebo			
	Basal	3 months	Basal	3 months
Energy (kcal/day)	1457.4 ± 332	1402.8 ± 223	1673.9 ± 455	1587.1 ± 429
CH (g/ day)	145.5 ± 46.1	152.6 ± 38.7	176.4 ± 82.2	162.7 ± 43.9
Fat (g/ day)	65.9 ± 20.2	68.9 ± 14.1	76.2 ± 20.1	67.2 ± 21.8
Protein (g/ day)	75.2 ± 15.9	72.4 ± 15.6	82.7 ± 23.4	84.8 ± 24.2
Total Fiber (g/ day)	8.6 ± 4.1	7.1 ± 4.5	9.6 ± 3.1	9.5 ± 3.6
Cholesterol (mg/day)	347.3 ± 213	461.5 ± 211	471.8 ± 137	389.1 ± 14

CH: Carbohydrates. No statistical differences.

in NAFLD patients, intestinal microflora contributes to the onset and progression of chronic liver damage by way of translocation of endotoxins from intestinal lumen to mesenteric circulation and through the direct production of ethanol. Endotoxins activate Kupffer cells in the liver and enhance the production of TNF alpha and IL-6. Our results did not support this hypothesis, because TNF alpha and IL-6 remained unchanged after treatment.

Lactic acid bacteria have been an integral component of the human diet. Colonization of the gastrointestinal tract, by these probiotics, results in a modification of gut flora and reduces the proinflammatory species[23]. In animals with alcoholic and non alcoholic steatohepatitis, treatments as lactobacilli, including VS#3, has a beneficial effect upon liver damage[8-9,21,24]. Recently VSL#3 probiotic treatment has shown a modulation effect on liver fibrosis in model mice with NAFLD[25]. Perhaps these beneficial effects of probiotics on liver function are not secondary to interleukin production and it is due to a reduction of the endotoxin mediated liver damage[26]. A decrease of plasma levels of oxidative/nitrosative stress could be other way to explain this improvement in liver function after probiotic treatment. Nevertheless, these studies have not demonstrated beneficial effects on cardiovascular risk factors, according with our data.

In conclusion, a tablet containing 500 million of *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* with a randomized clinical design, improved liver aminotransferases levels in patients with NAFLD. The results of this pilot study are in keeping with previous observations and suggest that, in some conditions in which intestinal microflora may be involved as a cofactor of NAFLD, the modulation of liver-gut axis may be a potential treatment with probiotics.

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