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Effect of probiotics supplementation on liver stiffness and steatosis in patients with NAFLD

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Abstract

Background and Aim: To compare the effects of probiotics on liver stiffness and steatosis in obese and non-obese patients with nonalcoholic fatty liver disease (NAFLD),the pragmatic clinical trial included 50 obese body mass index (BMI) ≥25 kg/m^2 and 50 non-obese NAFLD BMI <25 kg/m^2 age and sex-matched patients.

Materials and Methods: Fibroscan with controlled attenuated parameter (CAP) was done at day 0 and at the end of 6 months. Probiotics supplementation was provided for both groups for 6 months along with lifestyle modifications.

Results: At inclusion, both groups had comparable characteristics except BMI, metabolic syndrome and waist circumference (WC). Beneficial changes occurred in BMI (p=0.024), WC (p=0.045), ALT (p=0.024), total cholesterol (p=0.016), LDL (p=0.025) and triglyceride (p=0.021) of obese group, systolic blood pressure (p=0.003) and LDL level (p=0.018) in non-obese group. No significant change was observed in liver enzymes and glycemic profiles. Significant improvement in CAP was observed in both groups. But after adjusting for changes in BMI and WC, the change in CAP among non-obese participants were significantly higher compared to obese, mean change of 19.33±48.87 and 16.02±51.58 dB/m in non-obese and obese patients, respectively; p=0.044).

Conclusion: Probiotics improve CAP/ steatosis in both obese and non-obese NAFLD patients and improvement was higher in non-obese, irrespective of BMI change.

Keywords: Nonalcoholic steatohepatitis; nonalcoholic fatty liver disease; steatohepatitis; non-obese nonalcoholic steatohepatitis; probiotics.

What is already known?

Probiotic supplementation in patients with nonalcoholic fatty liver disease (NAFLD) has a variable impact on body mass index (BMI), liver enzymes, lipid profiles, and blood glucose levels.

What is new in this study?

This study reveals that the improvement in steatosis among non-obese NAFLD patients receiving probiotic supplementation is significantly greater than that in obese patients, regardless of BMI improvement.

What are the Future Clinical and Research Implications of the Study Findings?

Further clinical trials focusing on probiotic treatment for non-obese NAFLD patients can be pursued.Initiatives to gain a deeper understanding of the mechanisms underlying NAFLD in non-obese patients may be undertaken.

Introduction

Non-alcoholic fatty liver disease (NAFLD), a significant health concern responsible for hepatic and extrahepatic morbidity and mortality, has a global prevalence of 25.2%.[1,2] The prevalence of NAFLD is notably high in Asia, especially in the Middle East, where it has been reported to affect up to 60% of the population.[3] NAFLD encompasses a spectrum of liver conditions, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), which may progress to fibrosis, cirrhosis, and even hepatocellular carcinoma.[4] Although commonly associated with obesity and insulin resistance, NAFLD can occur at lower body mass indices (BMIs) in Asian populations.[2,5] Factors such as visceral obesity, high fructose and cholesterol intake, genetic predispositions, and gut dysbiosis are thought to contribute to the development of NAFLD in lean or non-obese individuals.[6,7] To manage NAFLD, various treatment strategies have been proposed, including pharmacological, non-pharmacological, and surgical interventions. Recently, probiotic therapy has emerged as a promising approach, especially for non-obese NAFLD patients.[8,9]

Probiotics are live microorganisms that confer health benefits, primarily by modulating the intestinal microbiota, producing antibacterial substances, enhancing epithelial barrier function, and reducing intestinal inflammation.[10] Numerous randomized clinical trials have evaluated the efficacy of probiotics in NAFLD, demonstrating beneficial effects.[11–15] Probiotic supplementation in NAFLD patients significantly im-

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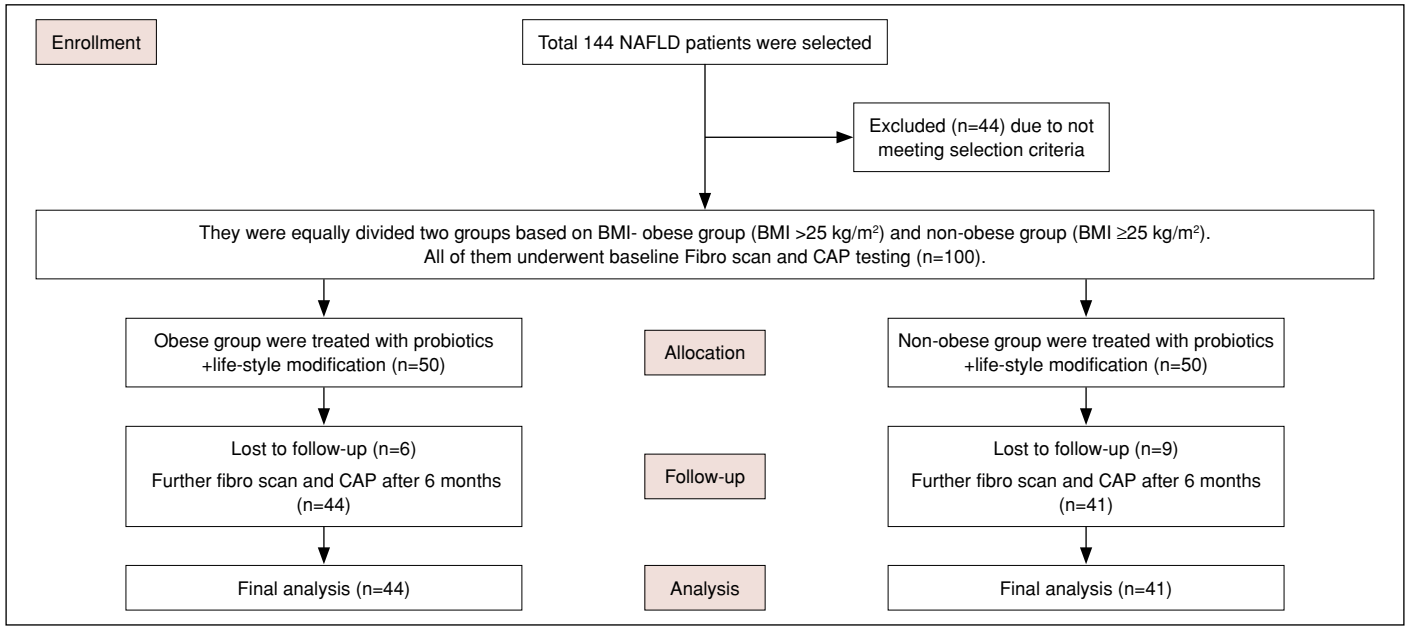

Figure 1. we have enrolled a total of 100 patients out of 144 initially selected patients. Forty-four patients were excluded. Six patients in obese group and 9 patients in non obese group were lost to follow up at the end of the study. So 44 patients in obese group and 41 patients in non obese group were included in the analysis.

pacts BMI, liver enzymes, lipid profiles, and blood glucose levels. While some studies suggest that probiotics, alongside lifestyle modifications, can improve both laboratory parameters and BMI,[2,12,14] others report no reduction in BMI with or without lifestyle interventions.[13] Furthermore, probiotics have been shown to improve liver histology, steatosis, and stiffness,[11,13,15] and to reduce the ultrasonographic grade of fatty liver.[12] However, one study noted only a slight reduction in liver stiffness.[4] Interestingly, probiotic supplementation has also been found beneficial in NAFLD patients with normal or low BMI.[8]

Given the rising prevalence of NAFLD in Asia and the higher mortality rates among lean individuals,[16,17] coupled with the role of dysbiosis in the pathogenesis of NAFLD, probiotic supplementation could be a viable option to improve hepatic inflammation, steatosis, and fibrosis. The conventional weight reduction strategy may not be suitable for these lean patients.[18] Therefore, this study hypothesized that probiotic supplementation might be particularly effective in improving liver stiffness and steatosis in non-obese patients with NAFLD compared to their obese counterparts. Consequently, this study aims to investigate the effects of probiotic supplementation on liver stiffness and steatosis in both obese and non-obese populations with NAFLD.

Materials And Methods

Formal ethical approval was obtained from the Institutional Review Board (IRB) of the university. After receiving written informed consent from each participant, we enrolled a total of 100 patients from an initial selection of 144. These patients, of both sexes and aged 18–67 years, participated in an open-level, head-to-head clinical trial. Six patients in the obese group and nine in the non-obese group were lost to follow-up by the end of the study (Fig. 1), resulting in 44 patients in the obese group and 41 in the non-obese group being included in the analysis. This study was conducted over 14 months at the Department of Hepatology in a tertiary care hospital. Patients with ultrasonographic evidence of fatty liver were divided into two groups based on the BMI cutoff value of ≥25 kg/m^2, as suggested by the Asia-Pacific criteria.[19] Non-obese NAFLD patients, defined by a BMI <25 kg/m^2, were compared with obese NAFLD patients (BMI ≥25 kg/m^2), with 50 patients in each group. Exclusions were patients with a history of alcohol consumption (≥20 g/day in men or ≥10 g/day in women), positive viral markers (hepatitis B, hepatitis C), known cases of secondary fatty liver (e.g., use of anabolic steroids, tamoxifen, anticonvulsants, antiarrhythmic drugs), CLD with a known etiology (e.g., Wilson's disease, autoimmune liver diseases, hemochromatosis, cirrhosis of the liver), pregnant women, or those suffering from any malignancies before baseline. Also excluded were those with acute or CLD disease. Patient allocation was performed by research physicians who were aware of the study groups; thus, no blinding was done. Each participant was prescribed a probiotic capsule (Protexin Balance by Novartis Pharma, containing Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus, Bifidobacterium breve, Lactobacillus acidophilus, Bifidobacterium longum, Lactobacillus bulgaricus) twice daily for six months, along with lifestyle modification advice and concurrent standard management. Lifestyle modification, including moderate exercise (half-hour walks each day) and dietary advice to avoid saturated fats, excessive sugar, soft drinks, fast food, and refined carbohydrates, was suggested for both groups. A diet chart for NAFLD was provided to every patient. Diabetic patients were treated with Gliclazide, Glimepiride, or Insulin. For dyslipidemia, non-pharmacological measures were initially advised, followed by pharmacological agents if necessary after three months. Hypertensive patients were managed with antihypertensive drugs, excluding ACE inhibitors, ARBs, and calcium channel blockers (Diltiazem). Fatty liver was diagnosed based on ultrasonographic criteria, then CAP and LSM were assessed using transient elastography in patients with NAFLD. Evaluations were performed on the right lobe of the liver through intercostal spaces with patients lying in the decubitus position, the right arm in abduction. The median LSM is expressed in kPa, and CAP in dB/m. All patients were advised to contact the team immediately if they had any queries or unusual events arose. Each patient was followed up monthly for three months, with a final

Table 1. Baseline characteristics of the obese and non-obese groups

Variable	Obese group (n=44)	Non-obese group (n=41)	p
Age (years)	39.7±10.3	38.1±11.0	0.497
Sex (male/female)	17 (38.6%)/27 (61.4%)	22 (53.6%)/19 (46.4%)	0.165
Diabetes	12 (24.0%)	14 (17.0%)	0.636
Hypertension	18 (21.0%)	11 (13.0%)	0.252
Metabolic syndrome	44 (51.77%)	29 (34.12%)	<0.001
BMI (kg/m^2)	27.98±2.56	22.74±1.60	<0.001
Waist circumference (WC, cm)	96.89±7.15	78.96±4.31	<0.001
Systolic blood pressure (SBP, mmHg)	130.57±16.11	129.02±16.74	0.666
Diastolic blood pressure (DBP, mmHg)	82.84±7.27	82.93±6.89	0.956
Alanine transaminase (ALT, U/L)	54.17±44.93	42.59±24.96	0.160
Aspartate aminotransferase (AST, U/L)	43.63±54.59	32.86±16.17	0.295
Gamma-glutamyl transferase (GGT, U/L)	47.11±8.23	53.64±49.01	0.578
Fasting blood sugar (FBS, mmol/L)	6.27±1.46	6.28±1.74	0.963
Blood sugar 2 hours after breakfast (mmol/L)	8.18±2.78	8.52±3.29	0.613
Serum cholesterol (mg/dl)	211.95±57.64	204.14±51.66	0.530
High-density lipoprotein (HDL, mg/dl)	41.14±8.50	46.25±17.28	0.095
Low-density lipoprotein (LDL, mg/dl)	130.18±33.58	118.63±31.79	0.142
Triglycerides (mg/dl)	219.59±116.39	231.32±180.54	0.731
Controlled attenuation parameter (CAP, dB/m)	301.23±45.85	285.71±57.37	0.178
Liver stiffness (kPa)	5.98±2.38	4.89±1.36	0.014

Data expressed as mean±SD and frequency (percentage). P value determined by Chi-Square test and unpaired t test as appropriate. BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT; Gamma-glutamyl trasferase; FBS: Fasting blood sugar; HDL: High density lipoprotein; LDL: Low density lipoprotein; CAP: Control attenuated parameter.

visit six months from the first visit. Each visit, occurring between 10:00 AM to 2:00 PM, consisted of clinical examinations, BP and Body BMI determinations, and information recorded into a pre-designed, pre-tested questionnaire. A pre-test was conducted in the aforementioned department for 10 patients. Serum was collected for Fasting Blood Sugar (FBS), 2-Hour After Breakfast Fasting (2HABF), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma-Glutamyl Transferase (GGT), fasting lipid profile, and fibroscan of the liver with CAP in the first and last visit. Study compliance was strictly monitored. FBS, 2HABF, and lipid profile for diabetic and dyslipidemic patients were assessed as needed. The primary parameters that were compared between the first and last visits are BP, WC,BMI, ALT, AST, GGT, TC, TG, HDL, LDL, FBS, 2HABF, LSM, CAP.

Anthropometric Measurement, Laboratory Test, Ultrasound (USG) Evaluation, and Elastography Scan

Anthropometric measurements were performed by a research physician with the assistance of a staff nurse using standard protocols and calibrated instruments. The weight and height of participants were measured with light clothes and without shoes. BMI was calculated as weight (kg) divided by height (m)^2. WC was measured with a nonelastic tape at a point midway between the lower border of the rib cage and the iliac crest at the end of normal expiration. All laboratory tests were conducted at the biochemistry and pathology department of the university. The sonographic feature of NAFLD was determined based on the presence of bright hepatic echotexture (compared with kidney), blurring of intrahepatic vasculature, and deep attenuation, either singly or in combination. All sonography was performed by a single experienced radiologist. Elastography scans were also performed by an expert elastographer with the FibroScan system (Echosens, Paris, France), using the M probe for standard examinations and the XL probe to increase the reliability of measurements in overweight patients.

Statistical Analysis

Statistical analysis was conducted using SPSS version 20 (SPSS Inc., Chicago, IL) and included the chi-square test, paired and unpaired t-tests. Analysis of Covariance (ANCOVA) was utilized for adjusting BMI and WC while assessing CAP and liver stiffness improvement in both obese and non-obese groups. A $p<0.05$ was considered statistically significant.

Results

Baseline Characteristics of Patients

The mean age of the study population was 38.94±10.64 years, ranging from 18 to 67 years, with 54.1% female respondents. The age and sex distribution were statistically similar between the obese and non-obese groups. Diabetes Mellitus (DM) and Hypertension (HTN) were found in a statistically similar proportion in both groups. Metabolic syndrome was significantly more prevalent in the obese group (51.77%) compared to the non-obese group (34.12%, $p<0.001$). The average BMI of obese and non-obese patients was 27.98±2.56 and 22.74±1.60 kg/m^2, respectively ($p<0.001$). The mean WC was also significantly higher in the obese group (96.89±7.15 cm) compared to the non-obese group (78.96±4.31 cm; $p<0.001$). Liver function tests were statistically similar between the groups. The average baseline CAP in the obese group (301.23±45.85 dB/m) was statistically similar to that in the non-obese

Table 2. Changes in transient elastography of liver, clinical variables and biochemical variables after 6 months of treatment

Variable	Obese group			Non-obese group		
	Baseline	After 6 months	p	Baseline	After 6 months	p
Transient elastography						
CAP (dB/m)	301.2±45.85	285.20±47.0	**0.048**	285.7±57.3	266.38±60.5	**0.018**
Liver stiffness (kPa)	5.98±2.38	5.43±1.94	0.163	4.89±1.36	4.68±1.53	0.443
Clinical variables						
BMI (kg/m^2)	27.98±2.55	27.36±2.66	**0.024**	22.74±1.60	23.25±2.42	**0.043**
Waist circumference (WC, cm)	96.89±7.14	94.83±6.89	**0.045**	78.96±4.30	80.60±7.02	0.071
Systolic blood pressure (SBP, mmHg)	131.05±15.98	131.98±14.14	0.687	129.02±16.74	124.27±13.21	**0.003**
Diastolic blood pressure (DBP, mmHg)	83.14±7.07	84.42±5.36	0.232	82.93±6.89	81.83±4.96	0.183
Biochemical variables						
ALT (U/L)	53.90±45.62	38.60±22.56	**0.024**	42.59±24.96	39.15±27.28	0.369
AST (U/L)	34.26±26.13	31.54±28.48	0.324	32.86±16.17	28.02±15.79	0.114
GGT (U/L)	48.95±51.95	41.53±30.64	0.353	49.40±44.63	37.50±22.21	0.247
Fasting blood sugar (FBS, mmol/L)	6.22±1.46	5.87±1.14	0.112	6.28±1.73	6.21±1.80	0.698
Blood sugar 2 hours after breakfast (mmol/L)	8.18±2.85	8.31±2.98	0.802	8.52±3.28	8.41±3.36	0.812
Serum cholesterol (mg/dl)	213.93±58.36	189.10±38.53	**0.016**	204.14±51.66	192.22±61.0	0.105
Serum HDL (mg/dl)	41.25±8.70	41.15±9.85	0.943	46.25±17.28	43.42±8.66	0.290
Serum LDL (mg/dl)	129.54±34.65	111.89±39.36	**0.025**	121.23±31.0	110.63±22.6	**0.018**
Serum triglycerides (mg/dl)	216.49±117.3	184.85±103.3	**0.021**	231.32 ±180.5	250.8±249.1	0.517

Data expressed as mean±SD. *: P value determined by paired t test. CAP: Control attenuated parameter; BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT; Gamma-glutamyl transferase; FBS: Fasting blood sugar; 2HABF: Blood sugar two hour after breakfast; HOMA-IR: Homeostasis model assessment insulin resistance; HDL: High density lipoprotein; LDL: Low density lipoprotein.

group (285.71±57.37 dB/m, p=0.178). Liver stiffness in the obese group (5.98±2.38 kPa) was significantly higher than in the non-obese group (4.89±1.36 kPa, p=0.014) (Table 1).

Changes in Transient Elastography, Clinical, and Biochemical Variables After 6 Months of Treatment

The mean CAP value in the obese group before and after intervention was 301.2±24.85 dB/m and 285.20±47.0 dB/m, respectively. In the non-obese group, these values were 285.7±57.3 dB/m and 266.38±60.5 dB/m, respectively. There was a significant decrease in CAP from its baseline value following intervention in both groups (p=0.048 and 0.018 respectively for obese and non-obese groups). The mean liver stiffness remained almost the same in both groups before and after the intervention. The average baseline and follow-up liver stiffness were 5.98±2.38 kPa and 5.43±1.94 kPa in the obese group (p=0.163), and 4.89±1.36 kPa and 4.68±1.53 kPa in the non-obese group (p=0.443) (Table 2).

The mean BMI decreased significantly in the obese group, reducing from 27.98±2.55 kg/m^2 at baseline to 27.36±2.66 kg/m^2 after six months (p=0.003). In contrast, the mean BMI in the non-obese group increased significantly from 22.74±1.60 kg/m^2 at baseline to 23.235±2.42 kg/m^2 (p=0.043). WC decreased significantly in the obese group (from 96.89±7.14 cm to 94.83±6.89 cm at baseline and after intervention, p=0.045). In the non-obese group, it increased from 78.96±4.30 cm to 80.60±7.02 cm over the same period, although the increase was not significant (p=0.071). Systolic BP reduced significantly in the non-obese group (p=0.003), while it remained nearly the same in the obese group (p=0.687) after the end of treatment.

ALT decreased significantly from 53.90±45.62 U/L at baseline to 42.59±24.96 U/L after 6 months of intervention in the obese group (p=0.024), but showed a non-significant reduction in the non-obese group (from 42.59±24.96 to 39.15±27.28 U/L, p=0.369). The obese group had a significant reduction in average serum cholesterol level (from 213.93±58.36 to 180.10±38.53 mg/dL, p=0.016), average Low-Density Lipoprotein (LDL) level (from 129.54±34.65 to 111.89±39.36 mg/dL, p=0.025) and average Triglycerides (TG) level (from 216.49±117.3 to 184.85±103.3 mg/dL, p=0.021). On the other hand, the non-obese group had a significant reduction only in serum LDL level (from 121.23±31.0 to 110.63±22.6 mg/dL, p=0.018). The rest of the parameters of liver function, glycemic profile, and lipid profile didn't show any significant change in either the obese or non-obese groups (Table 2).

Comparative Analysis of Clinical, Biochemical, and Transient Elastography Improvement in Obese and Non-Obese Groups

CAP improvement was higher in the non-obese group (19.33±48.87 dB/m) than in the obese group (16.02±51.58 dB/m), and the difference was significant (p=0.045). Liver stiffness improvement was significantly higher between groups of obese and non-obese (respectively 0.55±2.52 kPa and 0.21±1.77 kPa, p=0.014), although it was not significant within the group before and after intervention. These significance levels were achieved after statistical adjustment for improvements in BMI and WC of patients using ANCOVA. In the obese group, BMI and WCreduced, while in the non-obese group, BMI and WC increased at the end of the intervention period. Improvement in the glycemic profile,

Table 3. Comparison of anthropometric, biochemical, and elastography improvements between obese and non-obese groups

Variable	Obese	Non-obese	p
CAP (dB/m)	16.02±51.58	19.33±48.87	**0.044***
Liver stiffness (kPa)	0.55±2.52	0.21±1.77	**0.014***
BMI (kg/m^2)	0.61±1.97	-0.52±1.57	**0.005**
Waist circumference (cm)	2.07±5.46	-1.64±5.67	**0.003**
Fasting blood sugar (mg/dL)	0.36±1.40	0.07±1.09	0.306
ALT (U/L)	15.30±3.43	3.43±23.58	0.122
AST (U/L)	2.71±16.03	4.83±17.88	0.601
GGT (U/L)	7.41±39.92	11.90±51.22	0.726
Serum cholesterol (mg/dL)	24.83±62.21	11.91±43.55	0.299
LDL (mg/dL)	17.65±46.04	10.60±23.14	0.420
HDL (mg/dL)	0.10±8.74	0.36±9.90	0.903
TG (mg/dL)	31.64±82.38	-19.51±181.44	0.115

Statistically significant ($p<0.05$). Data expressed as mean±SD. P value determined by unpaired t-test; *: After adjusting for improvements in BMI and waist circumference (by repeated measures analysis of covariance [ANCOVA] with between subject effects); CAP: Control Attenuated Parameter; BMI: Body mass index; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT; Gamma-glutamyl transferase; HDL: High density lipoprotein; LDL: Low density lipoprotein

liver function tests, and lipid profile were statistically similar between the two groups (Table 3). CAP/steatosis improvement in the non-obese group was independent of weight loss or BMI reduction.

Probable Side Effects

Treatment was generally well-tolerated. One patient from both the obese and non-obese group developed diarrhea. Two patients from the obese group and one from the non-obese group developed dyspepsia. None of the side effects were statistically significant in proportion in any of the groups. There were no troublesome side effects to warrant the urgent withdrawal of drugs (Table 4).

Discussion

NAFLD is common among obese patients, but an increased incidence of NAFLD among non-obese patients is now observed.[20] The burden of NAFLD is increasing worldwide. Current NAFLD treatment focuses on the components of metabolic syndrome and aims to reverse liver injury. The principal treatment for NAFLD and non-alcoholic steatohepatitis (NASH) involves lifestyle modification through diet and exercise in both obese and non-obese patients.[21,22] Although several drugs have been tested, a universally accepted medication is yet to be found. This open-label pragmatic trial study assessed the effects of probiotics on non-obese NAFLD patients and compared them with obese NAFLD patients. This is the first trial of its kind carried out on Bangladeshi NAFLD patients. The effectiveness of probiotics was observed using transient elastography. The study demonstrated that six-month treatment with probiotics is associated with improved hepatic steatosis in NAFLD patients, irrespective of their weight.

A current challenge in many clinical and therapeutic investigations is outcome evaluation. Routine hematological tests are helpful in patients with NAFLD but not sufficient to assess the real effectiveness of treatment. Serum factor changes may not always correspond to variations in liver conditions. Conversely, needle liver biopsy provides the most conclusive evaluation for both disease diagnosis and follow-up but is difficult to perform repeatedly in all NAFLD patients due to its invasiveness and potential complications. Ultrasonography is commonly used for NAFLD diagnosis. FibroScan (transient elastography) and Controlled Attenuation Parameter (CAP) offer a promising non-invasive and rapid bedside method for diagnosing and quantifying hepatic fibrosis and steatosis, respectively. Elastography is the best-tolerated imaging technique, playing a role in staging fibrosis, steatosis, prognosis, surveillance, and treatment decisions. Therefore, it is reasonable to measure the outcome of probiotics in NAFLD patients using FibroScan.

Table 4. Comparison of probable side effects in obese and non-obese groups

Side effect	Obese (n=44)	Non-obese (n=41)	p
Diarrhea	1/43	1/40	1.000
Dyspepsia	2/42	1/40	1.000

In the current study, the proportion of female NAFLD patients was higher than male, aligning with the findings of Alam et al.,[17] who noted that females are predominantly affected by NAFLD in the country. The most significant changes in hepatic steatosis induced by probiotics were reflected by the decreased CAP in both non-obese and obese NAFLD patients. However, the improvement was more substantial in the former group ($p=0.044$). This is consistent with the observations of Mofidi et al.[8] were the first to observe improvements in hepatic steatosis and fibrosis in lean NAFLD patients using non-invasive tests like FibroScan with CAP. Their study included both a symbiotic and a placebo group, with the mean reduction in hepatic issues significantly greater in the symbiotic group than in the placebo group. Similarly, Malaguarnera et al.[18] noted the efficacy of probiotics on hepatic fat content in NASH patients. By the end of their study period, it was found that Bifidobacterium longum combined with fructo-oligosaccharides and lifestyle modification led to a significant reduction in AST, LDL cholesterol, steatosis, and the NASH activity index, compared to lifestyle modification alone.Malaguarnera et al.[18] regarding the efficacy of probiotics on hepatic fat content in NASH patients.

In this study, the mean liver stiffness, as indicated by liver fibrosis, showed a non-significant reduction in both groups. This finding aligns with the results of Wong and colleagues,[23] who observed no significant change in liver stiffness in biopsy-proven NASH patients after six months of probiotic treatment. Similarly, Alisi et al.[24] reported improvements in liver fibrosis, lobular inflammation, and hepatocyte ballooning in obese NASH children treated with a probiotic containing eight strains for four months. However, when comparing the dynamic reduction between the two groups, taking into account improvements in BMI and WC, the decrease in liver stiffness was significantly greater in obese NAFLD patients than in their non-obese counterparts. This suggests that probiotics, combined with lifestyle modifications, were substantially more beneficial for the obese patient group. A recent trial by Duseja and colleagues[11] supports this hypothesis.

BMI showed a statistically significant decrease in the obese group and a significant increase in the non-obese group. Both groups were advised to maintain a strict lifestyle and weight-reducing diet in addition to probiotics, but monitoring patient compliance was challenging. This might have led to the unexpected BMI increase in the non-obese group. However, a reduction of CAP was noted in both groups, with a significantly high-

er reduction in the non-obese group after adjusting for changes in BMI and WC This suggests that the changes in CAP were primarily the effect of probiotics rather than weight reduction. This finding is novel in this study. Nonetheless, Gao et al.[25] have previously shown that probiotics did not affect BMI, blood glucose level, and insulin resistance among NAFLD patients. This study also found no effect of probiotics on the glucose levels of both obese and non-obese patients. In a similar vein, Wong et al.[23] reported that there were no significant changes in fasting glucose levels in NAFLD patients before and after treatment with probiotics.

WC significantly decreased in obese patients and did not show any significant change in non-obese patients in the current study. Similar findings were reported by Famouri et al.[26] stating that a probiotics compound used in obese children has a significant effect on WC.

Regarding liver function tests, in the obese group, a reduction in the level of ALT was statistically significant (p=0.024), but reductions in AST and GGT levels were not significant. Meanwhile, in the non-obese group, none of the liver function tests showed any significant improvement during the treatment period. However, when improvements in both groups were compared, no statistically significant difference was noted between these groups. Mofidi et al.[8] found a significant decrease in AST in probiotic-treated lean NAFLD patients in comparison to those who were placebo-treated. However, no improvement was observed in ALT and GGT levels in any of the groups. Famouri et al.[26] found that there is a significant reduction of AST in both probiotics and placebo groups, but ALT was only reduced in the probiotics group. Gao et al.,[25] in a meta-analysis of nine RCTs, found that probiotics significantly improved ALT and AST levels in both children and adult NAFLD patients, irrespective of their weight. The findings of the present study are partially consistent with these studies in that ALT showed significant improvement in obese groups.

In the current study, there were significant changes in TC, LDL, and TG in the obese group treated with probiotics before and after intervention. This study found a significant improvement in the LDL level of the non-obese group. Also, the comparison of dynamic improvements between the two groups shows that improvements in any parameters of the lipid profile were statistically similar between obese and non-obese patients. Gao et al.,[25] in their meta-analysis, also reported improvements in the serum lipid profile with probiotics in NAFLD patients. Improvement in lipid parameters of blood using probiotics among NASH patients was noted previously by Malaguarnera et al.[18] They found that Bifidobacterium longum with fructo-oligosaccharides in NASH patients caused significant improvement of TC, LDL, and TG.

Probiotics were well tolerated with only a few minor side effects. None required treatment discontinuation due to the development of side effects. No cardiovascular events occurred, and none of the patients developed signs and symptoms of cirrhosis. In the current study, there was no case fatality that can be attributable to probiotics.

However, the main limitations of this study included a small sample size, short duration of trial and follow-up, lack of a control group, and lack of assessment of inflammatory markers. Other limitations include a lack of blinding and the inability to monitor patients' compliance with lifestyle modification advice, which could have led to potential bias of the effect measures. However, as both obese and non-obese groups were given probiotics and lifestyle modification, the effect of the absence of blinding could be ignored. But we had to resort to statistical means to adjust the changes in BMI and WC in both groups as we were unable to conduct strict monitoring of lifestyle modification. Another limitation was that gut microbial mapping was not done due to limited resources.

Conclusion

This study has shown that probiotic supplementation improves CAP significantly in both obese and non-obese NAFLD patients. CAP improvement was higher in the non-obese, irrespective of changes in their BMI.

Ethics Committee Approval: This study was reviewed and approved by the Institutional Review Board (No. BSMMU/2017/12512).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – PKD; Design – SA, PKD, MA, MJH; Supervision – SA; Materials – SA, PKD, MJH; Data Collection and/or Processing – SA, PKD, MA, MJH; Analysis and/or Interpretation – MJH; Literature Search – MJH; Writing – SA, PKD; Critical Reviews – SA.

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

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