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Original Article

Effect of Probiotics on Lipid Profile, Glycemic Control, Insulin Action, Oxidative Stress, and Inflammatory Markers in Patients with Type 2 Diabetes: A Clinical Trial

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Abstract

Background: The dramatic increase in the incidence of diabetes and its associated complications require a natural and safe solution to control and delay such complications. The present study tested the hypothesis that probiotics may affect biochemical indices of diabetic patients

Methods: Thirty four types 2 diabetic patients aged between 25 to 65 years, and diagnosed with diabetes for less than 15 years were selected for this single- blinded clinical trial. Using balanced block random sampling, the patients were divided into two groups of intervention (probiotics) and placebo. Blood samples tested for baseline glucose, insulin, TG, total cholesterol, LDL-C, HDL-C, malondialdehyde, high sensitive CRP (hs-CRP) and IL-6. After six weeks of experiment, fasting blood samples were re-tested and the data obtained were analyzed using SPSS software.

Results: There were no significant differences between anthropometric data including body mass index and waist to hip ratio in placebo and treatment groups. There was no significant difference in FBS, Serum TG concentration total cholesterol and LDL-C levels between placebo and treatment groups. HDL-C levels were slightly elevated after probiotic treatment, which were not statistically significant. Insulin, MDA and IL-6 levels were reduced and high sensitive CRP hs.CRP levels were elevated, although, not statistically significant.

Conclusion: The result of this study indicates a non- significant declining trend in the level of TG, MDA and IL-6 and insulin resistance after consumption of probiotics.

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Keywords ● Probiotic ● Diabetes ● Insulin resistance ● CRP

Introduction

Diabetes is a metabolic disease that leads to high blood sugar due to either insulin insufficiency, insulin resistance or both.[1] According to the World Health Organization at least 171 million people (2.8% of the world population) suffered from diabetes in year 2000.[1] This number will almost double by year 2030.[1]

It is expected that more than 70% of total diabetic patients in the world will be from developing countries by year 2030.[2] The

prevalence of type 2 diabetes in Iran ranges from 1.3% to 14.5% which will increase as the population ages in both males (10.6%) and females (11.3%).[2]

Vascular diseases are one of the most common causes of morbidity and mortality in diabetic patients.[3] Although, there is positive relation between insulin resistance and vascular disease,[4] the exact mechanisms by which diabetes leads to arthrosclerosis is not well- understood. Numerous studies have documented a role for inflammation in atherosclerosis.[5] C-reactive protein (CRP) and interleukin 6 (IL-6) the two most sensitive markers of inflammation have been elevated in patients with type 2 diabetes.[6] In addition, high CRP level is shown to be a risk factor for developing type 2 diabetes, which may be atherogenic.[7]

Hyperglycemia is also consistently associated with increased oxidative stress. Oxidative stress is a component of cellular damage and has an important role in the pathogenesis of a number of human diseases including atherosclerosis. Mechanisms that contribute to increased oxidative stress in diabetes may include not only increased non-enzymatic glycosylation and auto-oxidative glycosylation but also to decreasing antioxidant defence potential.[8]

FAO/WHO, define probiotics as live microorganisms which when administered in adequate amounts confer a health benefit on the host.[9] Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics. Animal studies showed that *Lactobacillus* GG treatment not only reduces glucose intolerance but also significantly decrease hyperglycemia in streptozotocin induced diabetes rats.[10]

Among other beneficial effects of probiotics and prebiotics, the reduction of blood lipids is of particular interest.[5] Lactobacilli and bifidobacteria are the primary probiotic bacteria which are associated with cholesterol reduction, although comparable effect may be produced by other lactic acid bacteria, such as enterococci.[11] It has also been reported that oral administration of heat killed Lactobacillus casei to non-obese diabetic (NOD) mice reduces the incidence of diabetes, but the mechanism underlying this effect has not been clarified.[12,13]

This study was designed to determine the effect of probiotics on lipid profile, glycemic control, insulin level, oxidative stress and inflammatory markers in patients with type 2 diabetes.

Patients and Methods

This single-blinded clinical trial comprised 40 patients with type 2 diabetes recruited from medical clinic affiliated with Shiraz University of Medical Sciences (SUMS) Shiraz Iran. Diabetic patients with fasting blood glucose ≥126 mg/dl, aged from 25 to 65 years, and diagnosed as having diabetes for less than 15 years were eligible for the study. Exclusion criteria were current smokers, subjects on non-steroidal anti-inflammatory drugs and multivitamin, as well as patients undergoing hormone replacement therapy, and those with any chronic diseases involving kidney, liver, and lung. The research was approved by the Ethics committee of SUMS, and written informed consent was obtained from all patients prior to commencement of the study. Subjects were initially studied during a screening visit

Study Design

After an overnight fast starting from 8 pm in previous evening baseline plasma samples were collected and analyzed for triglyceride, total cholesterol, LDL-C, HDL-C, glucose, insulin, malondialdehyde, hs-CRP and IL-6. Using balanced block random sampling, subjects were then divided into two groups of intervention (probiotics) and placebo. Patients in the intervention or treatment group received 1500 mg probiotic capsules twice daily, after lunch and evening meal for 6 weeks. The lactobacillus probiotics contained L. acidophilus, L. bulgaricus, L. bifidum, and L. casei. Patients in placebo group received 1500 mg capsules containing 1000 mg magnesium stearate twice daily for six weeks. Magnesium stearate is generally considered safe for human consumption at levels below 2500 mg/kg per day.[14] According to the FDA's subcommittee report on GRAS (generally recognized as safe) substances (SCOGS), adding magnesium stearate directly to human food was affirmed as GRAS.[15] After six weeks of experiment, fasting blood samples were collected and analyzed for all aforementioned parameters.

Methods of Data Gathering

Demographic data including age, sex, weight, height, body mass index (BMI), and waist to hip ratio (WHR) were measured before and after the intervention. Auto-analyzer Bio-Systems A-25 was used to determine the lipid profile and blood glucose concentration. ELISA method was employed to determine insulin levels, high sensitive CRP (hsCRP) and IL-6. Spectrophotometric method was used for determining the level of malondialdehyde (MDA).

Statistical Analysis

SPSS software, version 16 was used to test the data. Paired samples *t* test was used to compare

continuous variables within groups. Comparison between different groups was performed through two independent samples t-test. In the absence of normal distribution, comparison between groups was made using non-parametric Wilcoxon on signed ranks and Mann-Whitney tests. P values <0.05 was considered significant.

Results

The study was conducted on 34 patients, of which 26 were females and 8 males. Patients' characteristics are shown in table 1. The mean age in the placebo and treatment groups were 51.8±10.2 and 55.4±8 respectively. There were no significant differences in BMI and WHR between placebo and treatment groups (table 1).

Table 2 shows changes in biochemical markers after probiotic treatment. The fasting blood sugar did not change significantly after probiotic treatment (table2). Serum triglyceride concentration was reduced in probiotic treated group but the change was not significant (table2). There were no significant differences in total serum cholesterol, LDL-C, and HDL-C levels, between probiotic and placebo groups (table2). Fasting plasma insulin level did not change in probiotic group compared to placebo group (table2).

Although MDA and IL-6 levels were reduced in treatment group, but the changes were not statistically significant (table 2). There were an increase in CRP levels in treatment group compared to placebo, but the change was not significant (table2).

Insulin-sensitivity was determined through quantitative insulin sensitivity check index (QUICKI) and insulin-resistance by HOMA IR, FIRI, Bennett's Index and Ins/gluc ratio but there were no significant changes in these indices (table 2).

Discussion

Diabetic complication, such as cardiovascular disease on the one hand and the dramatic growth of diabetic incidence on the other, demands a

Table1: The mean anthropometric data in the placebo and treatment groups

Parameters	Placebo (mean±SD*) (n=18)	Treatment (mean±SD) (n=16)	P value
Hip (Cm)	102.16±8.58	107.25±5.81	0.055
Waist (Cm)	92.22±12.05	101.37±7.64	0.012
Weight (Kg)	68.55±7.95	74.56±9.95	0.064
Height (Cm)	158.72±7.59	163.56±9.08	0.105
WHR**	0.90±.08	0.94±.05	0.529
BMI (Kg/Cm^2)***	27.24±2.73	27.97±3.81	0.096

*Standard Deviation; **Waist to Hip Ratio; ***Body Mass Index

Table 2: The mean parameters in placebo and treatment groups

Parameters	Placebo group mean±SE before intervention	Plecebo group mean±SE after intervention	Treatment group mean±SE before intervention	Treatment group mean±SE after intervention	P value in plecebo group	P value in treatment group
FBG (mg/dl)	149.83±14.06	162.67±16.38	158.56±13.70	158.69±16.38	0.27	0.99
TG (mg/dl)	178.66±16.50	190.94±18.52	182.18±14.15	172.56±20.61	0.39	0.56
Chol (mg/dl)	172.72±11.49	184.83±13.07	185.56±10.12	191.25±10.22	0.10	0.45
LDL-C (mg/dl)	92.72±7.28	97.44±7.75	101.06±6.91	103.88±7.06	0.26	0.54
HDL-C (mg/dl)	40.89±2.82	43.44±3.39	41.38±1.97	42.94±1.91	0.14	0.28
Insulin (ng/ml)	0.25±0.02	0.25±0.02	0.41±0.16	0.35±0.11	1.000*	0.430*
MDA (µmol/L)	4.28±0.34	4.63±0.47	5.09±0.53	4.24±0.44	0.948*	0.301*
IL-6 (pg/ml)	4.38±0.80	3.09±0.43	4.51±0.45	3.83±0.35	0.038*	0.105*
hs-CRP (ng/ml)	2178.56±410.16	4060.30±1199.59	3174.87±701.77	4333.81±1256.6	0.071*	0.196*
Insulin action						
QUICKI[1]	0.1740±0.05281	0.1725±0.04821	0.0991±0.09542	0.1152±0.09006	0.96	0.77
HOMA IR[2]	1.7494±0.3042	1.8793±0.3151	2.9181±1.2697	2.2061±0.6598	0.37*	0.37*
FIRI[3]	1.5744±0.2738	1.6913±0.2836	2.6262±1.1428	1.9855±0.5938	0.37*	0.37*
Bennett's Index[4]	0.3010±0.0252	0.2946±0.0242	0.2602±0.0415	0.2716±0.0407	0.70	0.68
Ins/gluc ratio[5]	0.0019±0.0002	0.0018±0.0002	0.0030±0.0010	0.0028±0.0009	0.52*	0.37*

*Non-parametric Wilcoxon on signed ranks test; Insulin-sensitivity measure: [1]QUICKI (quantitative insulin sensitivity check index): 1/log (glucose0 (mg/dl))+log (insulin0 (mU/ml)); Insulin-resistance measures: [2]HOMA IR (homeostasis model for insulin resistance): Insulin0(mU/ml)×glucose0 (mmol/l)/22.5, [3]FIRI (fasting insulin-resistance index): Insulin0 (mU/ml)×glucose0 (mmol/l)/25, [4]Bennetts index: 1/log (glucose0 (mmol/l))×log (insulin0 (mU/ml)), [5]Insulin/glucose: Insulin0 (mU/ml)-to-glucose0 (mmol/l) ratio

natural and safe solution to control and delay these complications. A strong association has been found between the level of oxidative stress and risk of cardiovascular disease. Oxidative stress not only causes much pathopysiological complication but is also linked to insulin resistance which in turn causes diminished glucose uptake and disposal in peripheral tissues, and increasing glucose production in the liver. It has also been reported that postprandial hyperlipidemia and hyperglycemia are associated with increasing LDL-C oxidation and higher risk for cardiovascular disease.[4]

Studies showed that probiotic containing foods may reduce the concentration of serum lipid and decreases both fasting and postprandial blood sugars in human.[16,17] Mann and Spoerry reported that lactic acid bacteria are associated with a marked reduction in the total serum cholesterol.[17] Yun si et al, reported a significant reduction in fasting and postprandial glucose and decreasing HbA1c in probiotic (BNR17) treated rats.[18]

In the present study, we were not able to demonstrate any significant effect on fasting blood glucose after treating with probiotics. Serum triglyceride concentration was decreased but the change was not statistically significant. The reasons for these unexpected results can be related to either the small sample size or short duration of the study.

Gilliland et al. observed some strains of Lactobacillus acidophilus may decrease cholesterol absorption by enhancing the binding of cholesterol to the intestinal lumen.[19] Other possible cholesterol lowering properties of probiotics are deconjugation of bile by bile salt hydrolyses, binding of cholesterol to cellular surface and coprecipitation of cholesterol with deconjugated bile.[20] This study showed no significant improvement in serum total cholesterol, LDL-cholesterol and or HDL-cholesterol after treating diabetic patients with probiotics.

Yadav et al. in their study on diabetic rats reported a marked reduction in pancreatic tissue oxidative damage due to a significant decrease in lipid peroxidation.[21] In another study the same investigators showed that probiotic dahi not only decreases oxidative damage but also increases the antioxidant content and activities of catalase, glutathione peroxidase and superoxide dismutase in diabetic rats.[22]

The mechanism by which oxidative stress results in diabetic complications and tissue damage is the overproduction of the reactive oxygen species and reduction of the antioxidant defense function of the body. Lipid peroxidation is one of the main biological targets of oxidative stress, which leads to formation of secondary products such as malondialdehyde that exacerbates oxidative damage. MDA has been found to significantly increase in pathological conditions,[23] which is considered as a common oxidative stress biomarker in recent years.

The present study, showed a reduction in MDA levels in probiotic-treated group; however, the reduction was not statistically significant. Ejtahed et al.[24] showed a significant reduction in blood glucose and MDA level in type 2 diabetic patients after consuming probiotic yogurt. Songisepp et al. evaluated the functional efficacy of antioxidative properties of probiotic in healthy subjects and found a significant improvement in blood total antioxidant activity (TAA) and total antioxidant status (TAS) after receiving probiotics.[25] Harisa et al. also reported a significant decrease in MDA concentration after treating diabetic rats with L. acidophilus.[26]

Divergent evidence is available on the anti-inflammatory properties of Probiotics. While some studies reported beneficial effect,[27] others showed no effect at all. In this study, Interleukin-6 (IL-6) was reduced while CRP levels were elevated but the change was not statistically significant. Marschan et al.[28] examined the effect of probiotic bacteria on in vivo cytokine, antibody, and inflammatory responses in allergy-prone infants and showed that infants receiving probiotic had higher plasma levels of CRP, and IL-10 compared with those in the placebo group. Hatakka et al. studied the effect of Lactobacillus rhamnosus GG (LGG) on rheumatoid arthritis (RA) patients and reported an increase in serum IL-1 beta after LGG treatment with no significant change in IL-6, TNF-alpha, Myeloperoxidase (MPO), and IL-10.[29]

Conclusion

A reduction in oxidative stress and cardiovascular risk factor seems to be an ideal treatment strategy in type 2 diabetic patients. The result of this study demonstrated that a 6 weeks oral treatment with probiotics decreased the concentration of TG, MDA, and IL-6 level in type 2 diabetic patients; however the change were not statistically significant. These finding could warrant future studies to determine the therapeutic effects of probiotic on diabetic patients.

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Conflict of interest: None declared

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