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EFFECT OF PROBIOTIC FERMENTED MILK (KEFIR) ON SERUM LEVEL OF INSULIN AND HOMOCYSTEINE IN TYPE 2 DIABETES PATIENTS

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Abstract

Background. Probiotic fermented milk is one of the most beneficial foods. The main purpose of this study was to investigate the effect of probiotic fermented milk on the serum level of insulin and homocysteine in the type 2 diabetes patients.

Methods. This study was done in 60 patients with type 2 diabetes. The intervention group received 600 mL of probiotic fermented milk (kefir) daily and control group received 600 mL of conventional fermented milk daily for 8 weeks. Food intake, anthropometric indices, serum parameters were assessed at the beginning and at the end of the study. The statistical analysis was done by the use of SPSS software (Ver.13).

Results. The mean of serum insulin level did not reduce significantly after the intervention in probiotic fermented milk group, and there was no significant difference between the two groups. The mean of HOMA-IR decreased significantly in probiotic fermented milk group after intervention and there was a significant difference between the two groups after intervention. The mean of quickie increased in probiotic fermented milk group, but this increase was not significant. Also, there was not significant difference between the two groups after intervention. The mean of homocysteine level decresead significantly in patients with probiotic fermented milk and conventional fermented milk consumption.

Conclusions. By considering the effect of probiotic fermented milk on some risk factors of cardiovascular disease in diabetic patients, probiotic foods may be useful as an adjuvant therapy in diabetic patients.

Key words: Probiotic fermented milk, Insulin, Homocysteine, Type 2 diabetes.

INTRODUCTION

Type 2 diabetes is a metabolic disorder resulted from deficiency in insulin action, insulin secretion or both (1). In the world, diabetes is one of the most important healthcare and social-economic problems. It is estimated that the number of patients with this disease will be increased to 250 million by 2020 (2) and 366 million by 2030 (3). Insulin resistance has an important role in diabetes development (2). Homocysteine is a necessary and sulphur containing amino acid derived from the metabolic demethylation of dietary methionine (4, 5) and is involved in the metabolism of methionine (6). The increase of homocysteine known as risk factor for cardiovascular disease and high homocysteine may cause blood clots and finally heart attack or stroke (7). Recent studies showed that plasma homocysteine level is elevated in diabetic patients. High homocysteine level accelerates the risk of heart disease in diabetics (8). According to FAO/WHO definition, "probiotics are alive and non-pathogenic microorganisms that have beneficial effects on the host in appropriate dose". Probiotics have many properties such as reduction of serum cholesterol, immune adjuvant properties, anticarcinogenic activity, and stabilization of the gut mucosal barrier, improvement of the normal microflora, food allergies, and alleviation of intestinal bowel disease symptoms (9). Probiotics also are responsible for the production of vitamins such as folate which is involved in the homocysteine metabolism (10). Two species of probiotics, lactobacillus and bifidobacterum are common species of probiotic bacteria (11). Some of them improve insulin secretion and glucose resistance and some others cause weight loss. Studies showed that probiotics improve insulin sensitivity and systemic inflammation (12). Due to increasing prevalence of Type 2 diabetes and the useful effect of probiotics on homocysteine and insulin sensitivity, the aim of present study is to investigate the effect of probiotic

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fermented milk (kefir) on serum level of homocysteine and insulin in type 2 diabetic patients.

METHODS

Participants

The present study was a randomized double-blind placebo-controlled clinical trial which was conducted on patients with type 2 diabetes in Tabriz, Iran. Ethics committee of Tabriz University of Medical Sciences approved this clinical trial. Clinical trial number of this study was IRCT201307092017N14.

To calculate sample size, α value equal to 0.05 and a power of 80 % were considered. The sample size was selected in 23 subjects per groups. This number was finalized to 30 persons per group to adjust the anticipated dropout rate. Patients were recruited from community of diabetes patients in Tabriz. The inclusion criteria were considered as diabetic patients with fasting blood glucose ≥125 mg/dL, age 35 to 65 years, no insulin therapy, and illness duration less than 20 years. The exclusion criteria were pregnancy, conventional medical disorders such as thyroid, liver, gastrointestinal, cardiovascular, kidney and autoimmune systems disease and patients taking non-steroidal anti-inflammatory drugs, cigarette smoking, breast feeding, vitamin and mineral use, and hormone replacement therapy.

Study design

The eligible participants (30 patients per group) were randomly allocated to intervention or control groups by random block procedure produced by Random Allocation Software (RAS). Patients were matched on sex, age and duration of disease in each group. All patients refrained from using probiotic fermented milk and any probiotic food before the recruitment. Patients in the intervention group received 600 mL fermented milk (kefir) containing probiotics twice a day (lunch and dinner), and control group received 600 mL conventional fermented milk (dough) twice daily (lunch and dinner) during the eight weeks of intervention. All patients were requested not to alter their common dietary intakes, life style, other vitamin and minerals supplement consumption, medication and traditional medicine as an adjuvant therapy during the intervention. Also, the patients were asked to inform the researcher concerning the medical changes that occurred during the intervention. Patients received supply of fermented milk-kefir and conventional fermented milk weekly. The data related to anthropometric measurements, and fasting blood samples were gathered at the beginning and end of the study. Personal and demographic information was collected by questionnaire. Using a scale (Seca, Hamburg, Germany) with 0.1 kg divisions, body weight was measured without shoes and with light clothings. Using a stadiometer (Seca) with 0.1 cm accuracy, the height was measured. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters (kg/m^2). The fasting blood sampling for biochemical assessment was done from antecubital vein, from all patients after 10 to 12 hours of overnight fasting. Samples were centrifuged within 5-7 min of collection and the serum was kept at -70°C (SANYO, mdf-u33v, Japan, 2010). Serum insulin level was measured by commercially available enzyme-linked immunosorbent assay (ELISA), Monobind, USA). Homocysteine was measured by chemiluminescent immunoassay using ADVIA Centaur CP (Zimens, Germany). Insulin resistance was estimated through Homeostasis Model Assessment (HOMA) which was calculated by the fasting concentration of glucose (mg/dL) multiplied by Insulin (μU/mL) divided by 405.

Quantitative insulin sensivity check index (Quicki) was calculated by 1/[log insulin (μU/mL)+log glucose (mg/dL)].

Characteristics of intervention

According to standard Goldam factory protocol, the conventional fermented milk (dough) contained *Streptococcus thermophiles* and *Lactobacillus bulgaricus*. The probiotic fermented milk (kefir) included *Streptococcus thermophiles* and was enriched by *Lactobacillus casei*, *Lactobacillus acidophilus* and *bifidobacterium lactis* (DSM. Co, Australia). According to manufacturer's instructions, *Lactobacillus acidophilus* was cultured on clindamycin and MRS P=6.2 medium, *Lactobacillus casei* on vancomycin and MRS PH=6.2 medium and bifidobacterium lactis on strictly anaerobic medium with L- cysteine. Both types of fermented milk were manufactured weekly and were refrigerated at 4°C. Microbiological assessment for kefir was done on the first day and then was refrigerated for later analysis on the 7th, 14th and 21st day of storage. Table 1 shows the microbiological analysis of probiotic fermented milk (kefir) on the 1st, 7th, 14th and 21st day. Both types of fermented milk contained 0.3% fat and were produced in Tabriz (Goldam Dairy Co., Tabriz, Iran).

Table 1. Colony count of probiotic strains in probiotic fermented milk (29)

Probiotic strain	Colony count/mL 1	Colony count/mL 7	Colony count/mL 14	Colony count/mL 21
L.acidophilus	25×10^6	12×10^6	5×10^6	3×10^6
L.casei	15×10^6	10×10^6	4×10^6	2×10^6
B.lactis	8×10^6	6×10^6	2×10^6	0.5×10^6

Table 2. The demographic data in the probiotic fermented milk(kefir) and conventional fermented milk (dough) groups (29)

	probiotic fermented milk (kefir) (n=30)	conventional fermented milk (dough) (n=30)
Sex		
female no(%)	12(40)	14(46.66)
male no(%)	18(60)	16(53.33)
Body Mass Index mean±SD (kg/m^2)	28.89±4.77	27.47±3.55
Duration of Diseases (years) mean±SD	6.47±0.90	7.36±0.84

Table 3. Effect of probiotic fermented milk (kefir) and conventional fermented milk on serum levels of insulin ,HOMA-IR and Quicki

Variable	Period of study	Probiotic group (Mean±SD) (n=30)	Conventional group (Mean±SD) (n=30)	^{b}p
Insulin (micro IU/mL)	Baseline	16.80±9.58	13.74±5.69	0.13
	After intervention	14.41±4.46	14.48±8.65	0.05
^{a}p		0.16	0.26	
HOMA-IR	Baseline	7.05±4.83	6.30±3.03	0.17
	After intervention	4.93±1.81	7.38±4.94	<0.001
^{a}p		0.03	0.45	
Quicki	Baseline	0.29±0.22	0.29±0.18	0.20
	After intervention	0.30±0.15	0.29±0.24	0.07
^{a}p		0.82	0.10	

^{a}paired t-test. ^{b}Independent sample t-test.

Statistical analysis

The data were analyzed by SPSS Ver.13 (SPSS, Inc. USA). Kolmogorov-Smirnov test was used for assessing normality of variables distribution. Quantitative data were represented as mean ± standard deviation (SD). To compare within group changes before and after the study paired sample t-test was used. The comparison of biochemical indicators between the two groups was assessed using independent sample t-test. $p<0.05$ was considered statistically significant.

RESULTS

Baseline clinical characteristics

The study was conducted in 60 patients with type 2 diabetes mellitus: 30 persons received probiotic fermented milk and 30 persons received conventional fermented milk. Demographic data of patients consisting of sex, BMI, duration of disease are presented in Table 2.

Effect of probiotic fermented milk (kefir) and conventional fermented milk on serum levels of insulin, HOMA-IR and Quicki

Table 3 shows the serum level of insulin, HOMA-IR and Quicki indices in two groups. Serum level of insulin decreased in probiotic fermented milk group after intervention but it was not significant. There was no significant difference between the two groups in serum level of insulin after intervention. HOMA-IR was significantly decreased in probiotic fermented milk group after intervention. Also, comparison of HOMA-IR between the two groups showed a significant difference ($p<0.001$). Quicki increased non significantly in probiotic fermented milk group. There was no significant difference between the two groups after intervention in Quicki level.

Effect of probiotic fermented milk (kefir) and conventional fermented milk on serum level of homocysteine

The effect of probiotic fermented milk and conventional fermented milk on the serum level of homocysteine is shown in Table 4. According to Table 4, there was no significant difference in the serum level of homocysteine between the two groups after intervention. Serum homocysteine level decreased

Table 4. Effect of probiotic fermented milk(kefir) and conventional fermented milk on serum level of homocysteine

Variable	Period of study	Probiotic group (Mean±SD) (n=30)	Conventional group (Mean±SD) (n=30)	^{b}p
Homocysteine	Baseline	8.91±2.42	8.53±3.27	0.23
(μmol/L)	After intervention	7.35±2.18	6.91±2.10	0.88
^{a}p		<0.001	0.02	
Mean difference		1.55	1.61	

a paired t-test. b Independent sample t-test.

Table 5. Effect probiotic fermented milk and conventional fermented milk on BMI index

Variable	Period of study	Probiotic group (Mean±SD) (n=30)	Conventional group (Mean±SD) (n=30)	^{b}p
BMI (kg/m^2)	Baseline	28.74±4.75	27.30±3.64	0.21
	After intervention	28.18±4.77	27.47±3.55	0.25
^{a}p		0.29	0.97	

a paired t-test. b Independent sample t-test.

significantly after 2 months in both groups. The mean difference in serum level of homocysteine in each group is shown in Table 4.

Effect of probiotic fermented milk and conventional fermented milk on BMI index

Table 5 shows the mean of BMI in probiotic and conventional dough groups. There were not significant changes within and between groups in Body Mass Index in both groups.

DISCUSSION

This randomized controlled clinical trial was aimed to investigate the effect of probiotic fermented milk (kefir) on the serum level of insulin, homocysteine and BMI in Type 2 diabetes. According to our findings in the two groups, HOMA-IR decreased in probiotic group after intervention and the differences between the two groups were significant. Insulin serum level and Quicki changes were not significant in both groups. homocysteine serum level in both groups decreased after intervention. Changes of BMI during the study were not significant. Recent studies showed that probiotic bacteria in foods improve blood glucose level by inhibiting the production of reactive oxygen metabolites and cytokines that play a role in destruction of beta cells. (13). El Khamisy *et al.* demonstrated that insulin secretion increased in the groups that received *Bifidobacterium* and *Lactobacillus* as compared to control groups (14). One study on mice with high fructose diet showed reducing insulin resistance in mice with consumption of probiotic *L-reuteri* GMNL-263 (15). In other study conducted by Yadav *et al.* (2013), authors suggested that insulin resistance suppressed in mice with probiotic VSL#3 (16). In NAFLD study, the results showed that cardiovascular risk factors remained unchanged after patients treatment with probiotic administration (17). The effective mechanism of *Bifidobacterium adolescentis* on the insulin serum level is that probiotic bacteria enhanced production of GLP-1 that improves insulin sensitivity and modulates insulin secretion (18). Probiotics can modulate insulin resistance by inhibition of proinflammatory cytokines (19), since high blood glucose concentration, insulin resistance and dyslipidemia accompany with activation of inflammation pathways (20).

In fact, in this study in line with other studies, insulin serum level in the probiotic group decreased but this decrease was not significant. Our findings also showed that the serum level homocysteine decreased in the two groups. In a study by Naruszewiczs *et al.* (2002) consumption of probiotic containing *Lactobacillus plantarum* in smoking persons was shown reduced cardiovascular disease risk factors (21). In another study, Pompei *et al.* (2007) showed that administration of bifidobacteria was enhanced serum folate level in rats with folate deficiency. (22). Valentini *et al.* (2014) demonstrated that the serum level of homocysteine decreased in the group which used VSL#2 (23). One study in postmenopausal women with metabolic syndrome also indicated that probiotics reduced homocysteine serum level in *L-plantarum* group statistically (24). The effective mechanism is that some probiotic bacteria may contribute in folate synthesis (10). Food source of folate participate in conversion of homocysteine to methionine by giving methyl group (25). Our findings

were in agreement with the results of other studies. Comparison of BMI index between and within groups has not shown significant differences. Gobel *et al.* (2012) showed that probiotic intervention with Ls-33 was not shown any beneficial effect on parameters related to the metabolic syndrome in obese adolescents (26), but in the study conducted on obese persons, it was observed that BMI index decreased in the group with probiotic cheese consumption and hypocaloric diet (27). Fathi *et al.* showed kefir drink cause similar weight loss in comparison with milk in premenopausal obese women (28). However, our study was not consistent with Fathi *et al.* study in BMI index.

Overall, studies showed diet as a determinant factor in related to gut microbiota can affect multiple physiological functions in type 2 diabetes.

In our published data the beneficial effect of probiotic fermented milk on fasting blood glucose, HbA1c and lipid profile was reported (29). According to the present study, insulin resistance in Type 2 diabetes may be influenced by probiotics food consumption. However, in our study increased insulin sensitivity was not significant, but further studies can improve the positive effect of probiotic fermented milk in Type 2 diabetic patients.

In conclusion, in this study we showed that probiotic fermented milk consumption containing *L-acidopilus*, *L-casei*, *B-lactis* in type 2 diabetes patients decreases insulin serum level as well as HOMA-IR and increases Quicki, but only HOMA-IR decrease was statistically significant in the probiotic group and between the two groups. Serum level of homocysteine decreased in both groups. BMI did not change on probiotic consumption group after 2 months.

Conflict of interest

The authors declare that they have no conflict of interest.

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