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Study of Lactulose effects on intestinal probiotics and blood glucose status in type 2 diabetic patients

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A B S T R A C T

Oxidative stress plays a major role in pathogenicity and progress of diabetes. Among various useful foods with antioxidant effect, probiotics have been reported as effective reducers of oxidative stress. Intestinal bacteria have recently been recognized as a factor contributing to adjustment of body weight, insulin resistance, glucose metabolism, and other heart and metabolic risks. The aim of this study was to investigate the effect of Lactulose on the number of intestinal probiotics bacteria and blood sugar status in patients with type 2 diabetes. In a double-blind randomized controlled clinical trial that we performed in Endocrine Research Center of Tabriz University of Medical Sciences on patients with type 2 diabetes, we investigated the effect of Lactulose on the number of intestinal probiotics bacteria and blood sugar status in patients with type 2 diabetes. The mean ages of the intervention (Receiving Lactulose Syrup) and control group patients were 57.0±6.07 and 63.35±13.07 years, respectively (P=0.059). There was no significant difference between the patients in the two groups with respect to laboratory parameters before and after the study, and it was only mean secondary TG that was significantly higher in the intervention (Receiving Lactulose Syrup) group patients. There was no significant difference between the mean numbers of primary and secondary Bifidobacterium and Lactobacillus clooney count among the patients in the two groups. The mean secondary HbA1c and FBS was significantly lower in the intervention (Receiving Lactulose Syrup) group patients, and the mean secondary HDL was significantly higher in the intervention (Receiving Lactulose Syrup) group patients. No significant changes were observed in other laboratory parameters. No significant changes were observed in the clooney count of Lactobacillus and Bifidobacterium in the patients in the two groups. Except in FBS, no significant changes were observed in the laboratory parameters in the control group patients. The obtained results suggest that Lactulose had no significant, considerable effect in the patients under study except for decrease in Lactobacillus and increase in Bifidobacterium which was not significant.

Introduction

Probiotics are defined as living micro-organisms that can enhance the health of the host in different ways if adequate amounts of them are available in the host body.

Some of these mechanisms include the following: increasing the resistance to enteric pathogens, inhibiting the excessive growth of enteric bacteria, and regulating

the immune system. The probiotics mainly used are the lactobacillus bacteria or Bifidobacterium (1). Probiotics were initially used to improve the digestive system disorders (such as diarrhea, irritable bowel syndrome, constipation, and lactose intolerance) and to inhibit the excessive proliferation of intestinal pathogenic bacteria. However, recent studies have revealed that probiotics can have other advantages other than improving human health. One of the applications of probiotics is for improving metabolic disorders. The preparation of a probiotics should involve the use of a specific number of colonial forming units (CFUs) per dosage (2). Daily consumption of one million to one billion CFUs is reported as the minimum dosage for medical purposes (3). DM-2 is a metabolic disorder that is associated with high blood glucose, which is caused by resistance to insulin, and relative insulin deficiency (4). The most common type of diabetes is DM-2 that accounts for 90% of diabetic cases. Obesity is also the main cause of DM-2 in patients with genetic backgrounds (5, 6).

On the other hand, oxidative stress plays a major role in the pathogenesis and progress of diabetes. Among different useful foods with antioxidant effects, probiotics are known as the effective reducers of oxidative stress (7). Recently, Mahdinia et al. carried out a study to examine the effects of probiotics and yogurt on blood glucose and antioxidant status in DM-2 patients. Their findings revealed that probiotic yogurt considerably reduces blood glucose and HBA1C. In this study, the activities of "erythrocyte superoxide dismutase", "glutathione peroxidase" and "total antioxidant status" increased in the experimental group as compared to the control group but no changes were observed in the concentration of insulin and activity of "erythrocyte catalase" in the two groups. Consumption of probiotic yogurt reduces the level of FBS and increases the antioxidant status in DM-2 patients. These results indicate that probiotic yogurt is a promising important factor in controlling diabetes (8).

Lactulose is a disaccharide compound developed by Montgomery and Hudson for the first time (9). This substance is yeasted by the bacteria while passing through the intestines and is transformed into fatty acids with short chains, fatty amine acid, lactic acid, etc. (10). Lactulose may be accompanied by an increase in the colon Bifidobacterium and a decrease in the number of useful putrefactive bacteria. Therefore, production and absorption of toxic intestinal substances is inhibited by Lactulose (12).

It has been found out that the concentration of intestinal microbiota is associated with energy homeostasis, low-grade inflammation, and the subsequent disturbance of natural glucose tolerance. In animal species, changes in intestinal microbiota leads to a change in the metabolism of fatty acids in adipose tissues and hepatic tissues and ultimately results in obesity, resistance to insulin and diabetes (13). Recently, intestinal bacteria have been recognized as the factors involved in the regulation of body weight, resistance to insulin, glucose metabolism, and other cardiac and metabolic hazards (14). It was found out that a change in the composition and extent of colonization of intestinal bacteria leads to a change in glucose metabolism and hepatic function (15, 16). One of the ways of changing the composition of intestinal bacteria is the use of diets (17). Lactulose is one of the factors that can change the composition of intestinal bacteria for predominance of probiotics bacteria. Considering the positive effects of probiotics on metabolic conditions such as

diabetes, the objective of the present research project was to determine the effect of lactulose on probiotic bacteria and blood sugar status in DM-2 patients. There is no study carried out with exactly the same method in the medical data banks. Therefore, this novel theory can be considered an innovation in the field of controlling diabetes. Since the use of lactose for the treatment of intestinal conditions of diabetic patients (such as constipation) it was assumed that recommended use of Lactulose is harmless.

The aim of this research was to examine the effect of Lactulose on the number of intestinal probiotics bacteria and blood sugar status in DM-2 patients.

Materials and Methods

In a double blind randomized and controlled clinical trial that was carried out in Tabriz on patients with DM-2, the effect of Lactulose on the number of intestinal probiotic bacteria and blood sugar in DM-2 patients was investigated.

The target population included 40 diabetic patients that were randomly divided into the following two 20-groups: the control group and the experimental group. In order to determine the size of samples the ratio estimation formula was used and the study was carried out by assuming P=0.05, a difference level of 0.17 and significance level of 0.05 (based on RR calculations and a confidence interval of 95%) considering previous studies.

Members of both of the intervention (experimental) and control groups were examined for their age, gender, diabetes type and blood sugar. The results of the two groups were synchronized. The two 20-member groups were formed randomly using Rand-list. Informed consent of the patients was obtained prior to the study. In order to study the effect of Lactulose on the number of intestinal probiotic bacteria, 40 patients with DM-2 who aged between 30 and 70 years and had HbA_1c=6.8-8 were selected. The selected patients were randomly divided into the intervention and control groups through a double blink process.

Following the classification, the patients were coded in two groups and the coding was carried out by another person. The project and laboratory executives were blind to the classification of patients. Adult patients in the intervention group consumed 20-30 g/day of Lactulose in three months. Patients in the control group experienced no intervention.

The exclusion criteria for this research included the following: consumption of antibiotics; presence of acute and chronic digestive diseases leading to functional bowel disorders; an HbA_1c below 6.5% and over 8%; need for a change in the diabetes control method (such as the use of medicines or diets) during the research; consumption of medicines influencing enteric function (including anti-ulcer drugs, probiotic factors, laxatives, and hydragogue drugs); lack of a history of surgery on the digestive system and livers; lack of other systemic diseases that affect the digestive system (such as uncontrolled hypothyroidism, renal failure, liver failure, and advanced cardiac failure); and lack of consent for taking part in the research.

Contraindications for this drug include the presence of appendicitis or its signs, rectal bleeding with unknown causes, and ileus. The onset of the effects of the drug is seen in about 24-48 hours and the drug lacks systemic absorption. This drug was used

with a glass of water due to its osmotic effects and absorption of water by blood.
The number intestinal probiotic bacteria in both groups was determined by counting the number of bacteria in the excretion samples of patients and the effect of Lactulose on the levels of blood sugar in the control and experimental groups were compared.

After classifying the diabetic patients and obtaining their informed consent. The patients were classified into the intervention and control groups and their blood and excretion samples were obtained and transferred to the laboratory. At the laboratory the levels of blood glucose and HBA1C of patients were measured using a standard method. Two grams of excretion of the patients were obtained and the number intestinal probiotic bacteria were counted using the colony counter method.

In this method, the excretion samples of patients were immediately cultured in the broth media selected for probiotic bacteria. The samples were cultured in a specific medium using the standard pour plate method. The number bacteria was counted and recorded 24 to 48 hours later.

After 90 days, the blood and excretion samples of patients were obtained once again. The levels of glucose and HBA1C were measured and the number of probiotic bacteria was counted.

Ethical Considerations

The patients were assured that their participation in the study was fully voluntarily and secret and their names and addresses were going to remain confidential. Ultimate trusteeship was practiced to secure the personal information of participants throughout the study. The written consent of participants was obtained in accordance to the appendix.

Statistical Analysis

The collected data were analyzed by SPSS-17 statistical software. The collected data were expressed as percentage and mean ± SD. Continuous (quantitative) variables were compared by Independent samples and Paired t test. Categorical (qualitative) variables were compared by contingency tables and Chi-square test or Fisher's exact test. P-value ≤ 0.05 was considered statistically significant.

Results and Discussion

In this study, the effect of Lactulose on the number of intestinal probiotic bacteria and blood sugar status in DM-2 patients was examined and the following results were obtained:

The mean age of patients in the intervention and control groups was 57.0 ± 6.07 years and 63.35 ± 13.07 years, respectively (P=0.059). Five of the patients in the intervention group and 5 in the control group were male. Fifteen patients in the intervention group and 15 patients in the control group were female (p=1).

Results of laboratory experiments on patients of the two groups in the beginning and end of the research are presented in tables 1 and 2. Variations of laboratory parameters in patients of the intervention and control groups are also shown in tables 3 and 4.

No significant changes were observed in the mean levels of BS (blood sugar) two hours after eating, cholesterol levels and TG of patients in the intervention group. However, a significant increase was observed in the level of HDL. Moreover, no significant changes were observed in the number of lactobacillus and Bifidobacterium in patients

of the intervention group. Other than the decrease in FBS, no significant changes were observed in the laboratory parameters of patients in the control group.

Several experimental studies on living beings have revealed that the use of probiotic products can have very useful effects on the health and convenience of humans. The most important general mechanism of probiotics is their ability to change the potentially dangerous natural intestinal microflora to useful and healthy microorganisms (37-39).

Microorganisms in probiotics include acid lactic producers such as lactobacilli and Bifidobacterium that are highly available in yogurt and dairies along with yeasts. These microbes are not pathogenic and live in the stomach and the small intestine (40).

The ability of different microflora producing microbes in the digestive system reflects the metabolic ability of these microbes. The metabolic power of intestinal microflora is equal to that of the most active metabolic organ in the body being the liver. Hence, the digestive system not only has a role in human nutrition but also plays a vital role in human health due to its metabolic and endocrine activities (41).

Lactulose has been used since more than 40 years ago as a probiotic nutritional supplement for children to increase the number of lactobacilli in the intestines of newborns. However, the usefulness of this substrate for effective growth of these microorganisms has not been scientifically proved yet (42).

The effect of probiotics on the level of serum cholesterol is under investigation. The studies conducted in 1970 and 1980 showed a significant reduction in the level of serum cholesterol as a result of daily intake of fermented milk, but these studies are criticized for their methodologies. One of the points of criticism is that in most of the registered studies a great amount of yogurt (0.5-8.4 ml) is consumed. Recently, two controlled clinical trials showed that yogurt (200 ml/day) containing in vivo mediums of lactobacillus acidophilus or yogurt (375 ml/day) yeasted with lactobacillus acidophilus and fructo oligosaccharides (as a probiotic) reduce serum cholesterol by 2.9 and 4.4%, respectively (42).

In our study, the mean levels of primary and secondary cholesterol in patients of the intervention group were 169.0 ± 45.85 mg/dl and 157.50 ± 58.93 mg/dl, respectively. However, the changes were not significant. Moreover, the mean level of TG also reduced but the decrease was not significant. However, the level of HDL in patients of the experimental group increased significantly.
People's willingness to use probiotics is increasing because probiotics can contribute to the enhancement of human health. Probiotics are living microorganisms with positive effects on human health (43-46). Among the different probiotics, lactobacilli have drawn more attention. The most important effect of probiotics is their placement in the intestine to stimulate and clear it and therefore to inhibit the adherence of pathogens and prevent the toxic effect of toxins. Consumption of probiotics can have positive effects on the health of individuals (43, 45, 47).

Considering the positive effects of probiotics and their ability to reduce glucose levels their contribution to the treatment of diabetes is not unexpected. Optional treatments are often expensive and painful and therefore researchers are always searching for easier ways for treating diabetes.

Table.1 Evaluation of studied parameter at before of study

	Group		P
	Intervention	Control	
FBS	152.25 ± 31.43	158.30 ± 39.05	0.593
BS 2 hpp	212.40 ± 48.98	214.65 ± 46.14	0.882
HbA1C	7.82 ± .53	7.82 ± .64	0.979
TG	189.55 ± 71.15	148.15 ± 60.34	0.054
Cholesterol	169.00 ± 45.86	148.60 ± 32.62	0.113
HDL	39.70 ± 7.83	41.80 ± 8.12	0.410
Lactobacillus	$42*10^{11} \pm 18*10^{12}$	$67*10^{11} \pm 27*10^{12}$	0.646
Bifidobacterium	$41*10^{10} \pm 15*10^{11}$	$45*10^{11} \pm 13*10^{12}$	0.198

Table.2 Evaluation of studied parameter at after of study

	Group		P
	Intervention	Control	
FBS	132.85 ± 35.94	129.15 ± 26.40	0.713
BS 2 hpp	191.45 ± 46.79	207.30 ± 49.78	0.306
HbA1C	7.44 ± .55	7.62 ± .90	0.451
TG	171.75 ± 52.70	126.25 ± 47.79	0.007
Cholesterol	157.50 ± 58.93	141.30 ± 28.86	0.277
HDL	45.50 ± 8.15	44.70 ± 13.44	0.821
Lactobacillus	$56*10^{11} \pm 24*10^{12}$	$97*10^{10} \pm 30*10^{11}$	0.408
Bifidobacterium	$40*10^{10} \pm 85*10^{10}$	$49*10^{11} \pm 20*10^{12}$	0.323

Table.3 Evaluation of studied parameter at before and after of study in Intervention group

	Intervention Group		P
	Before	After	
FBS	155.28 ± 35.12	131.00 ± 31.18	0.041
BS 2 hpp	213.53 ± 46.98	199.38 ± 48.36	0.059
HbA1C	7.82 ± .58	7.53 ± .74	0.005
TG	168.85 ± 68.40	149.00 ± 54.74	0.133
Cholesterol	158.80 ± 40.62	149.40 ± 46.53	0.239
HDL	40.75 ± 7.95	45.10 ± 10.98	0.005
Lactobacillus	$55*10^{11} \pm 23*10^{12}$	$32*10^{11} \pm 17*10^{12}$	0.302
Bifidobacterium	$24*10^{11} \pm 98*10^{11}$	$26*10^{11} \pm 14*10^{12}$	0.965

Table.4 Evaluation of studied parameter at before and after of study in Control group

	Control Group		P
	Before	After	
FBS	158.30 ± 39.05	129.15 ± 26.40	<0.001
BS 2 hpp	214.65 ± 46.14	207.30 ± 49.78	0.056
HbA1C	7.82 ± .64	7.62 ± .90	0.009
TG	148.15 ± 60.34	126.25 ± 47.79	0.025
Cholesterol	148.60 ± 32.62	141.30 ± 28.86	0.119
HDL	41.80 ± 8.12	44.70 ± 13.44	0.056
Lactobacillus	$67*10^{11} \pm 27*10^{12}$	$97*10^{10} \pm 30*10^{11}$	0.466
Bifidobacterium	$45*10^{11} \pm 13*10^{12}$	$49*10^{11} \pm 20*10^{12}$	0.801

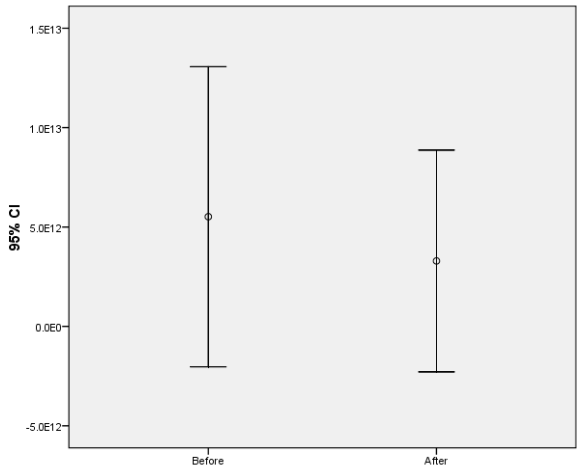

Chart.1 Distribution of primary and secondary lactobacillus cloni count in Intervention group

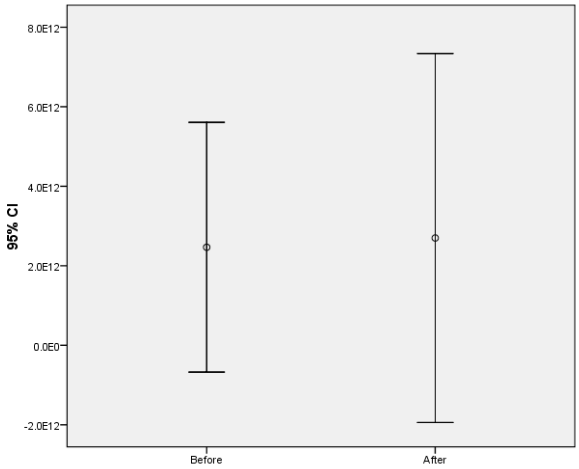

Chart.2 Distribution of primary and secondary Bifidobacterium cloni count in Intervention group

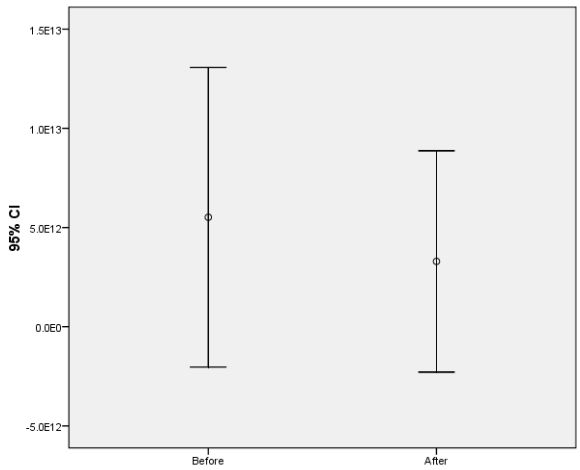

Chart.3 Distribution of primary and secondary Lactobacillus cloni count in Control group

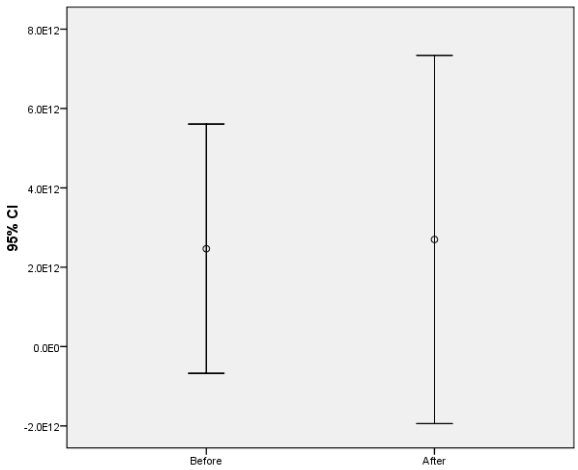

Chart.4 Distribution of primary and secondary Bifidobacterium cloni count in Control group

Recently, researchers have been intensely looking for different solutions and strategies to prevent the incidence of DM-2 or even postpone its onset. Damage to the antioxidant system is another complication caused by diabetes and therefore vitamins E and C are used to prevent or reduce the complications of diabetes.

In 2007, Laleye et al. reported that a fermentative native food in Nigeria, which is called Nono and contains lactobacilli, was considerably effective for the treatment of diabetes in rats that became diabetic through ALLOXAN (48). In another study that was carried out by Mihoko Tabuchi et al. in 2003 it was found out that GG lactobacillus that was orally fed to diabetic rats considerably reduced blood glucose through streptozotocin. Therefore, it was concluded that GG lactobacillus is capable of fighting diabetes (49).

In 2007, Hariom Yadav et al. studied the effect of a yeasty product named Dahi which contained lactobacillus casei and lactobacillus acidophilus on diabetic rats (50). According to researchers this effect is most probably caused by the increase in the number of lactobacilli in the small intestine as a result of consumption of probiotic food products that contain lactobacillus. It can increase the need of lactobacilli for glucose as the power supply for these microorganisms and their metabolism. The final consequence of this increase is the reduction in the concentration of glucose released in serum and other animal organs (49-51). However, understanding the mechanism of this effect calls for more studies to find out whether other lactobacilli have this effect or not. Further research on this finding will lead to a simpler way of preventing and treating diabetes.

No significant difference was observed between the experimental parameters of the two groups before and after the study. However, the level of secondary TG in the intervention group was significantly higher. No significant difference was also observed between the mean number of primary and secondary lactobacillus and Bifidobacterium in patients of the two groups.

The mean level of FBS and secondary HbA1c in patients of the intervention group was significantly lower whereas the mean level of secondary HDL in patients of the intervention group was significantly higher.

No significant difference was observed in other experimental parameters. In addition, no significant difference was observed in the number of lactobacilli and Bifidobacterium in patients of the two groups. Other than FBS, no significant difference was seen in the experimental parameters of patients in the control group.

Conclusion

Research results indicate that other than the decrease in the number of lactobacilli and the increase in the number of Bifidobacterium, which was not significant, Lactulose had no significant and considerable effect on the condition of patients under study.

Suggestions

Further studies with larger samples and longer courses are recommended.

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