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Effects of Probiotics on Gut Microbiota in Type 2 Diabetes Patients

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ABSTRACT

Objective: To study the effect of probiotics on gut microbiota in Type 2 diabetes patients and its clinical application value. **Methods:** Select Type 2 diabetes patients to take orally probiotics for 24 weeks, collect stool samples of subjects at the baseline and end of the trial, identify and analyze gut microbiota of each sample by 16srRNA high-throughput sequencing, and compare the changes of blood glucose, blood lipid and insulin resistance before and after the intervention. **Results:** A total of 75 patients completed clinical observations. 16srRNA high-throughput sequencing showed that the proportion of the subjects with increased Actinobacteria and Tenericutes at the end of the trial has increased (37.8% and 75.7% respectively). The genus level analysis showed that the number of subjects with increased intestinal probiotics and with decreased conditioned pathogens all increased. Cluster analysis before and after intervention showed that the gut microbiota of samples in the same group had a higher similarity. Compared with the subjects at the baseline status, at the end of the trial after the intervention, fasting blood glucose (FBG) of the subjects significantly decreased ($P<0.05$), the proportion of the subjects with triglyceride (TG) and cholesterol up to standard increased, and HOMA-IR was significantly improved ($P<0.05$). **Conclusions:** Probiotics can regulate the gut microbiota of Type 2 diabetes patients, promote fasting blood glucose (FBG) to reach the standard and improve insulin resistance, and help improve lipid metabolism.

1. Introduction

In recent years, the relationship between gut microbiota and Type 2 diabetes has attracted more and more attention from scholars both at home and abroad [1]. The changes of gut microbiota are closely related to insulin resistance, obesity, systemic inflammation and oxidative stress of the host [2]. Gut microbiota imbalance increases insulin resistance and thus affects the progress of glucose metabolism and its complications. This study is aimed to

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study the changes of gut microbiota in Type 2 diabetes patients by probiotics intervention and explore the clinical application value of this intervention method.

2. Materials and Methods

2.1 Research Objects

101 Type 2 diabetes patients who received treatment from the Department of Endocrinology and Metabolism, China-Japan Union Hospital of Jilin University from January 2017 to July 2017 were selected as subjects, meeting the requirements of 7.0%≤HbA1c≤10.0%, with the history of diabetes for one week to 26 years. All subjects were informed of the research plan and then participated in the trial, voluntarily followed the guidance of the researchers and signed informed consent. Exclusion criteria: patients with severe impairment of liver and kidney functions, heart failure, fever, infection, or acute diabetic complications, pregnant and lactating women, or patients with FBG no less than 13.3 mmol/L, or patients with severe hypertension (systolic blood pressure no less than 180mmHg and/or diastolic blood pressure no less than 110 mmHg) or patients researchers considered inappropriate for the trial. The diagnosis of Type 2 diabetes conforms to the diagnostic criteria of WHO diabetes formulated in 1999.

2.2 Research Plan

A special person is responsible to collect the information of the medical history of all subjects, and record their gender, age, course of diseases, height, weight and blood pressure. Require the subjects to have a regular diet and guide them to take the similar daily exercise, and on the basis of keeping the same hypoglycemic treatment plan unchanged, probiotics products were taken daily, including three Changle capsules taken orally three times a day and one bag of Jiangtangqi granule taken two times a day after mixing with water 1.5 hours before meals (all produced by Jilin Tiansanqi Pharmaceutical Co., Ltd.) for 24 weeks. Changle capsule is a health food mainly made from corn flour, rice flour, defatted milk powder, wheat flour, defatted soybean flour, Isomaltooligosaccharide, sugar, glucose and yeast extracts. It contains 1.2×10^8 CFU Bifidobacterium bifidum, 4.2×10^8 CFU Lactobacillus acidophilus, and 4.3×10^8 CFU Streptococcus thermophilus per 100g. The effective ingredients of Jiangtangqi granule are soybean flour, milk powder, cocoa powder, calcium powder, yam flour, konjac flour, spirulina powder, almond powder, Fructus Mume powder, licorice powder, etc. Take it before meals to increase the sense of satiety and the carbohydrates in it play a role similar to prebiotics. During the trial period, all subjects continued the original hypoglycemic, hypotensive, hypocholesterolemic treatment plan. A total of 101 subjects participated in the trial, the clinical observation of 75 subjects was completed and these subjects were safely discharged.

2.3 Testing Indicators

FBG, and blood glucose, blood lipid, liver function, kidney function, urine routine, fasting C-peptide (FCP), glycosylated hemoglobin (HbA1c) and other indicators 2 hours after meals of each subject were detected at the baseline and end of the trial. The feces of the subjects at the baseline and end of the trial were collected for 16srRNA high-throughput sequencing of gut microbiota (implemented by Shanghai Omicsspace Bioteh CO., LTD) to identify and analyze the intestinal microbial diversity of the samples. HOMA-IR = (FCP×FBG) / 22.5.

2.4 Statistical Methods

The data in normal distribution were expressed by means ± standard deviation (SD) and analyzed by paired t-test. The data in non-normal distribution were expressed by median (quartile) and analyzed by paired rank sum test. All data were analyzed by SPSS software.

3. Results

The clinical observation of 75 patients on the effect of the joint intervention on the general condition, blood glucose, blood lipid and insulin resistance of subjects was completed (with an average age of 56.5 ± 7.5 years), including 25 male and 50 female patients. The body weight, systolic and diastolic blood pressure of the subjects decreased at the end of the trial compared with those at baseline, but there was no statistical significance. The fasting blood glucose (FBG) significantly decreased, and the difference was statistically significant ($P<0.05$). The blood glucose and glycosylated hemoglobin decreased 2 hours after meals, but there was no statistical significance (Table 1). HbA1c<7.0% was defined as reaching the standard, and the HbA1c standard-reaching rate of subjects was 27.7% when the subject entered the group and 35.6% when the subject left the group. The effect of the joint intervention on blood lipid was not statistically significant (Table 1). Triglyceride (TG)<1.7mmol/L was defined as reaching the standard, the triglyceride standard-reaching rate was 38.6% when the subject entered the group and 52.5% when the subject left the group. The total cholesterol (TC)<5.2 mmol/L was defined as reaching the standard. The TC standard-reaching rate was 44.6% when the subject entered

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the group and 61.4% when the subject left the group. There was no statistical difference in fasting C-peptide (FCP) level between the subjects before and after the intervention. HOMA-IR was used to represent the insulin resistance level. HOMA-IR of the subjects decreased at the end of the trial after the intervention, the difference was statistically significant (P<0.05), and insulin resistance was improved.

Safety

During the visit, no severe hypoglycemia and cardiovascular and cerebrovascular diseases occurred in all subjects, and there was no significant change in liver function and renal function of all subjects before and after the joint intervention.

Results of gut microbiota sequencing

After the feces of all subjects were collected, the DNA sample and OUT generation were all completed by the sequencing company. A total of 70 effective samples were obtained, with "pre-" representing pre-intervention samples and "post-" representing post-intervention samples. a: The analysis of Alpha diversity is obtained by statistical software. The Chao index represents the abundance of flora, and the Shannon index represents the diversity of flora. The OUTs dilution curves of each sample are shown in Figure 1 and the Shannon dilution curves are shown in Figure 2. The curves with different colors represent different samples. With the increase of sequencing quantity, the dilution curves of each sample gradually become flat, indicating that the samples have sufficient test depth, rich species, and enough sequencing quantity to reflect the microbial information in the samples. The Runk-Abundance curve of each sample is shown in Figure 3. With the increase of the number of flora in each sample, the species abundance of each sample decreases. When the species abundance is lower than 1e-04, the curve is close to a plateau, and the genus of most samples is between 100 and 200. b: flora structure and cluster analysis. The flora structures of all samples at the phylum level are shown in Figure 3 and among them, Firmicutes, Bacteroidetes and Proteobacteria accounted for a higher proportion in each sample, and all of them are the dominant flora. Tenericutes accounted for a higher proportion in a total of 22 samples, including 8 samples (24.2%) in the pre-group and 14 samples (37.8%) in the post- group. Actinobacteria accounted for a higher proportion in a total of 32 samples, including 4 samples (12.1%) in the pre-group and 28 samples (75.7%) in the post- group. Cyanobacteria accounted for a slightly higher proportion in a total of 5 samples including two samples in the pre-group and three samples in the post- group. The above results showed that after the intervention, the proportion of flora in each sample changed and the abundance of Actinobacteria and Tenericutes increased. Cluster analysis showed that the pre-group represented the baseline status and the post- group represented the status at the end of trial, and the overall performance was that the samples in the pre-group and the samples in the post- group clustered first in the group. and the 9 samples in the post- group clustered together at the top of the tree diagram of phylum level similarity analysis shown in Figure 3, followed by 7 and 8 samples in the pre- group clustered and merged, indicating that the gut microbiota of the samples in the same group has high similarity. In the overall tree diagram, there was a crossed and clustered position between the pre- group and post- group, which is related to the complexity of gut microbiota in patients with diabetes and relatively many confounding factors. c: Genus level analysis showed that compared with the baseline status, the number of the subjects with increased intestinal probiotics and with decreased conditional pathogens all increased at the end of the trial. The subjects with increased Bifidobacterium under Actinobacteria in probiotics accounted for 62.2%. The subjects with increased L.Lactobacillus in Lactobacillus under Firmicutes increased by 21.6%. In terms of the conditional pathogens, the subjects with decreased Escherichia coli

Table 1. Effect of joint intervention on the subjects' blood glucose, blood lipid and insulin resistance

	Baseline	End of trial	t	P
FBG (mmol/L)	10.36±3.63	7.81±3.15*	6.68	<0.001
Blood glucose 2h after meals(mmol/L)	18.12±6.35	17.35±5.22	1.02	0.31
HbA1c (%)	8.14±2.47	7.82±1.46	1.21	0.23
TC (mmol/L)	5.38±1.27	5.27±1.16	0.99	0.33
TG (mmol/L)	1.86 (1.15, 2.87)	2.00 (1.30, 2.80)	-0.91	0.37
FCP (ng/ml)	1.25±0.75	1.10±0.68	1.15	0.26
HOMA-IR	0.68±0.21	0.55±0.17*	3.45	0.03

*P<0.05

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under Escherichia accounted for 29.7% and the subjects with decreased Enterococcus 43.2%. It is further indicated that probiotics can increase the proportion of beneficial bacteria in the gut microbiota of some diabetics and reduce the proportion of harmful bacteria.

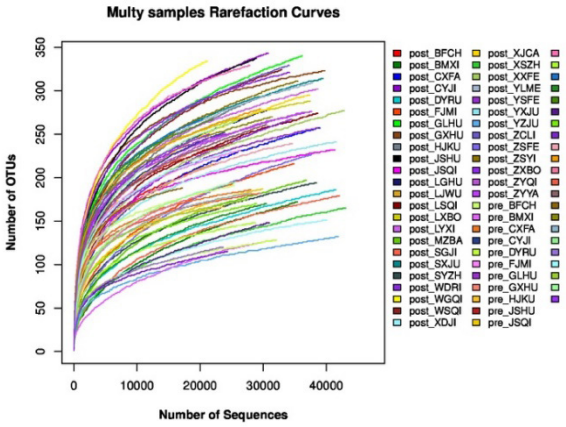

Figure 1. OUTs dilution curve of each sample

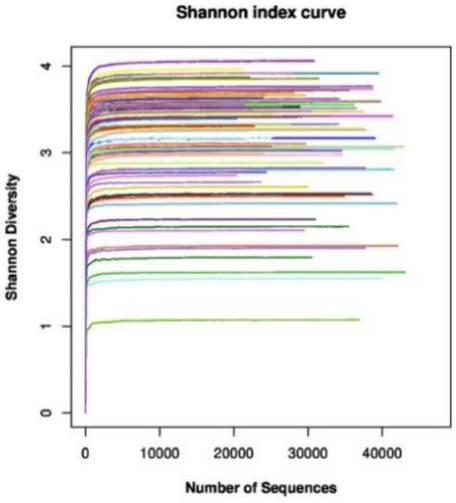

Figure 2. Shannon index curve of each sample

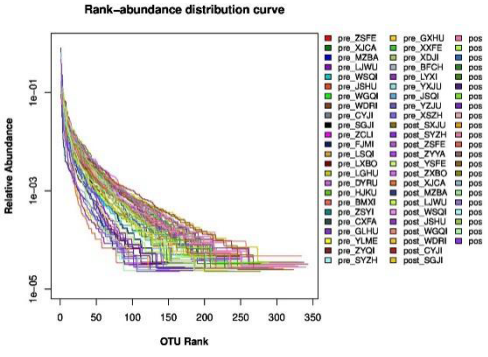

Figure 3. Runk-Abundance curve of each sample

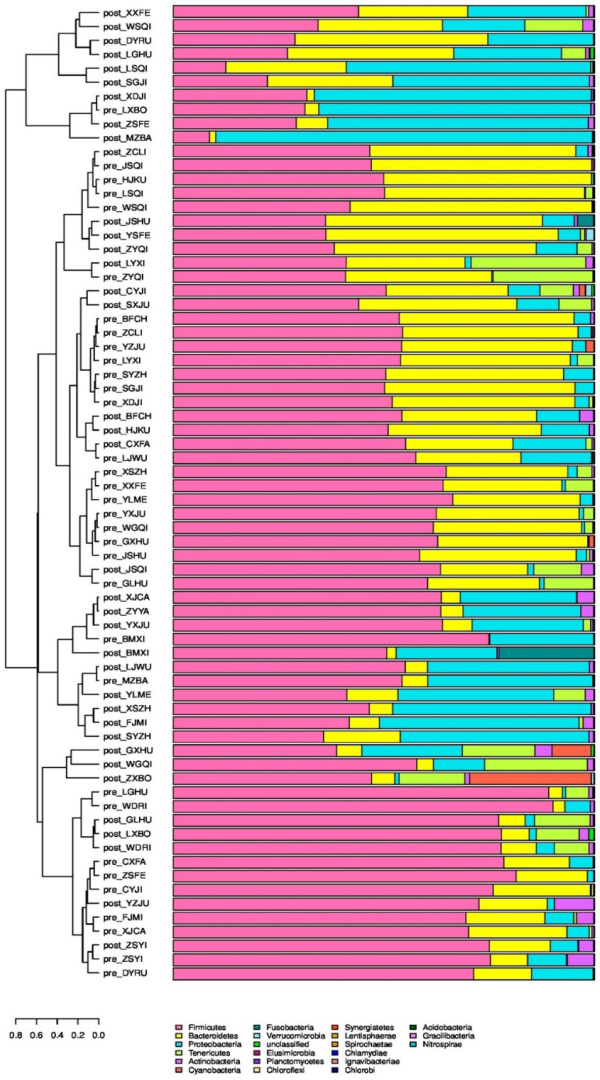

Figure 4. Tree diagram and histogram of phylum-level clustering of each sample

4. Discussions

Commensal intestinal bacteria and their metabolites are involved in metabolic abnormalities and diseases by affecting the host's metabolism and immune system. Studies have found that patients with abnormal glucose metabolism are accompanied by an imbalance of gut microbiota. On the one hand, the number of gram-negative bacteria increases causing the increases of lipopolysaccharide (LPS). After LPS enters the human body and is recognized by immune cells, it produces multiple inflammatory factors and induces the occurrence and development of systemic chronic inflammation [3,4]. On the other hand, the bacteria producing butyrate

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decreases, increasing insulin sensitivity, and weakening the barrier function to reduce inflammation and protect intestinal mucosal, and then inducing the occurrence and development of diabetes [5]. Metagenome technology refers to the technology to directly extract the total DNA from the environment to study the sum of microbial genes without the microbial culture stage. 16srRNA high-throughput sequencing technology used in this study is the main method of metagenome technology. The variable region of bacteria 16srRNA gene has the species specificity and the taxonomic characteristics of bacteria can be obtained by analyzing the sequence of the variable region. Therefore, this method is more accurate and specific than the traditional PCR method. In this study, Type 2 diabetes patients took orally probiotics for joint intervention for 24 weeks. The intestinal bacteria were classified and identified and accurately quantified by high-throughput sequencing at baseline and after intervention respectively. The results showed that all samples from all subjects had enough test depth and abundant species, reflecting the microbial information in samples. In this study, the Firmicutes, Bacteroidetes, and Proteobacteria were the dominant bacteria in the intestinal tract of the subjects with Type 2 diabetes. After 24 weeks of intervention, the number of the subjects with significantly increased Tenericutes, Actinobacteria, and Cyanobacteria in the samples increased, indicating that the flora structure of each sample changed after intervention. The study has shown that the proportion of Actinobacteria/Firmicutes and Firmicutes/Bacteroidetes in patients with Type 1 diabetes decreased, and that the beneficial bacteria producing butyrate decreased [6]. The sequencing results of this study were similar to the above. After the intervention, the proportion of Tenericutes, Actinobacteria and Cyanobacteria in some samples increased significantly, and the Lactobacillus under Firmicutes increased. Cluster analysis showed that most samples in the post- group and the pre- group could cluster in the group, indicating that the probiotics in this study changed the proportion of all types of bacteria in the gut microbiota of Type 2 diabetes patients and there were some similarities in the changes of flora.

Comparing the glucose and lipid metabolism indexes of subjects at the baseline and end of the trial, it was found that after the intervention, fasting blood glucose (FBG) was significantly improved, blood lipid standard-reaching rate increased, HOMR-IR index decreased, and insulin resistance was improved. At the end of the trial, the number of subjects with increased intestinal probiotics and with decreased conditional pathogens all increased. The subjects with increased Bifidobacterium under Actinobacteria in probiotics increased by 62.2%, and the subjects with increased L.Lactobacillus that belongs to Lactobacillus under Firmicutes increased by 21.6%. It is considered that these changes are related to probiotics intervention combined with this research plan. Changle capsule contains Bifidobacterium bifidum, Lactobacillus acidophilus, and Streptococcus thermophilus, without hypoglycemic components. The effect of Jiangtangqi granule is to increase the sense of satiety and reduce the intake of other foods by taking it before meals, so the diet variation of each patient can be well controlled. The carbohydrate in the granule plays a role similar to prebiotics, conducive to the absorption of probiotics in the Changle capsule and reproduction in the intestinal tract, but there is no composition of directly reducing blood glucose in the capsule. In this study, joint intervention by increasing probiotic intake is used to effectively supplement the probiotics/prebiotics and regulate the metabolism of fatty acids to regulate the gut microbiota of Type 2 diabetes patients, thus improving fasting blood glucose at the end of the trial. Animal studies showed that the rats fed a high-fat diet were fed with probiotics in advance, which has certain preventive effects on the formation and development of Type 2 diabetes in the rats. Moreover, the hypoglycemic effect of probiotics is related to the improvement of the structure of gut microbiota, inhibition of the reproduction of harmful bacteria, reduction of the expression of inflammatory factors TNF-a and IL-6 [7]. Amar, et al. found that Bifidobacterium and Lactobacillus could change the early bacterial translocation in the rats with diabetes induced by a high-fat diet, thereby reducing the expression of some cytokines such as TNF-α, IL-1b, PAI-1 and IL-6, and improving insulin sensitivity and glucose metabolism [8]. Therefore, now many scholars believe that probiotics, as a kind of active microorganisms regulating the intestinal microecological balance of the host, can improve energy metabolism in the body, reduce chronic inflammation and oxidative stress by changing gut microbiota and its metabolites. The application of probiotics has gradually become a research focus in preventing and controlling the occurrence and development of Type 2 diabetes [9].

In this study, after increasing the probiotics intake, no obvious abnormality found in the liver and kidney functions of patients, and the subjects had no obvious discomfort, so this method has better safety. Although the results showed that the gut microbiota changed after the intervention, and the glucose metabolism, insulin resistance and lipid metabolism were improved, the correlation analysis and attribution analysis could not be well made because of the less strict grouping and more

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confounding factors before intervention. Therefore, we should strictly group and increase the sample size in future study. Meanwhile, inflammatory indexes and oxidative stress indexes should be measured and determined for correlation analysis and attribution analysis. In conclusion, the intake of intestinal probiotics can regulate the gut microbiota of Type 2 diabetes patients, promote fasting blood glucose to reach the standard, and improve insulin resistance to help lipid metabolism reach the standard.

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