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Effect of Basic Periodontal Therapy Combined with Probiotics on Oral Microecology and Blood Sugar Control in Patients with Diabetes and Periodontitis

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BH and JL conceived the study. BH carried out the experiments and the data analysis. JCQ conducted experiments and modified the language of the article. TLH contributed to sample preparation. All authors discussed the results and contributed to the final manuscript.

Key words
Diabetes mellitus complicated by periodontitis, Probiotics, Intestinal flora, Oral flora

ABSTRACT

To explore the effect of basic periodontal therapy combined with probiotics on oral and intestinal flora, inflammatory factors and blood sugar control in patients with diabetes and periodontitis. A total of 72 diabetic patients with periodontitis who were treated in our hospital were taken as the expirmental objects, as the control group (n=36) and the study group (n=36). Patients in the control group received normal diabetes treatment and basic periodontal treatment, and the study group received probiotic treatment on the basis of the control group. The differences in oral and intestinal flora between the two groups of study subjects were compared, and changes in inflammatory factors, oxidative stress molecules, and blood glucose control related indicators were detected in each patient. There was no statistical difference in gender distribution, age distribution, BMI and HbA1c between the control group and the study group before treatment (P>0.05). After treatment, the PI (P=0.000), GI (P=0.000), PPD (P=0.031), and CAL (P=0.022) of the study group with diabetes and periodontitis were significantly different from the control group. The basic periodontal treatment combined with probiotics has a significant effect on the oral and intestinal flora, inflammatory factors and blood sugar control of patients with diabetes and periodontitis.

INTRODUCTION

Diabetes mellitus is a kind of endocrine system disease characterized by disorder of blood glucose metabolism, with manifestation of persistent hyperglycemia (Wintermeyer *et al*., 2019). It is the third major disease that severely threatens the lives and health of human beings, following cardiovascular disease and tumor. There are over 400 million people suffering from this disease, 90% of whom are patients with type 2 diabetes mellitus. The severity of diabetes mellitus is associated with the damage to the targeted organ caused by persistent hyperglycemia of the body, which mainly results in serious complications such as microvascular disease, renal lesions and foot lesions, thus threatening the lives of patients (Engel-Yeger *et al*., 2018). Periodontitis, the sixth major complication of diabetes mellitus, is closely related to glucose metabolism disorder of the body, and it is a kind of infectious disease caused by microbial disorders in the dental plaque. Failure of glucose control in diabetic patients is one of the important factors facilitating the development of periodontitis (Babaev *et al*., 2017). The incident rate of periodontitis in diabetic patients is triple of that of non-diabetics. Therefore, diabetes mellitus is one of the vital risk factors for periodontitis (Acharya *et al*., 2017). Changes of microorganisms in human bodies have great impact on the development of diseases. Probiotic treatment can modulate the level of microorganisms in the body, including those in the intestinal tract and oral cavity, and may have positive effects on the treatment of diabetes mellitus complicated by periodontitis (Tunapong *et al*., 2018). Hence, in this study, changes in the oral and intestinal flora, combined with changes in the inflammatory

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factors, oxidative stress molecules and indicators related to blood glucose control were compared between patients with diabetes mellitus complicated by periodontitis who received probiotic treatment and those who did not, so as to clarify the process of probiotic treatment for patients with diabetes mellitus complicated by periodontitis.

MATERIALS AND METHODS

Subjects

A total of 72 patients with diabetes mellitus complicated by periodontitis admitted to our hospital were enrolled as expirmental objects and divided into control group (n=36) and experimental group (n=36). General and clinical data including name, gender, hospitalization ID, height, weight, medical history and family history of diabetes mellitus were collected from patients in both groups. Patients in both groups underwent basic therapy of diabetes mellitus as well as initial periodontal therapy, which included periodontal scaling, subgingival curettage and oral administration of Nidazole drugs. Based on these therapies, patients in expermintal group received treatment with probiotics live combined bifidobacterium, lactobacillus and enterococcus capsules, oral, 2 pieces/time, 3 times/day. There were no statistically significant differences in the general data such as age and gender between the two groups of patients (p>0.05).

Comprehensive periodontal examination

Comprehensive periodontal examination was performed by a physician with intermediate professional title or above in the Department of Stomatology. The plaque index (PI), gingivitis index (GI), periodontal pocket depth (PPD) and clinical attachment loss (CAL) were examined.

Detection of oral and intestinal flora

At 3-4 h after meal, each patient rinsed the mouth with clear water for 2-3 min to remove residues. After drying up slightly, the oral mucosa was scraped with a sterile cotton swab to collect the oral flora, which were then stored in flora preservation solution. Fresh mid-posterior-segment fecal samples (5 g) were collected from each expirmental object and stored in a liquid nitrogen tank within 1 h. Next, the total bacterial DNA was extracted from the oral and intestinal flora samples. Then the bacterial 16S rRNA was amplified *via* polymerase chain reaction, purified and sequenced before analysis.

Detection of inflammatory factors and oxidative stress molecules

The levels of oxidative stress molecules superoxide dismutase (SOD), total antioxidant capacity (T-AOC) and malondialdehyde (MDA), and inflammatory indicators transforming growth factor-alpha (TGF-α), interleukin-1 beta (IL-1β), IL-6 and IL-4 were determined *via* enzyme-linked immunosorbent assay. During detection, 3 replicates were set for each expirmental object. After detection, the corresponding optical density values were read with a microplate reader, and converted into the actual concentration of indicators through the standard curves.

Detection of indicators related to blood glucose control

The levels of indicators related to blood glucose control venous blood glucose and glycosylated hemoglobin (HbA1c) were detected and analyzed in the Biochemistry Room of the Department of Clinical Laboratory of our hospital.

Statistical analysis

SPSS 22.0 was utilized for statistical analysis. Unpaired t test was adopted for intergroup comparison, and linear discriminant analysis Effect Size (LEfSe) was used for microbial diversity analysis. p<0.05 was considered to be statistically significant.

RESULTS

There were no statistically significant differences in the general data including gender and age distribution, body mass index (BMI) and HbA1c between the groups of patients before treatment (p>0.05) (Table I).

Table I. Comparison of general data between the two groups.

	Control group (n=36)	Experimental group(n=36)	p
Gender (n)			>0.05
Male	20	19	
Female	16	17	
Age (Y)	51.22±4.24	52.45±5.94	>0.05
BMI (kg/m^2)	26.83±5.38	27.21±4.82	>0.05
HbA1c (%)	7.8±1.1	8.1±0.9	>0.05

Table II shows effect of comprehensive periodontal examination in both groups. There were evident differences in the PI (p=0.000), GI (p=0.000), PPD (p=0.031), CAL (p=0.022) between the two groups of patients. The levels of inflammatory factors TGF-α (p=0.047), IL-1β (p=0.031) and IL-6 (p=0.026) in patients were significantly lower in experimental group than those in control group, while the level of IL-4 (p=0.004) was notably higher in experimental group than that in control group. The levels of SOD (p=0.000) and T-AOC (p=0.028) in patients were markedly higher in experimental group than those in control group. The levels of blood glucose (p=0.046) and HbA1c (p=0.045) in patients were obviously lower in

experimental group than those in control group, indicating that the blood glucose was evidently better controlled in experimental group. After treatment, the abundance of *Streptococcus oralis* (p=0.048) was notably lower in experimental group than that in control group, while the abundance of oral lactobacilli (p=0.000) and bacteroidetes (p=0.000) was markedly higher in experimental group than that in control group. The experimental group had significantly higher abundance of peptostreptococcaceae, tanticharoenia, prevotellaceae and corynebacteriaceae, while the control group had higher abundance of flavobacteriaceae, sphingobacteriales and asaccharobacter (Figs. 1, 2).

Table II. Effect of basic periodontal therapy combined with probiotics on periodontal parameters, inflammatory factors, oxidative stress molecules, blood glucose level and dominant oral flora in patients with diabetes and periodontitis.

Group	Control group (n=36)	Experimental group (n=36)	t	p
Periodontal parameters				
PI	2.01±0.16	1.32±0.21	11.32	0.000
GI	2.07±0.21	1.33±0.24	11.56	0.000
PPD	6.68±1.01	5.41±0.31	8.35	0.031
CAL	5.84±0.46	4.88±0.22	9.45	0.022
Inflammatory factors (ng/L)				
IL-1β	16.54±1.84	14.21±1.45	7.02	0.047
TGF-α	26.53±3.84	17.34±1.94	8.23	0.031
IL-6	29.47±3.62	21.24±2.74	8.98	0.026
IL-4	22.82±3.15	48.21±5.42	11.23	0.004
Oxidative stress molecules				
SOD (U/mL)	24.32±3.21	67.42±5.34	24.21	0.000
T-AOC (U/mL)	6.32±0.89	9.76±1.02	8.64	0.028
MDA (nmol/mL)	5.71±0.84	5.43±0.43	4.35	0.083
Blood glucose level				
Blood glucose (mmol/L)	7.32±0.87	7.02±0.64	7.36	0.046
HbA1c (%)	7.1±0.5	6.7±0.4	7.43	0.045
Oral flora				
Streptococcus oralis	7.54±0.85	5.22±0.92	7.12	0.048
Oral Lactobacilli	4.03±0.42	9.84±0.54	15.11	0.000
Flavobacteriaceae	3.28±0.24	4.83±0.82	5.32	0.068
Actinobacteria	5.83±0.83	5.79±0.74	1.83	0.773
Bacteroidetes	3.72±0.36	11.25±1.24	17.37	0.000

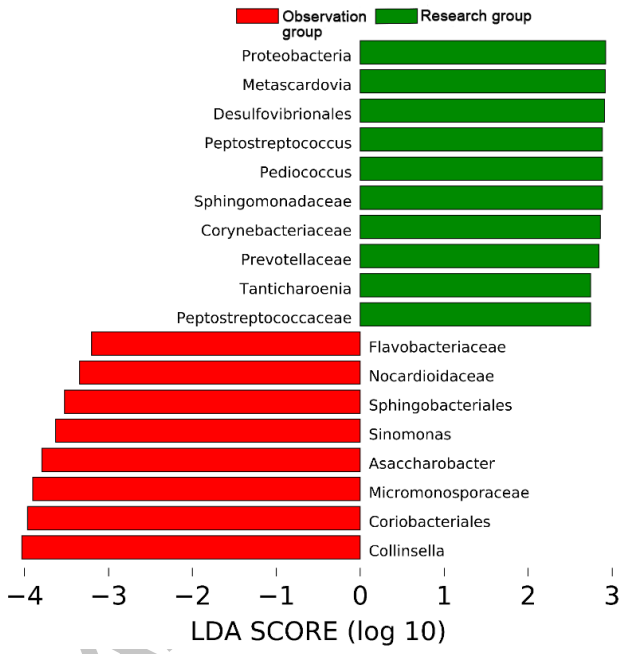

Fig. 1. LDA score of intestinal flora in both groups after treatment

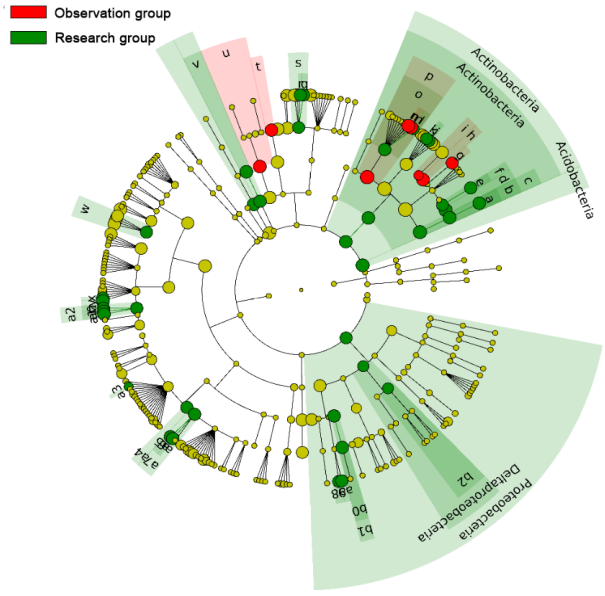

Fig. 2. LEfSe of intestinal flora in both groups after treatment.

DISCUSSION

Diabetes mellitus is a common type of endocrine system disease, which is triggered by persistent

hyperglycemia due to an absolute or relative deficiency of insulin secretion or decreased insulin sensitivity induced by multiple causes (Lu *et al*., 2018). Its morbidity rate is obviously increasing year by year, which is associated with the improvement of people's living standards, ageing of population and development of diagnostic technologies. Besides persistent disorder of blood glucose in the body, diabetes mellitus will also lead to complications involving multiple organs and systems in the patients, such as complications of the vascular system (He *et al*., 2018), urinary system (Strand-Holm *et al*., 2019), locomotor system (Hu *et al*., 2019) and eyes (Yoo and Oh, 2019). As one of the complications of diabetes mellitus, periodontitis severely harms the oral health of patients. It has been confirmed by expirmental that there is an evident bidirectional relationship between diabetes mellitus and periodontitis (Lee *et al*., 2019). Specifically, they facilitate each other, and the hyperglycemia of diabetic patients can obviously increase the risk of periodontitis. In terms of mechanism, diabetes mellitus may facilitate the occurrence of periodontitis through: (1) microvascular diseases or blood flow changes of periodontal tissues, (2) aggregation of advanced glycation end products in periodontal tissues, (3) local immune cell dysfunction (Bakshi *et al*., 2018), (4) disturbance of inflammatory factors, and (5) changes in the composition and abundance of microorganisms. These pathophysiological changes in diabetic patients may be the vital factors promoting the occurrence of periodontitis (Ziukaite *et al*., 2018). Developing and exploring new methods for treating diabetes mellitus complicated by periodontitis in the disordered biological processes of diabetic patients is of great significance for reducing the complications of this disease and improving the quality of life of patients (Hu *et al*., 2017).

Persistent hyperglycemia of diabetic patients will lead to disturbance of microbial abundance, and it is also an important factor promoting the occurrence of complications (Otero *et al*., 2019). Therefore, the targeted use of probiotic preparations to modulate the microorganisms of patients may be of critical significance for the treatment of complications. Probiotics are microorganisms colonizing human bodies, which are beneficial to the body and play important roles in immune activation, inflammation responses, the modulation of the homeostasis of local intestinal microenvironment and the maintenance of normal metabolic levels (Yousefi *et al*., 2019). It has been revealed that the deficiency of probiotics will lead to the malabsorption of many kinds of vitamins and trace elements, thus affecting the normal development of the body. Moreover, Lactobacilli and Bifidobacteria are very important for the maintenance of normal immune status of the body (Wang *et al*., 2019). Exogenous supplement of probiotics can correct the disturbance of microorganisms in patients with diabetes mellitus complicated by periodontitis, thereby inhibiting the progression of periodontitis and improving the symptoms of diabetes mellitus at the same time (Albuquerque-Souza *et al*., 2019). In this study, 72 patients with diabetes mellitus complicated by periodontitis were enrolled as expirmental objects. After comparing the changes in the oral and intestinal flora between patients who received probiotic treatment and those who did not, it was found that compared with that in control group, the abundance of *Streptococcus oralis* (p=0.048) markedly decreased and the abundance of oral Lactobacilli (p=0.000) and Bacteroidetes (p=0.000) obviously increased in experimental group. After treatment, the experimental group had significantly higher abundance of peptostreptococcaceae, tanticharoenia, prevotellaceae and corynebacteriaceae, while the control group had higher abundance of flavobacteriaceae, sphingobacteriales and asaccharobacter. These results suggest that probiotic treatment have relatively greater impact on the composition and abundance of oral and intestinal flora in patients with diabetes mellitus complicated by periodontitis.

Meanwhile, in this study, it was found that there were noticeable differences in the PI (p=0.000), GI (p=0.000), PPD (p=0.031) and CAL (p=0.022) in patients between the two groups after treatment. Besides, after treatment, the levels of TGF-α (p=0.047), IL-1β (p=0.031) and IL-6 (p=0.026) of patients were obviously lower in experimental group than those in control group, while the level of IL-4 (p=0.004) was notably higher in experimental group than that in control group. After treatment, the levels of SOD (p=0.000) and T-AOC (p=0.028) were markedly higher in experimental group than those in control group after treatment. Moreover, the levels of blood glucose (p=0.046) and HbA1c (p=0.045) of patients were evidently lower in experimental group than those in control group after treatment, implying that the blood glucose was obviously better controlled in experimental group. The above results indicate that probiotic treatment combined with initial periodontal therapy and basic therapy of diabetes mellitus obviously improves the control of inflammation level and blood glucose, and the symptoms of periodontitis in patients with diabetes mellitus complicated by periodontitis. Probiotic treatment can be taken as an effective adjuvant treatment method for patients with diabetes mellitus complicated by periodontitis.

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Not applicable.

Ethics statement

The experiment was often approved by the Ethics Committee of the the 305th Hospital of the Chinese People's Liberation Army Beijing, and all patients participating in this study provided written informed consent in accordance with the "Helsinki Declaration".

Statement of conflict of interest

The authors have declared no conflict of interest.

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