ORIGINAL RESEARCH

# Changes in Oral Subgingival Microbial Distribution and Community Structure in CP-T2DM Patients Before and After Combined Periodontal-Endodontic Treatment

Yongxi Ding, MD; Youliang Liu, MD


## ABSTRACT

**Objective** • This study aims to investigate the oral subgingival microbial community in patients with chronic periodontitis with type 2 diabetes mellitus (CP-T2DM) before and after combined periodontal-endodontic treatment.

**Methods** • A retrospective selection of 88 patients with CP-T2DM (CP-T2DM group) treated at our hospital from May 2021 to June 2022 was conducted. Additionally, 90 patients with CP were selected as the control group (CP group). The study compared the distribution of oral subgingival microbial communities between the two groups and analyzed differences in the distribution of oral subgingival microbial communities in patients with different clinical characteristics within the CP-T2DM group, both before and after treatment.

**Results** • The CP-T2DM group showed lower relative abundances of *Cilia* and *Streptococcus* while higher relative abundances of *Tannerella* and *Citrobacter* ($P < .05$) compared to the CP group. Furthermore, the relative abundance of *Cilia* was found to be negatively correlated with fasting blood glucose (FBG) and HbA1c, whereas the relative abundance of *Citrobacter* was positively correlated with FBG and HbA1c ($P < .05$).

**Conclusions** • Significant differences were observed in the oral subgingival microbial communities distribution between CP-T2DM and CP patients. The relative abundance of ciliate and citrate bacteria was found to be associated with the blood glucose level of patients. (*Altern Ther Health Med.* 2023;29(8):166-171).


**Yongxi Ding, MD,** School of Clinical Medicine, Hunan University of Chinese Medicine, Yiyang, Hunan, China. **Youliang Liu, MD,** Department of stomatology, Yiyang Stomatology Hospital, Yiyang, Hunan, China.

*Corresponding author: Youliang Liu, MD*
*E-mail: yongxiding@hotmail.com*

## INTRODUCTION

Type 2 diabetes (T2DM) is a metabolic disease primarily characterized by two related problems: insulin resistance in muscle, fat, and liver cells, and inadequate insulin production by the pancreas, leading to poor blood sugar control,[1-3] On the other hand, chronic periodontitis is a persistent inflammatory condition resulting from Gram-negative bacterial infection, commonly affecting the periodontal supporting tissues.[4-6]

Relevant studies have demonstrated that T2DM and chronic periodontitis (CP) can interact and exacerbate each other, possibly through shared inflammatory mechanisms. Diabetes is considered a risk factor for chronic periodontitis, as it not only contributes to tooth loss in diabetic patients but also disrupts blood sugar regulation, hastening disease progression.[7-10] The primary cause of chronic periodontitis is the dynamic interaction between plaque microbes and the host's immune and inflammatory responses.[11] Identifying the pathogenic bacteria present in patients with chronic periodontitis holds dual benefits; it aids in the treatment of the periodontal condition and has the potential to help regulate patients' blood glucose levels, thereby enhancing their overall well-being.[12]

The objective of this study was to explore the oral subgingival microbial community in patients with chronic periodontitis and type 2 diabetes mellitus (CP-T2DM) before and after combined periodontal-endodontic treatment. Additionally, the study aimed to assess its clinical significance, providing a robust foundation for the clinical management of CP-T2DM.

## METHODS

### Study Design

A total of 88 patients with CP-T2DM (CP-T2DM group) who received treatment at our hospital from May 2021 to June 2022 were retrospectively selected. During the same period, 90 patients with CP but without T2DM, treated in our hospital, were chosen as the control group (CP group).

### Inclusion and Exclusion Criteria

Inclusion criteria were as follows: (1) Patients diagnosed with CP based on the classification of the American Academy

of Periodontology[13]; (3) Patients diagnosed with T2DM according to the 1999 WHO diagnostic criteria for diabetes[14]; (4) Age of participants was 18 years or older; (5) No history of antibiotic use within the last 3 months; (6) Obtained informed consent from patients and their families.

Exclusion criteria were as follows: (1) Patients with invasive periodontitis, periodontal abscess, active caries, or other oral diseases; (2) Patients with complications such as malignant tumors, liver and kidney dysfunction, endocrine diseases, or other serious medical conditions; and (3) Pregnant or lactating women.

### Clinical Procedures

**Plaque Collection.** Samples were collected from six sites around the patient's implant on the lips and cheeks, near the lingual side, and at the distal axis angle. Patients gargled, and the sampling sites were kept moist. A sterile scraper was used to scrape gingival plaque, and the teeth were dried using an air gun. The tip of a sterile paper was gently inserted into the bottom of the gingival sulcus and left in place for 10 seconds. The sterile paper was carefully extracted and transferred into a 200 μL buffer solution. Subsequently, the samples were stored at -20°C for further analysis.

**PCR Detection.** The PCR primers were designed explicitly with 16S rRNA as the template. The primer sequences were 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3').

**Bacterial DNA Extraction.** Samples were retrieved from the refrigerator, thawed for 30 minutes, shaken, and thoroughly mixed. Subsequently, plaque DNA was extracted using the phenol-chloroform method.

**PCR Amplification Product Detection.** For gel electrophoresis, 3μl of amplification products were mixed with 2 g/dL agarose (0.5 mgEB/L). The voltage was set to 100V, and the standard controls used were pUC19 DNA/MspI and DNA Marker DL 2000. The amplification bands were observed under an ultraviolet detector to determine the relative abundance of *Ciliates*, *Streptococcus*, *Tannerella*, and *Citrobacter*.

**Blood Glucose Measurement.** Fasting blood glucose levels were measured in both patient groups.

**Determination of Glycated Hemoglobin.** Venous blood was collected from the patient's upper arm on an empty stomach. The collected blood was centrifuged at a speed of 4000 r/min for 10 minutes. After centrifugation, the supernatant was separated and measured using a glycated hemoglobin meter.

**Classification of Periodontitis Disease.** Periodontitis is classified into three categories: mild, moderate, and severe. (1) Mild periodontitis is characterized by an attachment loss of 1-2 mm, periodontal pocket depth less than 4 mm, and alveolar bone resorption not exceeding 1/3 of the root length. (2) Moderate periodontitis is marked by an attachment loss of 3-4 mm, periodontal pocket depth less than 6 mm, and alveolar bone resorption more than 1/3 of the root length but not exceeding 1/2 of the root length. (3) Severe periodontitis exhibits an attachment loss greater than 5 mm, periodontal pocket depth greater than 6 mm, alveolar bone resorption exceeding 1/2 of the root length, and, in some cases, extending up to 2/3 of the apex, resulting in noticeable tooth loosening.

### Treatment Method

Initially, both patient groups thoroughly cleaned the gums to ensure the absence of visible plaque, dental calculus, and pigmentation, which could interfere with subsequent experiments. After a one-week interval, subgingival scaling and root planning treatment were initiated. This procedure began with the administration of local anesthesia to the gingiva, followed by subgingival scaling using an ultrasonic handle and subsequent smoothing of the gingival root surface with a scraper. The periodontal pocket was then rinsed with 3% hydrogen peroxide and disinfected using 1% iodine glycerin until achieving a smooth and flat root surface with no residual dental calculus beneath the gum line.

### Statistical Analysis

Data analysis was conducted using SPSS 22.0 software (IBM, Armonk, NY, USA). The measurement data, such as age and body mass index, were expressed as mean ± standard deviation ($\bar{x} \pm s$). The *t* test or *F* test was utilized to analyze differences between the groups. For count data, including gender and periodontitis index, the data were presented as n (%). Differences between groups were analyzed using the $\chi^2$ test, and Pearson correlation analysis was employed to explore correlations between variables.

## RESULTS

### Clinical Characteristics

The clinical general data of the CP-T2DM group and CP group is presented in Table 1, and both groups were found to be comparable.

**Table 1.** Comparison of clinical general data between two groups

| Groups | No of cases | Gender | | Age (years old) | Body Mass Index (kg/m²) | Periodontitis graduation | | |
|---|---|---|---|---|---|---|---|---|
| | | Male | Female | | | Slight grade | Moderate grade | Heavy grade |
| CP-T2DM group | 88 | 40 (45.45) | 48 (54.55) | 54.49 ± 9.87 | 22.14 ± 2.01 | 21 (23.86) | 38 (43.18) | 29 (32.95) |
| CP Group | 90 | 39 (43.33) | 51 (56.67) | 52.78 ± 9.10 | 22.18 ± 1.94 | 23 (25.56) | 40 (44.44) | 27 (30.00) |
| $t/\chi^2$ | | 0.081 | | 1.202 | -0.135 | 0.191 | | |
| *P* value | | .776 | | .231 | .893 | .909 | | |

Note: CP-T2DM group: Group of patients with chronic periodontitis and type 2 diabetes mellitus. CP Group: Group of patients with chronic periodontitis. Body Mass Index (BMI) (kg/m²): Mean BMI of participants in kilograms per square meter.

### Comparison of Relative Abundance of Main Subgingival Microbial Genera

The relative abundance of ciliated bacteria and *Streptococcus* in the CP-T2DM group was significantly lower than in the CP group ($P < .05$). Conversely, the relative abundance of *Tannerella Forsythia* and *Citrobacter* in the CP-T2DM group was considerably higher than in the CP group ($P < .05$). However, the relative abundance of *Prevotella* and *Fusobacterium* between the CP-T2DM and CP groups showed no statistically significant difference ($P > .05$). Refer to Table 2 for details.

**Table 2.** Comparison of relative abundance of main subgingival microbial genera between CP-T2DM group and CP group (%)

| Category | CP-T2DM group (n = 88) | CP group (n = 90) | *t* | *P* value |
|---|---|---|---|---|
| *Prevotella* | 18.82 ± 3.32 | 18.01 ± 3.50 | 1.583 | .115 |
| *Fusobacterium* | 14.11 ± 2.81 | 13.92 ± 3.00 | 0.436 | .663 |
| *Porphyromonas* | 12.28 ± 3.01 | 12.05 ± 2.83 | 0.525 | .600 |
| *Treponema* | 7.82 ± 1.18 | 7.74 ± 1.20 | 0.448 | .654 |
| *Selenomonas* | 2.93 ± 0.88 | 2.90 ± 0.81 | 0.237 | .813 |
| *Actinobacillus* | 4.01 ± 1.00 | 3.84 ± 0.81 | 1.248 | .214 |
| *Leptotrichia* | 1.20 ± 0.55 | 2.89 ± 0.65 | -18.706 | .000 |
| *Tannerella forsythia* | 2.80 ± 0.88 | 2.01 ± 0.93 | 5.819 | .000 |
| *Streptococcus* | 1.43 ± 0.72 | 2.83 ± 0.82 | -12.094 | .000 |
| *Citrobacter* | 2.81 ± 0.81 | 2.14 ± 0.79 | 5.587 | .000 |

Note: Values are expressed as mean ± standard deviation (%); "n" denotes the number of participants in each group; "*t*" represents the *t* statistic; and "*P*" indicates the corresponding *P* value for the statistical comparison between the two groups.

### Comparison of Subgingival Microbial Genera in CP-T2DM Patients with Different Characteristics

The relative abundance of main subgingival microbial genera in CP-T2DM patients with different sex, ages, body mass index, and periodontitis was compared, and no statistically significant differences were found ($P > .05$). However, in CP-T2DM patients with fasting blood glucose levels ≥7.0 mmol/L, the relative abundance of *Citrobacter* was significantly lower compared to patients with fasting blood glucose levels <7.0 mmol/L ($P < .05$). Conversely, the relative abundance of *Citrobacter* was significantly higher in CP-T2DM patients with fasting blood glucose levels ≥7.0 mmol/L when compared to those with fasting blood glucose levels <7.0 mmol/L ($P < .05$).

Similarly, in CP-T2DM patients with $HbA_{1c}$ levels ≥6.5%, the relative abundance of *Citrobacter* was significantly lower than that in patients with $HbA_{1c}$ levels <6.5% ($P < .05$). Conversely, the relative abundance of *Citrobacter* was significantly higher in CP-T2DM patients with $HbA_{1c}$ levels ≥6.5% in comparison to those with $HbA_{1c}$ levels <6.5% ($P < .05$). Refer to Table 3 for detailed results.

### Analysis of Relationships

The relative abundance of subgingival microorganisms in the CP-T2DM group was found to be correlated with fasting blood glucose and $HbA_{1c}$ levels. Specifically, the results demonstrated that the relative abundance of ciliates exhibited a negative correlation with fasting blood glucose and $HbA_{1c}$ (r = -0.445 and -0.412, $P < .05$). Conversely, the relative abundance of *Citrobacter* showed a positive correlation with fasting blood glucose and $HbA_{1c}$ (r = 0.481 and 0.432, $P < .05$).

### Comparison of Relative Abundance of Main Subgingival Microbial Genera Before and After Treatment in CP-T2DM Group

In the CP-T2DM group, the relative abundances of *Cilia* and *Streptococcus* after treatment were (3.45 ± 0.71)% and (2.96 ± 0.80)%, respectively, which were significantly higher than those before treatment ($P < .05$). Conversely, the relative abundances of *Tannerella* and *Citrobacter* after treatment were (1.81 ± 0.71)% and (2.00 ± 0.77)%, respectively, which were significantly lower than those before treatment ($P < .05$). However, there was no statistically significant difference in the relative abundance of *Prevotella* and *Fusobacterium* before and after treatment in the CP-T2DM group (Table 4, $P > .05$).

## DISCUSSION

The human oral cavity harbors a diverse array of over 500 bacterial species, maintaining a dynamic balance under normal conditions. Disruption of this balance can occur due to factors like reduced immunity or the introduction of foreign substances into the oral cavity, leading to the development of diseases.[15-17] Subgingival microbes have been identified as the primary culprits behind periodontitis. Currently, the standard treatment for most periodontitis cases involves physical plaque removal from the teeth.[18-20] However, chronic periodontitis in individuals with T2DM tends to be more severe and prone to recurrence. Therefore, it becomes crucial to identify the specific pathogenic bacteria responsible for chronic periodontitis in patients with T2DM.

Related studies have indicated that the primary pathogenic bacteria found in subgingival plaque include *Porphyromonas gingivalis, Treponema denticola, Prevotella intermedia, Tannerella forsythii, Fusobacterium nucleatum*, and others.[21] These bacteria initially invade periodontal tissue, followed by alveolar bone and cementum, releasing significant quantities of bacterial toxins and their byproducts into the tissue.[22] The infected periodontal tissue can elicit a systemic immune response, leading to potential infections in other organs.[23-24] It has been reported that the detection rate of *Actinobacillus, Porphyromonas gingivalis*, and *Tannerella forsythii* in CP-T2DM patients was significantly higher than that in CP patients, suggesting that both CP-T2DM and CP patients share certain pathogenic microorganisms.[25]

In the current study, the relative abundance of *Cilia* and *Streptococcus* in the CP-T2DM group was found to be significantly lower than that in the CP group. In contrast, the relative abundance of *Tannerella* and *Citrobacter* was

**Table 3.** Comparison of relative abundance of main subgingival microbial genera in different patients of CP-T2DM group (%)

| Clinical Data | n | *Prevotella* | *Fusobacterium* | *Porphyromonas* | *Treponema* | *Selenomonas* | *Actinobacillus* | *Leptotrichia* | *Tannerella* | *Streptococcus* | *Citrobacter* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gender | | | | | | | | | | | |
| Male | 40 | 18.65 ± 3.12 | 13.96 ± 2.94 | 12.34 ± 2.84 | 7.90 ± 1.21 | 2.91 ± 0.95 | 3.96 ± 0.96 | 1.18 ± 0.48 | 2.82 ± 0.94 | 1.41 ± 0.65 | 2.77 ± 0.86 |
| Female | 48 | 18.96 ± 3.24 | 14.24 ± 3.01 | 12.23 ± 2.96 | 7.75 ± 1.31 | 2.95 ± 0.97 | 4.05 ± 0.99 | 1.22 ± 0.46 | 2.78 ± 0.91 | 1.45 ± 0.62 | 2.84 ± 0.91 |
| *t* | | -0.454 | -0.439 | 0.177 | 0.554 | -0.194 | -0.431 | -0.398 | 0.202 | -0.295 | -0.368 |
| *P* value | | .651 | .662 | .860 | .581 | .846 | .668 | .691 | .840 | .769 | .714 |
| **Age** | | | | | | | | | | | |
| <50 years old | 42 | 18.79 ± 3.03 | 14.17 ± 2.90 | 12.31 ± 2.85 | 7.79 ± 1.14 | 2.90 ± 0.94 | 3.97 ± 0.86 | 1.22 ± 0.51 | 2.77 ± 0.91 | 1.40 ± 0.54 | 2.82 ± 0.85 |
| ≥50 years old | 46 | 18.85 ± 2.96 | 14.06 ±2 .98 | 12.25 ± 2.96 | 7.85 ± 1.21 | 2.96 ± 0.97 | 4.05 ± 0.90 | 1.18 ± 0.33 | 2.83 ± 0.90 | 1.46 ± 0.50 | 2.80 ± 0.82 |
| *t* | | -0.094 | 0.175 | 0.097 | -0.239 | -0.294 | -0.425 | 0.441 | -0.311 | -0.541 | 0.112 |
| *P* value | | .925 | .861 | .923 | .812 | .769 | .672 | .661 | .757 | .590 | .911 |
| **Body Mass Index BMI** | | | | | | | | | | | |
| >22 kg/m$^2$ | 50 | 18.71 ± 3.60 | 14.03 ± 2.68 | 12.31 ± 2.99 | 7.78 ± 1.22 | 2.90 ± 0.91 | 3.95 ± 0.88 | 1.17 ± 0.36 | 2.77 ± 0.94 | 1.44 ± 0.49 | 2.84 ± 0.78 |
| ≤22 kg/m$^2$ | 38 | 18.96 ± 3.52 | 14.22 ± 2.75 | 12.24 ± 3.01 | 7.87 ± 1.31 | 2.97 ± 0.94 | 4.09 ± 0.93 | 1.24 ± 0.42 | 2.84 ± 0.99 | 1.42 ± 0.50 | 2.77 ± 0.80 |
| *t* | | -0.326 | -0.326 | 0.108 | -0.332 | -0.352 | -0.721 | -0.841 | -0.338 | 0.188 | 0.412 |
| *P* value | | .745 | .745 | .914 | .741 | .725 | .473 | .403 | 0.736 | .851 | .681 |
| **Periodontitis Graduation** | | | | | | | | | | | |
| Slight Grade | 21 | 18.44 ± 3.11 | 14.20 ± 2.93 | 12.18 ± 2.80 | 7.80 ± 1.21 | 2.91 ± 0.83 | 3.98 ± 0.93 | 1.17 ± 0.31 | 2.79 ± 0.82 | 1.45 ± 0.41 | 2.81 ± 0.71 |
| Moderate Grade | 38 | 18.91 ± 3.05 | 14.05 ± 2.60 | 12.32 ± 2.95 | 7.84 ± 1.28 | 3.04 ± 0.88 | 4.05 ± 0.99 | 1.22 ± 0.37 | 2.82 ± 0.85 | 1.40 ± 0.38 | 2.90 ± 0.74 |
| Heavy Grade | 29 | 18.98 ± 3.08 | 14.12 ± 2.81 | 12.30 ± 2.80 | 7.81 ± 1.30 | 2.80 ± 0.91 | 3.98 ± 0.92 | 1.20 ± 0.29 | 2.78 ± 0.88 | 1.49 ± 0.40 | 2.78 ± 0.80 |
| *F* | | 1.022 | 1.312 | 1.144 | 0.892 | 0.771 | 0.615 | 0.904 | 0.532 | 0.411 | 0.382 |
| *P* value | | .682 | .601 | .674 | .712 | .722 | .741 | .701 | .754 | .781 | .801 |
| **Fasting Blood Glucose** | | | | | | | | | | | |
| <7.0 mmol/L | 39 | 18.95 ± 2.29 | 14.22 ± 2.98 | 12.54 ± 2.91 | 7.69 ± 1.13 | 2.83 ± 0.91 | 4.05 ± 1.02 | 1.43 ± 0.31 | 2.79 ± 0.97 | 1.46 ± 0.32 | 2.42 ± 0.95 |
| ≥7.0 mmol/L | 49 | 18.72 ± 2.34 | 14.02 ± 3.01 | 12.07 ± 3.12 | 7.92 ± 1.21 | 3.01 ± 0.96 | 3.98 ± 1.05 | 1.02 ± 0.29 | 2.81 ± 0.99 | 1.41 ± 0.35 | 3.12 ± 0.90 |
| *t* | | 0.462 | 0.311 | 0.723 | -0.912 | -0.894 | 0.315 | 6.390 | -0.095 | 0.691 | -3.536 |
| *P* value | | .645 | .757 | .472 | .364 | .374 | .754 | .000 | .925 | .491 | .001 |
| **$HbA_{1c}$** | | | | | | | | | | | |
| >6.5 % | 37 | 18.95 ± 4.41 | 14.21 ± 2.95 | 12.13 ± 2.65 | 7.80 ±1.15 | 2.96 ± 0.81 | 4.11 ± 0.99 | 1.46 ± 0.42 | 2.81 ± 0.90 | 1.38 ± 0.41 | 2.44 ± 0.71 |
| ≥6.5 % | 51 | 18.73 ± 4.12 | 14.04 ± 3.00 | 12.39 ± 2.81 | 7.83± 1.21 | 2.91 ± 0.86 | 3.94 ± 0.90 | 1.01 ± 0.36 | 2.74 ± 0.88 | 1.47 ± 0.44 | 3.08 ± 0.78 |
| *t* | | 0.240 | 0.264 | -0.439 | -0.117 | 0.276 | 0.839 | 5.395 | 0.365 | -0.974 | -3.944 |
| *P* value | | .811 | .792 | .662 | .907 | .783 | .404 | .000 | .716 | .333 | .000 |

Note: The data is presented as mean ± standard deviation (%); The "*t*" values represent the *t* statistic, and "*P*" values indicate the corresponding *P* values for the statistical comparisons between the different categories.

**Table 4.** Comparison of relative abundance of main subgingival microbial genera before and after treatment in CP-T2DM group (%)

| Category | Before treatment (n = 88) | After treatment (n = 88) | *t* | *P* value |
|---|---|---|---|---|
| *Prevotella* | 18.82 ± 3.32 | 18.21 ± 3.40 | 1.204 | .230 |
| *Fusobacterium* | 14.11 ± 2.81 | 14.03 ± 2.97 | 0.184 | .855 |
| *Porphyromonas* | 12.28 ± 3.01 | 12.14 ± 3.00 | 0.309 | .758 |
| *Treponema* | 7.82 ± 1.18 | 7.84 ± 1.17 | -0.113 | .910 |
| *Selenomonas* | 2.93 ± 0.88 | 2.96 ± 0.85 | -0.230 | .818 |
| *Actinobacillus* | 4.01 ± 1.00 | 3.91 ± 0.78 | 0.740 | .460 |
| *Leptotrichia* | 1.20 ± 0.55 | 3.45 ± 0.71 | -23.501 | .000 |
| *Tannerella* | 2.80 ± 0.88 | 1.81 ± 0.71 | 8.213 | .000 |
| *Streptococcus* | 1.43 ± 0.72 | 2.96 ± 0.80 | -13.335 | .000 |
| *Citrobacter* | 2.81 ± 0.81 | 2.00 ± 0.77 | 6.799 | .000 |

Note: The data is presented as mean ± standard deviation (%).The "*t*" values represent the *t* statistic, and "*P*" values indicate the corresponding *P* values for the statistical comparisons between the relative abundances before and after treatment. A *P* value of less than .05 indicates a statistically significant difference between the two groups.

significantly higher in the CP-T2DM group compared to the CP group. However, there was no significant difference in the relative abundance of *Prevotella* and *Fusobacterium* between the CP-T2DM and CP groups. These findings suggest a significant difference in the distribution of oral subgingival microbial communities, including *Cilia, Streptococcus, Tannerella*, and *Citrobacter*, between CP-T2DM and CP patients.

$HbA_{1c}$, also known as glycosylated hemoglobin, is an essential indicator used for monitoring blood glucose in diabetic patients.[26-28] It provides an objective reflection of the recent average blood glucose level, irrespective of the impact of diet, surgery, trauma, or other factors.[29-32] The results of this study indicated that there was no significant difference in the relative abundance of the main subgingival microbial genera in CP-T2DM patients concerning gender, age, body mass index, and periodontitis.

Furthermore, in the CP-T2DM group, the relative abundance of ciliates in patients with fasting blood glucose ≥7.0 mmol/L was significantly lower than in patients with fasting blood glucose <7.0 mmol/L. Conversely, the relative abundance of *Citrobacter* was significantly higher in patients with fasting blood glucose ≥7.0 mmol/L compared to those with fasting blood glucose <7.0 mmol/L. The relative

abundance of ciliates in CP-T2DM patients with $HbA_{1c}$ ≥6.5% was found to be significantly lower than that in patients with $HbA_{1c}$ <6.5%. On the other hand, the relative abundance of *Citrobacter* was significantly higher in patients with $HbA_{1c}$ ≥6.5% compared to those with $HbA_{1c}$ <6.5%. These results suggest a correlation between the relative abundance of ciliates and Citrobacter with the fasting blood glucose level of the patients.

In this study, the relative abundance of the main genera of subgingival microorganisms in the CP-T2DM group was further correlated with fasting blood glucose and $HbA_{1c}$ levels. The results revealed a negative correlation between the relative abundance of ciliates and fasting blood glucose and $HbA_{1c}$ levels. Conversely, there was a positive correlation between the relative abundance of *Citrobacter* and fasting blood glucose and $HbA_{1c}$ levels. These findings indicate that as the fasting blood glucose level increases, the relative abundance of ciliates decreases while the relative abundance of *Citrobacter* increases.

When periodontal tissue is in a diseased state, its bacterial colony diversity may be significantly lower than that in a normal state.[33-34] *Prevotella* is a prominent bacterium found in the gingival plaque of patients with chronic periodontal disease. Its population increases and reproduces in the subgingival plaque of patients with severe periodontitis, and its abundance is closely associated with alveolar bone resorption and periodontal pocket depth.[35]

The results of this study revealed that the relative abundance of ciliates and *Streptococcus* in the CP-T2DM group after treatment was significantly higher than before treatment. Conversely, the relative abundance of *Tannerella* and *Citrobacter* after treatment was significantly lower than before treatment. However, there was no significant difference in the relative abundance of *Prevotella* and *Fusobacterium* before and after treatment in the CP-T2DM group. These findings provide valuable insights for clinical practice.

### Study Limitations

The present study has a few limitations that should be acknowledged. Firstly, the sample size was relatively small, which may have affected the generalizability of the results. Secondly, the study design was retrospective, and the data were collected from a single hospital, which may introduce bias and limit the ability to establish causal relationships. Additionally, the study focused on specific subgingival microbial genera, and other potentially relevant microorganisms were not considered. Future research should include a broader range of microbial species to gain a comprehensive understanding. Moreover, while efforts were made to control for confounding variables, the influence of other factors, such as diet, lifestyle, and medication use, could not be eliminated. Lastly, the study did not explore the long-term effects of the combined periodontal-endodontic treatment, warranting further investigation to assess treatment efficacy over time.

## CONCLUSION

In conclusion, this study highlights significant differences in oral subgingival microbial communities distribution between CP-T2DM and CP patients. Moreover, the relative abundance of ciliated bacteria and citrate bacteria was found to be associated with the blood glucose levels of patients. Following treatment, an increase in the levels of ciliated bacteria and *Streptococcus*, along with a decrease in the levels of *Tannerella* and *Citrobacter*, was observed in CP-T2DM patients. These findings provide valuable insights into the diagnosis and treatment of chronic periodontitis, laying a favorable foundation for future clinical management.

### CONFLICT OF INTEREST

The authors declare to have no conflict of interest.

### DATA AVAILABILITY STATEMENT

Due to the nature of this research, the study participants did not agree to their data being shared publicly, so supporting data is not available.

### FUNDING

This study did not receive funding in any form.

### REFERENCES

1. Brunton S. Pathophysiology of Type 2 Diabetes: The Evolution of Our Understanding. *J Fam Pract*. 2016 Apr;65(4 Suppl):supp_az_0416.
2. Hackett RA, Steptoe A. Type 2 diabetes mellitus and psychological stress - a modifiable risk factor. *Nat Rev Endocrinol*. 2017;13(9):547-560. doi:10.1038/nrendo.2017.64
3. Damanik J, Yunir E. Type 2 Diabetes Mellitus and Cognitive Impairment. *Acta Med Indones*. 2021;53(2):213-220.
4. Cardoso EM, Reis C, Manzanares-Céspedes MC. Chronic periodontitis, inflammatory cytokines, and interrelationship with other chronic diseases. *Postgrad Med*. 2018;130(1):98-104. doi:10.1080/00325481.2018.1396876
5. Li C, Lv Z, Shi Z, et al. Periodontal therapy for the management of cardiovascular disease in patients with chronic periodontitis. *Cochrane Database Syst Rev*. 2017;11(11):CD009197. doi:10.1002/14651858.CD009197.pub3
6. Zhang D, Liu W, Miao R, et al. Chronic Periodontitis is a Risk Factor of Renal Dysfunction in Patients with Type 2 Diabetes. *Exp Clin Endocrinol Diabetes*. 2021;129(6):407-412. doi:10.1055/a-0895-5275
7. Wu Wei, Hao Shuli, Zhou Yusen, et al.Effect of periodontal basic therapy on serum biochemical indexes and inflammatory cytokines in patients with type 2 diabetes mellitus and chronic periodontitis[J]. *Chin J General Practice*, 2020, 18(2):249-251,281.
8. Xi S, Jin Z. Establishment and correlation of diabetic periodontitis model [J]. *Xinjiang Yike Daxue Xuebao*. 2015;1(6):756-760.
9. Genco RJ, Borgnakke WS. Diabetes as a potential risk for periodontitis: association studies. *Periodontol 2000*. 2020;83(1):40-45. doi:10.1111/prd.12270
10. Jia W, Song X, Xu S, et al. Clinical significance of Th17/Treg imbalance in patients with type 2 diabetes mellitus and chronic periodontitis [J]. *Journal of Applied Stomatology*. 2020;36(6):886-890.
11. Preshaw PM, Taylor JJ, Jaedicke KM, et al. Treatment of periodontitis reduces systemic inflammation in type 2 diabetes. *J Clin Periodontol*. 2020;47(6):737-746. doi:10.1111/jcpe.13274
12. Niu Jiahui, li chuang, Li Rong, et al. Based on high-throughput sequencing technique, the changes of subgingival flora in patients with type 2 diabetes mellitus complicated with chronic periodontitis before and after periodontal treatment were explored [J]. *Xinjiang Yike Daxue Xuebao*. 2021;44(4):453-457.
13. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol*. 1999;4(1):1-6. doi:10.1902/annals.1999.4.1.1
14. Qian R. Significance of new diagnostic criteria and classification of diabetes [J]. *Journal of Clinical Internal Medicine*. 2000;17(3):133.
15. Chen Tingtao, Xin Hongbo, wangxin, et al. Application of artificial cultured oral bacteria transplantation in the treatment of oral diseases: CN105193857A[P].2015.
16. Baker JL, Bor B, Agnello M, Shi W, He X. Ecology of the Oral Microbiome: beyond Bacteria. *Trends Microbiol*. 2017;25(5):362-374. doi:10.1016/j.tim.2016.12.012
17. White T, Alimova Y, Alves VTE, et al. Oral commensal bacteria differentially modulate epithelial cell death. *Arch Oral Biol*. 2020;120:104926. doi:10.1016/j.archoralbio.2020.104926
18. Jian Z, Xiao D, Wang X, et al. Effect of orthodontic treatment on atrophic periodontitis and its influence on SF-36 score of patients [J]. *Journal of Practical Medicine*. 2019;35(24):3832-3836.
19. Jepsen S, Blanco J, Buchalla W, et al. Prevention and control of dental caries and periodontal diseases at individual and population level: consensus report of group 3 of joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. *J Clin Periodontol*. 2017;44(suppl 18):S85-S93. doi:10.1111/jcpe.12687
20. Cirelli JA, Fiorini T, Moreira CHC, Molon RS, Dutra TP, Sallum EA. Periodontal regeneration: is it still a goal in clinical periodontology? *Braz Oral Res*. 2021;35(suppl 2):e09. doi:10.1590/1807-3107bor-2021.vol35.0097
21. Wilson C, Lukowicz R, Merchant S, et al. Quantitative and Qualitative Assessment Methods for Biofilm Growth: A Mini-review. *Res Rev J Eng Technol*. 2017;6(4):http://www.rroij.com/open-access/quantitative-and-qualitative-assessment-methods-for-biofilm-growth-a-minireview-.pdf.
22. Rampelotto RF, Lorenzoni VV, Silva DDC, et al. Assessment of different methods for the detection of biofilm production in coagulase-negative staphylococci isolated from blood cultures of newborns. *Rev Soc Bras Med Trop*. 2018;51(6):761-767. doi:10.1590/0037-8682-0171-2018
23. Yu Z, Xue L, Teng Z. Effect of periodontal basic treatment on blood sugar control of diabetic patients [J]. *Journal of Practical Stomatology*. 2009;25(5):722-724.
24. Liccardo D, Cannavo A, Spagnuolo G, et al. Periodontal Disease: A Risk Factor for Diabetes and Cardiovascular Disease. *Int J Mol Sci*. 2019;20(6):1414. doi:10.3390/ijms20061414

25. Ma L, Pan YP, Zhang JQ. [Detection of putative periodontal pathogens of periodontitis with type 2 diabetes] [J]. *Chung Hua Kou Chiang Hsueh Tsa Chih*. 2010;45(6):337-341.
26. Zuo TT, Luo FY, Jin HY, et al. Determination of the bioaccessibility of cadmium in golden thread by physiologically based extraction test digestion using the in vitro/Caco2 cell model and subsequent risk assessment. *World J Tradit Chin Med*. 2021;7:240-245.
27. Wayne N, Perez DF, Kaplan DM, Ritvo P. Health Coaching Reduces HbA1c in Type 2 Diabetic Patients From a Lower-Socioeconomic Status Community: A Randomized Controlled Trial. *J Med Internet Res*. 2015;17(10):e224. doi:10.2196/jmir.4871
28. Rhee M. HbA1c and Diabetes: mismatches and Misclassifications. *J Clin Endocrinol Metab*. 2020;105(7):e2630-e2632. doi:10.1210/clinem/dgaa185
29. Vural Keskinler M, Takir M, Caklili OT, Oguz A. The Frequency and Determinants of HbA1c Variability in Type 2 Diabetic Patients. *Metab Syndr Relat Disord*. 2021;19(7):372-377. doi:10.1089/met.2020.0131
30. Papathanassiou E, Papaioannou AI, Papanikolaou I, et al. Glycated Hemoglobin (HbA1c) as a Predictor of Outcomes during Acute Exacerbations of Chronic Obstructive Pulmonary Disease. *COPD*. 2021;18(2):219-225. doi:10.1080/15412555.2021.1902491
31. Ohde S, Deshpande GA, Yokomichi H, Takahashi O, Fukui T, Yamagata Z. HbA1c monitoring interval in patients on treatment for stable type 2 diabetes. A ten-year retrospective, open cohort study. *Diabetes Res Clin Pract*. 2018;135:166-171. doi:10.1016/j.diabres.2017.11.013
32. Hemachoudary Y, Kumari P. Effect of Nutritional Anaemia on HbA1c Levels in Type 2 Diabates. *J Assoc Physicians India*. 2020;68(1):46.
33. Xiao Weizhuo, Zhang Long, Wang Xiaodong, et al. Metatranscriptome analysis of subgingival plaque in patients with chronic periodontitis [J]. *chinese medicine guides*, 2019, 16(21):21-23,42.
34. Chen Bin, Li Lili, zhangqian, et al. Study on the difference of subgingival flora between aggressive periodontitis, chronic periodontitis and periodontal healthy people [J]. *Chinese Journal of Stomatology*. 2020;55(7):466-474.
35. Su J, Peng X. Clinical study on the detection of periodontal pathogenic bacteria in saliva and subgingival plaque of chronic periodontitis patients with diabetes mellitus [J]. *Chinese Journal of Practical Stomatology*. 2018;11(2):79-83.