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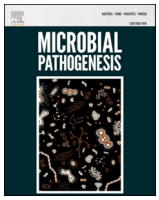

Impact of periodontitis on oral microbiota in pregnant women with gestational diabetes mellitus: A case-control study

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ABSTRACT

Objectives: This study aims to explore the distribution characteristics of oral microbiota in pregnant women with gestational diabetes mellitus (GDM) and its association with periodontitis.
Methods: This case-control study included 80 GDM women, divided into the periodontitis group (PD group, n = 40) and the periodontally healthy group (N group, n = 40). Unstimulated saliva samples were collected for 16S rRNA sequencing targeting the V3~V4 region. We used α-diversity and β-diversity to assess the richness and diversity of the oral microbiota and the variability between groups. The Mann-Whitney *U* test and linear discriminant analysis effect size (LEfSe) analysis were used to identify significant differences in microbial composition and to evaluate categorical differences between groups.
Results: Significant differences in α-diversity and β-diversity were observed between the PD group and the N group. The PD group exhibited a higher abundance of *Bacteroidota*, *Fusobacteriota*, *Spirochaetota*, *Synergistota*, and *Choroflexi* compared to the N group. LEfSe analysis identified *Spirochaetora* and *Tetraptera* as the taxonomic biomarkers in the PD group. Furthermore, activating environmental adaptation, terpenoid and polyketide metabolism, and the immune and endocrine systems pathways may be involved in the potential mechanisms of the PD group.
Conclusions: A significant correlation was observed between oral microbiota composition and periodontitis in pregnant women with GDM. *Spirochaetota* and *Tetraptera* are closely linked to the progression of periodontitis and may serve as biomarkers for early diagnosis. Targeting these microbial taxa could provide new strategies for preventing and managing periodontitis during pregnancy, potentially reducing adverse maternal and fetal outcomes.

1. Introduction

Periodontitis and gestational diabetes mellitus (GDM) are two common complications of pregnancy that can significantly impact the health of both the pregnant woman and the fetus. Periodontitis is a chronic inflammatory disease [1], which can influence the development and progression of systemic diseases such as atherosclerosis, diabetes, rheumatoid arthritis, and respiratory diseases [2–4], making it a major global public health concern [5]. During pregnancy, hormonal, dietary and lifestyle changes increase the risk of periodontitis from the outset, with pregnant women at particularly high risk of gingivitis from three months onwards [6,7]. This risk becomes even more pronounced in the third trimester, when hormonal fluctuations and metabolic changes peak, further exacerbating inflammation and gum bleeding [8]. It is estimated that up to 40 % of pregnant women suffer from this condition [9], which can lead to complications such as preterm delivery and low

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birth weight [10,11].

On the other hand, GDM is a metabolic disorder first identified during pregnancy and affects approximately 14 % of pregnancies worldwide [12,13]. In China, the incidence rate of GDM is approximately 14.8 %, representing a significant public health challenge [14]. GDM is associated with several adverse pregnancy outcomes and long-term health problems for both mothers and their offspring, including macrosomia, preterm birth, gestational hypertension, and an increased risk of developing type 2 diabetes mellitus [15,16], which have received much attention. Notably, there is a bidirectional relationship between GDM and periodontitis. Studies have shown that pregnant women with GDM have a significantly higher risk of developing periodontitis [17,18], and the presence of periodontitis may contribute to the onset and progression of GDM [19–21]. Given this association, it is necessary to understand the biological mechanism by which GDM complicates periodontitis in order to improve the health outcomes of pregnant women and their offspring.

The oral microbiota comprises a diverse community of microorganisms residing in the oral cavity, including bacteria, fungi, and viruses. Maintaining a balanced oral microbiota is essential for oral health, as imbalances can lead to the development of various oral diseases, including gingivitis, periodontitis, and oral mucosal disease [22,23]. Periodontitis is a common oral disease that is significantly influenced by an individual's oral microbiota. Numerous studies have shown marked differences in the composition of the oral microbiota between periodontitis and healthy individuals [24–26]. There is also an association between the oral microbiota and GDM [27,28]. Zhang et al. investigated the combined effects of periodontitis and GDM on the oral and gut microbiomes in the second trimester and found significant changes in both microbiomes when periodontitis coexisted with GDM [29]. However, there is still a lack of understanding of biomarkers in GDM women with periodontitis, especially in the third trimester, a critical period when hormonal and metabolic changes peak.

In this study, we employed high-throughput 16S rRNA sequencing to investigate the characteristics of the oral microbiota in GDM women with periodontitis, focusing specifically on the third trimester, with the aim of shedding light on the potential association between periodontitis and oral microbiota in this critical stage of pregnancy.

2. Materials and methods

2.1. Study design and participants

This case-control study was derived from a population-based cohort in Fujian Province, supported by the Startup Fund for Scientific Research of Medical University (grant number: 2022QH1190). The cohort recruited 302 women diagnosed with GDM through the Oral Glucose Tolerance Test (OGTT). The aim is to explore the association between dietary intake and periodontitis among these pregnant women. In the context of the study objectives, we expect to find significant associations between the composition of the oral microbiota and periodontitis in pregnant women with GDM. All participants were recruited during their third trimester between January 2023 and July 2023 from the maternity clinic of a maternal and child hospital in Fujian Province, China. The diagnostic criteria for GDM followed the guidelines of the International Diabetes and Pregnancy Study Group [30]. The inclusion criteria for the study were as follows: age ≥18 years, no use of antibiotics or probiotics in the past 3 months, no serious periodontal disease before pregnancy, no serious pregnancy complications or comorbidities, no history of smoking or alcohol consumption, and no communication problem.

All participants underwent a comprehensive oral examination conducted by a dentist using a periodontal probe (UNC-15; Hu-Friedy). The examination included recording bleeding on probing (BOP), probing depth (PD), and clinical attachment level (CAL) [31]. Periodontitis was diagnosed according to the following criteria: Mild periodontitis was defined as having ≥2 interproximal sites with CAL ≥3 mm, and ≥2 interproximal sites with PD ≥ 4 mm or one site with PD ≥ 5 mm with a positive BOP; moderate periodontitis was defined as ≥ 2 interproximal sites with CAL ≥4 mm, or ≥2 interproximal sites with PD ≥ 5 mm; severe periodontitis was defined as ≥ 2 interproximal sites with CAL ≥6 mm and ≥1 interproximal site with PD ≥ 5 mm [31]. Participants with mild, moderate, or severe periodontitis were classified as the periodontitis group (PD group), while those without periodontitis were classified as the periodontally healthy group (N group). This study included a total of 80 GDM women (40 periodontally healthy and 40 with periodontitis) (Supplementary Fig. 1).

This study was approved by the Human Research Ethics Committee of Fujian Maternity and Child Health Hospital (approval no. 2022YJ080). Written informed consent was obtained from all participants, and the study was conducted anonymously.

2.2. Data collection

To ensure a comprehensive understanding of the participants and to minimize the impact of confounding factors on the experimental results, the researchers used a structured questionnaire administered in face-to-face interviews to collect the following data: general demographic characteristics, and pregnancy-related information (including age, gestational age, educational level, and average personal monthly income), oral health-related behaviors (including the brushing frequency, frequency of dental floss using, and frequency of dental visit) and dietary intake. Participants' dietary intake was assessed by a validated semi-quantitative food frequency questionnaire (SQ-FFQ) [32], a survey of the subject's dietary data over the previous four weeks, including the types of food consumed, frequency of consumption, and amount of food consumed per time. Dietary energy was calculated based on Chinese food composition tables [33].

Indicators such as gestational age, pre-pregnancy body mass index (BMI), and OGTT blood glucose were obtained from the medical records.

2.3. Sample collection

Saliva samples were collected from all participants during their third trimester of pregnancy. Participants were instructed to refrain from eating, rinsing, brushing, drinking, or chewing gum for 1 h. Approximately 1.5 mL of unstimulated saliva was collected from each participant in sterile 3 mL tubes, which were then stored at −80 °C until DNA extraction.

2.4. DNA extraction, 16S rRNA sequencing, and raw data processing

Total DNA was extracted from saliva samples using the PowerSoil DNA Isolation Kit (Qiagen, Germany) according to the manufacturer's instructions. The quality and integrity of the extracted DNA were further assessed by 1 % agarose gel electrophoresis, which showed clear, intact high-molecular-weight DNA bands with no significant fragmentation, indicating the absence of substantial degradation. The V3-V4 hypervariable region of the 16S rRNA gene was amplified using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) in a PCR reaction. The PCR products were purified on AMpure XP magnetic beads (Beckman Coulter, USA) and barcoded for sample identification. A sequencing library was constructed by pooling the barcoded PCR products and sequenced on an Illumina NovaSeq 6000 platform using a paired-end strategy. Raw Reads were quality filtered by first removing low-quality sequences using Trimmomatic software (version 0.33) and removing primer sequences using Cutadapt software (version1.9.1) to ensure that only high-quality Clean Reads without primer contamination were retained. Afterwards, denoising was performed using QIIME2 to remove low-quality and erroneous sequences. process to remove low-quality and erroneous sequences. Next, double-ended sequences were spliced and chimeric sequences were removed using UCHIME (version 8.1) with USEARCH

(version 10.0). Finally, the OTUs were classified and annotated using the SILVA 138 database (http://www.arb-silva.de) and the RDP classifier (confidence threshold of 70 %).

2.5. Statistical and Bioinformatics analysis

Demographic data were analyzed using IBM SPSS (version 25), with significance set at $P < 0.05$. Continuous variables with normal distributions were expressed as mean ± standard deviation (SD) and compared between groups using an independent samples *t*-test. Categorical variables were described as numbers and frequencies, and compared using x^2 tests or Fisher's exact test.

Sequence analysis of the oral microbiota was performed using QIIME2 (version 2020.6) and R software (version 4.1.2). We used R software to generate a Venn diagram, illustrating the shared and unique operational taxonomic units (OTUs) between the periodontitis group (PD group) and the periodontally healthy group (N group). This provided a clear visualization of the overlap and differences in OTUs between the two groups. The sample size and sequence accuracy were determined by pan/core curve and rarefaction curves. The α-diversity indices, such as the Chao1 index, ACE index, PD whole tree, and Shannon index, were calculated using QIIME2 to assess the richness and evenness of the oral microbiota. The β-diversity was evaluated using weighted UniFrac distances, which involved performing the principal coordinate analysis (PCoA) and generating a heat map to visualize the differences in microbial composition between the groups. The Mann–Whitney *U* test was used to identify significant differences in the total microbial composition between the two groups. To detect significant species differences between the groups, we performed a linear discriminant analysis effect size (LEfSe) analysis with a linear discriminant analysis (LDA) score threshold of 4.0. This threshold was used to determine the strength of these differences and the level of group differentiation. Finally, we used PICRUSt2 (version 2.4.1) to analyze the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways to observe differences and changes in functional gene metabolic pathways in the microbial communities between the PD and N groups.

3. Results

3.1. Characteristics of GDM women

Table 1 presents the characteristics of the participants. No statistically significant differences were observed between the periodontitis group (PD group) and the periodontally healthy group (N group) in terms of general characteristics, pregnancy-related information, oral health-related behaviors, and OGTT blood glucose ($P > 0.05$).

3.2. Comparison of daily dietary intake between the two groups

The average daily dietary intake of GDM women was categorized based on total energy, grains, meat and poultry, seafood, vegetables and fruits, and dairy products. Independent sample *t*-tests revealed no statistically significant differences in dietary intake between the two groups ($P > 0.05$) (Table 2).

3.3. Comparison of oral microbiota characteristics between the two groups

A total of 5,179,237 sequences were generated, with the number of sequences per sample ranging from 39,872 to 67,769 (mean [SD] = 64,740 [3276]). After quality control and homogenization, a total of 26,929 OTUs were generated from all sequences. In the periodontitis group (PD group), there were a total of 16,753 OTUs, of which 13,424 were unique. In contrast, the control group (N group) had 13,505 OTUs. There were 3329 OTUs shared between the two groups (Supplementary Fig. 2). Pan/core curve and the rarefaction curves showed a gradual stabilization in the number of OTU species per sample with increasing sequencing depth, indicating that the sequencing depth was sufficient to capture and reflect the microbial diversity and composition of the samples (Fig. 1).

The α-diversity analysis demonstrated that the PD group exhibited significantly elevated levels of microbial richness and diversity compared to the N group: ACE index (756.07 ± 186.11 vs 610.91 ±

Table 1
The demographic and clinical characteristics of subjects (n = 80).

Characteristics	PD group (n = 40)	N group (n = 40)	t/χ^2	P[a]
	N (%)/M ± SD			
Age (y)	32.22 ± 3.37	30.80 ± 4.90	−1.514	0.134
Gestational age (wk)	30.75 ± 2.47	30.50 ± 2.47	−0.464	0.644
Pre-pregnancy BMI (kg/m^2)	23.41 ± 3.83	22.91 ± 3.13	−0.639	0.525
Educational level (y)			4.115	0.269
≤12	8(20)	14(35.0)		
13-15	11(27.5)	7(17.5)		
≥16	21(52.5)	19(47.5)		
Average personal monthly income (CYN)			7.287	0.202
<6000	15(37.5)	12(30.0)		
6000-8999	14(35.0)	9(22.5)		
9000-11999	6(15.0)	14(35.0)		
≥12000	5(12.5)	5(12.5)		
Brushing frequency (times/day)			2.673	0.263
≤1	3(7.5)	5(12.5)		
2	36(90.0)	31(77.5)		
≥3	1(2.5)	4(10.0)		
Frequency of dental floss using (times/day)			3.421	0.738
<1	31(77.5)	30(75.0)		
1	6(15.0)	5(12.5)		
≥2	3(7.5)	5(12.5)		
Frequency of dental visit (months/times)			5.398	0.145
<6 months	0(0)	1(2.5)		
6–12 months	0(0)	2(5.0)		
When oral problems occur	11(27.5)	5(12.5)		
Never	29(72/5)	32(80.0)		
OGTT (mmol/L)				
OGTT-0h	4.86 ± 0.42	4.92 ± 0.45	0.129	0.575
OGTT-1h	10.35 ± 1.42	10.26 ± 1.34	−0.303	0.763
OGTT-2h	8.54 ± 1.44	8.58 ± 1.45	0.563	0.897
PD (mm)	3.0 ± 0.49	1.64 ± 0.38	13.80	<0.05
CAL (mm)	3.52 ± 0.55	1.60 ± 0.32	18.96	<0.05

Abbreviations: BMI, body mass index; OGTT, Oral Glucose Tolerance Test, probing depth (PD), and clinical attachment level (CAL).
[a] *P*-values were obtained from independent samples *t*-test and χ2 test.

Table 2
Comparison of daily dietary intake between the two groups (n = 80)[a].

Dietary intake per day	PD group (n = 40)	N group (n = 40)	t	P[b]
Total energy (kcal)	1865.18 ± 329.46	1956.40 ± 345.06	1.029	0.230
Cereals(g)	161.78 ± 56.76	169.75 ± 56.78	0.628	0.532
Meat and poultry (g)	64.26 ± 36.79	56.83 ± 43.29	−0.827	0.411
Seafood(g)	84.60 ± 71.88	76.22 ± 52.31	−0.596	0.553
Vegetables and fruits(g)	480.79 ± 144.40	521.33 ± 172.89	1.138	0.259
Dairy products (g)	402.96 ± 154.64	423.32 ± 121.38	0.655	0.514

[a] Continuous variables were presented as mean ± SD.
[b] *P*-values were obtained from independent samples *t*-test.

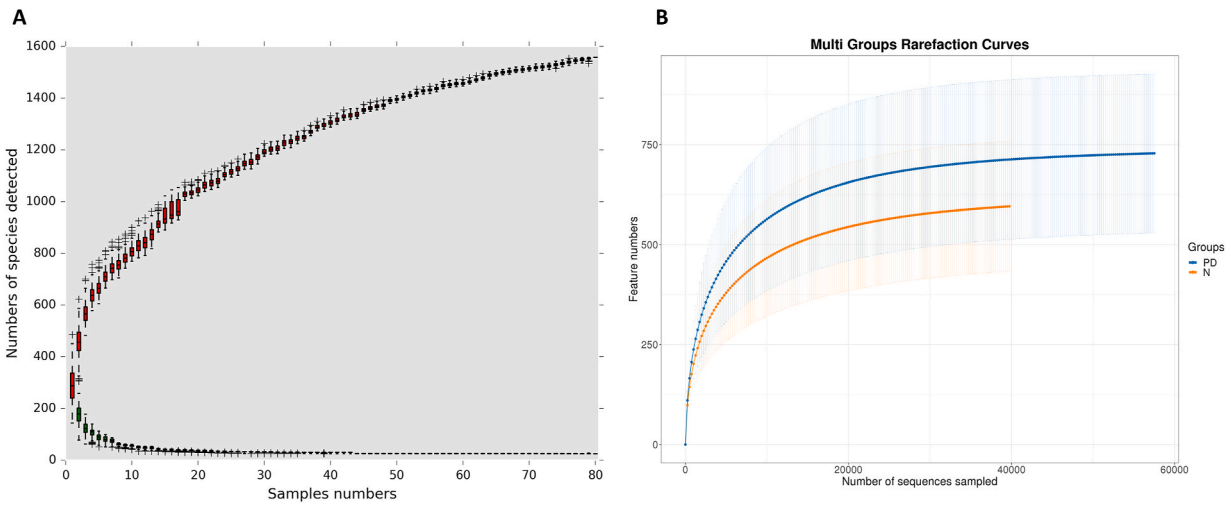

Fig. 1. Pan/core curve and rarefaction curves.
A: Pan/core curve, B: Rarefaction curves
The pan/core and rarefaction curves showed a gradual stabilization in the number of OTU species per sample with increasing sequencing depth, confirming that the sequencing was comprehensive enough to accurately reflect the microbial diversity and composition of the samples, thus ensuring the validity of our dataset.

160.34, $t = -3.743$, $P = 0.004$), Chao 1 index (773.84 ± 185.23 vs 628.68 ± 163.35, $t = -3.722$, $P = 0.005$), PD_whole_tree index (129.26 ± 37.50 vs 103.05 ± 38.15, $t = -3.097$, $P = 0.021$), and Shannon index (7.07 ± 0.69 vs 6.62 ± 0.59, $t = -3.122$, $P = 0.016$) (Fig. 2).

The weighted UniFrac PCoA and heatmap were used to show the differences in species diversity between samples. As shown in Fig. 3 A, samples from the PD group showed a notable separation, indicating a wider range of compositional variability compared to the N group. This observation suggests that the microbial community in the PD group was more diverse, with a higher β-diversity than that of the N group.

The weighted UniFrac heatmap showed a clear distinction between the groups: samples from the N group were tightly clustered, indicating similarity in species composition within the group (Fig. 3 B). In contrast, samples from the PD group were more dispersed, indicating a greater variation in species composition. This finding suggests that the PD group had a higher level of β-diversity, highlighting significant differences in

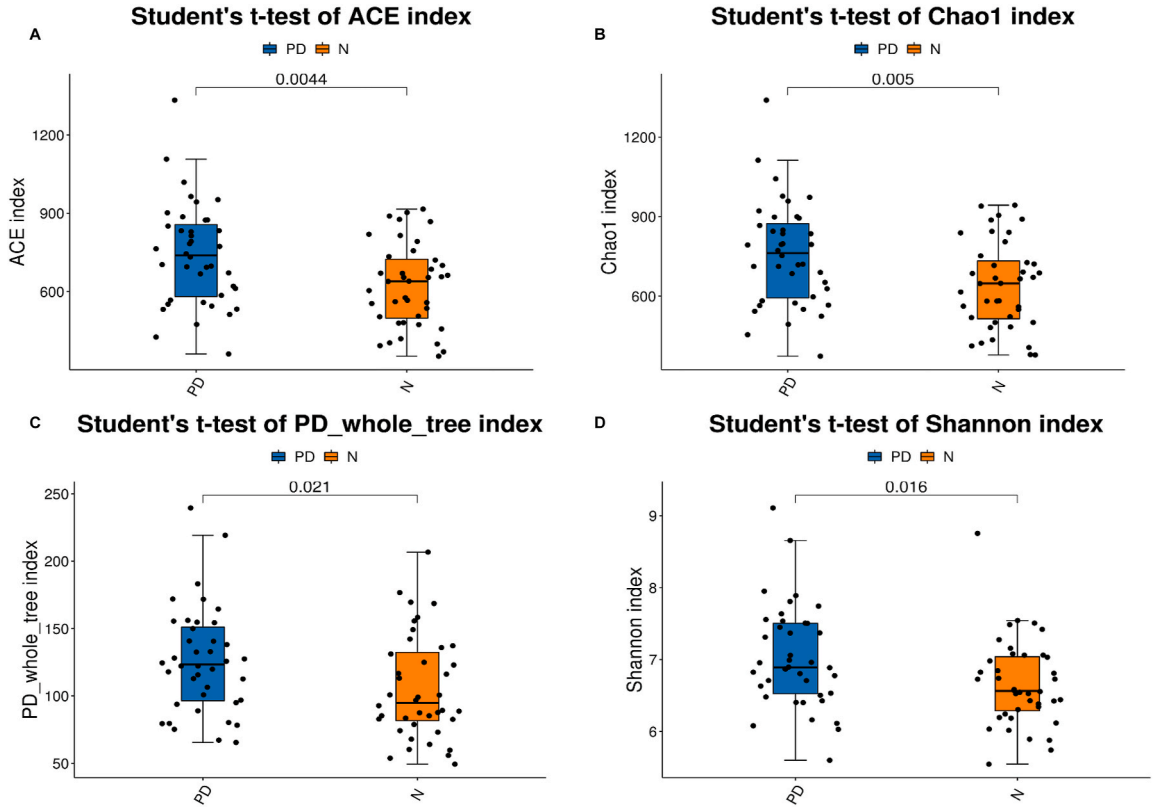

Fig. 2. Comparison of α-diversity in oral microbiota between PD and N groups.
A: ACE index, B: Chao1 index, C: PD whole tree index, and D: Shannon index.
PD group, periodontitis group; N group, periodontally healthy group. There was a significant difference in α-diversity in the PD group compared to the N group.

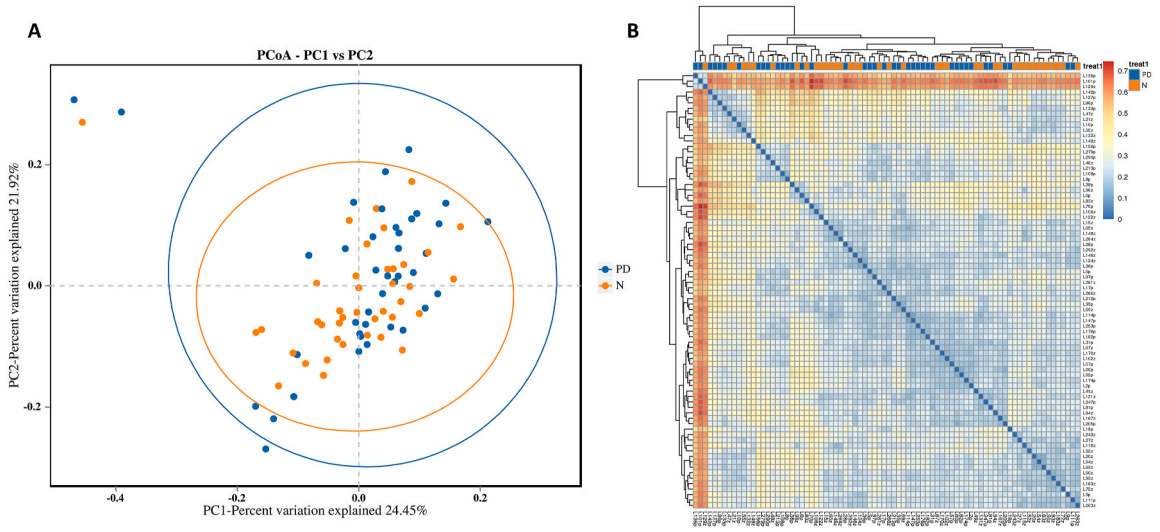

Fig. 3. Weighted UniFrac distance of PCoA and heatmap between PD and N groups.
A: Weighted UniFrac distance of PcoA, B: Weighted UniFrac distance of PCoA and heatmap.
PD group, periodontitis group; N group, periodontally healthy group. PCoA based on weighted UniFrac showed differences in oral flora composition between the PD group and the N group. Each point represents one sample. The distances between the different samples reflect the comparability of the two cohorts.

the microbial community structure between individuals with and without periodontitis.

Supplementary Fig. 3 shows the top ten relative abundances, highlighting significant differences at the phylum and genus level between the groups. Phylum-level relative abundance analysis revealed that the PD group had a higher abundance of *Spirochaetota* and *Synergistota*. Genus level relative abundance analysis revealed that *Porphyromonas* and *Fusobacterium* were higher in the PD group.

The Mann–Whitney *U* test revealed significant differences in abundance at the phylum and genus level between the groups (Fig. 4). In the PD group, *Bacteroidota*, *Fusobacteriota*, *Spirochaetota, Synergistota*, and *Choroflexi* were more abundant, whereas *Firmicutes*, *Actinobacteriota,* and *Patescibacteria* were less abundant at the phylum level ($P < 0.05$). At the genus level, *Fusobacterium*, *Porphyromonas*, *Treponema*, *Aggregatibacter*, and *Filifactor* showed higher relative abundances in the PD group. Conversely, *Veillonella*, *Rothia*, *Leptotrichia*, *TM7x*, *Actinomyces*, *Granulicatella*, *Oribacterium*, and *Corynebacterium* showed lower relative abundances in the PD group ($P < 0.05$).

LEfSe was used to analyze significant differences in species abundance between two groups to identify key biomarkers. The results indicated that the *Spirochaetota* phylum had a higher relative abundance in the PD group, whereas the *Firmicutes* phylum predominated in the N group. At the genus level, *Tetraptera* was dominant in the PD group, whereas *Negativicutes* was dominant in the N group. These results suggest that *Spirochaetota* and *Tetraptera* are important biomarkers of periodontitis (Fig. 5).

3.4. The gene function prediction of oral microbiota in the KEGG databases

We compared the functional characteristics and metabolic pathways of the oral microbiome between the PD and N groups, focusing on

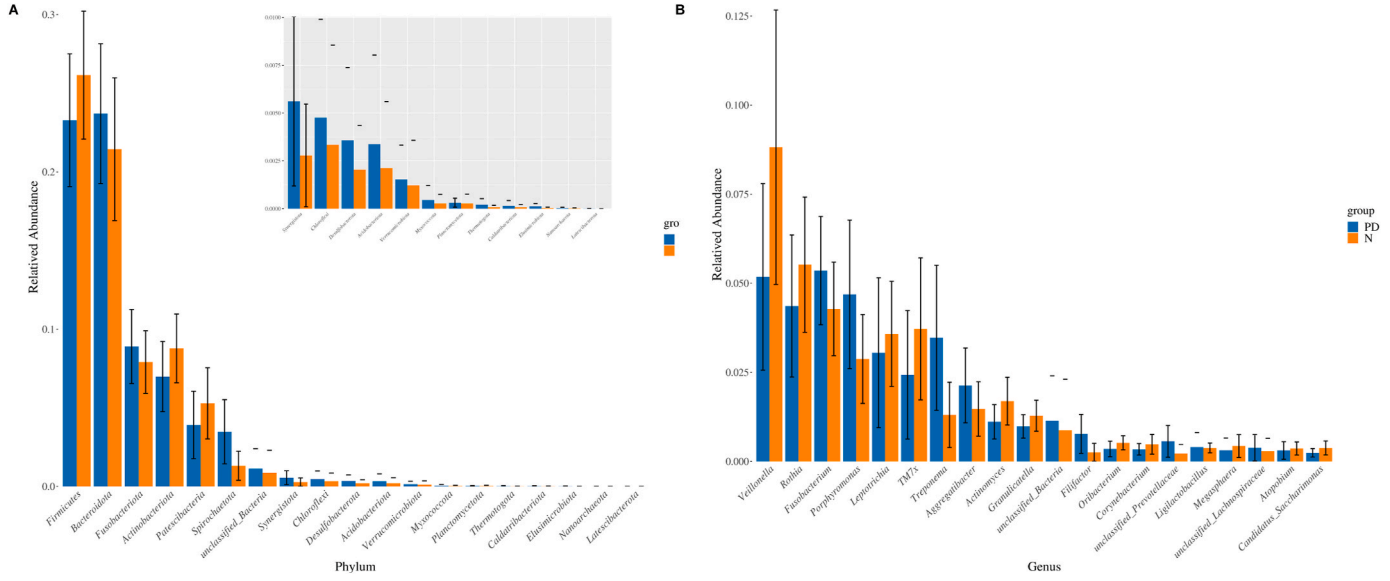

Fig. 4. Analysis of differences in oral microbiota composition between PD and N groups at the phylum and genus level.
A: phylum level, B: genus level.
The Mann-Whitney *U* test was used to analyze significant differences between the species of the two sample groups, corrected for p-values (FDR).

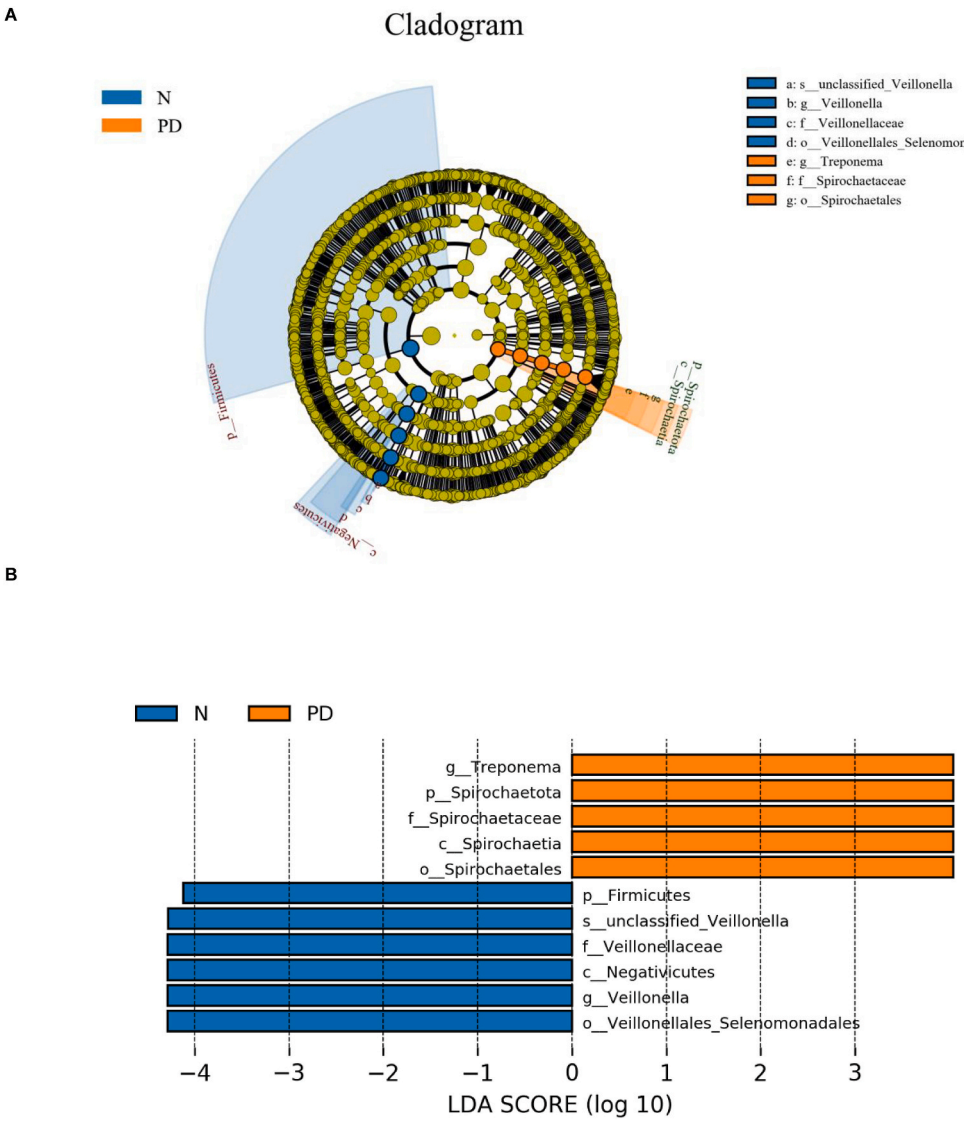

Fig. 5. Distinct abundance of taxa identified by LEfSe analysis between the two groups.
A: The circles radiating from inside to outside of the evolutionary branching diagram represent taxonomic levels from phylum to species. B: These several genera were distinguished using linear discriminant analysis (LDA) using LEfSe, spanning phylum to genus. The logarithmic LDA score was 4.0.

differences in KEGG pathway (Fig. 6). The results showed that the PD group had significantly increased activity in pathways related to environmental adaptation, terpenoid and polyketide metabolism, and immune and endocrine systems, contrasting with decreased activity in global metabolism pathways compared to the N group.

4. Discussions

In this study, we analyzed the oral microbiota in late pregnancy using 16S rRNA gene sequencing in two groups: 40 GDM women with periodontitis, and 40 GDM women who were periodontally healthy. Our findings revealed significant alterations in the oral microbiota and predicted functional genes in pregnant women with GDM and periodontitis. These alterations suggest a potential link between oral microbiota dynamics and the concurrent presence of GDM and periodontitis, providing novel insights into the mechanisms underlying periodontitis in pregnancy and highlighting opportunities for its prevention and management.

In the current study, α-diversity and β-diversity analyses revealed that the oral microbial community in pregnant women with GDM

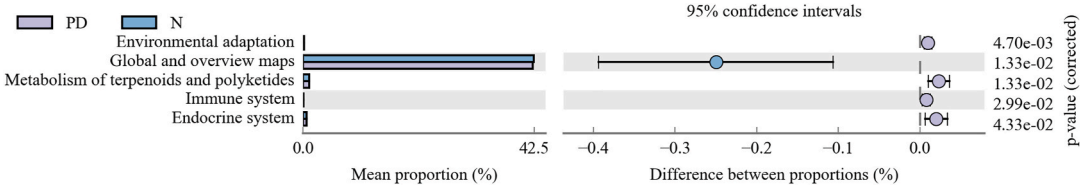

Fig. 6. The function prediction of the PD group and N group
It revealed that periodontitis alters the function of the oral microbial community.

belonging to the periodontitis group displayed significantly higher abundance and diversity than those in the periodontally healthy group. This finding is consistent with previous studies indicating an association between periodontitis and increased diversity and abundance of the oral microbial community [24,25,34–36]. However, Ye et al. found no significant differences in α-diversity and β-diversity between periodontitis and non-periodontitis pregnant women in their analysis of subgingival microbiota during mid-pregnancy [37], which contrasts with our findings. This discrepancy may be due to the different stages of pregnancy, as the composition of the oral microbiome may be altered by the changes in gestation [38,39]. The study by Zhang et al. reported that the microbial community richness was significantly lower in pregnant women with GDM and periodontitis in mid-pregnancy compared to those with GDM but without periodontitis [29]. Our findings differ from this study, and the discrepancy may be attributed to factors such as sample size, living environment, and dietary habits. Sample size has a direct effect on the stability and reliability of statistical analyses. Therefore, the larger sample size of our study may explain the discrepancy. In addition, the living environment and dietary habits of pregnant women vary by region, which could lead to regional differences in the composition and function of the oral microbiota.

At the phylum level, the periodontitis group showed significantly higher abundances of *Bacteroidota*, *Fusobacteriota*, *Spirochaetota, Synergistota*, and *Choroflexi* compared to the periodontally health group, which is consistent with previous studies [24,34]. For instance, Zhang et al. [29] performed 16S rRNA sequencing to analyze the subgingival microbiota in mid-pregnancy and also reported a significantly higher abundance of *Bacteroidetes*, *Spirochaetes*, *Tenericutes*, and *Synergistetes* in the periodontitis group compared to healthy controls. In addition, our results indicated an increased abundance of *Firmicutes*, *Actinobacteria,* and *Patescibacteria* in the GDM women without periodontitis. Abusleme et al. used 454 pyrosequencing to analyze samples from both periodontitis patients and healthy individuals, and identified a significant correlation between the *Actinobacteriota* phylum and oral health [25], supporting our findings. Although previous studies have often associated the *Firmicutes* phylum with periodontitis [34,36,40], our results provide an interesting contrast. OJ Park et al. observed that the *Firmicutes* phylum was prevalent in patients with gingivitis [41]. However, we observed an enrichment of *Firmicutes* in the periodontally healthy group. This difference may be due to the inclusion of individuals with gingivitis in our periodontally health control group, thus influencing the distribution of *Firmicutes*. Future studies should examine the microbiota of GDM women within the gingivitis and periodontitis categories. Identifying distinct microbial markers and mechanisms by comparing microbial composition between periodontal conditions will improve the understanding of periodontal disease in GDM.

At the genus level, we found significantly higher abundances of *Fusobacterium, Porphyromonas*, *Treponema*, *Aggregatibacter*, *Unclassified Prevotellaceae*, and *Filifactor* in the GDM with periodontitis group, while *Veillonella*, *Rothia*, *Leptotrichia*, *TM7x*, *Actinomyces*, *Granulicatella*, *Oribacterium*, and *Corynebacterium* were found in greater abundance in the periodontally healthy group, consistent with findings from previous studies in the general population [36,42,43]. For example, Tsai et al. found that the abundance of *Porphyromonas*, *Treponema*, *Aggregatibacter*, and *Filifactor* was significantly higher in the periodontitis group than in the healthy control group, whereas the abundance of *Actinobacteria* and *Corynebacterium* was significantly lower [43]. Ye et al. also reported increased *Porphyromonas*, *Treponema*, and *Filifactor* in pregnant women with periodontitis, while healthy women had more *Actinomyces* and *Corynebacterium* [37]. These findings highlight a strong association between periodontitis and the oral microbiota and emphasize the stability and variability of the microbiota in different populations and pathological conditions.

Our LEfSe analysis identified *Spirochaetora* as a key biomarker within the GDM cohort affected by periodontitis, consistent with the findings of Narita Y et al., who reported a significant increase in *Spirochaetora* abundance in periodontitis patients [44]. This supports the potential association between the *Spirochaetes* phylum and periodontitis, particularly in pregnant women with GDM. Periodontal pathogens are linked to inflammation and insulin resistance, which can worsen GDM. Interventions like probiotics or antimicrobial treatments targeting Spirochaetora could help reduce inflammation and improve blood sugar control in pregnant women with GDM. Future studies should investigate the role of *Spirochaetes* in the development of periodontitis and their potential influence on GDM and pregnancy outcomes. Such research could provide critical insights into the microbiological interactions between periodontitis and GDM, guiding the development of novel preventive and therapeutic strategies for pregnant women with GDM.

KEGG pathway analysis revealed that periodontitis alters the function of the oral microbial community. The increased activity of environmental adaptation pathways in the periodontitis group indicates a highly adaptive microbial community. Terpenes and polyketides, crucial secondary metabolites, play important roles in microbial defense, signaling, and host interactions [45]. Their increased metabolic activity may be detrimental to pathogen defense and anti-inflammatory responses in periodontitis. Furthermore, the increased activity in immune pathways in periodontitis highlights an inflammatory profile, suggesting that periodontitis-associated oral microbes may influence inflammation by modulating host immune responses. The significant activity in endocrine system pathways in the periodontitis group suggests a strong connection with systemic endocrine functions, underlining the association between periodontitis and overall health. Recent research has linked periodontitis not only to oral health, but also to systemic diseases such as diabetes and cardiovascular disease [1]. This study sheds light on the complex relationship between periodontitis and oral microbial metabolism, providing insights into the pathogenesis of periodontitis and potential new avenues for treatment.

This study has several limitations. Firstly, the absence of a control group consisting of pregnant women without GDM constrains our ability to understand the interplay between GDM and periodontitis comprehensively. Including such a group would provide valuable comparative information. Secondly, the inclusion of pregnant women with gingivitis in the periodontally healthy group may have influenced the results. Future studies could adopt a more refined grouping approach by categorizing participants into non-gingivitis, gingivitis, and periodontitis groups, which would improve the clarity and interpretability of the findings.

5. Conclusions

Our study has identified significant alterations in the oral microbiota of GDM women with periodontitis, providing novel insights into the mechanisms of GDM women with periodontitis. These findings provide valuable insights into the prevention and management of periodontitis during pregnancy. Future studies should include healthy pregnant women as controls and expanded sample sizes are needed to deepen our understanding of the interplay between oral microbiota, periodontitis, and GDM, and to help develop new strategies for prevention and treatment.

CRediT authorship contribution statement

Ru-Lin Liu: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Xiao-Qian Chen:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Jing Liu:** Methodology, Data curation. **Qing-Xiang Zheng:** Conceptualization. **Jia-Ning Li:** Data curation. **Yu Zhu:** Data curation. **Jia-Chen Liu:** Methodology. **Xiu-Min Jiang:** Conceptualization.

Patient consent

All participants provided informed consent.

Ethics approval

This study was approved by the Human Research Ethics Committee of Fujian Maternity and Child Health Hospital (approval no.2022YJ080).

Author declarations

The authors declare no conflict of interest.

Data sharing statement

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA008585) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human.

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2025.107607.

Data availability

I have share the link to the data.

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