Journal of Oral Microbiology

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/zjom20

Oral and gut microbial profiling in periodontitis and Parkinson's disease

Ekin Yay, Melis Yilmaz, Hilal Toygar, Nur Balci, Carla Alvarez Rivas, Basak Bolluk Kılıç, Ali Zirh, Bruce J. Paster & Alpdogan Kantarci

To cite this article: Ekin Yay, Melis Yilmaz, Hilal Toygar, Nur Balci, Carla Alvarez Rivas, Basak Bolluk Kılıç, Ali Zirh, Bruce J. Paster & Alpdogan Kantarci (2024) Oral and gut microbial profiling in periodontitis and Parkinson's disease, Journal of Oral Microbiology, 16:1, 2331264, DOI: 10.1080/20002297.2024.2331264

To link to this article: https://doi.org/10.1080/20002297.2024.2331264

JOURNAL OF ORAL MICROBIOLOGY
2024, VOL. 16, 2331264
https://doi.org/10.1080/20002297.2024.2331264

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Oral and gut microbial profiling in periodontitis and Parkinson's disease

Ekin Yay[a,b*], Melis Yilmaz[a,c*], Hilal Toygar[c], Nur Balci[c], Carla Alvarez Rivas[a,d], Basak Bolluk Kılıç[e], Ali Zirh[e], Bruce J. Paster[a,d] and Alpdogan Kantarci [a,d]

[a]Department of Applied Oral Sciences, The ADA Forsyth Institute, Cambridge, MA, USA; [b]Periodontist, Private Practice, Istanbul, Turkey; [c]Department of Periodontology, Istanbul Medipol University, Istanbul, Turkey; [d]Department of Oral Microbiology and Infection, Harvard School of Dental Medicine, Boston, MA, USA; [e]Department of Neurology, Istanbul Medipol University, Istanbul, Turkey

ABSTRACT

Objectives: We tested the hypothesis that Parkinson's disease (PA) alters the periodontitis-associated oral microbiome.

Method: Patients with periodontitis with Parkinson's disease (PA+P) and without PA (P) and systemically and periodontally healthy individuals (HC) were enrolled. Clinical, periodontal and neurological parameters were recorded. The severity of PA motor functions was measured. Unstimulated saliva samples and stool samples were collected. Next-generation sequencing of 16S ribosomal RNA (V1-V3 regions) was performed.

Results: PA patients had mild-to-moderate motor dysfunction and comparable plaque scores as those without, indicating that oral hygiene was efficient in the PA+P group. In saliva, there were statistically significant differences in beta diversity between HC and PA+P ($p = 0.001$), HC and P ($p = 0.001$), and P and PA+P ($p = 0.028$). The microbial profiles of saliva and fecal samples were distinct. Mycoplasma faucium, Tannerella forsythia, Parvimonas micra, and Saccharibacteria (TM7) were increased in P; Prevotella pallens, Prevotella melaninogenica, Neisseria multispecies were more abundant in PA+P group, Ruthenibacterium lactatiformans, Dialister succinatiphilus, Butyrivibrio crossotus and Alloprevotella tannerae were detected in fecal samples in P groups compared to healthy controls.

Conclusions: No significant differences were detected between Parkinson's and non-Parkinson's gut microbiomes, suggesting that Parkinson's disease modifies the oral microbiome in periodontitis subjects independent of the gut microbiome.

ARTICLE HISTORY
Received 16 January 2024
Revised 26 February 2024
Accepted 11 March 2024

KEYWORDS
Periodontitis; Parkinson's disease; microbiome; next-generation sequencing; pathogenesis

Introduction

Parkinson's disease (PA) is a progressive neurodegenerative disorder primarily seen with motor and non-motor features [1]. The pathological hallmark of Parkinson's disease is the accumulation of alpha-synuclein (α-synuclein) protein in the brain [2]. The disease is characterized by the loss of dopaminergic neurons and consequent diminished motor function of the basal ganglia, both leading to clinical features [3]. With the progression of the disease, symptoms such as tremors, muscle rigidity, bradykinesia, and postural instability occur [4]. Parkinson's disease is a multifactorial disease with risk factors such as age, genetic features, and gender. Consumption of dairy products, exposure to pesticides, history of traumatic brain injury, methamphetamine, and melanoma increase the risk of Parkinson's disease [5].

While Parkinson's disease is primarily considered a neurodegenerative disorder, recent studies have suggested that the microbiome plays a role in influencing the disease onset. The gut-brain axis has been the focus of the microbiology of Parkinson's disease. The dysbiosis in the gut microbiome results in increased mucosal permeability, oxidative stress, inflammatory reactions, and aggregation of α-synuclein in the enteric nervous system (ENS) [6]. Through the vagal nerve, α-synuclein pathology is suggested to spread from the ENS to the central nervous system [7]. The dysbiotic gut microbiome could also induce a chronic inflammation that triggers an immune response, which is attributed to the severity of Parkinson's disease [8].

Periodontitis (P) is a multifactorial chronic inflammatory disease with various contributing factors, including the oral microbiome [9]. In individuals with periodontitis, there is a shift in the composition of the oral microbiome towards a dysbiotic state, marked by an overabundance of pathogenic bacterial species such as *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* [10]. The oral microbiome harbors the most comprehensive and highest bacterial diversity in the human body after

CONTACT Alpdogan Kantarci akantarci@forsyth.org Department of Applied Oral Sciences, ADA Forsyth Institute, 245 First Street #6103, Cambridge, MA 02142, USA
*These authors contributed equally to this work.
Supplemental data for this article can be accessed online at https://doi.org/10.1080/20002297.2024.2331264

the gut microbiome. Besides the other surfaces in the oral cavity, saliva contains its microbiome, consisting of a diverse array of bacteria, viruses, fungi, archaea, and protozoa [11].

Inflammation and oral pathogens associated with periodontitis contribute to the development or exacerbation of several systemic conditions, such as cardiovascular disease, diabetes, respiratory diseases, rheumatoid arthritis, and Alzheimer's disease [12]. Another neurodegenerative disorder, Alzheimer's disease, is linked to periodontitis via inflammatory pathways as well as bacterial pathogens. *P. gingivalis* and the virulence factors of oral pathogens potentially migrate to the brain through systemic circulation or the vagal nerve and contribute to neuroinflammation or amyloid plaque formation, hallmarks of Alzheimer's disease [13]. Although poor oral health, worsening of periodontal health, or tooth loss were linked with Parkinson's disease [14,15], there is limited research on the mechanisms of the association between Parkinson's disease and periodontitis.

In a recent study, we reported an inflammatory relationship between Parkinson's disease and periodontitis, showing increased levels of Parkinson's disease-related inflammatory markers [16]. Some studies have explored the potential links between gut microbiome alterations and Parkinson's disease development or progression [17]. We recently analyzed the levels of 40 bacterial species of subgingival plaque samples from Parkinson's disease patients with periodontitis and demonstrated a significant alteration in the Parkinson's patients' oral microbiome [18]. In a recent study where oral and intestinal microbiota were evaluated in Parkinson's patients *(Jo S, Kang W, Hwang YS, et al. Oral and gut dysbiosis leads to functional alterations in Parkinson's disease. NPJ Parkinsons Dis. 7 July 2022;8(1):87.)*, the impact of periodontitis was not evaluated. Our study was focused on elucidating the impact of Parkinson's disease on the oral and gut microbiome in patients with periodontitis. Since Parkinson's disease is a neurodegenerative disorder that alters the gut microbiome due to the local inflammatory response, we tested the hypothesis that Parkinson's disease alters the periodontitis-associated oral microbiome compared to the gut microbiome.

Materials and methods

Study population

The study was approved by the human subject ethics board (date: 27 November 2019; Number: 1012) for use and access of human subjects in research following the Helsinki Declaration of 1975, as revised in 2013. Healthy individuals ($n = 17$) and patients diagnosed with Stage III, Grade B periodontitis [9] ($n = 18$) were enrolled. All individuals gave oral informed consent. The United Kingdom Parkinson's Disease Society Brain Bank criteria were utilized for diagnosing Parkinson's disease [19]. For the Parkinson's disease group, participants referred from the University Parkinson's Disease and Movement Disorders Center and who had Stage III, Grade B periodontitis ($n = 16$) were included in the study. All patients with Parkinson's disease underwent an assessment conducted by a neurologist experienced in movement disorders. Information regarding the duration and pharmacological treatment of Parkinson's disease was documented. Patients with Parkinson's disease included in this study did not have a diagnosis of Parkinson's Plus Syndrome and had undergone deep brain stimulation therapy at least 4 months ago. The evaluation of motor function severity in Parkinson's disease patients was performed using the Unified Parkinson's Disease Rating Scale (UPDRS) – part III [20]. The disease stage was assessed using the Hoehn and Yahr scale (H&Y) [21]. UPDRS and H&Y assessments were conducted by an experienced neurologist.

The general exclusion criteria were as follows: being over 75 years of age and younger than 18 years of age, use of antibiotics and/or anti-inflammatory, nonsteroidal anti-inflammatory drugs, steroids, smoking, excessive alcohol use, immunosuppressants, beta-blockers, calcium channel blockers, anticoagulants, and hormonal contraceptives within 3 months preceding the study; nonsurgical periodontal treatment during the previous 6 months, surgical periodontal treatment during the previous 12 months, having less than 20 natural teeth (excluding third molars), and having a systemic disease additional to Parkinson's disease.

Clinical periodontal parameters

The periodontal disease status was diagnosed based on the most recent classification of periodontal and peri-implant diseases and conditions [9]. Probing pocket depth (PPD), bleeding on probing (BOP), gingival recession (GR), clinical attachment loss (CAL), and plaque index (PI) were recorded. Two periodontists calibrated the measurements on 10 non-study volunteers before the study [22]. The probing depth values demonstrated good reproducibility, assessed by inter-examiner analysis ($\kappa = 0.892$) before the study. The reproducibility assessment showed that the mean of repeated probing measurements was within 1 mm for 90% of the sites.

Saliva and stool sample collection

To minimize the impact of circadian rhythm, saliva samples were collected in the morning between 8–11 am, following an overnight fast. Initially, the patients were instructed to rinse their mouths with distilled water thoroughly. They were told to sit comfortably and spit into the plastic tubes five times for a minute for ten minutes. Following a 10-minute centrifugation at 2800×g, the samples were placed in Eppendorf tubes and kept at −80◦C until the analysis [23].

The stool samples were collected using the CanvaxBiotech Stool Sample Collection & Stabilization Kit (Cordoba, Spain). Initially, samples were kept at room temperature, then transferred into Eppendorf tubes and kept at −80◦C.

Next-generation sequencing

To characterize the entire subgingival microbiome in both patient and healthy control groups, DNA extraction was performed following the manufacturer's instructions (MasterPureTM DNA Purification Kit, Epicentre, Madison, WI, USA). In summary, 1 µL of Ready-Lyse Lysozyme solution was added to each sample and incubated overnight. Subsequently, when mixed with 1 µL Proteinase K, the samples underwent a 30-minute incubation period to complete the lysis process. After purification, any remaining DNA was resuspended in 25 µL of TE Buffer. The purity and concentration of the DNA were assessed using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). The nucleic acids were stored at −80°C until further utilization. Metagenomic analysis focused on the 16S ribosomal RNA gene (16S rRNA). DNA extracted from subgingival plaque samples was sequenced using Next-generation sequencing of the V1V3 region of the 16S rRNA gene (ZymoBIOMICS® targeted metagenomic sequencing-Zymo Research, Irvine, CA). After evaluating the quality of the samples, samples were prepared for sequencing. Next-generation sequencing was performed, followed by a quality assessment of the obtained sequences. Absolute abundances were recorded. Bioinformatic analyses were performed by comparing saliva and fecal samples among the HC, P, and PA+P groups and between saliva samples from healthy, periodontitis, and PA+P groups and their related fecal samples. Additionally, whole saliva and fecal sample comparisons were made.

Statistical analysis

All statistical analyses were conducted using GraphPad Prism 9.4.0 software (GraphPad Software, Inc., San Diego, CA, USA). The data distribution was assessed using the Kolmogorov-Smirnov test. For normally distributed data, one-way ANOVA was used, while the Kruskal-Wallis test was utilized for not-normally distributed data. Bioinformatic analyses were performed for the NGS results. Differences were assessed at the operational taxonomic units (OTUs), genus, and family levels. Alpha and beta diversity analyses were performed to illustrate group variations and similarities. Differential abundance among the three groups was assessed using ANCOM-BC (Analysis of Compositions of Microbiomes with Bias Correction) and LefSe (Linear Discriminant Analysis Effect Size) analysis. The criterion for statistical significance was $p < 0.05$.

Results

Demographic and clinical parameters – Parkinson's severity results

The PA+P group consisted of 3 females and 13 males; the P group included 8 females and 10 males, and the healthy group had 7 females and 10 males (Table 1). Clinical periodontal parameters (PPD, BOP, GR, CAL, and PI) are presented in Table 1. Probing pocket depth and clinical attachment levels were higher in the P and PA+PD groups than in the HC group ($p < 0.0001$). BOP showed a statistically significant increase in the PA+P and P groups compared to HC. The oral hygiene habits among the participants

Table 1. Demographic and clinic periodontal parameters of the study groups. Data are shown as mean ± standard deviation. Kolmogorov-Smirnov test was performed for normality check. Differences between groups were determined using the one-way ANOVA and Kruskal-Wallis tests. A value of $p < 0.05$ was considered significant.

Demographic and Clinical Parameters	HC $n = 17$	P $n = 18$	PA+P $n = 16$	*p-value*
Age[#,†]	31.47 ± 7.1	39.11 ± 9.7	55.56 ± 10.1	<0.0001
Gender (M/F)[#,†]	10/7	10/8	13/3	0.0215
PPD (mm)[*,#]	1.60 ± 0.22	2.82 ± 0.65	2.68 ± 0.43	<0.0001
BOP (%)[*,#]	7.27 ± 4.75	48.44 ± 18.84	62.68 ± 26	<0.0001
GR (mm)[*,#]	0.1 ± 0.36	0.28 ± 0.21	0.53 ± 0.6	<0.0001
CAL (mm)[*,#]	1.7 ± 0.42	3.1 ± 0.7	3.2 ± 0.85	<0.0001
PI[*,#]	0.61 ± 0.37	1.5 ± 0.36	1.8 ± 0.47	<0.0001

*Statistically significant difference between HC and P ($p < 0.05$), # Statistically significant difference between HC and PA+P ($p < 0.05$), †Statistically significant difference between P and PA+P ($p < 0.05$), PPD: probing pocket depth, BOP: bleeding on probing, CAL: clinical attachment level, PI: plaque index, GR: gingival recession.

Table 2. Beta-diversity correlation coefficient matrix results of saliva samples.

Group 1	Group 2	Sample size	Permutations	*pseudo-F*	*p-value*	*q-value*
Healthy Control	Parkinson + Periodontitis	33	999	6.474797	0.003	0.006
	Periodontitis	35	999	1.397011	0.204	0.204
Parkinson + Periodontitis	Periodontitis	34	999	4.642107	0.004	0.006

were as follows: In the control group, all individuals brushed their teeth at least twice a day. In the PA+P group, 50% of individuals brushed their teeth at least twice daily, while this rate decreased to 16% in the P group. In the control group, the rate of not performing interdental cleaning was 11%, whereas in the P group, it increased to 88%, and in the PA+P group, it reached 93%. According to the Hoehn and Yahr scale, 5 patients (31.25%) were diagnosed with Stage 1, whereas 11 patients (68.75%) were with Stage 2 Parkinson's disease. The mean UPDRS Part III score was 18.8 ± 6.1. This outcome and the better oral care in PA+P patients compared to P patients indicated that individuals with Parkinson's disease exhibited mild to moderate motor impairment, suggesting that manual dexterity was not a confounding factor for oral hygiene. Anti-parkinsonian drugs used by Parkinson's patients were as follows: levodopa (n: 16), rasagiline (n: 9), pramipexole (n: 5), amantadine (n: 4), pribedil (n: 2), and apomorphine (n: 1).

NGS of oral and gut microbiome

A total of 699 species were detected with next-generation sequencing in all samples (Supplementary File 1). There were significant differences in the beta-diversity analysis of saliva between HC and P ($p = 0.01$), HC and PA+P ($p = 0.001$), and PA+P and P ($p = 0.017$) groups (Figure 1(a)). There were also significant differences in comparing healthy controls with periodontitis subjects with ($p = 0.008$) or without ($p = 0.008$) Parkinson's. However, no significant differences were detected between Parkinson's and non-Parkinson's gut flora in the Bray-Curtis dissimilarity metric ($p = 0.974$) (Figure 1(b)). Table 2 represents beta-diversity correlation coefficient matrix results of salivary microbiomes.In saliva, there was a significant difference between HC and PA+P ($p = 0.0142$) groups regarding alpha diversity Figure 2(a). Figures 2(b,d) present group significance plots of the beta-diversity correlation coefficient matrix in saliva and gut, respectively. The gut microbiome significantly differed when PA+P and HC groups were compared with alpha diversity measurements ($p = 0.0355$) (Figure 2(c)).

As expected, the saliva and gut communities were distinct. Figure 3 presents major differences between fecal and saliva samples. A heatmap including both environments presents family-level comparisons of relative abundances (Figure 3(c)). *Streptococcus spp.*, *Saccharibacteria_(TM7)_[G-1] bacterium HMT352* and *Saccharibacteria_(TM7)_[G-3] bacterium HMT351* were predominantly abundant in oral microbiome. Regarding relative abundances, *Faecalibacterium prausnitzii*, and *Subdoligranulum variabile* were the most dominant species in the gut microbiome of all groups (Supplementary File 3).

According to the LefSe, there was a significant difference in relative abundance in species level in saliva samples (Supplementary File 2). The species-based analysis can be found at the link to our NGS results. In summary, class *Bacilli*, order *Lactobacillales*, family *Streptococcaceae*, and *Streptococcus multispecies spp11_20* were increasing in salivary microbiome while *Erysipelotrichaceae Breznakia*, phylum *Elusimicrobia*, family *Elusimicrobiaceae*, and *Elusimicrobium minutum* were decreasing in gut microbiome. In saliva, we detected increasing abundances of *Rothia dentocariosa* and *Gamella sanguinis* in HC group; *Mycoplasma faucium (M. faucium), Tannerella forsythia (T. forsythia), Parvimonas micra (P. micra)* and *Saccharibacteria (TM7)* subspecies were increased in P group; *Prevotella pallens (P. pallens), Prevotella melaninogenica (P. melaninogenica)* and *Neisseria* multispecies were more abundant in PA+P group, all compared to other groups (Figure 4). Figure 5 shows the scatter plots of highlighted salivary bacteria. No statistically significant features were found when PA+P and P groups were compared. Relative abundances of *Saccharibacteria bacterium TM7 (HMT 346), (HMT349), (HMT356), (HMT355)* in saliva samples are shown in Figure 6(a) and scatter plots of those bacterial taxa in Figure 6(b).

According to the ANCOM analysis, the differential abundance of *Parabacteroides distasonis* in HC and *Ruthenibacterium lactatiformans* in P groups were detected in fecal samples compared to each other. *Dialister succinatiphilus* was detected in fecal samples in P groups compared to healthy controls. Additionally, a higher abundance of *Butyrivibrio crossotus* and *Alloprevotella tannerae* were found in the PA+P and P groups compared to the control group (Figure 7(a)). Figure 7(b) represents scatter plots of those selected bacteria. There were no significant differences in differential abundance in the gut microbiome profiles of the study groups.

Discussion

We have recently shown that Parkinson's disease alters the subgingival microbiome and increases the

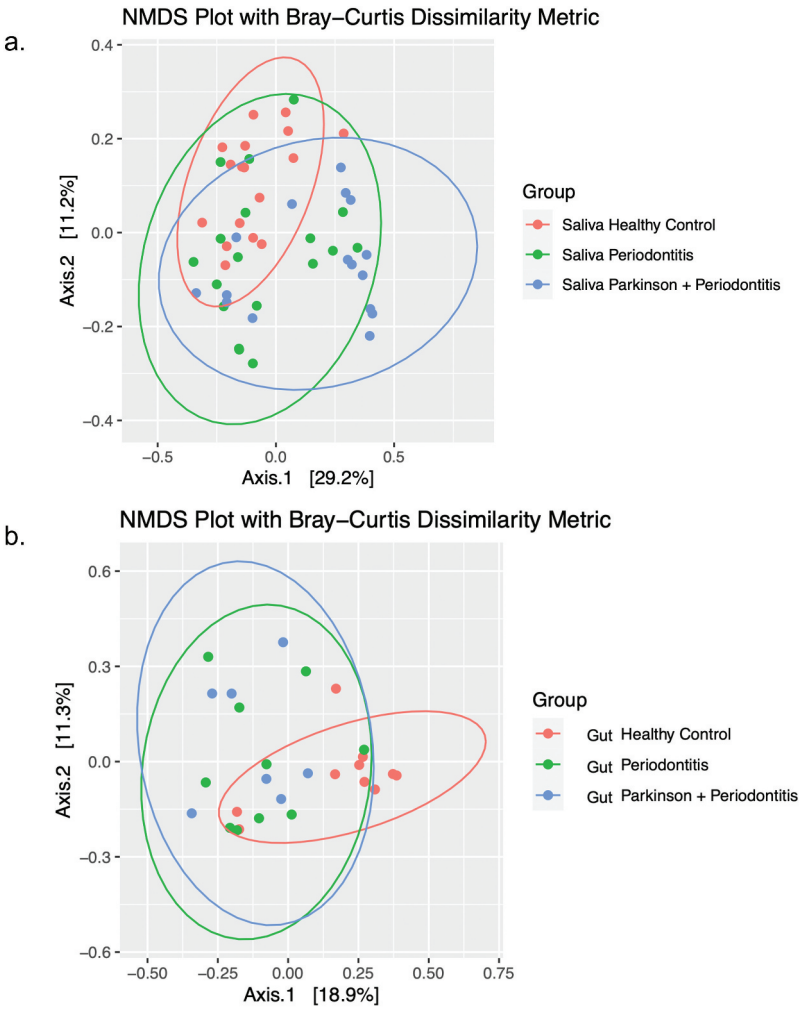

Figure 1. NMDS plots with bray-curtis dissimilarity metric. In our beta diversity analysis, the bray-curtis dissimilarity matrix was first calculated and then plotted separately by the PCoA and NMDS. These are beta diversity results for (a). saliva samples of three groups and (b). gut samples of the study groups.

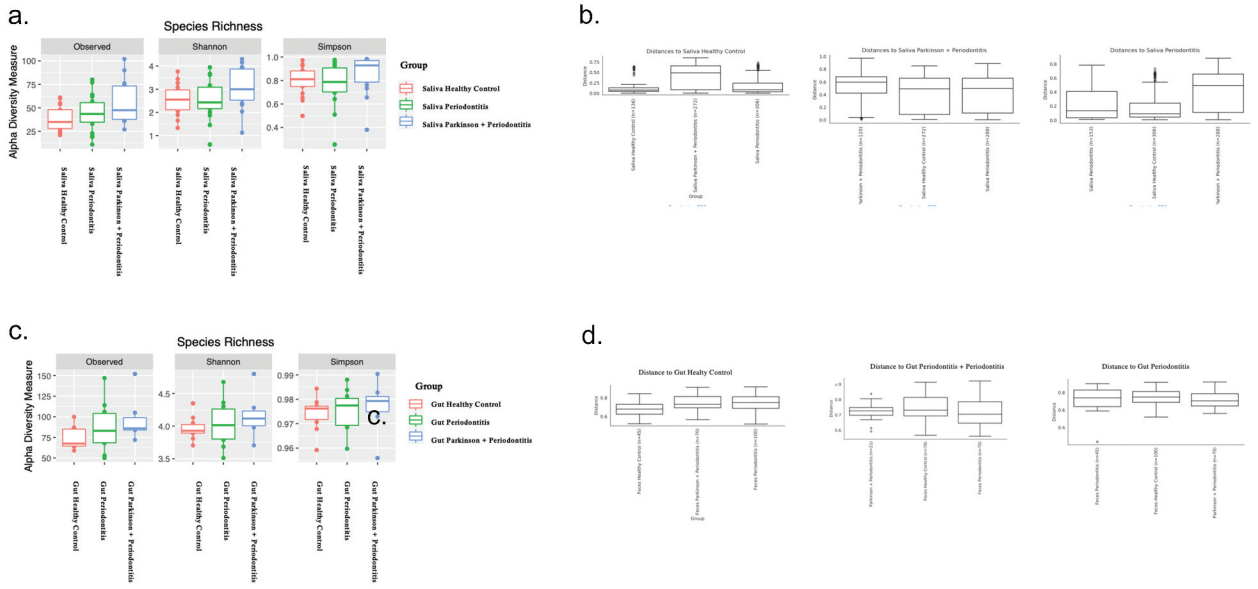

Figure 2. Species richness and distance plots. (a). alpha-diversity results of saliva samples. (b). group significance plots of beta-diversity correlation coefficient matrix in saliva samples. (c). alpha-diversity results of fecal samples. (d). group significance plots of beta-diversity correlation coefficient matrix in gut microbiome.

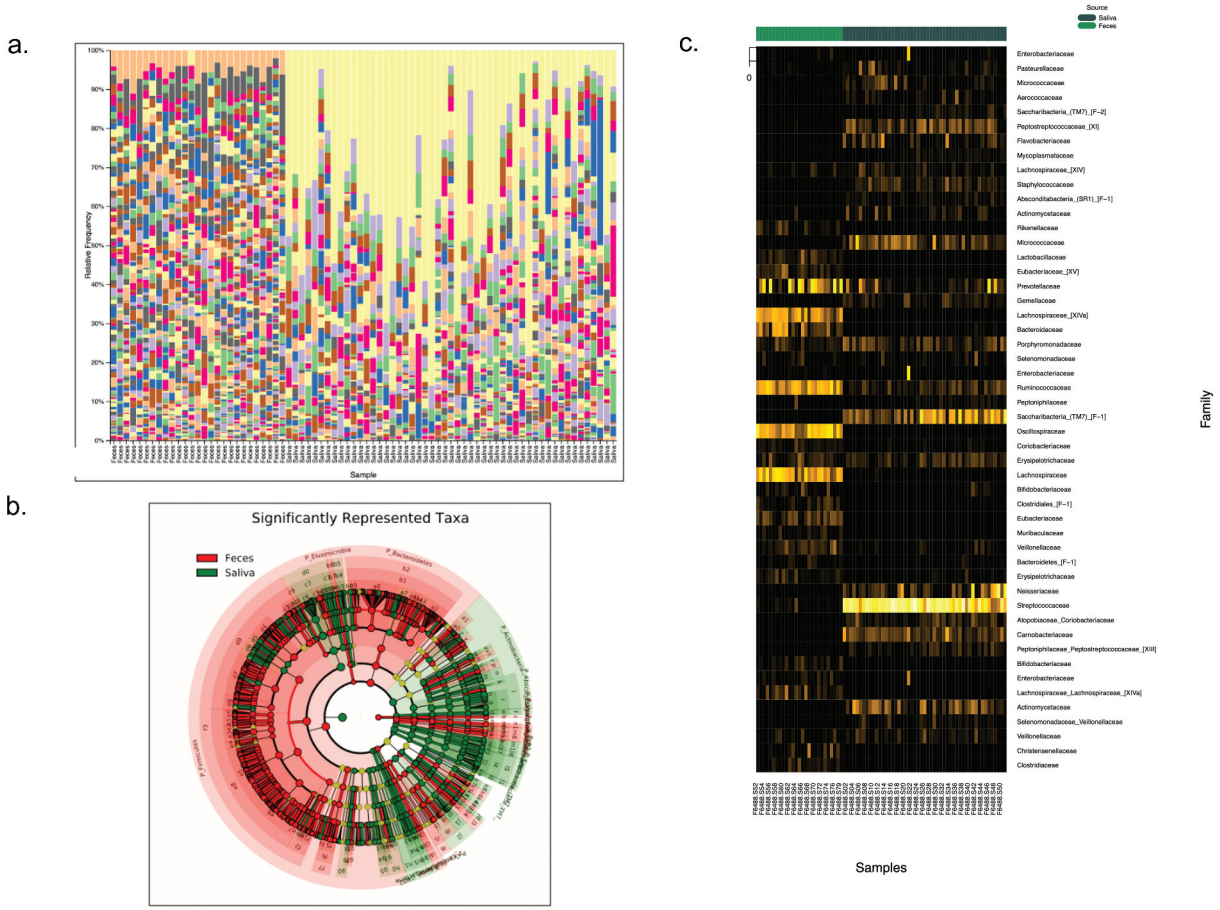

Figure 3. The species-level comparisons of fecal and saliva samples. (a). bar plots showing the difference in all of the samples. (b). LefSe cladogram representing the microbial differences in species level. (c). heatmap illustrates the family-level difference between two distinct communities.

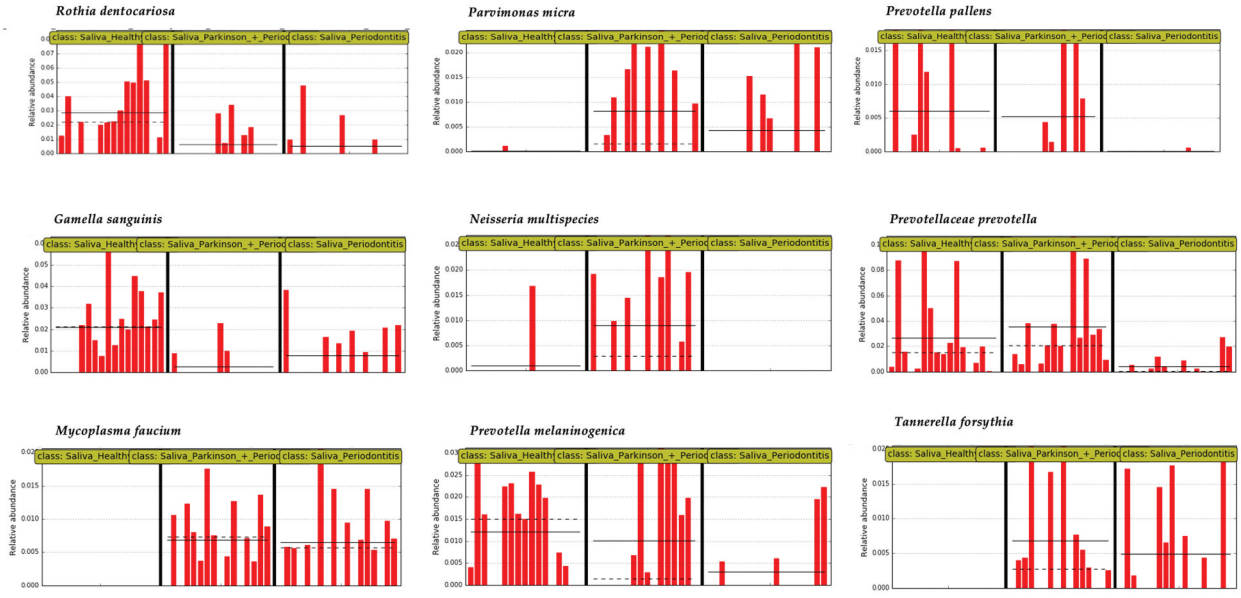

Figure 4. Abundance levels of nine salivary species in healthy control, Parkinson's and non-Parkinson's groups. LefSe differential abundance analysis was performed.

periodontal inflammatory burden [16,18]. However, the systemic relationship between the oral and intestinal microbiota has not been examined. Thus, we analyzed the saliva and stool samples of periodontitis patients with and without Parkinson's disease to test the hypothesis that Parkinson's disease alters the microbiome of these environments. We used next-generation sequencing to collect

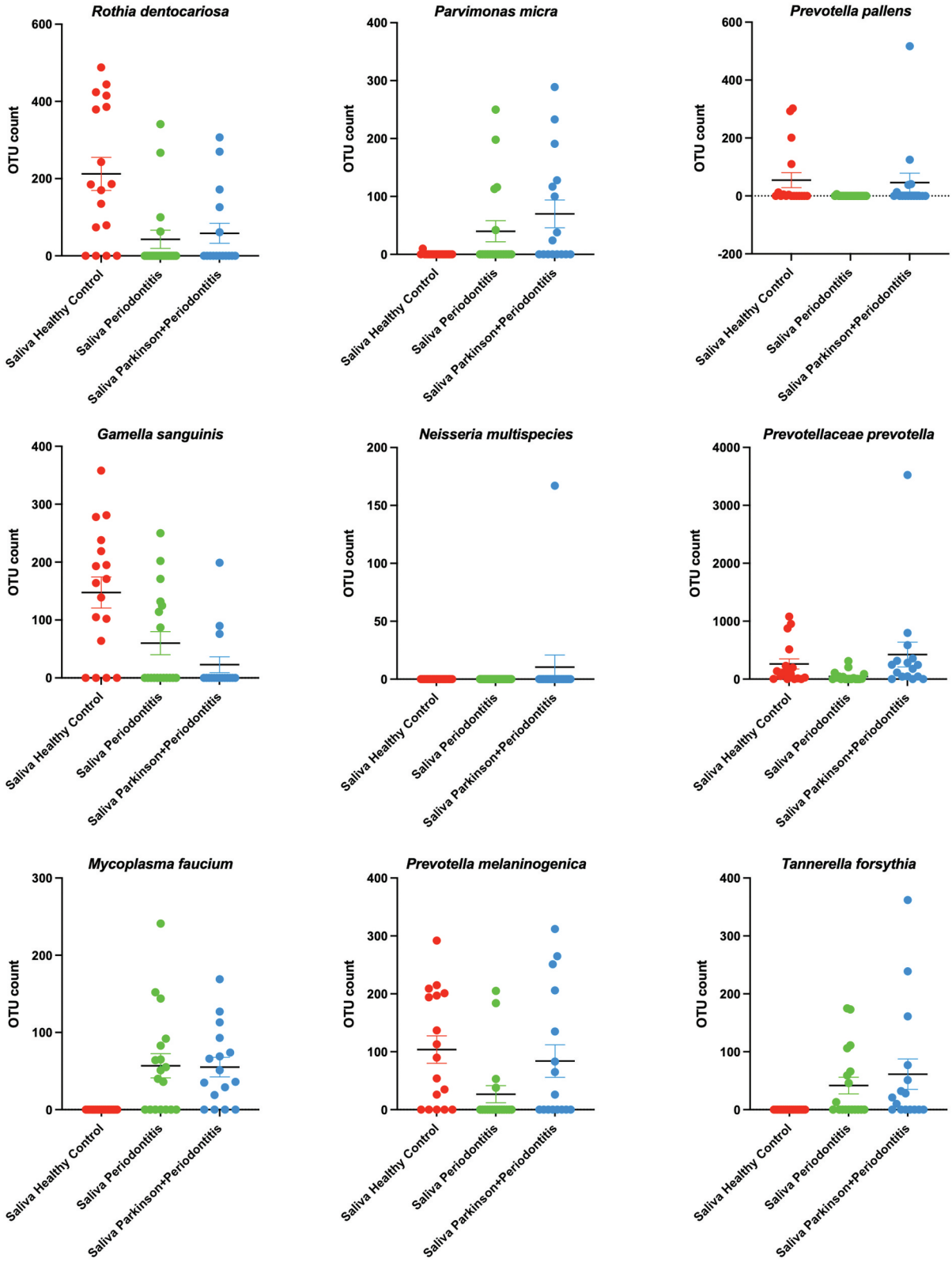

Figure 5. Scatter plots of the nine selected species in saliva.

information about microbial diversity, the interaction of species in family, genera, and species level, and co-occurrence or co-exclusion between microbial species among different samples. The data demonstrated that the oral microbiome in periodontitis was significantly changed with the impact of Parkinson's disease, differently from the gut microbiome. We also detected significantly diverse microbial profiles of saliva and stool samples, indicating that the microbiomes of the two environments originate disparately.

Our results showed a significant difference in gender in the PA+P group compared to the P and HC groups. Considering that Parkinson's disease affects

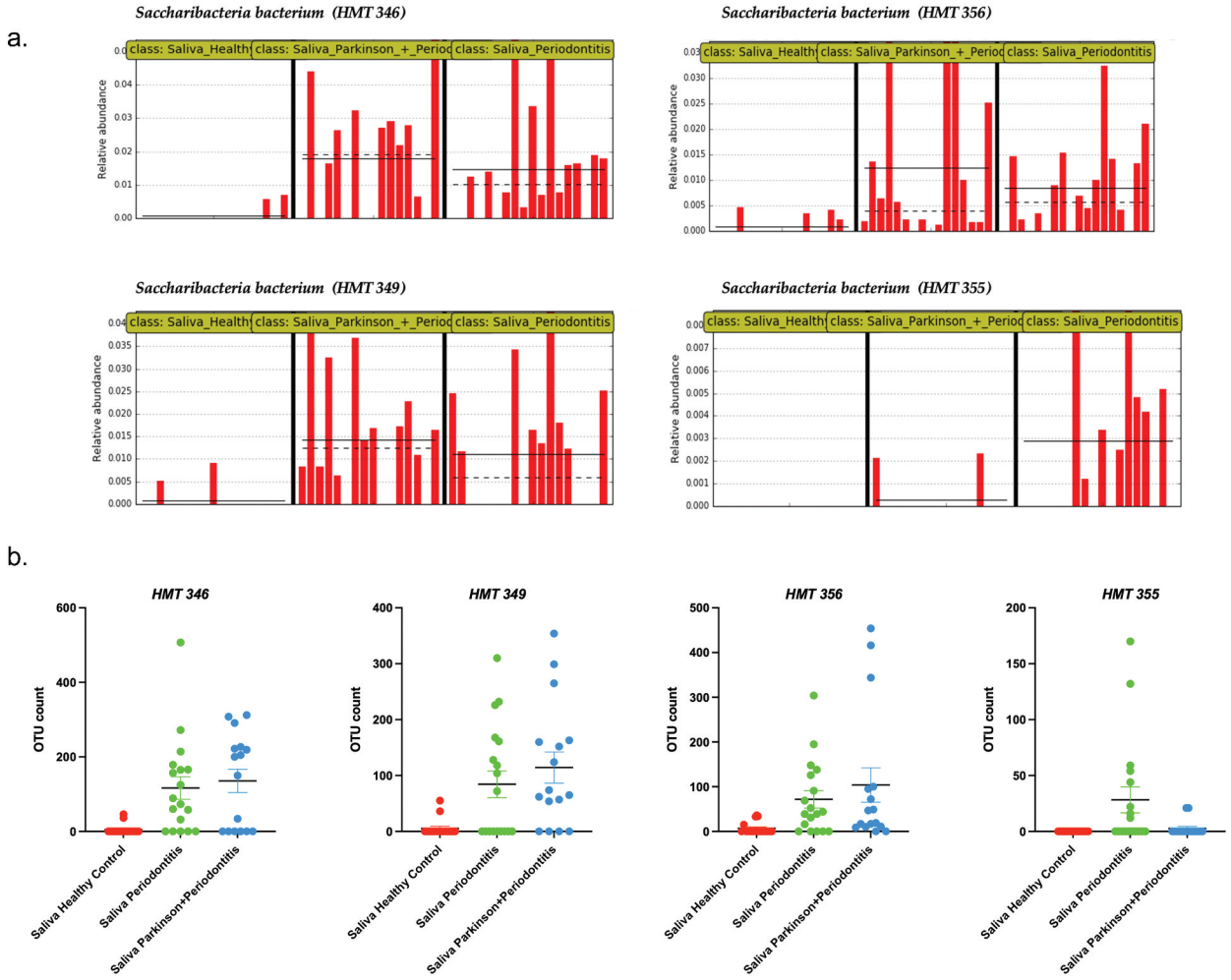

Figure 6. Abundance levels of four salivary species in all study groups. (a). relative abundances of *Saccharibacteria bacterium TM7 (HMT 346), (HMT349), (HMT356), (HMT355)* in saliva samples and (b). their scatter plot diagrams.

male individuals more than females [4], this result was expected [16]. Similarly, our results revealed a statistically higher average age in the PA+P group, consistent with previous studies that have reported an age-related increase in the prevalence of PA [4,16,24]. The UPDRS and H&Y scales used to evaluate the severity of Parkinson's disease showed that our study group was in the early stages and the motor dysfunction was not advanced. BOP, which indicates gingival inflammation, was higher in the PA+P group than in the P group, which may be linked to the fact that our PA group was not in an advanced stage and their oral hygiene practices were intact [16].

Many related symptoms of Parkinson's disease, including gastrointestinal problems, gender imbalance, and the wide range of drugs, may have an impact on the gut microbiome. In a study where the effects of anti-parkinsonian drugs on gut microbiome were studied, it was found that these drugs caused alterations in the gut microbiome of Parkinson's disease patients. The authors reported elevated levels of *Akkermansia, Lactobacillus*, and *Bifidobacterium* and reduced levels of *Lachnospiraceae* in the gut microbiome in patients with Parkinson's disease and suggested that such modifications were associated with the use of anti-parkinsonian drugs. However, they were unable to differentiate between different drugs since 90% of the study group were taking carbidopa/levodopa [25]. According to the available literature, the gut microbiome of Parkinson's disease patients may change and become more pathogenic due to the use of catechol-O-methyltransferase inhibitors and anticholinergic medications; however, similar effects were not observed with levodopa, dopamine agonists, monoamine oxidase inhibitors, or amantadine [26]. No such correlations were reported about the oral microbiome. Our study found a higher abundance of *Butyrivibrio crossotus* and *Alloprevotella tannerae* in the PA+P group compared to the control group, a highly novel observation suggesting the impact of periodontal disease. These results also may suggest an impact of anti-parkinsonian drugs on the gut microbiota in Parkinson's disease, where various medications are used.

We worked with saliva samples to provide microbial information about the oral cavity. The analysis of the salivary microbiome has gained attention as a non-

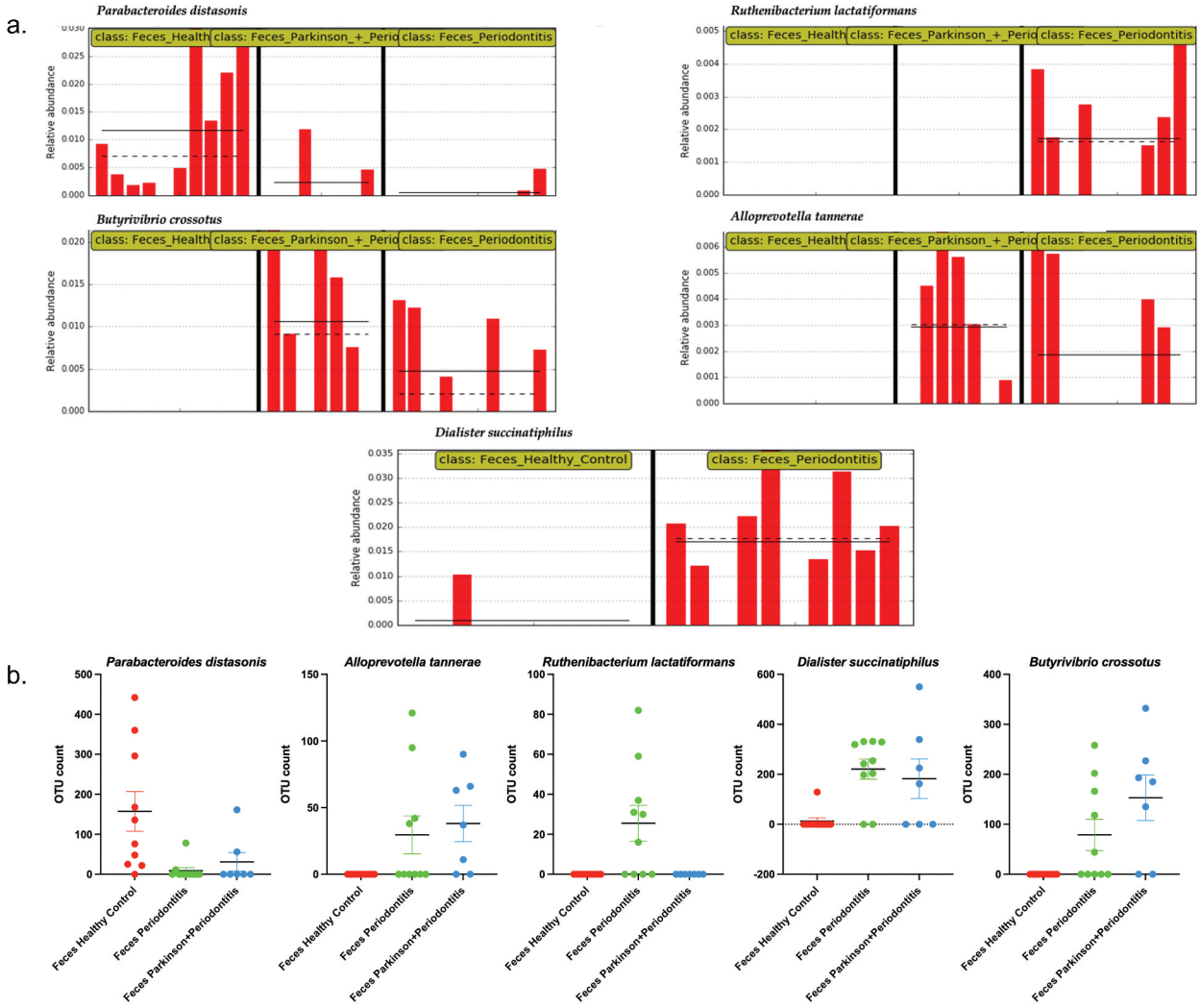

Figure 7. Abundance levels of five different gut microbiome species in healthy control, Parkinson's and non-Parkinson's groups. (a). Relative abundances of *parabacteriodes distasonis, Ruthenibacterium lactatiformans, butyrivibrio crossotus, alloprevotella tannerae, and dialister succinatiphilus* that were detected in stool samples. LefSe differential abundance analysis was performed. (b). scatter plots of five species from the gut microbiome.

invasive method for assessing oral and systemic health [27]. Changes in the salivary microbiome have been linked to periodontal disease, dental caries, and systemic diseases like diabetes and cardiovascular disease [28]. Thus, salivary changes can shed light on the early detection of specific microbial changes in the oral cavity, which helps diagnose systemic diseases such as Parkinson's disease. Parkinson's disease caused modifications in the oral microbiome of periodontitis patients in line with previous studies that demonstrated microbial changes in the salivary oral microbiome of Parkinson's patients [29,30]. Since the Parkinson's disease patients in our study had motor functions that enabled them to apply daily oral hygiene, the current data supported that the oral microbiome changes were not related to the periodontal status but rather a consequence of Parkinson's disease-related pathogenesis.

The bacterial taxa found in higher abundance in healthy subjects in this study were health-related species. *Rothia dentocariosa*, a Gram-positive coccal-to-rod shape bacterial species that is a part of the common member of the oral microbiome [31], and *Gemella sanguinis*, which is a Gram-positive facultative anaerobic bacterium [32], was found in higher abundances in HC. We also demonstrated consistent results with the periodontitis group with *M. faucium*, *P.micra*, *T. forsythia*[11], and *Saccharibacteria* species (*HMT 346, HMT 349, HMT 356*, and *HMT 355)* being higher in PA+P and P groups and at the minimum levels in HC [33]. *P. melaninogenica* was found higher in the PA+P group compared to P, which aligns with the findings in the subgingival plaque of PA patients [18]. Current results also revealed higher *Prevotella pallens* and *Neisseria* in the saliva of the PA group. *P. pallens*, commonly found in the oral cavity, is a Gram-negative bacterium [34]. Other *Prevotella* species are associated with conditions ranging from periodontal disease to cariotic lesions and endodontic infections [35]. Detecting higher levels of the family *Prevotellaceae* in our previous and current studies might suggest a role in Parkinson's disease-associated periodontitis.

Recently, there has been strong evidence that dysbiotic changes in the gut may impact various diseases, such as rheumatological diseases [36], stroke [37], and Alzheimer's disease [38], through the brain-gut axis. Dysbiotic gut microbiota leads to local inflammation, stimulating peripheral inflammation through the enteric nervous and circulatory systems, which disrupts the blood-brain barrier, resulting in neuroinflammation. Considering that alpha-synuclein aggregates, a key hallmark of the disease, are detected in the ENS earlier than in the *Substantia nigra*, and non-motor symptoms precede motor symptoms in the course of the disease, a detailed examination of gut microbiota may be significant for the early diagnosis of the disease [39]. In our fecal samples, higher abundances of *Butyrivibrio crossotus* were found in the PA+P and P groups compared to the control. *Butyrivibrio* belongs to the *Lachnospiraceae* family, producing short-chain fatty acids (SCFA), which are crucial in maintaining intestinal homeostasis [40]. An increase in *Butyrivibrio* was observed in the stool samples of Parkinson's disease patients compared to healthy individuals [41]. At the same time, decreased levels were reported in Parkinson's patients [42]. Our results revealed a higher presence of *Butyrivibrio crossotus* in the gut microbiota of individuals with periodontal disease than in healthy individuals. The higher presence of these bacteria in the gut compared to healthy individuals in disease conditions suggests an effort to regulate intestinal balance by producing SCFAs.

In an animal study, the oral administration of *P. gingivalis*, a keystone pathogen for periodontal disease, resulted in alterations in the gut microbiota with an increase in the amount of endotoxin in the serum and a decrease in the amount of tjp-1 and occlusion, which may all play a role in intestinal permeability [43]. Thus, periodontitis may cause dysbiosis in the intestinal microbiota [44]. The difference in gut microbiome between our healthy and the study groups can be easily explained by the impact of periodontitis on the gut microbiome independent of Parkinson's disease (Figure 1(b)). The Gram-negative anaerobic bacterium *Dialister succinatiphilus* has been reported to be isolated from human feces; however, its clinical significance is not fully understood [45]. In the fecal samples of individuals with autism spectrum disorder and obesity, increased species of *Dialister succinatiphilus* were reported [46]. In our results, the high prevalence of *Dialister succinatiphilus* was detected in the fecal samples of individuals with periodontitis. Meanwhile, *Ruthenibacterium lactatiformans (R. lactatiformans)*, a Gram-negative and lactate-producing bacterium [47], was higher in individuals with periodontal disease compared to other groups. This species is thought to contribute to the progression of the disease in multiple sclerosis patients by triggering mitochondrial dysfunction [48], where elevated abundances were reported in the gut microbiota of individuals with major depressive disorder [49]. Considering the impact of mitochondrial dysfunction on the pathogenesis of Parkinson's disease, our results may suggest a role for *R. lactatiformans* in the bidirectional mechanism between the two diseases. *Parabacteroides distasonis (P. distasonis)* is a Gram-negative, anaerobic member of the healthy gut microbiota. It has been reported that the abundance of *P. distasonis* decreases in various diseases such as obesity, inflammatory bone disease, and multiple sclerosis [50]. In an animal study, components of the commensal bacterium *P. distasonis* were used to treat mice with colitis, decreasing intestinal inflammation [51]. We found higher levels of *P. distasonis* in the fecal samples of healthy participants, which is consistent with the literature.

Our study is the first to comprehensively assess the microbiological basis of the brain-gut-mouth axis in Parkinson's disease patients with periodontal disease. Our results did not reveal a connection between these microbiotas; however, given that the microbiota may change daily and the potential effects of various medications, a more dynamic relationship may be possible. Our study was cross-sectional; therefore, we cannot show the progression of the disease process and make any causal inferences. Another limitation was a restricted sample size due to the rigorous inclusion and matching standards between study groups. However, full microbiome sequencing is costly and generates extensive data for bioinformatic analyses. Our data enabled clear comparisons between groups, which need to be validated in larger and independent cohorts.

Conclusion

Our findings demonstrate that Parkinson's disease impacts the oral microbiome in periodontitis independent of the gut microbiome. Specific and distinct oral and gut microbial species were associated with Parkinson's disease.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the no funding [R01AG062496].

ORCID

Alpdogan Kantarci http://orcid.org/0000-0002-2679-9100

References

[1] Stoker TB, Greenland JC. Parkinson's Disease: Pathogenesis and Clinical Aspects [Internet]. Brisbane (AU): Codon Publications; 2018. doi:10.15586/codonpublications.parkinsonsdisease.2018

[2] Sulzer D. Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease. Trends Neurosci. 2007;30(5):244–250. doi: 10.1016/j.tins.2007.03.009

[3] DeMaagd G, Philip A. Parkinson's disease and its management: part 1: disease entity, risk factors, pathophysiology, clinical presentation, and diagnosis. P T. 2015;40(8):504–532. doi: 10.3389/fneur.2012.00151

[4] Kalia LV, Lang AE. Parkinson's disease. Lancet. 2015;386(9996):896–912. doi: 10.1016/S0140-6736(14)61393-3

[5] Ascherio A, Schwarzschild MA. The epidemiology of Parkinson's disease: risk factors and prevention. Lancet Neurol. 2016;15(12):1257–1272. doi: 10.1016/S1474-4422(16)30230-7

[6] Yang D, Zhao D, Ali Shah SZ, et al. The role of the gut microbiota in the pathogenesis of Parkinson's disease. Front Neurol. 2019;10:1155. doi: 10.3389/fneur.2019.01155

[7] Braak H, Rüb U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. J Neural Transm (Vienna). 2003;110(5):517–536. doi: 10.1007/s00702-002-0808-2

[8] Zhu M, Liu X, Ye Y, et al. Gut microbiota: a novel therapeutic target for Parkinson's disease. Front Immunol. 2022;13:937555. doi: 10.3389/fimmu.2022.937555

[9] Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: consensus report of workgroup 2 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. J Periodontol. 2018;89 Suppl 1(S1):S173–S182.

[10] Socransky SS, Haffajee AD, Cugini MA, et al. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134–144. doi: 10.1111/j.1600-051x.1998.tb02419.x

[11] Deo PN; Deshmukh R. Oral microbiome: Unveiling the fundamentals. J Oral Maxillofac Pathol. 2019;23(1):122–128. doi: 10.4103/jomfp.JOMFP_304_18

[12] Bui FQ, Almeida-da-Silva CLC, Huynh B, et al. association between periodontal pathogens and systemic disease. Biomed J. 2019,42 (1):27–35. doi: 10.1016/j.bj.2018.12.001.

[13] Jungbauer G, Stähli A, Zhu X, et al. Periodontal microorganisms and alzheimer disease – a causative relationship? Periodontol 2000. 2022;89(1):59–82. doi: 10.1111/prd.12429.

[14] Cicciù M, Risitano G, Lo Giudice G, Bramanti E. Periodontal health and caries prevalence evaluation in patients affected by Parkinson's disease. Parkinsons Dis. 2012;2012:541908. doi: 10.1155/2012/541908

[15] Lyra P, Machado V, Proença L, et al. Parkinson's disease, periodontitis and patient-related outcomes: a cross-sectional study. Medicina (Kaunas). 2020;56(8). doi: 10.3390/medicina56080383.

[16] Yilmaz M, Yay E, Balci N, et al. Parkinson's disease is positively associated with periodontal inflammation. J Periodontology. 2023;94(12):1425–1435. doi: 10.1002/JPER.23-0274

[17] Romano S, Savva GM, Bedarf JR, et al. Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. NPJ Parkinsons Dis. 2021;7(1):27. doi: 10.1038/s41531-021-00156-z

[18] Yay E, Yilmaz M, Toygar H, et al. Parkinson's disease alters the composition of subgingival microbiome. J Oral Microbiol. 2023;15(1):2250650. doi: 10.1080/20002297.2023.2250650

[19] Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry. 1988;51(6):745–752. doi: 10.1136/jnnp.51.6.745

[20] Martínez-Martín P, Gil-Nagel A, Gracia LM, et al. Unified Parkinson's disease rating scale characteristics and structure. The cooperative multicentric group. Mov Disord. 1994;9(1):76–83. doi: 10.1002/mds.870090112

[21] Hoehn MM, Yahr MD. Parkinsonism: onset, progression, and mortality. 1967. Neurology. 1998;50(2):318 and 316 pages following. doi: 10.1212/wnl.50.2.318

[22] Gürlek Ö, Gümüş P, Nile CJ, et al. Biomarkers and bacteria around implants and natural teeth in the same individuals. J Periodontol. 2017;88(8):752–761. doi: 10.1902/jop.2017.160751

[23] Cağlayan F, Miloglu O, Altun O, et al. Oxidative stress and myeloperoxidase levels in saliva of patients with recurrent aphthous stomatitis. Oral Dis. 2008;14(8):700–704. doi: 10.1111/j.1601-0825.2008.01466.x

[24] Armstrong MJ, Okun MS. Diagnosis and treatment of parkinson disease: a review. JAMA. 2020;323(6):548–560. doi: 10.1001/jama.2019.22360

[25] Hill-Burns EM, Debelius JW, Morton JT, et al. Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome. Mov Disord. 2017;32(5):739–749. doi: 10.1002/mds.26942

[26] Chen ZJ, Liang CY, Yang LQ, et al. Association of Parkinson's disease with microbes and microbiological therapy. Front Cell Infect Microbiol. 2021;11:619354. doi: 10.3389/fcimb.2021.619354

[27] Liao C, Chen X, Fu Y. Salivary analysis: an emerging paradigm for non-invasive healthcare diagnosis and monitoring. Interdiscip Med. 2023;1(3). doi: 10.1002/INMD.20230009. 3

[28] Diao J, Yuan C, Tong P, et al. Potential roles of the free salivary microbiome dysbiosis in periodontal diseases. Front Cell Infect Microbiol. 2021;11:711282. doi: 10.3389/fcimb.2021.711282

[29] Mihaila D, Donegan J, Barns S, et al. The oral microbiome of early stage Parkinson's disease and its relationship with functional measures of motor and non-motor function. PLoS One. 2019;14(6):e0218252. doi: 10.1371/journal.pone.0218252

[30] Fleury V, Zekeridou A, Lazarevic V, et al. Oral dysbiosis and inflammation in Parkinson's disease. J Parkinsons Dis. 2021;11(2):619–631. doi: 10.3233/JPD-202459

[31] Georg LK, Brown JM. Rothia, gen. nov. An aerobic genus of the family Actinomycetaceae. IJSEM. 1967;17(1):79–88. doi: 10.1099/00207713-17-1-79

[32] Collins MD, Hutson RA, Falsen E, Sjöden B, Facklam RR. Description of gemella sanguinis sp. nov., isolated from human clinical specimens. J Clin Microbiol. 1998;36(10):3090–3093. doi: 10.1128/JCM.36.10.3090-3093.1998

[33] Bor B, Bedree JK, Shi W, et al. Saccharibacteria (TM7) in the human oral microbiome. J Dent Res. 2019;98 (5):500–509. doi: 10.1177/0022034519831671
[34] Könönen E, Eerola E, Frandsen EV, et al. Phylogenetic characterization and proposal of a new pigmented species to the genus Prevotella: Prevotella pallens sp. nov. Int J Syst Bacteriol 1998;48 Pt 1(1):47–51. doi: 10.1099/00207713-48-1-47
[35] Könönen E, Fteita D, Gursoy UK, et al. Prevotella species as oral residents and infectious agents with potential impact on systemic conditions. J Oral Microbiol. 2022;14(1):2079814. doi: 10.1080/20002297.2022.2079814
[36] Zhao T, Wei Y, Zhu Y, et al. Gut microbiota and rheumatoid arthritis: from pathogenesis to novel therapeutic opportunities. Front Immunol. 2022;13:1007165. doi: 10.3389/fimmu.2022.1007165
[37] Meng C, Deng P, Miao R, et al. Gut microbiome and risk of ischaemic stroke: a comprehensive Mendelian randomization study. Eur J Prev Cardiol. 2023;30 (7):613–620. doi: 10.1093/eurjpc/zwad052
[38] Chen C, Liao J, Xia Y, et al. Gut microbiota regulate Alzheimer's disease pathologies and cognitive disorders via PUFA-associated neuroinflammation. Gut. 2022;71 (11):2233–2252. doi: 10.1136/gutjnl-2021-326269
[39] Lebouvier T, Neunlist M, Bruley des Varannes S, et al. Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. PLoS One. 2010;5(9):e12728. doi: 10.1371/journal.pone.0012728
[40] Vacca M, Celano G, Calabrese FM, et al. The controversial role of human gut lachnospiraceae. Microorganisms. 2020;8(4). doi: 10.3390/microorganisms8040573.
[41] Tan AH, Chong CW, Lim SY, et al. Gut microbial ecosystem in parkinson disease: new clinicobiological insights from multi-omics. Ann Neurol. 2021;89(3):546–559.
[42] Vascellari S, Palmas V, Melis M, et al. Gut microbiota and metabolome alterations associated with Parkinson's disease. mSystems. 2020;5(5). doi: 10.1128/mSystems.00561-20
[43] Nakajima M, Arimatsu K, Kato T, et al. Oral administration of P. gingivalis induces dysbiosis of gut microbiota and impaired barrier function leading to dissemination of Enterobacteria to the liver. PLoS One. 2015;10(7):e0134234. doi: 10.1371/journal.pone.0134234
[44] Bao J, Li L, Zhang Y, et al. Periodontitis may induce gut microbiota dysbiosis via salivary microbiota. Int J Oral Sci. 2022;14(1):32. doi: 10.1038/s41368-022-00183-3
[45] Morotomi M, Nagai F, Sakon H, Tanaka R. Dialister succinatiphilus sp. nov. and Barnesiella intestinihominis sp. nov., isolated from human faeces. Int J Syst Evol Microbiol. 2008;58(Pt 12):2716–2720. doi: 10.1099/ijs.0.2008/000810-0.
[46] Zhang Q, Zou R, Guo M, et al. Comparison of gut microbiota between adults with autism spectrum disorder and obese adults. PeerJ. 2021;9:e10946. doi: 10.7717/peerj.10946
[47] Shkoporov AN, Chaplin AV, Shcherbakova VA, et al. Ruthenibacterium lactatiformans gen. nov., sp. nov., an anaerobic, lactate-producing member of the family Ruminococcaceae isolated from human faeces. Int J Syst Evol Microbiol. 2016;66(8):3041–3049. doi: 10.1099/ijsem.0.001143
[48] Esmael A, Talaat M, Egila H, et al. Mitochondrial dysfunction and serum lactate as a biomarker for the progression and disability in MS and its correlation with the radiological findings. Neurol Res. 2021;43(7):582–590. doi: 10.1080/01616412.2021.1893567
[49] Kovtun AS, Averina OV, Angelova IY, et al. Alterations of the composition and neurometabolic profile of human gut microbiota in major depressive disorder. Biomedicines. 2022;10(9). doi: 10.3390/biomedicines10092162
[50] Cui Y, Zhang L, Wang X, et al. Roles of intestinal parabacteroides in human health and diseases. FEMS Microbiol Lett. 2022;369(1). doi: 10.1093/femsle/fnac072
[51] Kverka M, Zakostelska Z, Klimesova K, et al. Oral administration of parabacteroides distasonis antigens attenuates experimental murine colitis through modulation of immunity and microbiota composition. Clin Exp Immunol. 2011;163(2):250–259. doi: 10.1111/j.1365-2249.2010.04286.x