Dysbiosis of Oral Microbiota Associated with Palmoplantar Pustulosis

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Keywords

Palmoplantar pustulosis · Oral microbiome · Dysbiosis · Periodontal diseases

Abstract

Background: Dysbiosis of oral microbiota is implicated not only in oral inflammatory lesions, but also in a variety of extraoral diseases. The etiology of palmoplantar pustulosis (PPP) remains unclear; however, it has been suggested that chronic inflammation caused by periodontopathic bacterial infection may play a role. **Objectives/Methods:** To determine whether patients with PPP have altered diversity and composition of oral microbiota, we conducted the 16S rDNA analysis using saliva samples collected from 21 outpatients with PPP and 10 healthy individuals. **Results:** We found that the proportion of bacteria in the phylum Proteobacteria was significantly lower in PPP patients ($p = 0.025$). At the genus level, patients with PPP had a significantly lower abundance of *Neisseria* ($p = 0.014$), which best accounted for the observed decrease in Proteobacteria. We also identified multiple minor genera and species that were represented at a significantly higher level in the PPP group, several of which have been associated with periodontal diseases. **Conclusion:** Our results suggest a possible link between PPP and dysbiosis of oral microbiota, particularly the lower abun-

dance of *Neisseria*, the most predominant genus of Proteobacteria in healthy oral microbiota. Probiotics that improves oral dysbiosis may be beneficial for patients with PPP as an adjunctive therapy.

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Introduction

The human oral cavity harbors more than 700 different species of resident microorganisms and is the second most diverse microbial community in the body. The growth of periodontal pathobionts, local odontogenic infection, and chronic inflammation have been linked to changes in the diversity and composition (dysbiosis) of the oral microbiota [1, 2]. In addition, oral dysbiosis may also contribute to a variety of systemic or autoimmune diseases [3–6], including atherosclerosis [7, 8], coronary artery disease [9, 10], diabetes mellitus [11, 12], rheumatoid arthritis [13, 14], systemic lupus erythematosus [15, 16], and Sjögren's syndrome [17, 18]. The oral microbiome serves as a source of pathogenic microorganisms, bioactive metabolites, and proinflammatory molecules for extraoral tissues.

Palmoplantar pustulosis (PPP) is a refractory skin disorder in which crops of multiple sterile pustules appear recurrently and bilaterally on the palms and soles. The pustules form erythematous, scaly plaques with chronic pain and/or itch, which seriously impairs the quality of life of the patients. The etiological factors for PPP remain unclear; however, it has been suggested that smoking and dental metal hypersensitivity trigger and/or exacerbate PPP [19–22]. Furthermore, accumulating evidence suggests that chronic odontogenic infection, in particular periapical lesions, has a close pathogenetic relationship with PPP [23–25].

To evaluate the role of oral microbiota in the pathogenesis of PPP, we conducted a comparative analysis of oral microbial profiles in patients with PPP and healthy individuals. The results of this study highlight specific oral dysbiosis in patients with PPP and suggest the possibility of adjunctive oral probiotics that improves the taxonomic composition of the oral microbial community in combination with the current pharmacotherapy for PPP.

Materials and Methods

For further details, see the online supplementary material (see www.karger.com/doi/10.1159/000511622 for all online suppl. material,) (Fig. 1). The main characteristics of PPP patients are summarized in online supplementary Table 1.

Results

Periodontal Health Status of the Subjects

Periodontal disease (probing depth ≥ 4 mm) was found in 42.1% of the PPP patients and half of the healthy controls (HC), and the difference was not significant ($p = 0.714$ in two-tailed Fisher's exact test). No significant difference in the number (5.75 [PPP] vs. 2.00 [HC], $p = 0.0789$ in two-tailed Welch's t test) and the mean depth (4.41 mm [PPP] vs. 4.00 mm [HC], $p = 0.0931$ in two-tailed Welch's t test) of periodontal pockets between PPP and HC was found. We thus reasoned that the enrolled PPP and HC subjects had a similar periodontal health status.

PPP Patients Have Dysbiosis of Oral Microbiota

In next-generation sequencing-based 16S rDNA amplicon analysis, averages (SD) of 17,621 (4,683; PPP) and 20,805 (4,985; HC) clean reads were obtained per sample,

Fig. 1. Flowchart of the Materials and Methods.

clustered into operational taxonomic units with 97% similarity, and assigned taxonomically. Of 198 operational taxonomic units (OTUs) obtained, 103 OTUs (52.0%) were assigned taxonomically at the genus level and 42 OTUs (21.2%) at the species level. A total of 14 phyla, 22 classes, 36 orders, 59 families, 96 genera, and 42 species were represented in the saliva samples (online suppl. Table 2). Generally, biodiversity of a microbial community has been assessed from 2 aspects: species diversity within a single ecosystem (α -diversity) and differences in overall diversity between different environments (β -diversity). There was no significant difference in the standard estimates of α -diversity between PPP patients and HC, as measured by species richness ($p = 0.521$ in two-tailed Welch's t test; Fig. 2a), evenness ($p = 0.601$ in two-tailed Welch's t test; Fig. 2b), and diversity ($p = 0.998$ in two-tailed Welch's t -test; Fig. 2c; $p = 0.381$ in two-tailed Welch's t test; Fig. 2d). However, β -diversity analysis revealed a clear separation between the PPP and HC microbial communities at the phylum and the genus levels (Fig. 2e, f), suggesting that PPP patients and HC have distinct oral microbial compositions.

Fig. 2. α - and β -Diversity comparisons of the oral microbiomes of the PPP and HC groups. **a–d** α -Diversity comparisons based on the species richness (**a**), species evenness (**b**), and species diversity (**c, d**). The mean values and outliers are shown by cross marks and open circles, respectively. Species richness, defined as the number of species identified in each sample, is the simplest measure of biodiversity. Pielou's evenness is a good measure of "relative evenness" for each community. Species diversity (Shannon index and

Simpson index) is a more complex measure that takes into account both species richness and evenness. **e, f** β -Diversity comparisons using principal coordinate analysis at the phylum (**e**) and the genus (**f**) levels. The proportion of variance explained by each principal coordinate (PCo) axis is denoted in the corresponding axis label. PPP and HC groups show clear separation as shown by dashed circles.

Decreased Proportion of Proteobacteria in PPP

We next investigated the taxonomic composition of the oral microbiota of PPP patients and HC. At the phylum level, a significant decrease in the proportion of Proteobacteria ($p = 0.0251$ in two-tailed Welch's t test) and a significant increase in the proportion of Synergistetes ($p = 0.0485$ in two-tailed Welch's t test) were observed in patients with PPP (Fig. 3a–c). Proteobacteria, along with Bacteroidetes and Firmicutes, was the most predominant phylum in HC; however, in patients with PPP, the relative abundance of Proteobacteria was decreased to nearly half that of Bacteroidetes and Firmicutes (Fig. 3a). Hierarchical cluster analysis revealed that PPP and HC were separated into 4 different clusters based on the phylum composition of the oral microbiota: one HC-specific cluster characterized by a higher proportion of Proteobacteria

and 3 PPP clusters with higher relative abundance of Bacteroidetes or Firmicutes (Fig. 3d).

Reduced Abundance of *Neisseria* in PPP

The differences in oral microbiota composition were further confirmed at the genus level. We found that patients with PPP had a significantly reduced abundance of the genus *Neisseria* than HC did ($p = 0.0140$ in two-tailed Welch's t test; Fig. 4a, b). *Neisseria* was the most predominant genus within Proteobacteria in healthy oral microbiota (Fig. 4a), and this reduction accounted for a large percentage of the observed decrease in Proteobacteria in PPP patients (Fig. 4b). Furthermore, the relative abundance of the rare genera within Firmicutes and Synergistetes was significantly increased in PPP patients: *Dialister* ($p = 0.0356$ in two-tailed Welch's t test), *Schwartz-*

(For legend see next page.)

Fig. 4. Taxonomic composition analysis of the oral microbiomes of the PPP and HC groups at the genus level. **a** Comparisons of the genus-level abundance within Proteobacteria of PPP and HC are shown in bar plots. The genera representing <0.1% of the total oral microbes of HC are included in “others.” **b–e** Comparisons of the

relative abundance of the genera with significant differences between PPP and HC. The genera within Proteobacteria (**b**), Firmicutes (**c, d**), and Synergistetes (**e**) are shown in box-and-whisker plots. The mean values and outliers are shown by cross marks and open circles, respectively.

Fig. 3. Taxonomic composition analysis of oral microbiomes of the PPP and HC groups at the phylum level. **a** The relative abundances of oral bacterial phyla of PPP and HC are represented by pie charts. The phyla representing <0.5% of the total oral microbes of HC are included in “others.” **b, c** Box-and-whisker plots show the comparisons of the relative abundance of the phyla with significant differences between PPP and HC. The mean values and outliers are shown by cross marks and open circles, respectively. **d** Hierarchical clustering of the phylum-level composition of the oral bacterial community of PPP and HC. The clustering results are repre-

sented as a dendrogram. Bar plots indicate the phylum-level composition of oral microbiota of each subject. Four different clusters (1–4 in the dendrogram) were identified. The cluster highlighted in blue corresponds to the HC microbiome characterized by a higher relative abundance of Proteobacteria. The red cluster represents a group of PPP patients harboring a higher relative abundance of Bacteroidetes. The clusters shown in green and magenta include PPP patients with a higher relative abundance of Firmicutes.

Table 1. The species with distinct relative abundance between PPP and HC groups

Phylum	Class	Order	Family	Genus	Species	Relative abundance (PPP), %	Relative abundance (HC), %	Abundance ratio (PPP/HC)	p value (Welch's t test)
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	<i>Prevotella</i>	Not identified	2.926	1.918	1.53	0.039
Firmicutes	Bacilli	Gemellales	Gemellaceae	Not identified	Not identified	6.489	3.284	1.98	0.100
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	<i>Catonella</i>	Not identified	0.139	0.243	0.57	0.053
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	<i>Filifactor</i>	Not identified	0.155	0.020	7.62	0.083
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	<i>Dialister</i>	Not identified	0.172	0.061	2.83	0.036
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Not identified	Not identified	0.001	0.000		0.095
Firmicutes	Clostridia	Clostridiales	Veillonellaceae	<i>Schwartzia</i>	Not identified	0.012	0.003	4.51	0.035
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	<i>Delftia</i>	Not identified	0.002	0.000		0.031
Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	<i>Desulfobulbus</i>	Not identified	0.007	0.000		0.042
Proteobacteria	Gammaaproteobacteria	Pasteurellales	Pasteurellaceae	<i>Actinobacillus</i>	<i>parahaemolyticus</i>	1.427	0.580	2.46	0.075
Synergistetes	Synergistia	Synergistales	Dethiosulfovibrionaceae	TG5	Not identified	0.081	0.011	7.39	0.046

ia ($p = 0.0351$ in two-tailed Welch's t test) and *TG5* ($p = 0.0461$ in two-tailed Welch's t test), all of which have been associated with periodontal disease and endodontic lesions (Fig. 4c–e) [26–28].

Higher Proportion of Periodontopathic Bacterial Species in PPP Patients

We found 11 species with distinct relative abundance between PPP patients and HC (Table 1). Several of these species, such as *Prevotella* sp., *Dialister* sp., *Schwartzia* sp., and *TG5* sp., were members of genera that are closely associated with odontogenic infection [26–29]. Three species were detected only in patients with PPP, 2 of which (unidentified species within the family Veillonellaceae and *Desulfobulbus* sp.) belong to taxa known as periodontal pathobionts [28, 30]. The overall results are illustrated schematically in Figure 5.

Post Hoc Power Analysis

Twenty-one PPP patients and 10 HC were enrolled in this study. In the analysis of Proteobacteria, the mean proportion and the SD were 20.0 and 9.56 (PPP) versus 29.7 and 9.92 (HC), respectively. The effect size (d) was calculated to be 1.00. Similarly, in the analysis of *Neisseria*, the mean proportion and the SD were 9.82 and 6.23 (PPP) versus 19.7 and 9.50 (HC), respectively. The effect size (d) was calculated to be 1.33. On the basis of these statistics, we obtained the statistical power values of 0.711 (Proteobacteria) and 0.917 (*Neisseria*).

Discussion/Conclusion

A significantly lower proportion of Proteobacteria was observed in PPP patients and appears largely attributable to the reduction of the genus *Neisseria*, although no specific species responsible for the decrease in *Neisseria* was identified in this study. Notably, an abundance of *Neisseria* is related to healthy periodontal conditions [31–34]. Most species within *Neisseria*, except for patho-

Fig. 5. Schematic summarizing the shift in oral bacteria in PPP patients. Taxa in each taxonomic hierarchy are shown by circles. The average relative abundance of the taxon in PPP (the numerical value in or below the circle) is represented by the size of the circle. Changes in the relative abundance of the taxa between PPP and HCC are expressed as the base 2 logarithm of the ratio of the average relative abundance (PPP/HC). The weight of the outline for each circle represents the statistical significance of the taxon (two-tailed Welch's t test) between PPP and HC.

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genic *N. gonorrhoeae* and *N. meningitides*, are nonpathogenic commensal bacteria in the human oral cavity. We speculate that the reduction of resident *Neisseria* permits the colonization and/or growth of other bacteria directly involved in PPP pathogenesis; these may include the periodontal pathogenic taxa that were found to be increased in PPP patients in this study. *Neisseria* is a dominant producer of acetaldehyde in the oral cavity [35, 36]. Alternatively, *Neisseria* is an important contributor to the oral bacteria-mediated reduction of nitrate (NO_3^-) to nitrite (NO_2^-) [37, 38], which can be converted to physiologically active nitric oxide (NO) in the stomach. Dysregulation of this NO_3^- - NO_2^- -NO pathway may cause PPP.

The phylum-level analysis also suggested that PPP was associated with a significantly higher proportion of Synergistetes, implicated in periodontitis and peri-implantitis [39–41]. The genus *TG5*, which was found to be significantly increased in PPP patients, has a close phylogenetic relationship with cluster A species, a periodontopathic bacterial group within the phylum Synergistetes [42–44]. Synergistetes has a potential role in oral dysbiosis through the generation of cyclodipeptide metabolites with quorum-sensing and/or bactericidal/bacteriostatic activity [45]. This induction of dysbiosis by Synergistetes may also be associated with PPP.

Although we could not find the causality between oral dysbiosis and PPP in this study, our findings suggest the possibility of oral probiotics that supplements *Neisseria* and/or improves oral dysbiosis as an add-on therapy. In fact, previous randomized controlled trials have demonstrated that oral probiotics using lozenges containing probiotic bacterial strains has beneficial effects on oral microbiome [46–48].

The small numbers of patients and controls examined in this study may limit its conclusions and generalizability, although the sample size yielded statistical power values at the recommended levels in a post hoc power analysis. In addition, we could find no evidence to suggest the causal relationship between oral dysbiosis and PPP in this study. Further confirmation studies with larger cohorts are needed to establish the causality. Mechanistic investigation will also be essential for a better understanding of the impact of periodontal disease and oral microbiome on PPP. Oral dysbiosis can affect the gut microbiota directly through saliva and indirectly through blood flow [49]; however, there have been no reports on the intestinal microbiome of PPP patients. Further comparative studies are required to elucidate whether PPP patients have specific dysbiosis of gut microbiota.

In conclusion, we found dysbiosis of oral microbiota, particularly, the significant decrease in the genus *Neisseria* and the concomitant increase in bacteria within the periodontopathic taxa, in the PPP cohort.

Key Message

Microbiome analysis reveals altered composition of oral microbiota in patients with palmoplantar pustulosis.

Statement of Ethics

This case-control observational study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and the strengthening of the reporting of observational studies in epidemiology (STROBE) guidelines. Ethical approval for this study was obtained from the Institutional Review Board of Takanawa Clinic (approval No.: 2016-1). A signed informed consent form was obtained from each participant prior to inclusion in this study.

Conflict of Interest Statement

Y.K., Y.S., K.K., M.M., and K.A. are employees of Takanawa Clinic (Tokyo, Japan). T.A. and T.N. have advisory roles in conducting clinical research in Takanawa Clinic.

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Author Contributions

Y.K., Y.S., K.K., M.M., and K.A. contributed to conception and design, contributed to data analysis and interpretation, and critically revised the manuscript. T.A. contributed to conception and design and critically revised the manuscript. T.N. contributed to conception and design, contributed to data analysis and interpretation, and drafted the manuscript. All authors have read and approved the final paper.

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