

【歯学系 (Dentistry)】

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Dissertation Abstract

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論 文 題 名 Title of Doctoral Dissertation	Relationship of salivary microbiome with the worsening of the periodontal health status in young adults: A 3-year cohort study 若年者における唾液中の細菌叢と歯周状態の悪化との関係：3年間のコホート研究			
論文内容の要旨（2000字程度） Dissertation Abstract (approx. 800 words)				
<u>Background</u>				
Periodontal pathogens detached from the subgingival microbiome are identified in saliva. Their presence or absence is associated with periodontal status. Recently, 16S rRNA gene analysis has been used extensively for classification and identification of the salivary microbiome. Besides, the salivary microbiome detected by 16S rRNA gene analyses has the potential to reflect subgingival plaque-derived bacteria representing periodontal diseases. However, the relationship between the salivary microbiome and the worsening of the periodontal health status detected by 16S rRNA gene analyses, especially in young adults, is not well characterized.				
<u>Objective</u>				
The objective of this study was to determine whether the salivary microbiome is associated with the worsening of the periodontal health status among Japanese young adults.				
<u>Materials and methods</u>				
In this 3-year cohort study, data from systemically healthy and non-smoking young university students (18-22 years) of Okayama University who underwent oral health examinations at baseline (2013) and follow-up (2016) were analyzed. The periodontal condition was assessed using the community periodontal index (CPI). The percentage of teeth exhibiting bleeding on probing (%BOP) among the ten representative teeth for CPI scoring system was calculated. The simplified oral hygiene index (OHI-S) was used to assess the level of dental plaque and calculus. Participants were asked to fill in the questionnaire about their general health condition and lifestyle habits during baseline and follow-up examinations. For salivary microbiome analysis, we randomly collected unstimulated saliva samples from selected students at follow-up. The salivary microbiome was assessed through 16S rRNA metagenomic sequencing. Two communities in the saliva microbiome were constructed based on the k means clustering algorithm; an unsupervised learning algorithm. Worsening of periodontal health status was defined as increase in %BOP or increase in probing pocket depth (PPD) from <4 mm to ≥4 mm. Comparisons of demographics and clinical parameters between the worsening and non-worsening groups were performed by the unpaired t-test, chi-square test, or Mann-Whitney U test as applicable. A p<0.05 was considered statistically significant.				

Results

Of 2,205 first-year students who volunteered to receive an oral examination and completed the questionnaire at baseline (2013), 457 participants (217 males and 240 females) with a mean age of 18.2 ± 0.43 years at baseline were followed at 2016. Among 457 students, 69 provided salivary samples for microbiome analysis.

When we defined the worsening of the periodontal health status as an increase in %BOP in the group of participants who provided salivary samples ($n=69$), OHI-S scores were significantly different between the worsening and non-worsening groups ($p<0.05$). However, when we defined the worsening of the periodontal health status as $PPD \geq 4$ mm, no significant differences were found in variables.

Using 16S rRNA metagenomic sequencing analysis, we obtained a total of 10,915,034 reads. Among them, 4,155,030 quality-passed reads (mean \pm SD: $60,217 \pm 17,205$) from regions V3 and V4 of the bacterial 16S rRNA gene were used for analysis. The sequences were assigned to 203 species-level OTUs. There were 73 genera and 13 phyla. Of them, *Streptococcus sp.* and *TM7 [G-1]* were most abundant in all participants. The type of community in the salivary microbiome clustered by statistical analysis and diversity was not significantly associated with the worsening of the periodontal health status in cases of increasing %BOP and PPD ($p>0.05$). The prevalence of *Campylobacter rectus*, *Dialister invisus*, *Prevotella shahii*, *Streptococcus parasanguinis*, or *Dialister pneumosintes* was significantly higher in the worsening group than in the non-worsening group ($p<0.05$) in the cases with increased %BOP. The prevalence of *Streptococcus salivarius*, *Prevotella histicola*, *Selenomonas sp.*, *Lachnoanaerobaculum orale*, *Stomatobaculum longum*, *Prevotella nigrescens*, *Actinomyces lingnae*, *Actinomyces oris*, or *Actinobaculum sp.* was significantly higher in the worsening group than those in the non-worsening group ($p<0.05$) in the cases with increased PPD.

Discussion

In this study, the prevalence of species including *Prevotella* was significantly higher in the worsening group than that in the non-worsening group. Our results support a previous study, which found that *Prevotella* was associated with periodontal diseases. Other species in our study may indirectly contribute to the worsening of the periodontal health status. However, an association between these species and periodontal disease was not observed in other studies.

The diversity in the microbiome in saliva was not associated with the worsening of the periodontal health status in this study. Wherein, previous study estimated an association between the loss of diversity and progression of periodontal disease. Another study also showed the association of subgingival microbial diversity to periodontal disease. Therefore, when collecting subgingival plaque instead of saliva, the diversity might be observed between the worsening and non-worsening groups.

The type of community in the salivary microbiome clustered by statistical analysis was not significantly associated with the worsening of the periodontal health status in this study. Our finding was inconsistent with a previous cross-sectional study that showed a significant association between salivary microbiome communities and periodontal diseases status. This discrepancy may be explained by differences in study design (prospective cohort study vs. cross-sectional study) and targeted age group (young adults vs. elderly people).

Conclusion

In conclusion, our 3-year cohort study suggested that the worsening of the periodontal health status was associated with some species, but not the type of community nor diversity in the salivary microbiome among Japanese young adults.