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学位論文の題目	Relationship of salivary microbiome with the worsening of 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)
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学位論文内容の要旨

Background

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.

Objective

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.

Materials and methods

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 ≥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 ± SD: 60,217 ± 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.

論文審査結果の要旨

　歯肉縁下の細菌叢に含まれる歯周病原菌は唾液中にも検出される。その存在は、歯周状態と関連する。一方、次世代シークエンスによる網羅的遺伝子解析の発展で、唾液中の口腔内細菌叢の解析が進んでいる。しかし、唾液中の口腔内細菌叢と歯周状態の悪化との関係を調べた報告は乏しい。本研究は、日本の若年者において、唾液中の口腔内細菌叢と歯周状態の悪化との関連を調べることを目的として行われた。

　デザインは3年間のコホート研究とした。18～22歳の岡山大学の学生の中で、ベースライン時（2013年）と、再評価時（2016年）に歯科健診を受診した者を分析した。歯周状態は、Community Periodontal Indexを用いて調べた。プロービング時出血の割合（%BOP）を計算した。口腔清掃度の評価には、Oral Hygiene Index-Simplified（OHI-S）を使用した。自己記入式質問票調査を行い、口腔保健行動を調べ、さらにフォローアップ時に無作為に抽出した69名の学生から安静時唾液を採取して、唾液中の細菌のDNAを抽出した。16S rRNA遺伝子特異的プライマー（V3-V4領域）を用いてPCR増幅を行い、次世代シーケンサーで網羅的遺伝子解析を行った。主要構成細菌の割合からk means clustering algorithmに基づき、唾液中の口腔内細菌叢をcommunityに分類した。また、Shannon Indexを用いて多様性を調べた。歯周状態の悪化は、%BOPの増加と歯周ポケット深さの増加（4 mm未満から4 mm以上）の2つの評価基準を用いた。

　得られた結果は以下の通りである。

1) %BOPが増加したことは、ベースライン時のOHI-Sのスコアと有意に関連していた。

2) 歯周ポケット深さが増加したことと有意に関連のある項目はなかった。

3) %BOPが増加した群では、*Campylobacter rectus*、*Dialister invisus*、*Prevotella shahii*、*Streptococcus parasanguinis*、および*Dialister pneumosintes*の割合が有意に高かった。

4) 歯周ポケット深さが増加した群では、*Streptococcus salivarius*、*Prevotella histicola*、*Selenomonas* sp.、*Lachnoanaerobaculum orale*、*Stomatobaculum longum*、*Prevotella nigrescens*、*Actinomyces lingnae*、*Actinomyces oris*、および*Actinobaculum* sp.の割合が有意に高かった。

5) 歯周状態の悪化と口腔内細菌叢の多様性およびcommunityの違いの間に有意な関連はなかった。

　以上のことから、日本の若年者において、歯周状態の悪化は、いくつかの口腔内細菌と関連があったが、口腔内細菌叢の多様性とcommunityの違いとの間に有意な関連はなかったことが示された。本研究は、3年間のコホート研究によって歯周状態の悪化と口腔内細菌叢との関連を調べることで、歯周病悪化の機構に関する最新の知見を示すとともに、将来の歯周病の予防に貢献するものである。

　また、本論文はすでにInternational Journal of Environmental Research and Public Healthに掲載されており、国際的にも評価されている。

　よって、審査委員会は本論文に博士（歯学）の学位論文としての価値を認める。