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Prediction of periodontopathic bacteria in dental plaque of periodontal healthy subjects by measurement of volatile sulfur compounds in mouth air

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ABSTRACT

Objectives: The aim of this study was to determine whether measurements of volatile sulfur compounds (VSCs) are useful to predict colonization of periodontopathic bacteria. For this purpose, we assessed the relationships among distributions of 4 species of periodontopathic bacteria in tongue coating and dental plaque, oral conditions including VSC concentration in mouth air, and smoking habit of periodontal healthy young subjects.

Methods: The subjects were 108 young adults (mean age, 23.5 ± 2.56 years) without clinical periodontal pockets. Information regarding smoking habit was obtained by interview. After VSC concentration in mouth, air was measured with a portable sulfide monitor (Halimeter®), non-stimulated saliva flow and dental caries status were assessed, and tongue coating and dental plaque samples were collected from the subjects. The tongue coating samples were weighed to determine the amount. The colonization of *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, and *Treponema denticola* in both tongue coating and plaque samples was investigated using species-specific polymerase chain reaction assays.

Results: Significant relationships were observed between the colonization of periodontopathic bacteria in tongue coating and plaque samples, especially that of *P. gingivalis*. VSC concentration showed the most significant association with colonization of *P. gingivalis* in both tongue coating and dental plaque. Logistic regression analysis demonstrated that the adjusted partial correlation coefficient [Exp(B)] values for VSC concentration with the colonization of *P. gingivalis*, *P. intermedia*, and *T. denticola* in dental plaque were 135, 35.4 and 10.4, respectively. In addition, smoking habit was also shown to be a significant variable in regression models [Exp(B) = 6.19, 8.92 and 2.53, respectively]. Therefore, receiver operating characteristic analysis was performed to predict the colonization of periodontal bacteria in dental plaque in the subjects divided by smoking habit. Based on our results, we found cut-off values that indicated likelihood ratios (LR) within the efficient range for positive findings in both groups.

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Conclusion: The present results demonstrated that measurement of VSC concentration in mouth air is a useful method to predict the presence of colonization of some periodontopathic bacteria in dental plaque.

1. Introduction

Some periodontopathic bacteria, *e.g.*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia* and *Treponema denticola* are highly capable of producing volatile sulfur compounds (VSC), which are considered to be a primary cause of oral malodor.[1–5] In some studies, high levels of VSC in mouth air have been occasionally found in periodontal healthy individuals.[6,13,14] On the other hand, oral malodor has been shown to be significantly related to periodontal status in both clinical and epidemiological studies.[6–12] Furthermore, recent studies have found a relationship between VSC level in mouth air and VSC-producing periodontopathic bacteria on the tongue.[13,15–17]

Tongue coating has been suggested to be an important reservoir of oral microorganisms for later attachment to tooth surfaces, thus microorganisms harboured on the tongue may contribute to plaque formation,[18–22] and there may be a relationship between microflora in tongue coating and dental plaque. We speculated that colonization of periodontopathic bacteria not only in tongue coating but also in dental plaque samples could be predicted by measurement of VSC in mouth air. In this study, we examined the prevalence of periodontopathic bacteria in both tongue coating and dental plaque samples obtained from young subjects without clinical periodontal pockets. From the results, we attempted to develop simple prediction models to show colonization of periodontopathic bacteria in dental plaque by measurement of VSC in mouth air.

2. Materials and methods

2.1. Subjects

The present subjects were recruited from students attending a dental university and those at a school for dental hygienists. Each provided informed consent according to a protocol approved by the Ethics Committees of Iwate Medical University School of Dentistry (no. 01027) and agreed to participate in the study. To exclude those with periodontitis, oral examinations were carried out, during which a well-trained examiner measured the periodontal pocket depths of all present teeth. At the same time, dental caries status was assessed according to the method of the World Health Organization.[23] Finally, 108 volunteers (mean 23.5 ± 2.55 years old; 68 males, 40 females) were enrolled. None had pockets greater than 3 mm deep or attachment loss at any site in the mouth, and the average number of DMFT was 8.44 ± 5.42 (range 0–18). Within 1 week after the oral examination, measurement of VSC concentration in mouth air, assessment of saliva flow amount and collection of oral specimens were carried out. Prior to the oral examinations, information regarding smoking habit was obtained from each subject by interview, which showed that 47 had a smoking habit and 61 did not, of whom 55 had never smoked and 6 had a previous smoking experience but had stopped. Similarly, it was confirmed that none were suffering from other oral or systemic diseases, nor had received antibiotic medication within 1 month prior to the examination day.

2.2. Measurement of VSC concentration

VSC concentration in mouth air was measured using a portable sulfide monitor (Halimeter® RH-17K; Interscan Corp., Chatsworth, CA, USA). This instrument responds to all 3 volatile sulfur compounds [hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), dimethyl sulfide (CH_3SCH_3)], and gives a reading for total VSC concentration. Individual VSC concentration was obtained according to the manufacture's manual, with a slight modification. In brief, the subjects were instructed to refrain from using scented products on the examination day, as well as from such oral activities as smoking, drinking, eating, chewing gum, tooth brushing, and mouth rinsing at least 2 h before testing. After the subject closed the mouth with breathing through the nose for 2 min, a disposable plastic straw attached to the air inlet of the monitor was inserted approximately 4 cm into the nearly closed mouth. The maximum value of 3 separate measurements was used as the individual VSC concentration. VSC concentrations were transformed to logarithms (log VSC) for further analysis.

2.3. Assessment of saliva flow amount

For assessing saliva-flow amount, the subjects were instructed to deposit non-stimulated saliva into a plastic tube for a period of 5 min, after which the volume of accumulated saliva was measured.

2.4. Collection of tongue coating and plaque samples

Tongue coating samples were collected as previously reported.[6,13] Briefly, after removing saliva from the tongue dorsum with cotton and a stream of air, any tongue accretion between the lingual papillae was removed carefully from the circumvallate papilla to the apex of the tongue dorsum by scratching with a sterile toothbrush. Collected samples were immersed in sterile phosphate-buffered saline (PBS; pH 7.4) and dispersed by sonication on ice. After centrifugation and washing with PBS for 3 times, we determined the amount of each tongue coating sample by measuring absorbance of the dispersed suspension sample with a calibration curve, which was obtained in a preliminary examination.[22] Dental plaque samples were taken with a sterile dental probe from the lingual surfaces of first or second molars of both sides.