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Title

Novel oral biomarkers predicting oral malodor

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Statement of clinical relevance
Most oral malodor comes from the oral cavity and has various influencing factors. In this study, we propose new oral function markers predicting oral malodor.

Abstract
Objective. We sought new markers to predict oral malodor.
Study design. Seventy-five adults complaining of oral malodor were classified into three groups clinically; no oral malodor, physiological oral malodor, and periodontitis-derived oral malodor. In addition to conventional clinical

parameters, seven salivary components, occlusal force, and lip-closing force were compared among the groups.
Results. Concerning the salivary components, cariogenic bacteria, occult blood, leukocytes, and ammonia differed significantly among the groups. Multiple logistic regression analyses showed that tongue coating scores and ammonia levels were significantly associated with genuine oral malodor, including physiological oral malodor and periodontitis-derived oral malodor, and the tongue coating score, plaque index, and occult blood level were significantly associated with periodontitis-derived oral malodor. Occlusal force and lip-closing force did not differ among the groups. However, there was a statistically significant interaction between occlusal force and lip-closing force in oral malodor in women ($p = 0.019$).
Conclusions. Novel salivary markers, ammonia levels, and occult blood levels may predict genuine oral malodor and periodontitis-derived oral malodor, respectively. An interaction effect between occlusal force and lip-closing force on oral malodor was observed in women.

Introduction

Oral malodor (halitosis) is an important health problem that affects physical and emotional health, and social life. Approximately 90% of oral malodor is associated with oral conditions; some is caused by systemic diseases, including otolaryngological infections, gastrointestinal disorders, hepatic diseases, and diabetes. Causes of oral malodor associated with oral conditions include periodontitis, poor oral hygiene, tongue coating, deep caries, inadequately fitted restorations, endodontic lesions, and low salivary flow.[1–4] Oral malodor originating from the oral cavity is subclassified as physiological or pathological oral malodor.[5] Patients with physiological oral malodor have no specific diseases or pathological conditions that can result in oral malodor, while patients with pathological oral malodor have diseases of the oral cavity that cause malodor. The leading causes of physiological and pathological oral malodor are tongue coating and periodontal disease, respectively.

The primary malodorous compounds that cause oral-derived malodor are volatile sulfur compounds (VSCs), such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide (CH_3SCH_3).[6] VSCs are produced in the oral cavity during the metabolism of the sulfur-containing amino acids cysteine and methionine by oral bacteria.[7, 8] Many Gram-negative anaerobes are important producers of VSCs.[9] On the other hand, Gram-positive bacteria may promote VSC production by Gram-negative bacteria.[10] Methylamine, dimethylamine, propionic acid, butyric acid, indole, skatole, and cadaverine may cause oral malodor,[11] although they are found at lower concentrations than VSCs and have a lesser effect on oral malodor. The human oral cavity contains more than 500 bacterial species that interact with each other and with host tissues, suggesting that multiple bacteria may be related to malodor production. The presence or absence of oral malodor is evaluated by a combination of an organoleptic test (OLT) and measurement of the VSC concentration by instrumental analysis. The results of the OLT are prioritized in determining oral malodor, but there is a correlation between the OLT scores and VSC concentrations measured by gas chromatography.[1, 12]

Since many salivary factors reflect the oral condition, such as oral bacteria, pH, buffer capacity, blood components, and inflammatory reactions, saliva analysis is often performed for risk diagnosis and screening of dental caries and periodontal diseases.[13–15] The amounts of mutans streptococci and lactobacilli, pH, and buffer capacity are used as markers for assessing the risk of dental caries.[13] Periodontopathic bacteria, occult blood, leucocytes, and cytokines are markers for assessing the risk of periodontal disease.[14] Ammonia is a marker of oral hygiene.[15] Factors associated with periodontal disease and oral hygiene may predict oral malodor. Gram-positive

bacteria including cariogenic bacteria may also predict oral malodor. However, salivary components that predict oral malodor have not been studied. Therefore, this study sought new markers of the risk of oral malodor using a multi-test device that analyzes cariogenic bacteria, pH, buffer capacity, occult blood, leukocytes, protein, and ammonia in saliva. The relationships of occlusal and lip-closing force with oral malodor were also investigated. Lip-closing force is associated with the likelihood of habitual mouth breathing, which has been reported as a cause of oral malodor in children;[16] however, the effect of mouth breathing on oral malodor in adults is unclear. Occlusal force is related to salivary flow, which may be a risk factor for oral malodor.[1] This study also examined devices that can be implemented quickly at the chairside without burdening the patient. These devices might be used for screening for oral malodor, such as at health events where it is difficult to perform oral examinations.

Materials and Methods

Study population

The study population consisted of 96 patients (43 men and 53 women, mean age 46.5 ± 19.1 years), who presented as outpatients at the Oral Malodor Clinic of Fukuoka Dental College Medical and Dental Hospital, between January 2017 and December 2019. All participants understood the nature of the research project and provided written, informed consent to participate in the study. Permission for this study was obtained from the Ethics Review Committee for Clinical Research of Fukuoka Gakuen (No. 322). In total, 75 adult patients (34 men and 41 women, mean age 50.4 ± 16.4 years) were analyzed. Patient inclusion criteria were aged over 20 years, visiting the clinic in the morning before eating, drinking, tooth cleaning and smoking after waking up, and no missing test items.

Assessment of oral malodor

The severity of oral malodor was rated using the OLT and gas chromatography. For the OLT, the subject was instructed to exhale through the mouth with moderate force into a Teflon sampling bag (GL Science, Tokyo, Japan) for 2–3 s to prevent dilution of the mouth odor by the lungs and the air in the examination room. This procedure was repeated until approximately 1 L of breath sample was obtained. The OLT score was estimated by two or three evaluators using a scale of 0–5 (0, absence of odor; 1, questionable odor; 2, slight malodor; 3, moderate malodor; 4, strong malodor; 5, severe malodor),[5] and the mean score was used for the analysis. The presence of OLT scores ≥ 2 among the three evaluators always exceeded 75% ($\kappa = 0.50$). A gas chromatograph (model GC2014; Shimazu Works, Kyoto, Japan) was used to assay the concentrations of H_2S, CH_3SH, and CH_3SCH_3 in mouth air. The total VSC concentration was defined as the sum of the H_2S, CH_3SH, and CH_3SCH_3 concentrations. The threshold level for oral malodor was defined as a ≥ 2 OLT score, indicating slight malodor.

Clinical examination

Periodontal health, plaque control, the degree of tongue coating, salivary flow, and mucosal moisture levels were evaluated. Periodontal health was assessed using the average probing pocket depth (PPD) and the percentage of bleeding on probing (BOP) values. The PPD and BOP were measured at six points around each tooth. The presence of ≥5 mm PPD with BOP[1] or the presence of ≥10% BOP[17] was recorded as the presence of periodontitis. Plaque control was evaluated using the Silness and Löe Plaque Index (PlI).[18] The degree of tongue coating was determined based on the tongue-coating score (TCS) on a scale of 0–4.[19] The flow rate of stimulated saliva was

measured using the chewing gum test.[20] The flow rate of resting saliva was measured as per a previous study.[1, 21] The moisture levels of the tongue and buccal mucosa were measured using an electronic device (Mucus; Life, Saitama, Japan).[1] For the moisture levels, the measurements were repeated three times, and the average was used for analysis.

Salivary component test

Salivary components related to dental caries, periodontitis, and oral hygiene were measured using a salivary multi-test system (SPOTCHEM ST ST-4910; ARKRAY, Kyoto, Japan) according to the manufacturer's instructions.[22] Each subject was instructed to rinse the oral cavity with 3 mL of distilled water for 10 s. The examiner dropped one 10-μL sample on each of seven pads on the test strip, which analyzed cariogenic bacteria, pH, buffer capacity, occult blood, leukocytes, protein, and ammonia. The color change of in each pad of the test strip (i.e., change in reflectance) was assessed for the specified wavelength. Assessments of pH, buffer capacity, occult blood, leukocytes, protein, and ammonia were performed after 1 min. Assessment of cariogenic bacteria was performed by measuring the change in reflectance after 1 and 5 min. Regarding the reliability of this salivary multi-test system, a moderate to high correlation with conventional methods ($p < 0.01$) was observed for all seven test items.[22]

Occlusal force

An occlusal force meter (GM10; Nagano Keiki, Tokyo, Japan) was used to measure the occlusal force.[23] Maximum occlusal forces were measured three times each at the left and right first molars (including prostheses and dentures), and the averages were recorded for each person. In cases where the first molar consisted of an implant, these were excluded from the examination, and the second molar of the same side was assessed.

Lip-closing force

Lip-closing force was measured using a digital force gauge (Lipplekun; SHOFU, Kyoto, Japan) according to the manufacturer's instructions. The subject was instructed to relax and sit in a chair. The examiner stood face-to-face with the subject, and inserted a plastic button attached to the Lipplekun in the vestibule and pulled parallel to the floor. The measurement was repeated three times, and the average was used for analysis.

Statistical analysis

The normal distribution of data was assessed using the Shapiro–Wilk test. To evaluate the mean differences between population subgroups, analysis of variance (ANOVA) was used. In cases where variance equality (as indicated by Levene's test) was not achieved, Welch's ANOVA was used. In cases with significant ANOVA results, the Games–Howell post-hoc test was conducted to make pairwise comparisons of the subgroup means, as the Games–Howell test does not assume equal sample sizes (or variances). The Kruskal–Wallis test was used to evaluate the median differences between population subgroups. When the Kruskal–Wallis test results were significant, the Mann–Whitney *U* test was conducted to make pairwise comparisons of the subgroups with the Bonferroni correction applied (significance level of $p < 0.016$). To evaluate the interaction effect between occlusal force and lip-closing force on oral malodor, two-way ANOVA was used. Multiple logistic regression analyses with backward stepwise selection were used to identify potential predictors of genuine oral malodor or periodontitis-

derived oral malodor. Significant results were those with (adjusted) significance levels of $p < 0.05$. All statistical analyses were conducted using IBM SPSS software (ver. 22.0; SPSS Japan, Tokyo, Japan).

Results

Classification of oral malodor in the study population

Table 1 summarizes the demographic and clinical characteristics of the three study groups. Among the 75 study patients, 21 had an OLT score < 2 and were classified as having no oral malodor. The remaining 54 patients, with OLT scores ≥ 2, were diagnosed as having genuine oral malodor and were subsequently subdivided according to the presence of periodontitis. In total, 24 patients were diagnosed as having physiological oral malodor, and 30 patients were diagnosed as having oral malodor derived from periodontitis. In terms of the clinical parameters, the average PPD, BOP, TCS, and plaque index differed significantly among the three groups. There were no significant differences in the number of teeth, stimulated salivary flow, resting salivary flow, tongue moisture, oral mucosal moisture, or smoking status among the three groups.

Salivary components

All the salivary test items tended to be lower in the no oral malodor group compared to the genuine oral malodor groups (Table 2). Cariogenic bacteria, occult blood, leukocytes, and ammonia differed significantly between the three oral malodor groups. There was no significant difference in cariogenic bacteria levels between any two groups. Occult blood levels were higher in the periodontitis-derived oral malodor group than in the other two groups, and there was a significant difference compared to the no oral malodor group ($p = 0.002$). Leukocyte levels were also higher in the periodontitis-derived oral malodor group compared to the other two groups, and there was a significant difference compared to the no oral malodor group ($p = 0.002$). Ammonia levels were significantly lower in the no oral malodor group than in the other two groups ($p < 0.001$ *vs* the physiological oral malodor group and $p = 0.003$ *vs* the physiological oral malodor group).

Occlusal force and lip-closing force

Occlusal force and lip-closing force differed significantly according to patient sex ($p < 0.01$). Table 3 lists the mean (± SD) occlusal force and lip-closing force in the three oral malodor groups by sex. The mean occlusal force was 293.9 ± 153.5 N for men and 213.2 ± 104.6 N for women. Although occlusal force tended to be higher in the men in the physiological oral malodor group and in the women in the no oral malodor group compared to the other groups, there was no difference among the three groups. The mean lip-closing forces were 12.3 ± 2.6 N in men and 9.7 ± 2.4 N in women, and there was no significant difference among the three groups when considering the men only or the women only. The interaction effect between occlusal force and lip-closing force on oral malodor was also examined (Figure 1). While there was no interaction effect between occlusal and lip-closing force on oral malodor ($p = 0.400$) in men, in women this interaction was significant ($p = 0.019$). A comparison of the women in the weak (< 200 N) and strong (≥ 200 N) occlusal force groups revealed that body mass index ($p = 0.025$) was lower in the women in the weak group than in those in the strong group, while saliva leukocyte levels ($p = 0.021$) were higher in the women in the weak group than in those in the strong group (data not shown).

Multiple logistic regression analysis

Multiple logistic regression analyses with backward stepwise selection were conducted using the seven salivary components in addition to the clinical parameters that showed significant differences between the groups to determine the available markers to diagnose oral malodor. TCS and salivary ammonia levels differed significantly between the no oral malodor group and the genuine oral malodor group, which consisted of the physiological and the periodontitis-derived oral malodor groups (Table 4). On the other hand, TCS, PlI, and the occult blood levels in saliva were significantly higher in the periodontitis-derived oral malodor group compared with the other two groups consisting of the no oral malodor group and the physiological oral malodor group (Table 5).

Discussion

The study subjects were diagnosed based on their oral malodor level and the presence of periodontitis. Periodontitis is the most common cause of pathological halitosis derived from the oral cavity. In terms of the clinical parameters, except for the periodontitis parameters, the TCS and PlI differed significantly among the three groups (Table 1). Tongue coating has been recognized as a main site of oral malodor release in previous studies.[24, 25] In particular, patients with periodontitis easily accumulate a tongue coating.[2, 26] In this study, the multiple logistic regression analysis showed that the TCS was a significant variable associated with the periodontitis-derived oral malodor in addition to the genuine oral malodor (Tables 4 and 5). Furthermore, the TCS of the periodontitis-derived oral malodor group was higher than that of the physiological oral malodor group, although there was no statistical difference after Bonferroni correction ($p = 0.038$, i.e. $p > 0.016$, Table 1). Although periodontopathic bacteria were reportedly detected in the tongue coating of patients with periodontitis-derived oral malodor, there is no unified opinion regarding the contribution of periodontopathic bacteria in the tongue coating to oral malodor.[27, 28] The clinical approach reported that a combination of tongue cleaning and periodontal treatment was needed to reduce periodontitis-derived oral malodor, and the effect of tongue cleaning alone on reducing oral malodor was less.[29] Although the tongue coating is a major site of oral malodor, there have been few studies on the factors that influence tongue coating accumulation, which need to be clarified based on basic approaches such as pathology, morphology, histology, and microenvironment.

Methyl mercaptan is an important marker of the oral malodor derived from periodontitis. Several periodontopathic bacteria produce VSCs.[9] Some previous comparative studies have reported that the methyl mercaptan/hydrogen sulfide ratio increased with the severity of periodontal disease.[26, 30] Pham et al.[29] showed that the methyl mercaptan level in the periodontal disease group was significantly higher than that in the gingivitis group. By contrast, Awano et al.[31] reported no significant difference in the methyl mercaptan level between halitosis subjects with and without periodontal pockets, although the methyl mercaptan levels of the two halitosis groups were higher than that of the non-halitosis group. In this study, the level of methyl mercaptan was significantly higher in individuals with periodontal disease, and in those with physiological oral malodor, than in those without oral malodor, but there was no significant difference in methyl mercaptan level between the periodontitis-derived oral malodor and physiological oral malodor groups. The methyl mercaptan/hydrogen sulfide ratio was lower than in previous reports, likely because our periodontitis group included gingivitis cases, and because tongue cleaning is now more widespread and some patients had already presented to general dental clinics for periodontal treatment (or were in a maintenance period).

Neither occlusal force nor lip-closing force independently affected oral malodor (Table 3). We considered lip-closing force as a candidate marker because mouth breathing has been reported to cause oral

malodor in children based on examinations and a history of perennial allergic or chronic rhinitis, or by self-reporting.[16, 32] Similarly, occlusal force has been reported to be correlated with salivary flow, which is an important factor in oral malodor.[33, 34] Previous studies have focused mainly on the influence of occlusal force and lip-closing force on the oral function of children and the elderly. On the other hand, the subjects in this study were adults complaining of oral malodor with no deterioration in oral function. The results indicate that neither occlusal force nor lip-closing force independently contributed to oral malodor in healthy adults. However, a significant interaction effect between occlusal force and lip-closing force on oral malodor was observed in women (Figure 1). The results imply that oral malodor occurs in individuals with weak occlusal force and strong lip-closing force, and in those with strong occlusal force and weak lip-closing force. In other words, oral malodor in women can be predicted based on the imbalance between lip-closing and occlusal force. Regarding the difference between men and women, sex differences in the strength and direction of the lip-closing force have previously been reported.[35] Concerns regarding oral malodor often cause psychological stress,[19, 36] and may affect facial expressions, posture, and the facial and occlusal muscles.[37] To clarify the mechanism underlying the interaction effect between occlusal force and lip-closing force on oral malodor, it may be necessary to investigate systemic factors, such as mental health status, posture, and lifestyle.

Several salivary parameters were significantly related to oral malodor (Table 2). Cariogenic bacteria, occult blood, leukocytes, and ammonia differed significantly among the three groups. The method of assessing cariogenic bacteria was based on resazurin dye, which is converted to the fluorescent molecule resorufin by reducing molecules derived from bacterial metabolism.[38] Levels of cariogenic bacteria were higher in the periodontitis-derived oral malodor group than in the no oral malodor group, but did not differ significantly (p = 0.072). Occult blood and leukocytes were quantitated by measuring hemoglobin peroxidase activity and leukocyte esterase activity, respectively,[22] and both were significantly higher in the periodontitis-derived oral malodor group than in the no oral malodor group (p = 0.002 for occult blood, and p = 0.002 for leukocytes). Occult blood and leukocytes were also higher in the periodontitis-derived oral malodor group than in the physiological oral malodor group, although not significantly after Bonferroni correction (p = 0.021 for occult blood, and p = 0.036 for leukocytes, i.e. $p > 0.016$). The multiple logistic regression analyses showed that the occult blood, TCS, and PlI could potentially indicate periodontitis-derived oral malodor. BOP, an index of periodontitis, is high in patients with oral malodor.[1, 39] Various salivary occult blood tests have been correlated with BOP when measured by oral examinations,[40] and bleeding in the oral cavity can be detected more quickly compared with a BOP examination. Ammonia levels were statistically different in all the combinations between the two groups. The multiple logistic regression analyses showed that ammonia levels and TCS could be used to detect genuine oral malodor, including physiological oral malodor and periodontitis-derived oral malodor (Table 4). Detection of ammonia in the current study was based on the chromogenic reaction of bromocresol green.[22] Ammonia levels measured by a portable ammonia-monitoring device showed a correlation with the total levels of VSCs measured with gas chromatography in a previous study.[41] Our method of measuring ammonia using the salivary multi-test system is useful for assessing genuine oral malodor.

Although there are independent measuring devices for both occult blood and ammonia, the saliva multi-system used in this study is unique in that it is possible to test quickly for seven items simultaneously using one clinical sample. It could prove useful at healthcare events, and in situations where there is no dental unit, because it enables the risk of oral malodor to be evaluated in an environment where oral examinations are not possible. In

this study, eating and drinking, oral cleaning, and smoking, which affect bad breath, were prohibited from the time of waking up to when the examination was carried out. It may be easier to implement if it is used in combination with tests performed on an empty stomach, such as gastroscopy and blood tests. The effectiveness of saliva testing to diagnose oral malodor should be further investigated under additional test conditions.

The limitations of this study were the small number of subjects and the study population being limited to healthy adults. In particular, occlusal force and lip-closing force were estimated to have a greater effect on oral malodor in children than in adults, but the number of underage patients was not sufficient for analysis. The salivary multi-test system used in the study measured the cariogenic bacterial level, but not that of periodontopathic bacteria. It may have been more suitable to measure the level of periodontopathic bacteria that directly produce VSCs as a predictive marker for periodontitis-derived oral malodor.

In conclusion, we analyzed the effects of occlusal force, lip-closing force, and salivary components on the clinical diagnosis of oral malodor to find new markers of the risk of oral malodor. Ammonia level was useful for diagnosing the risk of genuine oral malodor, and the occult blood level was effective for diagnosing the risk of periodontitis-derived oral malodor. Furthermore, an interaction effect between occlusal force and lip-closing force on oral malodor was observed in women.

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Authors' contributions

Takaesu Y, Suzuki N, and Hanioka T designed the study, analyzed the data, and wrote the manuscript. Suzuki N, Watanabe T, and Shimazu A measured oral malodor and carried out the oral cavity clinical assessments. The tests for occlusal force and lip-closing force, and saliva components were performed by Takaesu Y, Naito M, and Yatabe N. Yoneda M and Hirofuji T critically evaluated the analytical results, advised on data interpretation, and contributed to the discussion section of the manuscript.

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Figure legends

Figure 1. Interaction effect between occlusal force and lip-closing force on oral malodor in men (A) and women

(B). Open circles are values for the no oral malodor group and closed circles are those for the genuine oral malodor group. The solid and dotted line show the changes in the average value for the genuine and no oral malodor groups, respectively. The bars show mean ± standard error. In women, there was a significant interaction effect between occlusal force and lip-closing force ($p = 0.019$).

Table 1. Demographic and clinical characteristics of the study subjects (median [IQR] or number (%)).

Characteristic	No oral malodor group (n = 21)	Physiological oral malodor group (n = 24)	Periodontitis-derived oral malodor group (n = 30)
Age (years)	48 [31–60]	56 [41.5–66]	54.5 [39.5–64]
Female (%)	15 (71.4)	12 (50.0)	16 (46.7)
Oral malodor			
OLT score[a]	1.0 [1.0–1.5][b, d]	2.5 [2.25–3.0][d]	3.0 [2.5–3.5][b]
Hydrogen sulfide (ng/10 mL)[a]	0.35 [0.12–0.8][b, d]	2.86 [1.4–4.5][d]	2.62 [1.31–4.49][b]
Methyl mercaptan (ng/10 mL)[a]	0.14 [0.06–0.37][b, d]	1.41 [0.73–2.13][d]	1.72 [0.98–3.26][b]
Dimethyl sulfide (ng/10 mL)[a]	0.21 [0.0–0.36][b, d]	0.73 [0.42–1.2][d]	0.86 [0.5–1.48][b]
Total VSCs (ng/10 mL)[a]	0.63 [0.31–1.75][b, d]	5.41 [2.51–7.67][d]	5.33 [3.06–8.76][b]
Clinical parameters			
Number of teeth	28 [26–29]	27 [25–28]	27 [26–28]
Average PPD (mm)[a]	3.0 [3.0–3.0][b]	3.0 [2.97–3.04][c]	3.21 [3.07–3.59][b, c]
BOP (%)[a]	2.87 [1.92–6.55][b]	3.18 [0.66–4.89][c]	16.0 [9.57–23.7][b, c]
TCS[a]	1.0 [1.0–1.0][b, d]	2.0 [1.0–2.0][d]	2.0 [2.0–2.0][b]
Stimulated salivary flow (mL/5 min)	9.5 [5.0–11.5]	8.5 [6.75–11.3]	7.75 [4.63–9.88]
Resting salivary flow (g/1 min)	0.13 [0.1–0.27]	0.1 [0.06–0.21]	0.12 [0.06–0.18]
Moisture of tongue	28.3 [27.5–29.0]	28.4 [27.5–29.3]	28.2 [27.3–29.1]
Moisture of buccal mucosa	28.6 [28.1–29.1]	28.7 [27.8–29.0]	28.5 [28.0–29.3]
PlI[a]	0.21 [0.17–0.38][b]	0.29 [0.17–0.5]	0.38 [0.29–0.61][b]
Smoking status			
Non-smoker (%)	13 (61.9)	15 (62.5)	20 (66.7)
Past-smoker (%)	6 (28.6)	6 (25.0)	7 (23.3)
Smoker (%)	2 (9.5)	3 (12.5)	3 (10.0)

[a] Significant difference between the three groups by Welch's ANOVA or Kruskal–Wallis test ($p < 0.05$).

[b] Significant difference between the no oral malodor group and the periodontitis-derived oral malodor group by Mann–Whitney *U* test ($p < 0.016$).

[c] Significant difference between the physiological oral malodor group and the periodontitis-derived oral malodor group by Mann–Whitney *U* test ($p < 0.016$).

[d] Significant difference between the no oral malodor group and the physiological oral malodor group by Mann–Whitney *U* test ($p < 0.016$).

PlI, plaque index; PPD, probing pocket depth; BOP, bleeding on probing; TCS, tongue coating score; OLT, organoleptic test; VSCs, volatile sulfur compounds.

Table 2. Salivary components of the three groups (median [IQR]).

Salivary test item	No oral malodor group (n = 21)	Physiological oral malodor group (n = 24)	Periodontitis-derived oral malodor group (n = 30)
Cariogenic bacteria[a]	35 [20–52]	46 [32–70.8]	57 [25.5–77.3]
pH	54 [47–65]	55.5 [45–66.3]	56.5 [47.3–68.8]
Buffer capacity	33 [21–45]	43.5 [32.8–57.5]	47.5 [33–57.8]
Occult blood[a]	14 [12–20][b]	17 [14.5–26]	33.5 [15.3–52.8][b]
Leukocytes[a]	53 [41–67][b]	60 [49.8–70]	75 [60–84][b]
Protein	40 [32–62]	52 [45.8–60]	62 [42.5–80.8]
Ammonia[a]	63 [55–74][b, c]	79.5 [73.8–84.3][c]	76.5 [68.5–84.8][b]

[a] Significant ($p < 0.05$) difference among the three groups by Welch's ANOVA or Kruskal–Wallis test.

[b] Significant ($p < 0.016$) difference between the no and periodontitis-derived oral malodor groups by Mann–Whitney *U* test.

[c] Significant ($p < 0.016$) difference between the no and physiological oral malodor groups by Mann–Whitney *U* test.

Table 3. Occlusal force and lip-closing force of the three groups (average ± SD).

Clinical test item	No oral malodor group (n = 21)	Physiological oral malodor group (n = 24)	Periodontitis-derived oral malodor group (n = 30)
Occlusal force (N)			
Male	236.9 ± 134.6	373.3 ± 148.0	255.7 ± 147.7
Female	259.7 ± 108.7	180.1 ± 71.9	191.6 ± 112.2
Lip-closing force (N)			
Male	13.0 ± 2.0	12.1 ± 2.38	12.3 ± 3.07
Female	9.93 ± 2.17	9.69 ± 2.33	9.42 ± 2.73

No significant difference in any group comparison.

Table 4. Significant variables associated with the genuine oral malodor by multiple logistic regression analysis.

Variable	Crude Odds Ratios (95% CI)	Adjusted Odds Ratios (95% CI)	*P*-value
Sex			
Male	1.00	1.00	
Female	0.37 (0.13–1.10)	1.11 (0.26–4.66)	0.888
Age (years)			
< 40	1.00	1.00	
≧ 40	1.94 (0.66–5.71)	1.04 (0.99–1.08)	0.131
TCS			
0 or 1	1.00	1.00	
2 or 3	18.9 (4.80–74.6)	22.3 (4.42–112.3)	0.000
Ammonia			
< 80	1.00	1.00	
≧ 80	8.82 (1.87–41.6)	5.90 (1.01–34.3)	0.048

Table 5. Significant variables associated with the periodontitis-derived oral malodor by multiple logistic regression analysis.

Variable	Crude Odds Ratios (95% CI)	Adjusted Odds Ratios (95% CI)	*P*-value
Sex			
Male	1.00	1.00	
Female	0.58 (0.23–1.48)	0.92 (0.26–3.30)	0.898
Age (years)			
< 40	1.00	1.00	
≧ 40	1.12 (0.40–3.14)	0.99 (0.95–1.03)	0.457
TCS			
0 or 1	1.00	1.00	
2 or 3	6.84 (2.22–21.1)	6.35 (1.55–26.1)	0.010
PlI			
< 0.3	1.00	1.00	
≧ 0.3	4.00 (1.50–10.7)	4.57 (1.30–16.0)	0.018
Occult blood			
< 30	1.00	1.00	
≧ 30	8.14 (2.74–24.2)	12.6 (2.82–56.4)	0.001

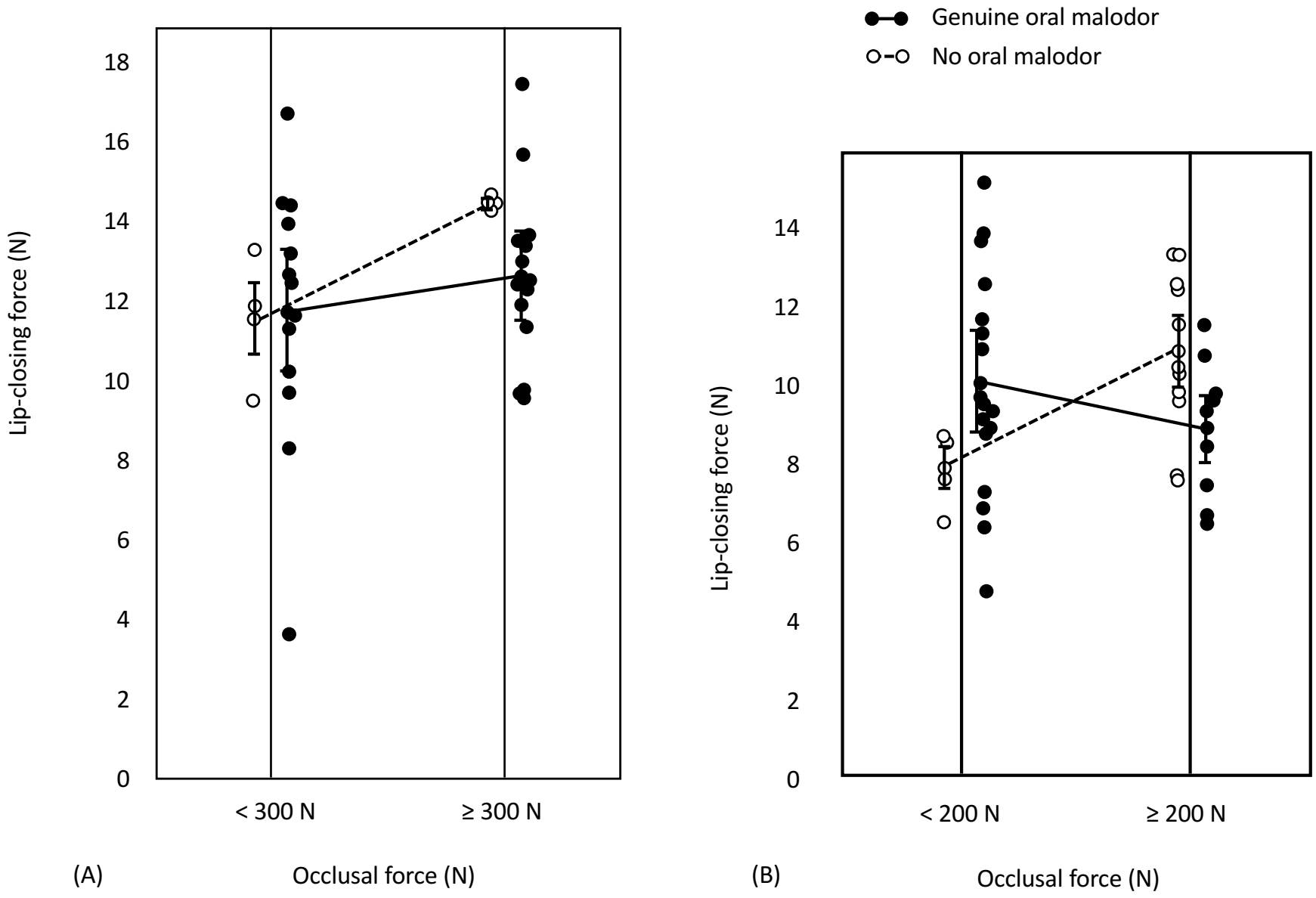
Genuine oral malodor
No oral malodor
Lip-closing force (N)
18
16
14
12
10
8
6
4
2
0
< 300 N
≥ 300 N
(A)
Occlusal force (N)
Lip-closing force (N)
14
12
10
8
6
4
2
0
< 200 N
≥ 200 N
(B)
Occlusal force (N)

Figure 1.