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Saliva viscosity as a potential risk factor for oral malodor

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Abstract

Objectives. The objective of this study was to assess whether saliva viscosity, measured by a viscometer, was a predictor of oral malodor. ***Materials and methods.*** The subjects were 617 patients who visited an oral malodor clinic. The organoleptic test (OT) was used for diagnosis of oral malodor. An oral examination assessed the numbers of teeth present and decayed teeth as well as the presence or absence of dentures. Further, periodontal pocket depths (PD), gingival bleeding, dental plaque and tongue coating were investigated. Unstimulated saliva were collected for 5 min. Saliva viscosity was measured with a viscometer. Logistic regression analysis with oral malodor status by OT as a dependent variable was performed. Possible confounders including age, gender, number of teeth present, number of decayed teeth, number of teeth with PD ≥ 4 mm, number of teeth with bleeding on probing, presence or absence of dentures, plaque index, area of tongue coating, saliva flow rate, saliva pH and saliva viscosity were used as independent variables. ***Results.*** Saliva viscosity ($p = 0.047$) along with the number of teeth with PD ≥4 mm ($p = 0.001$), plaque index ($p = 0.037$) and area of tongue coating ($p < 0.001$) were significant variables for oral malodor. Subjects with a higher number of teeth with PD ≥ 4 mm (OR = 1.32), plaque index (OR = 2.13), area of tongue coating (OR = 3.17) and saliva viscosity (OR = 1.10) were more likely to have oral malodor compared to those with lower values. ***Conclusions.*** The results suggested that high saliva viscosity could be a potential risk factor for oral malodor.

Key Words: *oral malodor, organoleptic test, saliva viscosity, viscometer*

Introduction

According to the Survey on Trends in Health and Welfare conducted in 1999 [1], sticky saliva was one of the common oral health–related problems in Japanese people. Saliva is a mucous secretion from the major and minor salivary glands, which covers the surface of the oral cavity. It is a complex aqueous mixture of high molecular weight mucins, lipids and other components such as proteins and bioactive molecules [2].

The mucins, which are secreted from all sero-mucous salivary glands, are very important substances for various kinds of saliva characteristics. They play a major role in the rheological properties of saliva, such as viscosity, elasticity and stickiness [3]. Viscosity is related to energy dissipated during flow. In saliva, two types of mucins are present, designated MUC5B and MUC7 [3]. It is reported that MUC5B may contribute to viscosity of saliva [4]. Besides mucin, viscosity of saliva is closely linked with various factors such as the flow rate, pH, dry weight of solids, protein content, glycoproteins, and proline-rich protein composition [5].

Several studies have found a relationship between saliva viscosity and the incidence of dental caries and periodontal disease [6,7]. A considered mechanism of the association is that the increase in saliva viscosity reduces bacterial clearance from the oral cavity and increases the risk of infectious diseases [8].

As for oral malodor, sticky saliva is a common subjective symptom among oral malodor patients [9]. However, there are very few studies that investigated the relationship between saliva viscosity and oral malodor. According to a previous study that examined 96 oral malodor patients aged 15–76 years, no association between saliva viscosity and oral malodor was found. However, saliva viscosity was evaluated by

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its appearance in the study [10]; ideally, a viscometer should be used to accurately measure the viscosity of saliva [7]. Therefore, the objective of this study was to assess whether saliva viscosity, measured by the viscometer, is associated with oral malodor.

Materials and methods

Subjects

The study subjects were oral malodor patients, who visited the Fresh Breath Clinic, Dental Hospital, Tokyo Medical and Dental University from April 2009 to March 2012. A total of 617 patients' data (223 males and 394 females) were used for the analysis, after excluding subjects who were edentulous and had missing values on study variables. All participants agreed to join the study and signed the informed consent form. This study followed the guidelines in this investigation and the study protocol was approved by the Ethics Committee for Human Research, Tokyo Medical and Dental University (Approval No. 132).

Oral malodor measurement

Patients were instructed not to have food or drink and to refrain from their oral hygiene practice on the morning of the appointment to reproduce genuine oral malodor. In addition, to exclude confounding smells, they were told to stop eating strong smelling foods for at least 48 h, using strong scented perfumes for 24 h and smoking or drinking alcohol for 12 h before the day of malodor assessment. Measurements were conducted between 9 and 11 am.

The organoleptic test (OT) [11] was used for clinical diagnosis of oral malodor in this study. Prior to malodor measurement, patients were instructed to close their mouth for 3 min and breathe through their nose. The OT was performed by trained dentists. The standardization of examiners was carried out with the T&T Olfactometer® (Daiichi Yakuhin Sangyo Co., Tokyo, Japan), an odor solution kit for examining the olfactory sense, to achieve the consistency of judgment [12]. Oral malodor judgment was made based on a 0–5 score, referring to previous criteria [11], where a score of 0 represented absence of odor, 1 barely appreciable odor, 2 slight malodor, 3 moderate malodor, 4 strong malodor and 5 severe malodor. Patients with scores of 0 or 1 were classified as normal, while those with scores of 2 or higher were classified as oral malodor.

Oral examination

All subjects received a standard oral examination after the oral malodor measurement. The numbers of teeth present and decayed teeth as well as the presence or absence of dentures were assessed (excluding 3rd molars). Periodontal pocket depths (PD) were measured at six sites on each tooth with a periodontal probe (PCP UNC 15 Hu-Friedy: Hu-Friedy Mfg. Co., Inc., Chicago, IL). The deepest pocket was recorded for each tooth. Gingival bleeding was evaluated when the bleeding was observed following the pocket depth measurement. Plaque Index by Silness & Löe [13] criteria was used for oral hygiene status. The area of tongue coating was evaluated based on the modified Oho et al. [14] criteria. The scores of tongue coating were as follows: 0, no observable tongue coating; 1, less than one-third of tongue dorsum with coating; 2, one-third to two-thirds of tongue dorsum with coating; 3, more than two-thirds of tongue dorsum with coating.

Collection of saliva

Unstimulated whole saliva was obtained by asking the subjects to spit saliva into a disposable paper cup for 5 min. The collection was performed between 9 and 11 am. Flow rate of saliva (mL/min) was calculated by weighing the volume of saliva.

Saliva viscosity measurement

Following the collection of unstimulated whole saliva, saliva viscosity, in units of centipoise (cP), was measured using the Brookfield DV-II+ Pro Viscometer with cone-plate (Brookfield Engineering Laboratories, Stoughton, MA). All measurements were carried out at shear rate 90 (1/s), speed 12 (rpm) and temperature 37°C, using 0.5 ml volume of saliva in conformity with the previous study [15,16]. The viscometer was calibrated using a standard calibration liquid (Brookfield viscosity standard, 4.2 cP at 37°C) before the measurement.

Statistical analyses

The independent sample *t*-test and chi-square test were used to detect the differences of characteristics between subjects with and without oral malodor. Pearson's correlation coefficients were calculated to analyze the association between salivary viscosity and other variables. Logistic regression analysis was performed with oral malodor status (0 = non-oral malodor, 1 = oral malodor) by OT as the dependent variable. Age, gender, number of teeth present, number of decayed teeth, number of teeth with PD ≥ 4 mm, number of teeth with bleeding on probing, presence or absence of dentures, plaque index, area of tongue coating, saliva flow rate, saliva pH, and saliva viscosity were used as independent variables. Multi-collinearity among variables was evaluated by computing the variance inflation factor (VIF). The VIF of above 5 was regarded as possible

Table I. Characteristics and bivariate association tests of the malodor and non-malodor groups.

Parameters	Malodor (n = 452) mean ± SD/n (%)	Non-malodor (n = 165) mean ± SD/n (%)	p-value
Age	51.19 ± 14.25	48.92 ± 14.85	0.082
Gender (male)	175 (38.7)	48 (29.1)	0.030
Number of teeth present	25.54 ± 3.77	25.76 ± 3.69	0.528
Number of decayed teeth	0.28 ± 1.06	0.19 ± 0.85	0.336
Number of teeth with PD ≥ 4 mm	1.48 ± 2.85	0.50 ± 1.11	< 0.001
Number of teeth with bleeding	3.86 ± 4.63	2.55 ± 3.56	< 0.001
Presence of dentures (yes)	41 (9.1)	18 (10.9)	0.536
Plaque Index	0.45 ± 0.43	0.26 ± 0.29	< 0.001
Area of tongue coating	1.94 ± 0.78	1.21 ± 0.74	< 0.001
Saliva flow rate (mL/min)	0.35 ± 0.35	0.41 ± 0.31	0.038
Saliva pH	6.88 ± 0.36	6.94 ± 0.41	0.077
Saliva viscosity (cP)	4.35 ± 2.59	3.91 ± 1.78	0.019

multi-collinearity that could inflate the standard errors of coefficients. Since all values of VIF were below 2 for explanatory variables in the regression, no suspicion of multi-collinearity was detected.

Statistical analysis was carried out using the SPSS 21.0 software (Japan IBM, Tokyo, Japan). The level of significance was set at $p < 0.05$ for all tests.

Results

Characteristics of subjects by oral malodor status

The mean age of all subjects was 50.6 ± 14.4 years. The mean ages of malodor (n = 452) and non-malodor (n = 165) groups were 51.2 ± 14.3 and 48.9 ± 14.9 years, respectively (Table I). A significant difference was not detected in mean age between the malodor and non-malodor groups ($p = 0.082$). About 40% of subjects (38.7%) were male in the malodor group and 29.1% in the non-malodor group. A significantly higher proportion of males was observed in the malodor group than in the non-malodor group ($p = 0.030$).

The mean numbers of teeth present and decayed teeth in all subjects were 25.6 ± 3.75 and 0.25 ± 1.01, respectively. There were no significant differences in mean numbers of teeth present and decayed teeth by oral malodor status. The number of teeth with PD ≥ 4 mm ($p < 0.001$) or with bleeding ($p < 0.001$) was significantly higher in the malodor group than in the non-malodor group. No significant difference in the proportion of denture wearers by oral malodor status was found. Plaque index ($p < 0.001$) and area of tongue coating ($p < 0.001$) were significantly higher in the malodor group than in the non-malodor group.

Mean saliva flow rate in the oral malodor group was significantly lower than that in the non-malodor group ($p = 0.038$), but a significant difference was not detected regarding saliva pH between the two groups. Subjects in the malodor group had a significantly higher mean saliva viscosity than those in the non-malodor group ($p = 0.019$).

Correlation between saliva viscosity and other variables

Significant correlation coefficients of saliva viscosity were detected with saliva flow rate and saliva pH (Table II). Saliva viscosity was negatively related with saliva flow rate (-0.107, $p = 0.007$) and saliva pH (-0.140, $p < 0.001$).

Association of saliva viscosity and other variables with oral malodor

As presented in Table III, the number of teeth with PD ≥ 4 mm ($p = 0.001$), plaque index ($p = 0.037$), area of tongue coating ($p < 0.001$) and saliva viscosity

Table II. Correlation coefficients between saliva viscosity and other variables.

	Saliva viscosity	
	Correlation coefficient	p-value
Age	0.032	0.421
Gender	−0.062	0.125
Number of teeth present	−0.045	0.263
Number of decayed teeth	0.072	0.075
Number of teeth with PD ≥ 4 mm	0.025	0.535
Number of teeth with bleeding	0.004	0.913
Presence of dentures	0.017	0.680
Plaque Index	0.056	0.163
Area of tongue coating	−0.036	0.372
Saliva flow rate	−0.107	0.007
Saliva pH	−0.140	< 0.001

Table III. Logistic regression analysis on oral malodor.

		95% CI		
	OR	Lower	Upper	*p*-value
Age	1.00	0.98	1.02	0.906
Gender	0.83	0.53	1.30	0.421
Number of teeth present	1.00	0.92	1.08	0.995
Number of decayed teeth	1.06	0.85	1.33	0.587
Number of teeth with PD ≥ 4 mm	1.32	1.12	1.57	0.001
Number of teeth with bleeding	0.94	0.89	1.01	0.069
Presence of dentures	0.51	0.21	1.25	0.142
Plaque Index	2.13	1.05	4.36	0.037
Area of tongue coating	3.17	2.37	4.24	< 0.001
Saliva flow rate	0.77	0.41	1.46	0.420
Saliva pH	0.77	0.44	1.34	0.347
Saliva viscosity	1.10	1.00	1.21	0.047

(p = 0.047) had significant associations with oral malodor status. Subjects with a higher number of teeth with PD ≥ 4 mm (OR = 1.32), plaque index (OR = 2.13), area of tongue coating (OR = 3.17) and saliva viscosity (OR = 1.10) were more likely to have oral malodor compared to those with lower values. Age, gender, number of teeth present, number of decayed teeth, number of teeth with bleeding, presence of dentures, saliva flow rate and saliva pH did not show significant relationships with oral malodor.

Discussion

Saliva viscosity as well as the number of teeth with PD ≥ 4 mm, plaque index and area of tongue coating were significant predictors of oral malodor, even after adjusting other possible confounders. Periodontal pockets, dental plaque and tongue coating have been demonstrated as risk factors for oral malodor in many studies [17–21].

Volatile Sulfur Compounds (VSCs) such as hydrogen sulfide and methyl mercaptan increase with the number of >3 mm periodontal pockets [17,18]. Plaque possesses potential for oral malodor formation, which is thought to harbor a higher proportion of the Gram-negative anaerobic bacteria that engage in proteolytic and aminolytic processes to produce VSCs [19,20]. Tongue coating, composed of desquamated epithelial cells, bacteria, blood components and other nutrients, is a major cause of oral malodor production [21].

However, saliva viscosity has not been reported as a risk factor for oral malodor in a previous study, in which viscosity was only evaluated visually. As saliva has a non-Newtonian trait of biological fluid [16], use of the viscometer is preferable to measure small-volume and low-viscosity saliva samples. This is the first study demonstrating a significant association of saliva viscosity measured by the viscometer and oral malodor. We used the Brookfield viscometer, which has been widely used for the measurement of viscosity of fluid like saliva [16,22].

Saliva viscosity is reported to be negatively related with saliva flow rate: the higher the viscosity, the lower the flow rate [15]. A similar result was found in this study. Zussman et al. [5] showed an age-related increase in saliva viscosity, accompanied by a reduction in saliva flow rate. Lower flow rate of saliva, which results in the drying of the oral cavity, is also one of the risk factors for oral malodor [21]. As bivariate analysis showed a lower saliva flow rate in the malodor group, a significant influence of higher saliva viscosity on oral malodor may be intermediated by the lower flow rate of saliva.

Another factor affecting saliva viscosity is the pH. The viscosity of salivary is pH dependent and greatest at pH 4.0 [23]. pH was negatively related with saliva viscosity in this study. pH is also the major regulating factor in the formation of oral malodor because acidity inhibits the production of odors while neutrality and alkalinity favor it [24]. However, if the viscosity becomes higher at lower pH, this condition may not be favorable for VSCs production. Further research will be needed to clarify this relationship.

Wearing complete acrylic dentures can change salivary viscosity by changing the temperature and pressure inside the space between the denture and the surface support [22]. Wearing dentures is also related to the presence of oral malodor [25]. However, no association between the presence of dentures and oral malodor was found in this study. This is partly because edentate subjects with complete dentures are excluded from the analysis.

Measurement of saliva viscosity requires careful attention, because the saliva viscosity is influenced by various factors. Circadian rhythm may affect the results, as there exists a significant within-subject variation in the viscosity of unstimulated saliva [15]. Briedis et al. [26] reported that saliva viscosity could increase immediately after eating, drinking coffee or under stress, although they found no significant variation of saliva viscosity by age or gender. Smoking is also considered to increase the viscosity of saliva [27]. To exclude the influence of such factors on viscosity, the collection of unstimulated saliva was standardized following the next rules in this study; (1) the collection was performed between 9 and 11 am, (2) the patients must not consume any food or drink in the morning on the appointment day, (3) smoking or drinking alcohol was prohibited for at least 12 h prior to the measurement.

In addition, since the handling and preservation procedures used for saliva collection have considerable effects on the biochemical and biophysical

properties of saliva [23], saliva collection should also be standardized. As saliva viscosity decreases upon storage within a few hours [23], we preserved saliva in a cold environment with ice and minimized the time between collection and measurement to ~2 h at most.

This study first revealed that higher saliva viscosity could be a potential risk factor for oral malodor. However, there is also a limitation in this study because only OT was used for oral malodor assessment and actual concentrations of VSCs such as hydrogen sulfide, methyl mercaptan and dimethyl sulfide were not measured. The relationship of saliva viscosity with oral malodor may differ depending on the individual gas, thus future research should examine the association of saliva viscosity with each VSCs gas measured by a device such as gas chromatography.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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