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Cadaverine as a Putative Component of Oral Malodor

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Abstract. Whereas previous studies have shown correlations between volatile sulphur compounds (VSC) and bad breath levels, it is probable that other compounds found in the oral cavity may contribute to oral malodor. In the present investigation, the possibility that diamines (cadaverine and putrescine) are associated with oral malodor parameters was assessed. Saliva samples from 52 subjects were analyzed for cadaverine and putrescine by HPLC. Oral malodor of whole mouth, tongue, and saliva of the subjects was recorded by an experienced judge on a continuous 10-cm scale; peak and steady-state VSC intra-oral levels were measured by the Interscan 1170 sulphide monitor. Log-transformed VSC and diamine levels were compared with odor judge measurements by Pearson analysis and stepwise forward multiple regression. Putrescine scores were not significantly associated with odor judge parameters or with VSC levels ($p > 0.1$). However, highly significant correlations ($p \leq 0.003$) were found between cadaverine levels and all three odor judge assessments. In contrast, associations between cadaverine and VSC measurements were non-significant. In an attempt to correlate odor judge results in terms of both VSC and diamines, we carried out stepwise forward multiple regression. Results showed that VSC and cadaverine both factor significantly in explaining each of the odor judge measurements, with multiple r values ranging from 0.545 ($p = 0.0002$) to 0.604 ($p < 0.0001$). The results suggest that cadaverine levels are associated with oral malodor, and that this association may be independent of VSC.

Key words. Diagnostic Tests, Halitosis, Ornithine Decarboxylase, Periodontal Diseases, Saliva.

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Introduction

Oral malodor is a common problem affecting most of the adult population (Tonzetich, 1973, 1977). In the large majority of cases, the origin of bad breath is the oral cavity itself, resulting from microbial putrefaction of food residues and host components. Patients with degenerative periodontal diseases tend to have increased numbers of leukocytes, exfoliated cells, and bacteria in their mouths, as well as higher malodor levels (Tonzetich and Kestenbaum, 1969; Tonzetich, 1973; Kostelc *et al.*, 1981). Most previous investigations have concentrated on the relationship between volatile sulphur components (VSC) and oral malodor (Tonzetich and Richter, 1964; Tonzetich, 1971). We have recently shown that VSC levels, recorded by a portable sulphide monitor, show significant correlations with bad breath, as measured by judges (Rosenberg *et al.*, 1991a,b).

In the present study, we have examined the possibility that cadaverine and putrescine, two malodorous diamines associated with bacterial putrefaction, are associated with bad breath. Regression analyses were carried out to compare the associations of cadaverine and putrescine with malodor and dental-related parameters in a clinical study comprised of 52 subjects.

Materials and methods

Patient population

The study consisted of 52 patients (mean age, 37.9 ± 11.9 years; 35 females), ranging from 11 to 63 years. Among the subjects, 43 complained of bad breath, having responded to a newspaper article on the subject. Exclusion criteria included smokers, subjects suffering from systemic disease

or taking antibiotics, and denture wearers. The study included parameters not reported here, including tests of self-estimation of oral malodor, and psychological data (in preparation).

On the day of the examination, the participants were instructed to refrain from using scented personal products. Subjects were also requested to refrain from oral activities, including drinking, eating, chewing gum, and mouthrinsing, two hours prior to appointment. The appointment for each subject was randomly scheduled on one of three days during a 12-day period.

Clinical examination

All the participants were scored for plaque accumulation and gingival health of all tooth surfaces (including wisdom teeth) by the Plaque Index (PI; Silness and Löe, 1964) and Gingival Index (GI; Löe and Silness, 1963). Probing depth measurements were recorded for the 30 subjects who were examined on days 2 and 3 of the study (N = 30) with a Michigan O probe, at six reference loci on the buccal and lingual sides of each tooth, including wisdom teeth. All participants were subjected to whole-mouth probing in order to identify shallow and deep pockets for sampling of plaque for the BANA test.

Volatile sulphide concentrations

VSC measurements were made by means of a portable industrial sulphide monitor (model 1170, 1.0 ppm full-scale, Interscan Corp., Chatsworth, CA), zeroed on ambient air before each measurement. A disposable 6.5-mm-circumference plastic straw was inserted into the air inlet of the monitor. Patients were instructed to bring their slightly opened mouths over the straw so that it extended approximately 4 cm into the oral cavity , and to breathe through their noses during the measurement. Both peak (maximum) and steady-state levels attained were determined in parts *per* billion (ppb) sulphide equivalents.

Odor judge assessment

Organoleptic assessment was made by an odor judge with extensive experience (M.R.), who was blinded from all other parameters. Odor judge malodor scores were recorded on a continuous unmarked 10-cm scale, marked at both ends as no odor and maximal odor, respectively. Results were scored independently for (i) whole mouth malodor, (ii) tongue malodor, and (iii) saliva malodor. For measurement of whole mouth malodor, subjects were requested to breathe out from the mouth, at *ca.* 10 cm from the nose of the judge. For measurement of tongue malodor, the subjects were instructed to extend their tongues and lick their wrists in a perpendicular fashion. Five seconds later, the odor judge assessed the odor from a distance of *ca.* 3 cm. For measurement of saliva malodor, the subjects were asked to expectorate into Petri dishes, which were incubated for approx. 5 min at 37°C; the dishes were then assessed by the odor judge at a distance of *ca.* 4 cm. Expectoration was carried out post-probing in order to potentiate saliva odor levels. The same saliva samples were then used immediately for BANA testing, and 100-µL samples were frozen at -20°C for subsequent diamine analysis (below).

BANA assay

The BANA reagent card (Perioscan™, Oral-B Laboratories, Redwood City, CA; Loesche *et al.*, 1990a,b) was used according to manufacturer's instructions. Results were recorded as either strong, dark blue spots (score = 2), weak, light blue spots (score = 1), or no color change (score = 0). For each subject, the average score was calculated for four samples, taken from a deep pocket, a shallow pocket, tongue dorsum, and saliva, in the following manner: Subgingival plaque was removed by curette from teeth presenting a shallow pocket (< 4 mm) and a deep pocket (≥ 4 mm), following local supragingival plaque removal. Subgingival plaque samples were then deposited directly onto the reagent card. A disposable plastic blade was used to obtain tongue samples by scraping the posterior tongue dorsum, and scrapings were applied directly onto the reagent card. Samples (*ca.* 10 µL) of expectorated saliva, taken following probing, were similarly applied onto the reagent card. Results comparing the BANA tests with dental and oral malodor parameters have been described separately (Kozlovsky *et al.*, 1994).

Diamine analysis

For high-performance liquid chromatography (HPLC) analysis, saliva samples were first derived by use of O-phthaldialdehyde reagent. O-phthaldialdehyde reagent solution was prepared by dissolution of 50 µg of O-phthaldialdehyde in 4.5 mL methanol, together with 0.5 mL borate buffer (0.9 M, pH = 9.5) and 50 µL of ß-mercaptoethanol. The solution was diluted 1:10 in methanol and kept at -20°C. To saliva samples (20 µL) were added 80 mL of borate buffer and 25 µL of the diluted reagent solution. The mixture was stirred briefly and injected (80 µL) into the HPLC system. The mobile phase of the HPLC was composed of a filtered, de-gassed 30:70 mixture of 12.5 mM sodium phosphate buffer (pH = 7.2)/acetonitrile. The HPLC system consisted of an Eldex 9600 pump (Eldex Laboratories, Inc., CA), an injection valve equipped with a 20-µL sample loop, and a C18 reverse-phase column (250 x 4 mm Lichrosphere 100 PR-18, 5 µm, Merck, Darmstadt, Germany). Samples were injected at a flow rate of 1 mL/min. Detection was carried out by a UV detector (Spectro Monitor 3,200, LDC Analytical, FL) at a wavelength of 231 nm.

Statistical analyses

Pearson correlation coefficients were used to determine the level of association of the various parameters. Stepwise forward multiple-regression analysis was carried out as

Table 1. Pearson correlation coefficients comparing diamine levels with malodor and dental parameters

	Log [cadaverine]	Log [putrescine]
Malodor Parameters		
Log Peak VSC	r = 0.137	0.112
	p = *1.0*[a]	*1.0*
Log Steady-state VSC	0.057	-0.007
	1.0	*1.0*
Whole mouth (odor judge)	0.375	-0.015
	0.027	
Tongue (odor judge)	0.453	0.127
	<0.009	*1.0*
Saliva (odor judge)	0.477	0.073
	<0.009	*1.0*
Dental Parameters		
PI	0.596	0.066
	<0.009	*1.0*
GI	0.568	0.031
	<0.009	*1.0*
Mean Probing Depth	0.471	0.109
	0.036	*1.0*
Mean BANA Score	0.409	0.006
	0.009	*1.0*

[a] Original p values have been multiplied nine-fold to adjust for multiple comparisons.

indicated. Statistical analyses were performed by SPSS software (SPSS Inc., Chicago, IL).

Results

Cadaverine levels in the 52 saliva samples tested ranged from 0 to 1395.3 μM, with a mean of 371.6 ± 307.6 μM (SD). Putrescine concentrations averaged 109 ± 92.7 μM, ranging from 21.4 to 599.4 μM. Putrescine and cadaverine levels, as well as VSC measurements, were log-transformed in order to obtain near normal distributions.

Pearson correlation coefficients comparing diamine levels with malodor and dental parameters are presented in Table 1. Log cadaverine scores were most highly associated ($p < 0.009$) with odor judge measurement of tongue and saliva, as well as with PI and GI scores. Significant correlations were also observed between cadaverine and the BANA test ($p = 0.009$), whole mouth odor judge scores ($p = 0.027$), and mean probing depth ($p = 0.036$). In contrast, no significant associations were found between cadaverine and VSC levels ($p = 1.0$).

Although log cadaverine and log putrescine scores were significantly associated with one another ($r = 0.482$; $p < 0.001$), log putrescine levels, *per se*, were not found to be significantly associated with any of the malodor or dental parameters (Table 1).

In order to relate each of the three odor judge measurements to both VSC levels and diamine concentrations, stepwise forward multiple-regression analysis was carried out (Table 2). In all three cases (whole mouth, tongue, and saliva), both VSC and log cadaverine levels factored into the regression equation, yielding multiple r values ranging from 0.545 (tongue; $p = 0.0002$) to 0.604 (saliva; $p < 0.0001$). In the case of whole mouth and tongue odor, log steady-state VSC values were not significant following entry of log peak VSC, whereas in the case of saliva, log steady-state levels were entered, and log peak VSC was subsequently left out of the regression equation. Putrescine levels were not significant in any of the analyses.

Stepwise forward multiple-regression analysis of dental indices (PI and GI) in terms of log cadaverine and log putrescine scores is summarized in Table 3. Both PI and GI were best explained by equations combining a positive association with log cadaverine scores and a negative association with log putrescine levels, yielding highly significant ($p < 0.0001$) multiple r values of 0.648 and 0.632, respectively.

Table 2. Stepwise forward multiple-regression analysis of odor judge assessments in terms of VSC and diamine concentrations

		Coefficient for				
Dependent Variable	Intercept	Log Peak VSC	Log Steady-state VSC	Log [cadaverine]	Log [putrescine]	Multiple r
Whole mouth	-9.38	5.86	N.E.[a]	1.55	N.E.	0.556
	0.0029[b]	*0.0009*	*0.3576*	*0.0180*	*0.0513*	*0.0001*
Tongue	-5.43	2.82	N.E.	1.30	N.E.	0.545
	0.0066	*0.0101*	*0.5099*	*0.0023*	*0.4345*	*0.0002*
Saliva	-14.38	N.E.	6.91	2.86	N.E.	0.604
	0.0002	*0.2104*	*0.0015*	*0.0004*	*0.1890*	*<0.0001*

[a] Not entered into the equation due to lack of significance.

[b] Numbers in italics represent levels of significance.

Table 3. Stepwise forward multiple-regression analysis of dental parameters in terms of diamine concentrations

Dependent Variable	Intercept	Coefficient for Log [cadaverine]	Coefficient for Log [putrescine]	Multiple r
Plaque Index	-0.34	0.99	-0.49	0.648
	0.4114[a]	*<0.0001*	*0.0239*	*<0.0001*
Gingival Index	0.00	0.80	-0.44	0.632
	0.9973	*<0.0001*	*0.0155*	*<0.0001*

[a] Numbers in italics represent levels of significance.

Discussion

The present investigation presents initial evidence in support of a role for cadaverine as a component of oral malodor. Cadaverine and putrescine are common bacterial degradation products, and may be produced in saliva as the result of decarboxylation [*i.e.*, of lysine and ornithine (Hayes and Hyatt, 1974)], or, in the case of putrescine, by transamination. Log-transformed cadaverine levels were significantly associated with whole mouth odor ($p < 0.027$), tongue odor ($p < 0.009$), and saliva odor ($p < 0.009$). No significant associations were found when comparing cadaverine with VSC measurements. Multiple-regression analysis indicated that both VSC and cadaverine levels factored significantly in accounting for odor judge measurements. In contrast, no association was found between putrescine and any malodor-associated parameters. The data raise the possibility that cadaverine elaboration in the oral cavity is carried out independent of VSC production, with both factors contributing to the overall oral malodor. This premise is further supported by preliminary *in vitro* data (not shown) showing that saliva samples, inoculated into growth medium, elaborate cadaverine over time, concomitant with appearance of malodor. Cadaverine has been previously shown to be a component of human dental plaque (Hayes and Hyatt, 1974) and is a malodorous product of bacterial putrefaction of meat and fish. Microbial production of cadaverine has been attributed to decarboxylation of lysine (Gale, 1946).

In contrast to cadaverine, putrescine levels, taken alone, did not correlate with any of the dental or malodor-associated parameters. Multiple-regression analysis of PI and GI in terms of cadaverine and putrescine showed that, whereas cadaverine contributed a highly significant positive association, putrescine contributed a significant negative association. These results raise the possibility that putrescine elaboration is inversely related to a factor of cadaverine production which detracts from cadaverine's overall association with PI and GI.

Most previous investigations which have studied the gaseous components contributing to oral malodor have concentrated on volatile sulphur compounds, primarily hydrogen sulphide and methyl mercaptan (Tonzetich and Richter, 1964). Correlations have been found between odor judge ratings and sulphide levels, by use of both gas chromatography and portable monitor techniques (Schmidt *et al.*, 1978). In one study (Tonzetich *et al.*, 1967), indole, methylamine, and cadaverine were added to bulk saliva, with no subsequent increase in odor or gas chromatographic detection of these components in the headspace. However, the gases contributing to oral malodor may be released into the oral headspace when saliva dries out on the mucosal surfaces (Rosenberg and McCulloch, 1992: I. Kleinberg, personal communication). Furthermore, different loci within the oral cavity elaborate odors with different characteristics, suggesting that a wider variety of gases contribute to oral malodor than was previously assumed. In this context, the highly significant correlations comparing cadaverine levels with PI, GI, probing depth, and tongue malodor suggest that both the periodontium and the tongue dorsum may be sites of cadaverine production. The association found here between cadaverine levels and the BANA test suggests that BANA-hydrolyzing micro-organisms present at those loci may be involved in cadaverine production. Experiments are under way to examine the relationship between cadaverine production by oral microbiota and malodor production at different sites in the oral cavity.

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References

Gale EF (1946). The bacterial amino acid decarboxylases. *Adv Enzymol* 6:1-32.

Hayes ML, Hyatt T (1974). The decarboxylation of amino acids by bacteria derived from human dental plaque. *Arch Oral Biol*

19:361-369.

Kostelc JG, Zelson PR, Prety G, Tonzetich J (1981). Quantitative differences in volatiles from healthy mouths and mouths with periodontitis. *Clin Chemist* 27:842-845.

Löe H, Silness J (1963). Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 21:533-551.

Loesche WJ, Bretz WA, Lopatin D, Stoll D, Rau CF, Hillenburg KL, *et al.* (1990a). Multi-center clinical evaluation of a chairside method for detecting certain periodontopathic bacteria in periodontal disease. *J Periodontol* 61:189-196.

Loesche WJ, Bretz WA, Kerchensteiner D, Stoll Y, Socransky SS, Hujoel P, *et al.* (1990b). Development of a diagnostic test for anaerobic periodontal infections based on plaque hydrolysis of benzoyl-DL-arginine-naphthylamine. *J Clin Microbiol* 28:1551-1559.

Rosenberg M, McCulloch CAG (1992). Measurement of oral malodor: current methods and future prospects. *J Periodontol* 63:776-782.

Rosenberg M, Kulkarni GV, Bosy A, McCulloch CAG (1991a). Reproducibility and sensitivity of oral malodour measurements with a portable sulphide monitor. *J Dent Res* 70:1436-1400.

Rosenberg M, Septon I, Eli I, Brenner S, Gelernter I, Gabbay J (1991b). Halitosis measurement by an industrial sulphide monitor. *J Periodontol* 62:487-489.

Schmidt NF, Missan SR, Tarbet WJ, Cooper AD (1978). The correlation between organoleptic mouth-odor ratings and levels of volatile sulfur compounds. *Oral Surg Oral Med Oral Pathol* 45:560-567.

Silness J, Löe H (1964). Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 24:747-759.

Tonzetich J (1971). Direct gas chromatographic analysis of sulphur compounds in mouth air in man. *Arch Oral Biol* 16:587-597.

Tonzetich J (1973). Oral malodour: an indicator of health status and oral cleanliness. *Int Dent J* 28:309-319.

Tonzetich J (1977). Production and origin of oral malodour: a review of mechanisms and methods of analysis. *J Periodont* 48:13-20.

Tonzetich J, Kestenbaum RC (1969). Odor production by human salivary fraction and plaque. *Arch Oral Biol* 14:815-827.

Tonzetich J, Richter VJ (1964). Evaluation of volatile odoriferous components of saliva. *Arch Oral Biol* 9:39-45.

Tonzetich J, Eigen E, King WJ, Weiss S (1967). Volatility as a factor in the inability of certain amines and indole to increase the odour of saliva. *Arch Oral Biol* 12:1167-1175.