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Original Article

Age-dependent aggravation of oral malodor and periodontal disease in dogs

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ABSTRACT — Halitosis or oral malodor is correlated with the concentration of volatile sulfur compounds (VSCs) produced in the oral cavity by metabolic activity of periodontal pathogenic germs. Our previous study demonstrated that VSCs in canine breath air can be measured using a portable VSC monitor. The aim of this study was to assess the association between oral malodor and periodontal disease in dogs. Forty-three laboratory Beagle dogs (1-16 years of age, 24 males, 19 females) were included in this study. Oral halitosis was evaluated by the organoleptic test score (OS) and by measuring the oral levels of VSCs: hydrogen sulfide (H_2S; HS), methyl mercaptan (CH_3SH; MM), and dimethyl sulfide (CH_3SCH_3; DMS) using OralChroma™. The calculus index (CI) and the gingival index (GI) were measured as periodontal parameters. Oral levels of halitosis parameters (OS, HS, MM, CI, and GI) in Group 2 dogs (7-16 years of age) were significantly higher than those in Group 1 dogs (1-6 years of age). In addition, significant positive relationships were found between oral malodor and periodontal disease, both of which are age-dependent in dogs. The present study suggested that aging is an important factor for oral malodor and periodontal disease in dogs.

Key words: Volatile sulfur compound, Portable gas chromatograph, Malodor, Periodontal disease, Dog

INTRODUCTION

Oral malodor (halitosis) and periodontal disease are very common in adult humans and companion animals. Oral malodor is primarily the result of microbial metabolism of amino acids from local debris in the oral cavity (Scully *et al*., 1994). The most common cause of oral malodor is increased levels of intraoral volatile sulfur compounds (VSCs), such as hydrogen sulfide (H_2S; HS), methyl mercaptan (CH_3SH; MM), and dimethyl sulfide (CH_3SCH_3; DMS), contrary to the traditional belief that amines and ammonia are the most important sources (Tonzetich and Carpenter, 1971). As even markedly low concentrations of VSCs were found to be toxic to perio-

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dontal tissues (Johnson *et al*., 1992), VSCs may not only be associated with oral malodor, but may also function in the etiology of periodontal diseases (Morita and Wang, 2001; Ratcliff and Johnson, 1999).

Quantification of oral malodor is a problem that has delayed scientific investigations into its causes and treatments. There are three generally accepted methods for the assessment of oral malodor: organoleptic measurement, gas chromatography (GC), and portable sulfide monitoring (Rosenberg *et al*., 1991). The portable sulfide monitor, a practical VSCs measuring device, accurately measures the amount of VSCs in a sample of mouth air (Tangerman and Winkel, 2008; Salako and Philip, 2011). The advantages of this monitor include the ease of use by non-skilled individuals, non-invasiveness, low possibility for cross infection, portability, relatively inexpensive cost, and rapid turnaround time of 1 to 2 min between measurements. The OralChroma™, a portable gas chromatograph for VSCs, which provides information on sulfide levels, has been recently developed for human use.

Recently, we reported that OralChroma™ could be used to measure oral levels of VSCs in canine breath air (Iwashita *et al*., 2017). As mentioned above, oral malodor and periodontal disease are very common in both humans and dogs, and there is an association between malodor and periodontal disease in humans (Yaegaki and Sanada, 1992). However, it remains to be investigated whether this is also true in dogs. In the present study, we examined the associations among clinical parameters, including oral malodor and periodontal disease, in 43 Beagle dogs.

MATERIALS AND METHODS

Animals

Forty-three laboratory Beagle dogs (19 females and 24 males, 1-16 years old, 9.0-16.5 kg body weight) with no systemic disorders were used in this study. The dogs recruited for the present study were selected among all the Beagle dogs housed at two research facilities by applying the following exclusion criteria: (a) age less than 1 year, (b) current participation in an experiment, (c) dental prophylaxis or treatment, or (d) treatment with an antimicrobial agent at least 3 months before the study. Both research facilities housed the dogs in similar conditions (21 ± 2°C room temperature and 55 ± 5% humidity, with a 12-hr light/dark cycle), including diets fed, which consisted of commercial dry pellets and water ad libitum. All experimental protocols were approved by the Animal Experiment Committee of Azabu University.

Periodontal examination

The periodontal condition was evaluated by measuring the calculus index (CI, scored in accordance with the modified Ramfjord index; scale 0 to 3) (Ramfjord, 1967) and the gingival index (GI, scored in accordance with the Loe gingivitis index; scale 0 to 3) (Loe and Silness, 1963) by a single researcher (M.S.), as previously described (Iwashita *et al*., 2017).

Measurement of oral malodor in dogs

Oral malodor was evaluated by the organoleptic test score and by measuring the levels of VSC using OralChroma™. The organoleptic test scores were determined by a single researcher (M.S.) blinded to the organoleptic examination. A score of 0-3 was given, where 0 represented absence of odor, 1 was barely noticeable odor, 2 was moderate malodor, and 3 was strong malodor. The three VSCs, HS, MM, and DMS were measured using an objective method with a portable sulfide monitor (OralChroma™, CHM-2, FIS Inc., Hyogo, Japan), as described previously (Iwashita *et al*., 2017). Briefly, oral air samples were taken from dogs by inserting a sterile disposable plastic 1-mL syringe into the oral cavity between the lips for 30 sec. Then, the plunger was pulled slowly, pushed in again, and pulled for a second time before removal from the mouth. The sample of mouth air (1.0 mL) was injected into the inlet of the OralChroma™. Three successive samples were collected and analyzed by the monitor, which provided the mean VSC concentration.

Statistical analysis

Descriptive statistics were presented as means ± standard deviation (SD) and analyzed by PRISM statistical software (GraphPad software, San Diego, CA, USA). The Mann-Whitney U test was used to determine significant differences between the 2 groups in clinical, microbiological, and VSC levels. Pearson rank correlation analyses were used to analyze the relationships among the clinical parameters. The level of significance was set at 5%.

RESULTS

Influence of age on clinical parameters

In order to investigate the influence of age on the periodontal conditions and oral malodor, dogs were classified into two groups: Group 1 (1-6 years old, n = 11) and Group 2 (7-16 years old, n = 32). The comparison of clinical parameters between Group 1 and Group 2 is presented in Table 1. No significant difference in oral bacterial number was observed between the two groups ($p > 0.05$).

Table 1. Influence of age on clinical parameters.

	Group I (Age: 1-6)	Group II (Age: 7-16)	p value
Body weight (kg)	11.4 ± 0.9	12.5 ± 2.2	0.034
Oral bacteria count (10^7 CFU)	3.2 ± 2.5	3.4 ± 2.4	0.16 (NS)*
Calculus index	1.0 ± 0.5	2.1 ± 0.6	0.000004
Gingival index	0.8 ± 0.6	1.8 ± 0.8	0.0003
Organoleptic score	1.5 ± 0.7	2.9 ± 0.3	0.00005
HS (ppb)	108.5 ± 99.2	523.7 ± 509.6	0.000010
MM (ppb)	52.9 ± 41.3	181.7 ± 179.3	0.0005
DMS (ppb)	24.5 ± 16.4	34.5 ± 30.4	0.18 (NS)*
∑VSC (ppb)	185.9 ± 132.4	739.9 ± 656.3	0.00006

Data are expressed as means ± standard deviation (SD). * NS: No significant difference (P > 0.05).

Table 2. Pearson correlation coefficients among clinical parameters.

	Age	Body weight	OBC	CI	GI	OS	HS	MM	DMS	ΣVSC
Age	-	-0.0336 NS*	0.2282 NS*	**0.7301** (p < 0.01)	**0.6023** (p < 0.01)	**0.7317** (p < 0.01)	**0.4804** (p < 0.01)	**0.4900** (p < 0.01)	0.0910 NS*	**0.5061** (p < 0.01)
Body weight			-0.0689 NS*	-0.0078 NS*	0.0710 NS*	0.2619 NS*	-0.1067 NS*	-0.2343 NS*	0.0409 NS*	-0.1433 NS*
OBC				**0.3134** (p < 0.05)	**0.3022** (p < 0.05)	-0.2401 NS (p > 0.05)	**0.3478** (p < 0.05)	**0.4018** (p < 0.01)	0.1410 NS*	**0.3826** (p < 0.02)
CI					**0.7372** (p < 0.01)	**0.6997** (p < 0.01)	**0.5684** (p < 0.01)	**0.4648** (p < 0.01)	-0.0212 NS*	**0.5623** (p < 0.01)
GI						**0.4955** (p < 0.01)	**0.4824** (p < 0.01)	**0.3412** (p < 0.05)	0.2657 NS*	**0.4756** (p < 0.01)
OS							**0.3789** (p < 0.02)	**0.3195** (p < 0.05)	0.1261 NS*	**0.3837** (p < 0.02)
HS								**0.7626** (p < 0.01)	0.2021 NS*	**0.9852** (p < 0.01)
MM									0.0471 NS*	**0.8586** (p < 0.01)
DMS										0.2134 NS*
∑VSC										-

OBC: oral bacteria count, CI: calculus index, GI: gingival index, OS: organoleptic test score, HS: hydrogen sulfide, MM: methyl mercaptan, DMS: dimethyl sulfide, VSC: volatile sulfur compounds. *NS: No significant difference (P > 0.05). A numbers of bold face mean a significant correlation.

In contrast, CI and GI among the periodontal parameters were significantly ($p < 0.05$) higher in Group 2 than in Group 1. The organoleptic score in Group 2 was also significantly higher than that in Group 1. In addition, oral levels of HS, MM, and ΣVSC (HS+MM+DMS) in Group 2 were significantly higher than those in Group 1, whereas there was no significant difference in DMS concentration between the two groups (Table 1).

The coefficient of correlation among clinical parameters

We further investigated possible associations among clinical parameters in dogs. The results are summarized in Table 2. No significant relationships were observed between body weight and other clinical parameters. Significantly positive and moderate to strong relationships were found between the age and organoleptic score ($r = 0.7301$), VSC (HS, MM, and ΣVSC) concentrations ($r = 0.4804$-0.5061), and periodontal parameters (CI and GI, $r = 0.6023$-0.7301). Significantly positive but mild to moderate relationships ($r = 0.3022$-0.4018) were observed between the oral bacteria count, and periodontal parameters (CI and GI) and VSC levels (HS, MM, and ΣVSC).

DISCUSSION

Oral malodor and periodontal disease are very common in adult humans and dogs. In the previous study (Hirai *et al*., 2013), we observed a positive correlation between age and periodontal disease in 176 privately owned pet dogs. In the present study, we examined the association between age and oral malodor in 43 laboratory Beagle

dogs. Oral malodor was evaluated by the organoleptic test and VSC monitor.

The result that CI and GI were age-dependent in the current study is consistent with our previous study reporting the age-dependency of periodontal disease in privately owned pet dogs (Hirai *et al*., 2013). In addition, halitosis parameters, OS, and VSCs (except DMS) levels were significantly higher in adult dogs than in younger dogs in the present study. These results demonstrate that oral malodor is positively correlated with periodontitis and age, consistent with the results of prior studies (Villa *et al*., 2014). These results also suggest that aging is an important risk factor of oral malodor and periodontal disease in dogs.

Among VSCs, there was no significant relationship between DMS and other VSC parameters. The VSC produced by intraoral bacteria were reported to vary according to bacteria species. Kuroshita *et al*. (2010) noted a positive relationship between *Porphyromonas gingivalis* and VSC level of HS and MM, and between *Aggregatibacter actinomycetemcomitans* and VSC levels of HS and DMS. The results of the current study are consistent with those of a previous study (Kuroshita *et al*., 2010). The strong correlation (r = 0.9852) between HS and ΣVSC is consistent with HS being the main component of ΣVSC. In contrast, the small ratio of DMS to ΣVSC, weak association with HS and MM, and low OS suggest that DMS mays play a minor role in oral malodor in dogs. Further studies are needed to clarify whether DMS is the optimal indicator of oral malodor in dogs.

Periodontal diseases are caused by pathogenic bacteria locally colonized in the dental calculus (Mandel and Gaffar, 1986; Coignoul and Cheville, 1984; Gupta *et al.,* 2016). In the current study, a positive correlation was observed between oral bacterial counts, and both periodontal parameters (CI and GI) and halitosis parameters (HS, MM, and ΣVSC). Although it is unclear why no association between oral bacteria count and periodontal parameters and halitosis parameters was observed, one possible reason is that pathogenic bacteria for oral malodor and periodontal disease are minor species among the oral bacteria. In a future study, the number of periodontal pathogenic bacteria that produce VSC, such as *Porphyromonas gingivalis, Treponema denticola,* and *Bacteroides forsythus,* should be examined.

To the best of our knowledge, this is the first study to analyze oral malodor and periodontal conditions in dogs with a focus on age. It is generally accepted that age is an important factor for the development of oral malodor and periodontitis in humans (Villa *et al*., 2014; Takeshita *et al.,* 2012; Panov, 2016; Van der Velden, 1984). The present study demonstrated a significant positive relationship between oral malodor and periodontal disease, both of which are age-dependent in dogs. Furthermore VSC, especially HS and MM, were suggested to play an important role in oral malodor and periodontal disease in adult dogs.

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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Coignoul, F. and Cheville, N. (1984): Calcified microbial plaque. Dental calculus of dogs. Am. J. Pathol., **117**, 499-501.

Gupta, S., Jain, P., Kumra, M., Rehani, S., Mathias, Y., Gupta, R., Mehendiratta, M. and Chander, A. (2016): Bacterial viability within dental calculus: an untrodden, inquisitive clinic-pathomicrobiological research. J. Clin. Diagn. Res., **10**, ZC71-ZC75.

Hirai, N., Shirai, M., Kato, Y., Murakami, M., Nomura, R., Yamasaki, Y., Takahashi, S., Kondo, C., Matsumoto-Nakano, M., Nakano, K. and Asai, F. (2013): Correlation of age with distribution of periodontitis-related bacteria in Japanese dogs. J. Vet. Med. Sci., **75**, 999-1001.

Iwashita, N., Sugita, K., Shirai, M., Murata, S., Yanagisawa, S., Goto, S., Takagi, Y. and Asai, F. (2017): Application of a portable gas chromatograph for quantitative measurement of canine oral malodor. Fundam. Toxicol. Sci, **4**, 23-29.

Johnson, P.W., Yaegaki, K. and Tonzetich, J. (1992): Effect of volatile thiol compounds on protein metabolism by human gingival fibroblasts. J. Periodontal Res., **27**, 553-561.

Kuroshita, R., Amano, S., Takahashi, S., Sakagami, H., Ohmori, Y. and Watanabe, S. (2010): Relation between count of periodontopathic bacteria in the tongue coat of healthy subjects and the concentration of volatile sulfur compounds in their breath. Ped. Dent. J., **20**, 57-64.

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

Mandel, I.D. and Gaffar, A. (1986): Calculus revisited. A review. J. Clin. Periodontol., **13**, 249-257.

Morita, M. and Wang, H.-L. (2001): Association between oral malodor and adult periodontitis: a review. J. Clin. Periodontol., **28**, 813-819.

Panov, V. (2016): Bad breath and its association with age and gender. Scr. Sci. Med. Dent., **2**, 12-15.

Ramfjord, S.P. (1967): The Periodontal Disease Index (PDI). J. Periodontol., **38**, 602-610.

Ratcliff, P.A. and Johnson, P.W. (1999): The Relationship Between Oral Malodor, Gingivitis, and Periodontitis. A Review. J. Periodontol., **70**, 485-489.

Rosenberg, M., Septon, I., Eli, Ilana, Bar-Ness, R., Gelernter, I., Brenner, S. and Gabbay J. (1991): Halitosis Measurement by an Industrial Sulphide Monitor. J. Periodontol., **62**, 487-489.

Salako, N.O. and Philip, L. (2011): Comparison of the Use of the

Halimeter and the Oral Chroma™ in the Assessment of the Ability of Common Cultivable Oral Anaerobic Bacteria to Produce Malodorous Volatile Sulfur Compounds from Cysteine and Methionine. Med. Princ. Pract., **20**, 75-79.

Scully, C., Porter, S. and Greenman, J. (1994): What to do about halitosis. Regular use of toothbrush and dental floss. BMJ, **308**, 217-218.

Takeshita, T., Suzuki, N., Nakano, Y., Yasui, M., Yoneda, M., Shimazaki, Y., Hirofuji, T. and Yamashita, Y. (2012): Discrimination of the oral microbiota associated with high hydrogen sulfide and methyl mercaptan production. Sci. Rep., **2**, 215.

Tangerman, A. and Winkel, E.G. (2008): The portable gas chromatograph OralChroma™: a method of choice to detect oral and extra-oral halitosis. J. Breath Res., **2**, 1-6.

Tonzetich, J. and Carpenter, P.A.W. (1971): Production of volatile sulphur compounds from cysteine, cystine and methionine by human dental plaque. Arch. Oral Biol., **16**, 599-607.

Van der Velden, U. (1984): Effect of age on the periodontium. J. Clin. Periodontol., **11**, 281-294.

Villa, A., Zollanvari, A., Alterovitz, G., Cagetti, M.G., Strohmenger, L. and Abati, S. (2014): Prevalence of halitosis in children considering oral hygiene, gender and age. Int. J. Dent. Hyg., **12**, 208-212.

Yaegaki, K. and Sanada, K. (1992): Biochemical and clinical factors influencing oral malodor in periodontal patients. J. Periodontol., **63**, 783-789.