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ORIGINAL ARTICLE

Clinical oral malodor measurement with a portable sulfide monitor

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OBJECTIVE: The aim of this study was to evaluate the clinical ability of Breathtron® by comparing it with other malodor measurement procedures: the organoleptic test (OT) and gas chromatography (GC).
SUBJECTS AND METHODS: Patients were 475 patients who visited a fresh breath clinic. Oral malodor was measured with the OT, GC, and Breathtron®. Correlation analysis and two linear regression analyses were conducted to examine the relationship of the Breathtron® values with OT scores and volatile sulfide compound (VSC) concentrations by GC: i.e. the regression of Breathtron® on OT and the regression of Breathtron® on total VSCs by GC. Receiver operating characteristics (ROC) analysis was conducted to investigate the sensitivity and specificity of Breathtron®.
RESULTS: The Breathtron® values were significantly correlated with OT and VSCs by GC. In the regression analysis, predicted Breathtron® values were 199.3 and 520.1 ppb for OT scores 1 and 2, and reasonably close to total VSCs by GC between 550 and 750 ppb. The ROC analysis demonstrated that Breathtron® is a useful and valuable adjunct measurement tool.
CONCLUSIONS: Breathtron® is a simple, rapid and reliable appliance for screening oral malodor if an appropriate malodor threshold level is chosen.
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Keywords: oral malodor; organoleptic test; gas chromatography; Breathtron®

Introduction

Oral malodor (bad breath or halitosis) is the fourth most common dental complaint among Japanese people, according to the national survey on health and welfare conducted in 1999 (Ministry of Health, Labour and Welfare, 1999). Approximately 15% of people who had dental problems suffered from bad breath. Hence oral malodor is deemed to be a subject of considerable public interest in Japan.

A number of intra- and extra-oral factors cause oral malodor, but in almost 90% of cases, the oral cavity is the origin of the malodor (Delanghe *et al*, 1999). Extra-oral origins include upper and lower respiratory tract conditions, gastrointestinal disorders and various systemic diseases (Attia and Marshall, 1982). It has been reported that anaerobic and mainly gram-negative bacteria within the oral cavity degrade amino acids in food debris, desquamated cells from oral mucosa, salivary proteins, leucocytes, dental plaque and microbial putrefaction to produce volatile sulfide compounds (VSCs) (Kostelc *et al*, 1980; Tonzetich and McBride, 1981; Attia and Marshall, 1982; Persson *et al*, 1990). VSCs, in particular hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulfide [$(CH_3)_2S$] are recognized as the predominant contributors to oral malodor (Tonzetich and Richter, 1964). The intensity of halitosis has been demonstrated to be significantly related to intra-oral VSCs level (Replogle and Beebe, 1996; Oho *et al*, 2001).

Malodor measurement is complex and is influenced by various elements such as gaseous molecular species, sampling procedures and judgment standards. It is also subject to the sensitivity, specificity and accuracy of the appliance used. Currently, three types of malodor assessment methods, gas chromatography (GC), the organoleptic test (OT), and sulfide monitoring are commonly used in halitosis research. A new sulfide monitoring instrument, Breathtron® has become popular in Japan because of its portability, simplicity and efficiency (Iwakura *et al*, 2002a,b; Washio *et al*, 2005), but its measurement ability in a clinical setting has not been fully investigated. Thus, the aim of this study was to evaluate the ability of Breathtron® in a clinical setting by comparing it with two other malodor measurement procedures.

Subjects and methods

The subjects were 475 patients, ranging in age from 16 to 80 years (mean age: 46.1 ± 14.4), who visited a fresh

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Table 1 Demographic characteristics of subjects

Age group	*Gender*		*Total number*
	Male, n (%)	*Female, n (%)*	
–29	18 (26.1)	51 (73.9)	69
30–39	26 (25.2)	77 (74.8)	103
40–49	22 (23.7)	71 (76.3)	93
50–59	30 (24.6)	92 (75.4)	122
60–	31 (35.2)	57 (64.8)	88
Total	127 (26.7)	348 (73.3)	475

breath clinic at a dental hospital (Tokyo Medical and Dental University). Table 1 describes demographic characteristics of the patients by age-group and gender. Oral malodor was measured with three types of methods: OT, GC, and Breathtron®.

Patients who agreed to participate in the study signed an informed consent form. The Ethical Committee for Human Research of the Tokyo Medical and Dental University approved the study protocol.

Malodor measurement

The procedure for oral malodor measurement was explained to the patients at their first visit. To reproduce genuine oral malodor, patients were advised to have no food or drink and to refrain from their usual oral hygiene practice on the morning of the appointment. They were also instructed 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 malodor assessment day to exclude confounding smells. Measurements were conducted between 9 and 11 o'clock in the morning because morning breath odor has been used as a model to investigate other offensive mouth breath (van Steenberghe *et al*, 2001). Patients closed their mouth for 3 min prior to each malodor measurement and breathed only through their nose.

Organoleptic test

The OT was performed by trained dentists. The standardization of examiners was done with the T&T Olfactometer® (Daiichi Yakuhin Sangyo Co., Tokyo, Japan), an odor solution kit for examining the olfactory sense, to calibrate the consistency of judgment before the measurements (Kawamoto *et al*, 2002; Murata *et al*, 2002). Judges rated the malodor on a 0–5 score, referring to previous criteria (Rosenberg *et al*, 1991a,b; Rosenberg and McCulloch, 1992) 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 and 1 were diagnosed as normal whereas those with scores of 2 and higher were diagnosed as having malodor. Examiners were blind to both the VSC concentrations by GC and the Breathtron® values, to avoid possible judgment biases. If two judges gave different scores, the mean score was used as the representative score for that patient.

Gas chromatography

A GC-8A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame photometric detector was used for the GC analysis. It has an auto-injection system with a 10 ml Teflon® (Du Pont, Tokyo, Japan) sample loop and a column packed with 25% 1,2,3-tris (2-cyanoethoxy) propane on an 80/100 mesh Shimalite AW-DMCS-ST support system at 60°C. The Teflon® tube was directly inserted into the oral cavity of a patient through the lips and teeth for the malodor measurement, and 20 ml of mouth air was aspirated with a syringe connected to the outlet of the auto-injector. Following the aspiration, a 10 ml sample of air was transferred to the column and chromatographed by a sulfur chemiluminescence detector that specifically responded to sulfur. The VSCs gases H_2S, CH_3SH and $(CH_3)_2S$ were determined by their characteristic retention times, and quantities were calculated by comparing their peak areas with those of dilutions of standard gases of H_2S, CH_3SH and $(CH_3)_2S$ that were prepared with a PD-1B permeater (Gastec Company, Kanagawa, Japan). Outcomes were shown as concentrations of H_2S, CH_3SH and $(CH_3)_2S$ (ng 10 ml^{-1}). Based on the olfactory threshold levels (H_2S > 1.5 ng 10 ml^{-1}, CH_3SH > 0.5 ng 10 ml^{-1} and $(CH_3)_2S$ > 0.2 ng 10 ml^{-1}) proposed by Tonzetich (1977), patients were classified as either normal or having malodor.

Breathtron®

Breathtron® (Yoshida, Tokyo, Japan), which is a semi-conductor type sulfide monitor, is composed of an air intake, sensor detector, control panel, digital display and printer. The semi-conductor sensor is based on a thick ZnO membrane that has a high specificity for VSCs (Shimura *et al*, 1996). The disposable mouthpiece, which has a build-in filter to eliminate other volatile compounds (like ketone and alcohol in toothpaste and mouth wash) is inserted into an end of the Teflon® tube connected to the monitor inlet. Breathtron® requires 1 min and 45 s for warm-up before operation, 45 s for measurement and 1 min and 30 s for each succeeding measurement. Measurements were performed by directly inserting the disposable mouthpiece into the patient's oral cavity. The patients closed their mouth tightly and breathed through their nose during the measurement. The aspiration rate of mouth air was 40–60 ml min^{-1}, and the Breathtron® values were presented in units of ppb.

Statistical analysis

Data analysis was done with the Statistical Package for Social Science (SPSS version 14J, SPSS Japan, Tokyo, Japan). Mean OT score, VSCs concentration by GC and the Breathtron® values were calculated by age and gender. Analysis of variance (ANOVA) was used to investigate the mean differences of the OT scores, VSC concentrations by GC and the Breathtron® values among different groups, followed by a Bonferroni multiple comparisons procedure.

As the distributions of VSCs concentration by GC and the Breathtron® values were not normal, natural log

transformations were made. The distribution after transformation was diagnosed with a normal probability plot and the Shapiro–Wilk test. The association among Breathtron® values, OT scores and VSC concentrations by GC was analyzed using Pearson correlation coefficients.

To further examine the relationship of the Breathtron® values with OT scores and VSC concentrations by GC, two linear regression analyses were conducted: a regression of Breathtron® values on OT scores as the independent variable and a regression of Breathtron® values on the total VSC concentrations [H_2S + CH_3SH + $(CH_3)_2S$] by GC as the independent variable. In the regression analysis, the total VSC concentrations by GC were converted into ppb to make them comparable to the Breathtron® values, assuming that the mouth air temperature and pressure were 37°C and 1013 hPa, respectively. In addition, receiver operating characteristics (ROC) analysis was conducted to investigate the sensitivity and specificity of Breathtron® compared with OT scores as an oral malodor diagnosing identifier.

Results

The kappa statistics computed between two examiners for the OT score ranged from 0.76 to 0.84 and showed good reproducibility. The mean OT scores, VSC concentrations by GC and the Breathtron® values were 1.88 ± 0.75 (OT), 6.57 ± 7.06 (H_2S), 3.43 ± 5.50 (CH_3SH), 0.98 ± 2.78 [$(CH_3)_2S$] and 779.9 ± 720.8 (Breathtron®), respectively. No statistically significant difference was found for any of the mean values of measurements by age-group. Similarly, gender showed no statistically significant difference, except that the OT scores for males (mean ± s.d.: 2.06 ± 0.80) showed a slightly higher mean value than those of females (1.81 ± 0.72) ($P < 0.01$).

As seen in Table 2, Breathtron® values were significantly correlated with OT scores ($r = 0.610$, $P < 0.01$) and VSC concentrations by GC. Among correlations with VSCs gases, the correlation with H_2S was the highest ($r = 0.687$, $P < 0.01$) followed by total VSCs ($r = 0.682$, $P < 0.01$), CH_3SH ($r = 0.584$, $P < 0.01$), and $(CH_3)_2S$ ($r = 0.506$, $P < 0.01$). The correlations of OT scores with VSCs gases were similar to those of Breathtron®. The correlations among VSCs gases by GC were all high and ranged from 0.75 to 0.90.

The following two linear regression equations were obtained: $Y = 4.34 + 0.96X$ (Y: natural log of the Breathtron® value, X: OT score, adj $R^2 = 0.37$, $P < 0.001$) (Figure 1). $Y = 2.12 + 0.68$X (Y: natural log of Breathtron® value, X: natural log of total VSCs concentration by GC, adj $R^2 = 0.47$, $P < 0.001$) (Figure 2).

The Breathtron® values predicted by OT scores from the first regression equation were 199.3, 322.0 and 520.1 ppb for OT scores of 1, 1.5 and 2, respectively. The Breathtron® values were also predicted by total

Table 2 Correlation coefficients among Breathtron®, organoleptic test (OT) and volatile sulfide compounds (VSCs) by gas chromatography (GC)

		GC			
	OT	*H_2S*	*CH_3SH*	*$(CH_3)_2S$*	*Total VSCs*
Breathtron®	0.610**	0.687**	0.584**	0.506**	0.682**
OT		0.615**	0.588**	0.525**	0.627**
GC					
H_2S			0.852**	0.758**	0.970**
CH_3SH				0.868**	0.944**
$(CH_3)_2S$					0.856**

**$P < 0.01$.

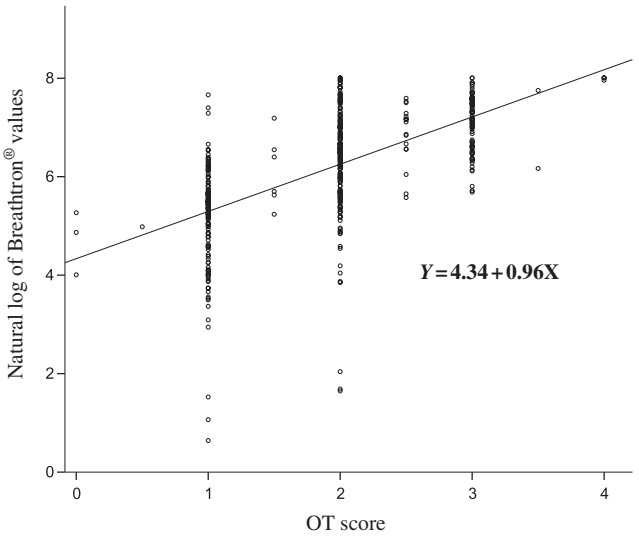

Figure 1 Scatterplot of Breathtron® values *vs* organoleptic test (OT) scores and the regression line of Breathtron® values on OT scores as the independent variable ($Y = 4.34 + 0.96X$)

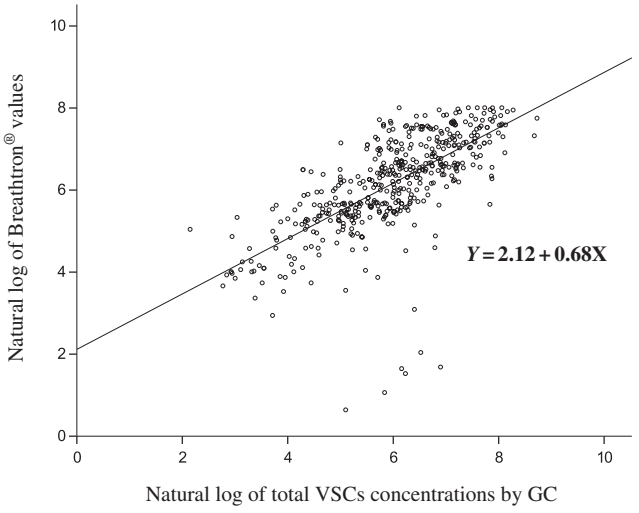

Figure 2 Scatterplot of Breathtron values *vs* total volatile sulfide compound (VSC) concentrations by gas chromatography (GC) and the regression line of Breathtron® values on the total VSC concentrations [H_2S + CH_3SH + $(CH_3)_2S$] by GC as the independent variable ($Y = 2.12 + 0.68X$)

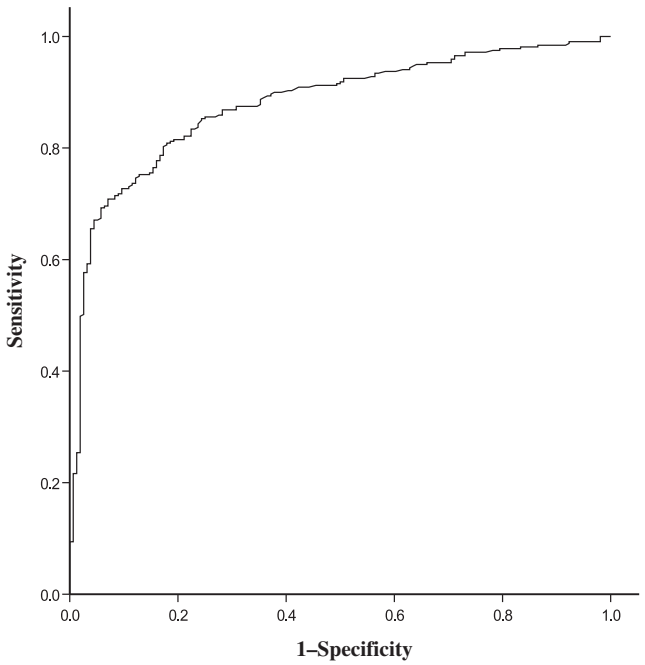

Figure 3 Receiver operating characteristics curve of the Breathtron® values, where the organoleptic test score was used as an oral malodor diagnosing identifier

VSC concentrations by GC from the second regression. Breathtron® displayed slightly higher values than corresponding total VSC concentrations by GC up to approximately 500 ppb of VSCs concentration, presented reasonably close values between 550 and 750 ppb, and then exhibited relatively lower values for total VSC concentrations by GC that were >750 ppb.

Figure 3 illustrates the ROC curve of the Breathtron® values, where the OT score was used as an oral malodor diagnosing identifier. The curve closely follows the left-hand border and the top border of the ROC space, demonstrating that Breathtron® is an accurate oral malodor diagnosing appliance. According to the ROC, when the cut-off point of Breathtron® is selected between 250 and 400 ppb, the range of sensitivity is 0.8–0.9 and the specificity is 0.6–0.8.

Discussion

It is generally accepted that the human nose is capable of detecting differences in the strength or concentration of odor molecules (Engen, 1964). As halitosis is a multifactorial symptom (Klokkevold, 1997), it is difficult to diagnose patients with a single parameter with a single assessment, other than the OT. Therefore, the OT is believed to be the most reliable and practical method for clinically diagnosing oral malodor and is regarded as a kind of reference standard of oral malodor measurement (Rosenberg, 1995), although some subjectivity is still expected even after a rigorous calibration.

The measurement of VSCs by GC allows a consistent and quantitative determination of VSCs gases (Tonzetich *et al*, 1967; Murata *et al*, 2002). GC has a couple of advantages: (i) separation and quantitative measurement of each of the VSCs gases; and (ii) the ability to measure very low concentration of VSCs gases. In contrast, the main disadvantages of GC are: (i) a relatively high cost; (ii) the need for skilled personnel; (iii) cumbersomeness and lack of portability; and (iv) the time required for detection and measurement (Rosenberg and McCulloch, 1992).

Halimeter® (Interscan Corp, Chatworth, CA, USA) is a popular sulfide monitor and has been widely used for oral malodor research because it is relatively inexpensive and easy to use (Rosenberg *et al*, 1991a,b; Silwood *et al*, 2001; Furne *et al*, 2002). Breathtron® could become an attractive alternative choice for measuring oral malodor as it is more portable (W150 × D203 × H150 mm, 2 kg) compared with Halimeter® (W254 × D267 × H114, 3.6 kg), simple and easy to use, for that reason, we evaluated its oral malodor measurement ability by comparing with the OT and GC. Breathtron® values yielded highly significant correlations with OT scores, and with each VSCs gas concentration and total VSCs concentration by GC. These correlations were comparable with previous reports (Rosenberg *et al*, 1991b; Furne *et al*, 2002; Iwakura *et al*, 2002a,b). Among these correlations, the correlation with H_2S was the highest and that with $(CH_3)_2S$ was the lowest, suggesting that the Breathtron® value was possibly most influenced by H_2S concentration. A sulfide monitor like Halimeter® was reported to have high sensitivity for H_2S, but only low sensitivity for CH_3SH (Silwood *et al*, 2001). Thereby, Breathtron® may have characteristics similar to that of Halimeter®. Further study would be required to confirm these characteristics.

Two linear regression analyses determined predicted values of Breathtron® for OT scores and total VSCs concentration by GC. The predicted Breathtron® values corresponding to OT scores of 1, 1.5 and 2 were 199, 322 and 520 ppb, respectively. Considering that an OT score of 2 is used as the oral malodor classification criteria, the oral malodor threshold level of Breathtron® would roughly range from 300 to 500 ppb.

Volatile sulfide compounds are the main gases that are routinely measured for assessing oral malodor and Breathtron® also responses to these gases. Thus comparing values from Breathtron® with total VSC concentrations by GC could provide reference standards for Breathtron® values. H_2S, CH_3SH and $(CH_3)_2S$ are the major components of VSCs, which are used to document the existence of oral malodor. Although other kinds of VSCs gases may exist, we regarded the sum of those three gases as the total VSCs concentration. The regression of Breathtron® values on total VSCs concentration by GC revealed that the estimated slope was not 1, nor was the intercept 0. In other words, the two types of measurements were not perfectly associated. Consequently, Breathtron® values between 550 and 750 ppb reflect total VSCs concentration by GC fairly well, but Breathtron® values below 550 ppb overestimate the equivalent VSCs concentration by GC, and Breathtron® values above 750 ppb are likely to underestimate total VSC concentrations.

The area under ROC curve is a reflection of how well Breathtron® distinguishes between patients with oral malodor and those without oral malodor. The greater the area the better the diagnosis with Breathtron®. The ROC curve indicated that Breathtron® could be a useful and valuable adjunct oral malodor screening appliance. The sensitivity and specificity were computed using specific cut-off points of the Breathtron® values. In the manufacturer's instructions and previous studies, patients with values of 250 ppb and below were categorized as normal and patients with values above this threshold were categorized as having malodor (Iwakura *et al*, 2002a,b). However, the results of this study suggest that the malodor threshold level of 250 ppb is too low. A sensitivity of 0.9 at the cut-off point of 250 ppb is very high and preferable, but the specificity of 0.6 seems a little low. For instance, about 90% of patients with oral malodor would be correctly classified as oral malodor patients, but 40% of patients without oral malodor would be incorrectly classified as having oral malodor. Taken together with the results of the linear regression, a threshold level between 350 and 400 ppb, where sensitivity and specificity are both around 0.80 and corresponding OT scores are between 1.5 and 2, would be appropriate in screening oral malodor in a clinical setting.

To date, the OT and VSCs measurements by GC have been widely used to assess oral malodor in numerous studies (Yaegaki and Coil, 2000; Koshimune *et al*, 2003; Lee *et al*, 2003; Awano *et al*, 2004; Greenman *et al*, 2005; Roldán *et al*, 2005). However, neither GC nor OT is amenable to quantitative measurement of large patient populations, and both these procedures are technically difficult and time-consuming. The use of Breathtron® would be a possible solution to this problem, because it is: (i) compact and portable; (ii) easy and simple to use; (iii) non-invasive, aspirating a small volume of mouth air (40–60 ml); (iv) inexpensive; (v) has a short sampling and turnaround time; and (vi) easy maintenance. Based on the above beneficial characteristics and its high sensitivity and specificity, Breathtron® is an alternative oral malodor screening instrument even if it lacks the ability to distinguish among the specific VSCs gases.

In summary, the results of this study suggest that Breathtron® constitutes a simple, rapid and reliable appliance for screening oral malodor, if an appropriate malodor threshold level is chosen. Breathtron® is particularly useful for field surveys of mass malodor measurement as well as for an oral malodor screening instrument in clinical dental practices because of its portability, ease of use and quickness. However, further clinical studies are needed to investigate the malodor measurement ability of Breathtron® in different sample groups and in oral conditions such as periodontal disease.

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