Clinical Application of a VSCs Monitor for Oral Malodour Assessment

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Purpose: The purpose of this study was to evaluate a volatile sulfur compounds (VSCs) monitor's ability to assess oral malodour in patients with and without periodontal disease in comparison with the assessment by gas chromatography (GC) or organoleptic testing.

Materials and Methods: Ninety-nine patients' mouth air was measured by GC, a VSCs monitor (Breathtron™) and organoleptic test. Patients who had a periodontal pocket depth of 4 mm and more for at least two tooth surfaces were assigned to the periodontal disease group.

Results: Total VSCs value by the Breathtron™ was higher in periodontal disease group than that in non-periodontal disease group, and it showed statistically significant correlations with specific VSCs gases from GC and with the organoleptic measurement. The Breathtron™ had high sensitivity in both groups.

Conclusion: The Breathtron™ can be a reliable instrument for the diagnosis of halitosis. However, the Breathtron™ should be used properly for measuring VSCs that are related to periodontal disease.

Key words: Oral malodour assessment, volatile sulfur compounds (VSC)

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Oral malodour may be caused by several intra- and extra-oral factors (Tangerman, 2002; Murata et al, 2002). Volatile sulfur compounds (VSCs), especially hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide ($(CH_3)_2S$), are the prominent elements of oral malodour (Tonzetich, 1971). The assessment of VSCs is an important criteria for classification of halitosis (Murata et al, 2002), and the measurement of VSCs is useful for diagnosing and monitoring the benefits of therapy for halitosis patients.

There are three main methods of VSCs assessment - gas chromatography (GC), organoleptic measurement and sulfide monitoring (Yaegaki and Coil, 2000). GC is considered the gold standard for measuring concentration of VSCs in mouth air, since it can measure the levels of specific VSCs gases. However, GC is not appropriate for chair-side clinical use because it requires a costly large-scale system, a long run time and an experienced operator. Recently, a portable gas chromatography (Oral Chroma™, Abilit Corporation, Osaka, Japan) has been used to investigate VSC levels (Aizawa et al, 2005). Although a previous study has reported high sensitivity to measure all VSCs in the mouth air (Hanada et al, 2003), nevertheless such a device still needs to be clinically tested for the measurement ability. The simplest approach to measurement of oral malodour are the organoleptic ratings by human judges (Shimura et al, 1996). This method closely simulates the everyday situations in which bad breath is detected, but it is subjective and requires trained odour judges whose reliability has been questioned (Rosenberg et al, 1991a; Schmidt et al, 1978).

Between these extremes a portable sulfide monitor - e.g. the Halimeter™ (Interscan Corporation, Chatsworth, California, USA) (Rosenberg et al, 1991a; Rosenberg et al, 1991b; Furne et al, 2002) and VSCs monitors (Shimura et al, 1996) have been reported to be inexpensive, easily used devices for measurement of VSCs concentration. However, all sulfide monitors analyse the total sulfur content of patients' mouth air, not specifically for VSCs. The Halimeter™ has a

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low sensitivity for CH_3SH, a significant contributor to halitosis caused by periodontal disease (Yaegaki and Coil, 2000; Brunette, 2002).

Therefore, this study evaluated the ability of a new VSCs monitor (Breathtron™, New Cosmos Electric Company, Osaka, Japan) to assess oral malodour in patients with or without periodontal disease and compared its results with those of gas chromatography and organoleptic testing.

MATERIALS AND METHODS

The subjects were 99 patients (29 males and 70 females, mean age 44.8 ± 15.0 years, mean number of teeth 25.4 ± 4.9) who visited the Fresh Breath Clinic, Dental Hospital, Tokyo Medical and Dental University. At the first visit, a questionnaire about their chief complaint, their malodour history and their dental and medical history related to malodour were administered first, followed by periodontal examinations.

Subjects were instructed how to prepare themselves (see below) before the assessment of malodour at the second visit. Each patient's mouth air was measured by three methods: GC, the Breathtron™ and an organoleptic test. Patients enrolled in the study signed an informed consent form, and this study was approved by the Ethical Committee for Human Research, Tokyo Medical and Dental University.

Periodontal Examination

Two calibrated dentists examined the patients' periodontal status. Both labial (buccal) and lingual surfaces of each tooth were probed using a manual periodontal probe, PCP UNC15 Hu-Friedy (Hu-Friedy Mfg. Co. Inc., Chicago, Illinois, USA). On each tooth surface, three sites (distal, middle and mesial) were explored. Patients were divided into two groups: a periodontal disease group with periodontal probing depths of 4.0 mm and over in at least two tooth sites and a non-periodontal disease group with probing depths of 4.0 mm and over in less than two tooth sites (Figueiredo et al, 2002).

Malodour Assessment

Patients were instructed to abstain from eating strong-smelling foods for at least 48 hours, from using scented cosmetics for 24 hours and from smoking for 12 hours before the assessment appointment. To maximise oral VSCs, patients were advised not to ingest any food or drink and to omit their usual oral hygiene practice on the morning of the assessment day. Measurements were conducted between 9 and 11 o'clock in the morning to evaluate the morning breath odour that can be used as a model to investigate other offensive breath odours (van Steenberghe et al, 2001). Patients were instructed to close their mouth for 3 minutes in an upright position prior to each sample collection (GC, Breathtron™ and organoleptic test) and breathe through their nose during the measurements.

1. Gas Chromatography (GC)

The gas chromatography analysis was carried out using a GC8A gas chromatograph (Shimadzu, Japan), equipped with a flame photometric detector. It has a Teflon column packed with 25% 1,2,3-Tris(2-Cyanoethoxy)Propane (TCEP) on 80/100 mesh Shimalite AW-DMCS-ST support system at 60 °C, and an auto-injection system with a 10 ml sample loop. After patients closed their mouth for three minutes, the Teflon tube connected to the auto-injector was inserted into the centre of the oral cavity through the lips and teeth, while the mouth remained closed. Following aspiration of 20 ml of mouth air with the syringe connected to the outlet of the auto-injector, a 10 ml sample of air was transferred to the column and chromatographed. A sulfur chemiluminescence detector that specifically responds to sulfur was used. The system was connected to a computerised recorder. VSCs were identified by their characteristic retention times, and quantities were determined by comparing their peak areas with those of dilutions of standard gases. Standard gases of H_2S, CH_3SH and $(CH_3)_2S$ were prepared with a PD-1B permeater (Gastec Company, Japan). Before the assessment, the ambient air was checked by GC to indicate 0 ng/10 ml. Results are given as concentrations of H_2S, CH_3SH and $(CH_3)_2S$. Using olfactory threshold levels suggested by Tonzetich (Tonzetich, 1977) (H_2S>1.5 ng/10 ml, CH_3SH >0.5 ng/10 ml and $(CH_3)_2S$>0.2 ng/10 ml), the subjects were identified as belonging to either a normal group or a malodour group.

2. Breathtron™

The portable VSCs monitor (Breathtron™, New Cosmos Electric Company, Osaka, Japan) is shown in Fig 1. The system is composed of a gas intake part, sensor detector, control panel and digital display of the output. This monitor is a semiconductor type VSCs sensor based on a ZnO thick film with a special filter inside the disposable mouthpiece. The special filter

Fig 1 VSCs monitor (Breathtron™)

was developed to trap ketone and alcohol smells in toothpaste, mouth rinse and mouth air, and has been described in more detail in a previous study (Shimura et al, 1996). The VSCs monitor's rate of inhaled air is 50-80 ml/min. It requires one minute 45 seconds for warming up before operation, 45 seconds for monitoring and one minute 30 seconds for purging the system. Measurement was performed by inserting the disposable mouthpiece with Teflon tube, which was connected to the monitor inlet, into the oral cavity. The subject was asked to breathe through his/her nose during measurement. Operation time is indicated by the digital display on the control panel. This instrument provides and prints out a digital read-out of the total VSCs concentration in gas aspirated from the oral cavity. The manufacturer recommends annual re-calibration. According to the manufacturer's instructions and previous studies, subjects with levels below 250 ppb were classified as belonging to the normal-odour group and subjects with levels above this threshold were classified as belonging to the malodour group (Iwakura et al, 2002a; Iwakura et al, 2002b).

3. Organoleptic Measurement

The organoleptic score was measured by two trained judges. Odour judges' sensitivity of smell was standardised by the T&T Olfactometer™ (Daiichi Yakuhin Sangyo Co., Tokyo, Japan), an odour solution kit for measuring the olfactory sense, before the experiments to maintain judges' consistency (Murata et al, 2002; Kawamoto et al, 2002). Judges were asked to rate on an integer 0-5 score, based on previous work (Rosenberg et al, 1991; Rosenberg and McCulloch, 1992) where 0 represented absence of odour, score 1 was given for barely noticeable odour, 2 for slight malodour, 3 for moderate malodour, 4 for strong malodour and 5 for severe malodour. Subjects with scores of 0, 1 and 2 were placed in the normal-odour group, while those with scores greater than 2 were placed in the malodour group. Both judges were blind to the VSCs concentration from both the GC and VSCs monitor in order to prevent any bias. If two judges gave different scores a mean score was used as the representative score for that patient.

Statistical Analysis

Data were analysed using the Statistical Package for Social Science (SPSS version 11). Inter-examiner reliability for organoleptic measurement between two judges was assessed by the kappa statistic. Unweighted kappa reliability for the organoleptic measurement showed a high correlation (k=0.80). Because of the non-normal distribution of VSCs values from GC and the Breathtron™, a transformation to natural logarithms was performed. The resulting normal distributions were checked by the Kolmogorov-Smirnov test. An independent-sample t-test was used to test for differences of each GC measurement, VSCs level of the Breathtron™ and organoleptic scores between the periodontal disease group and non-periodontal disease group. Pearson correlation co- efficients were determined between the Breathtron™ readout and the other two methods. A p-value of less than 0.05 was considered significant. The ability of the Breathtron™ to detect halitosis cases (sensitivity) and its ability to identify non-halitosis cases (specificity) were calculated using the GC and organoleptic measurements as identifiers of halitosis.

RESULTS

The subjects in this study suffered from bad breath for several months or years. Eighty-nine percent of the subjects answered that their daily life was disturbed by terrible malodour. In all subjects, there was no medical diseases history, such as sinusitis, diabetes mellitus and hepatic cirrhosis, which are considered to be non-oral causes of malodour (Scully et al, 1997). The number of patients being diagnosed as having periodontal disease (13 males and 22 females, mean age 51.5 ± 15.6 years) was 35, and 64 patients were considered to be without periodontal disease (16 males and 48 females, mean age 41.2 ± 13.5 years). The age of the periodontal disease

Table 1 Number of patients with malodour diagnosed with GC, the Breathtron™ and organoleptic test

Measurement	Periodontal disease group (N = 35) N (%)	Non-periodontal disease group (N = 64) N (%)	Total (N = 99) N (%)
GC			
H_2S	29 (82.9)	45 (70.3)	74 (74.7)
CH_3SH	30 (85.7)	50 (78.1)	80 (80.8)
$(CH_3)_2S$	29 (82.9)	45 (70.3)	74 (74.7)
Breathtron™	28 (80.0)	50 (78.1)	78 (78.8)
Organoleptic score	25 (71.4)	45 (70.3)	79 (70.7)

Table 2 Differences between the periodontal- and non-periodontal-disease groups (N = 99)

Parameters	Periodontal disease group(N=35) Mean (SD)	Non-periodontal disease group(N=64) Mean (SD)	Total (N=99) Mean(SD)
GC			
Concentration of H_2S (ng/10ml)	9.9 (9.0)	4.8 (4.4)	6.6 (6.8)
		*	
Concentration of CH_3SH (ng/10ml)	6.5 (7.0)	2.3 (2.4)	3.8 (5.0)
		**	
Concentration of $(CH_3)_2S$ (ng/10ml)	1.4 (1.2)	0.8(0.7)	1.0 (0.9)
		*	
Breathtron™			
VSCs level (ppb)	880 (771)	698 (602)	762 (669)
Organoleptic score	2.2 (0.8)	2.2 (0.8)	2.2 (0.8)

* Significant difference at P<0.05

** Significant difference at P<0.01

group was significantly higher than that of non-periodontal disease group.

Table 1 indicates the number of patients diagnosed as having malodour by GC, the Breathtron™ and organoleptic tests. The Breathtron™ showed a similar diagnosing pattern to GC and organoleptic tests, and 70-80% of patients were diagnosed as having malodour. In all measurements, more halitosis patients were diagnosed in the periodontal disease group.

Comparisons of malodour measurements for the periodontal and non-periodontal disease groups are shown in Table 2. The mean specific VSCs levels from GC and mean total VSCs level from the Breathtron™ were all higher for the periodontal disease group than for the non-periodontal disease group. Concentrations of H_2S, CH_3SH and $(CH_3)_2S$ were significantly different between two groups.

VSCs levels measured by the Breathtron™ were significantly correlated with all specific VSCs values

Table 3 Pearson correlations between the Breathtron™ and GC or organoleptic scores

Parameters	Periodontal disease group (N=35)	Non-periodontal disease group (N=64)	Total (N=99)
GC			
Concentration of H_2S	r = 0.78 **	r = 0.78 **	r = 0.79 **
Concentration of CH_3SH	r = 0.66 **	r = 0.69 **	r = 0.67 **
Concentration of $(CH_3)_2S$	r = 0.62 **	r = 0.65 **	r = 0.63 **
Mean organoleptic score	r = 0.42 *	r = 0.61 **	r = 0.56 **

* Significant correlation at P<0.05

** Significant correlation at P<0.01

Table 4 Sensitivity and specificity of the Breathtron™

	Periodontal disease group (N=35)	Non-periodontal disease group (N=64)	Total (N=99)
Sensitivity (%)			
GC			
H_2S	90	89	89
CH_3SH	89	94	92
$(CH_3)_2S$	82	86	84
Organoleptic measurement	88	91	90
Specificity (%)			
GC			
H_2S	44	56	52
CH_3SH	50	79	68
$(CH_3)_2S$	50	86	78
Organoleptic measurement	40	53	48

by GC and also with the organoleptic scores in both periodontal and non-periodontal disease groups (Table 3). Overall, correlations with specific GC gases were almost the same in both groups, and correlation with organoleptic scores was lower in the periodontal disease group than in the non-periodontal disease group.

The sensitivity and specificity of the Breathtron™ are shown in Table 4. The Breathtron™ showed a high sensitivity for detecting malodour in both the periodontal and non-periodontal-disease groups. On the other hand, the specificity was lower than the sensitivity in both groups, and the specificity in the periodontal disease group was lower than in the non-periodontal disease group.

DISCUSSION

In this investigation, most of the patients who came to our clinic were women. Previous studies also found a predominance of female patients at their breath odour clinic (Rosenberg and Leib, 1995; Oho et al, 2001; Tanaka et al, 2003). One of the probable reasons is that women tend to be more anxious with respect to bad breath in comparison with men (Oho et al, 2001).

The present study examined the measurement ability of a VSCs instrument in patients in a clinical situation with morning breath odour, which is universally accepted as the standard for investigating breath odour (van Steenberghe et al, 2001). It has been postulated that a decrease in salivation during sleep promotes proliferation of the oral bacteria responsible for the release of the offending gases in morning bad breath (Rosenberg and McCulloch, 1992; McDowell and Kassebaum, 1993).

The Breathtron™ showed the same malodour-diagnosing pattern as GC and the organoleptic tests in both groups. Oral malodour was more common in patients with periodontal disease, regardless of the measurement methods.

Periodontal disease causes high concentrations of VSCs in mouth air, with consequent quantitative changes in bad breath (Yaegaki, 1995). The intensity of the odour increases with the severity of periodontal disease (Yaegaki and Sanada, 1992a). In this study, we used the criterion of 4 mm pocket depth to separate the periodontal disease group from the non-periodontal disease group. Previous studies reported that VSCs concentration increased with the pocket depth and was higher in patients with probing depths of 4 mm or more than in subjects with probing depths of less than 4 mm (Yaegaki and Sanada, 1992a; Yaegaki and Sanada, 1992b). Our results also demonstrated that periodontal disease group patients had significantly higher level of VSCs. CH_3SH is the main component of VSCs in patients with periodontal involvement. The CH_3SH concentration is significantly higher in patients with periodontal disease than in orally healthy individuals (Yaegaki and Sanada, 1992a; Yaegaki and Sanada, 1992b). Similar results were obtained in this study.

The present study of Breathtron™ supports the concept that the VSCs levels measured by a sulfide monitor show a higher level in periodontal disease patients than in non-periodontal disease patients, although the difference was not statistically significant. The Breathtron™ displays total VSCs concentration from mouth air, but it cannot distinguish between the proportions and different species of VSCs. This might be one of the reasons why it did not show a significant difference between the periodontal and non-periodontal disease groups. High variance of the readouts is considered another reason. H_2S, CH_3SH and $(CH_3)_2S$ concentrations measured by GC detected statistically significant differences between the two groups, indicating that GC has a higher ability to identify specific VSCs than the Breathtron™.

The Breathtron™ values were significantly and positively related with all specific gases from GC and also with organoleptic scores. The correlation coefficients between the VSCs monitor and GC were highest for H_2S and lowest for $(CH_3)_2S$. The strength of association was almost the same in both the periodontal and non-periodontal disease groups. The association with organoleptic scores was weaker compared to that with GC. However, it was still significant. These results indicate that the Breathtron™ level has the association with the measurement by GC and organoleptic test irrespective of periodontal status of patients.

The Breathtron™ had a high sensitivity for VSCs measurements both in periodontal disease and non-periodontal disease groups but a low specificity, especially in the periodontal disease patients. The percentage of periodontal disease patients without halitosis that were diagnosed as non-halitosis cases was only 50%. The other 50% were scored as having halitosis. Because the Breathtron™ had a low specificity in the periodontal-disease group, the Breath-tron™ should be used cautiously for measuring VSCs that are related to periodontal disease.

Although the periodontal pocket examination used in the current study has some limitations for diagnosing periodontal disease, periodontal pocket depth itself does indicate a tendency for periodontal disease. Four millimetres pocket depth has been used repeatedly to identify periodontal disease patients in previous malodour studies (Figueiredo et al, 2002; Yaegaki and Sanada, 1992a; Yaegaki and Sanada, 1992b). Nevertheless, the criterion of periodontal disease used in the current study seems rather strict. There is a possibility of over-diagnosis of periodontal disease. Further clinical studies might be necessary for thoroughly evaluating the ability of the Breathtron™, targeting the patient with periodontal disease.

Overall, Breathtron™ showed a relatively high association with specific GC gases and a high sensitivity in both periodontal and non-periodontal patients. In addition, advantages of the Breathtron™ include its portability, reproducibility, no need for skilled personnel, relative inexpensiveness, non-invasiveness, low

likelihood of cross-infection and a rapid turnaround time of one to two minutes between measurements (Rosenberg et al, 1991b). In contrast to the other instruments, the Breathtron™ is more easily used. Its total processing time is shorter, and the digital output of the Breathtron™ can be printed out immediately. Another greatest advantage is that it could be conveniently used in the clinical setting and for field surveys.

To understand VSCs measurement precisely, a more detailed evaluation of the sulfide monitor should be undertaken in the future, including the measurement of mouth air of healthy people. Nevertheless, the Breathtron™ appears to be a reliable instrument for diagnosis of halitosis when used properly.

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