Variation in oral volatile sulphur compound formation

Alix Young, Grazyna Jonski and Gunnar Rölla

Department of Cariology, the Oral Research Laboratory and the Faculty of Dentistry, University of Oslo, Norway

Young A, Jonski G, Rölla G. Variation in oral volatile sulphur compound formation. Acta Odontol Scand 2002;60:321–324. Oslo. ISSN 0001-6357.

It has been suggested that the level of orally produced volatile sulphur compounds (VSCs) can be of use when monitoring oral malodour or in determining patients at risk of periodontal disease in longitudinal studies. It is not known, however, to what extent the level of VSC in mouth air is a stable, individual characteristic over time. *Aim:* The hypothesis to be tested was that the level of VSC in mouth air is an individual trait that is stable over time. *Methods:* Two groups of dental students participated in the study ($n = 30$ and $n = 11$). The amount of available substrate for VSC formation was standardized by rinses with 6 mM aqueous solutions of cysteine (pH 7.2). Part 1 used a Halimeter® to measure 'morning breath' and response to cysteine rinses. Part 2 measured response to cysteine rinsing using a gas chromatograph. Repeated measurements provided information concerning the longitudinal intra-individual variations in level of oral VSC formation. *Results:* Both groups showed large intra-individual variations in oral VSC. The differences were enhanced by cysteine rinses. *Conclusion:* The hypothesis was not supported. Oral VSC levels cannot be taken as diagnostic criteria in a normal population because of marked intra-individual variations over time. □ *L-cysteine; oral malodour; volatile sulphur compounds*

Alix Young, Department of Cariology, Faculty of Dentistry, University of Oslo, P.O. Box 1109, NO-0317 Blindern, Oslo, Norway. Tel. +47 22 85 2190, fax. +47 22 85 2344, e-mail. alixr@odont.uio.no

Volatile sulphur compounds (VSCs) are major components of oral malodour (1, 2, for review see 3). Oral VSC formation is caused by bacterial catabolization of sulphur containing organic substrates in periodontal pockets and on the dorsum of the tongue (1, 4). More than a hundred bacteria have been identified in periodontal pockets that are capable of producing VSC (5). It is well established that VSC can penetrate pocket epithelium and damage the underlying tissue (6, 7). It is thus possible that oral VSC formation may be an important aspect in the etiology of periodontal disease. It appears that especially methyl mercaptan may be involved in the pathogenesis of periodontal disease and other inflammatory diseases (6, 7).

Several authors have examined the relationship between oral malodour and periodontal diseases (6–11). There are clear indications that the VSC levels in mouth air correlate with the extent of periodontal breakdown (6, 7, 11). It has been shown that the VSC levels increase with the number and depth of periodontal pockets (12, 13). Some of the studies (9, 10) were performed using the portable sulphide monitor (i.e. Halimeter®). This instrument is suited mainly for screening purposes; it does not allow specific measurement of methyl mercaptan (3) and is therefore not suited for studies aimed at examining correlation between VSC and oral malodour formation.

It is conceivable that, in the future, longitudinal changes in the level of oral VSC in patients may be used to monitor response to periodontal treatment (13), and it has been suggested that this also applies in regard to prediction of future development of periodontal diseases (14). However, little information is available concerning the consistency of the individual level of oral VSC, as this would be essential in longitudinal studies such as those mentioned above. The hypothesis examined in the present paper was that the level of oral VSC in a healthy subject is a distinctive, individual characteristic which is stable over time. The longitudinal intra-individual variations in the level of oral VSC were monitored in two test panels consisting of healthy, young individuals with no complaints of oral malodour. Cysteine is a well-known substrate for oral VSC formation (15, 16). Mouth rinses with aqueous solutions of cysteine were therefore also performed in the test groups in an attempt to standardize individual substrate availability and thus exclude this as a cause for variation.

Materials and methods

Test subjects

The test panel for part 1 consisted of 30 volunteer dental students (age range 20–25 years) who were involved in the study over a period of 3–10 weeks. In part 2, a group of 11 volunteer graduate students (age range 28–38 years) were tested over a 3–6-week period. All subjects were in good general health, did not use any medication and had relatively good oral hygiene. None of the test subjects complained of oral malodour or had reason to believe that they suffered from oral malodour.

Subjects were instructed not to use toothpaste when brushing their teeth on the evenings prior to test days and to refrain from eating, drinking, smoking, and from their normal oral hygiene routine on the morning of test days. Testing was generally carried out between 0900 and 1000 h.

Part 1

Oral VSC measurements were carried out using a portable sulphide monitor (Halimeter RH17E, Interscan, Chatsworth, Calif., USA), excluding expired air. The Halimeter was used in this study because Kleinberg & Codipilly (14) used this apparatus in their original cysteine challenge model. A vacuum pump transferred the mouth air sample directly via a straw to the instrument sensors. The electrochemical sensors recorded the total amount of VSC in the mouth air sample in parts per billion (ppb). Two values were recorded. 'Morning breath' was measured first and used as the baseline value. Following this, the test panel rinsed with 5-ml aliquots of buffered aqueous 6 mM L-cysteine at pH 7.2 (Sigma Chemical Co. St. Louis, Mo., USA) (16). The rinse was expectorated after 30 s, and total mouth air VSC measurements were then recorded as follows. After rinsing with cysteine, the subjects were instructed to keep their mouth closed for 90 s. A straw was then inserted into the subject's mouth and positioned above the posterior part of the dorsum of the tongue so that it was not touching the oral mucosa or the tongue. The subjects were asked to keep their mouth open about 1.5 cm and to avoid breathing in or out during sampling. The peak value was recorded and the measurements made in triplicate. A variation of less than 10% was accepted and the mean ppb value calculated.

Part 2

The graduate students were tested using a gas chromatograph (GC) on from three to six separate occasions (i.e. $n = 3$ to 6) over a period of approximately 1 month. Upon presentation at the laboratory, the subjects rinsed for 30 s with 5 ml of the same cysteine solution as in part 1 of the study, and were instructed to keep their mouth closed for 90 s. Air samples were then rapidly aspirated from the back of the mouth using a 10-ml syringe attached to a mouthpiece and analysed directly for VSC in the GC

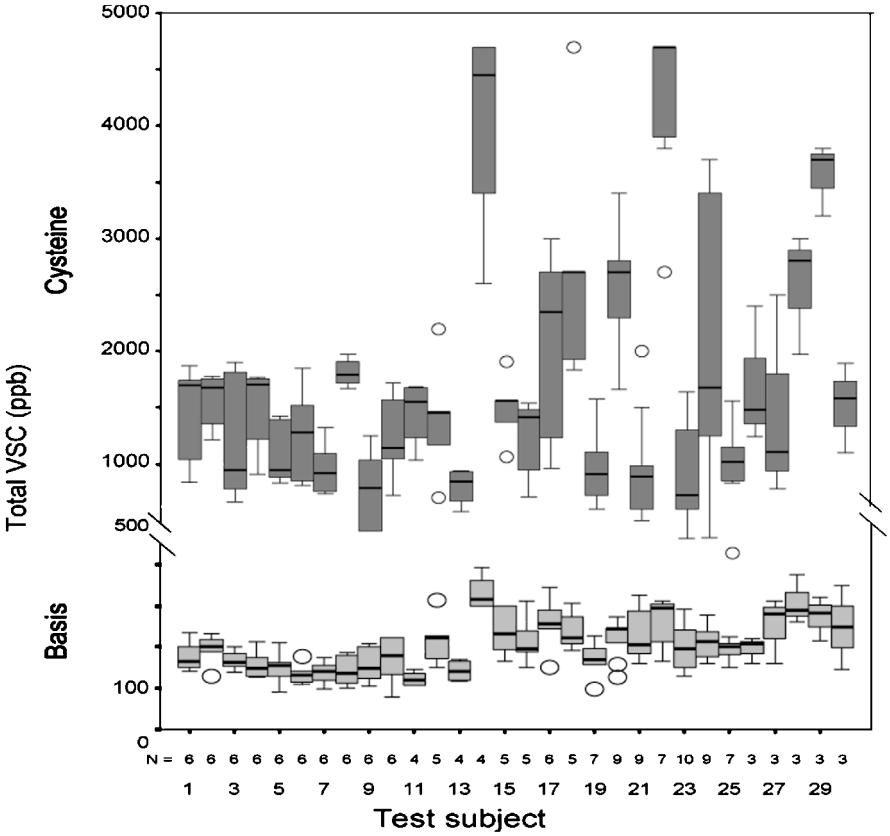

Fig. 1. Box plots of results obtained during a period of 3–10 weeks from total VSC analyses of 30 dental students using a Halimeter® Basis 'morning breath' levels (ppb) of total VSC production are shown in the lower part of the scale (light grey boxes), and total VSC production following rinsing with an aqueous cysteine solution (5 ml, 6 mmol for 30 s) is shown in the upper part of the scale (dark grey boxes). Median, 25% and 75% levels, and whiskers showing maximum values and minimum values, are represented. The total number of rinses for each subject (*n*) is shown. Several outlying values (1.5–3 box lengths from the upper or lower edge of the box) are shown represented by open circles.

(Shimadzu 14B, Shimadzu, Japan). The GC was equipped with a flame photometric detector, a 12 ft × $^1/_8$ inch Teflon column packed with 5% polyphenyl ether—0.05% phosphoric acid on 40/60 mesh Chromosorb T, and an auto-injection system with a 3-ml sample loop. Column conditions were temperature 70°C, nitrogen gas flow rate 32 ml/min, hydrogen gas flow rate 125 ml/min, air flow rate 43 ml/min (13). The measurements were made only once per subject per test day.

Results

Oral malodour was easily detected organoleptically in all test subjects immediately following cysteine rinses. Results of part 1 of the study are shown as box plots in Fig. 1. Intra-individual variations can be seen in 'morning breath', in particular following cysteine rinsing. This is illustrated by the length of the grey boxes representing 50% of the measurements, and the whiskers, demonstrating the full spread of measurements as measured by a Halimeter.

Results of part 2 of the study can be seen in Fig. 2. Individual VSC levels varied after cysteine rinsing as measured by GC, as can be seen by the size of the grey boxes and corresponding whiskers. Five of the 11 test subjects showed considerable variations (nos. 8, 10, 11, 12, and 14), one showed clear variation while the remaining 5 subjects showed minor variations.

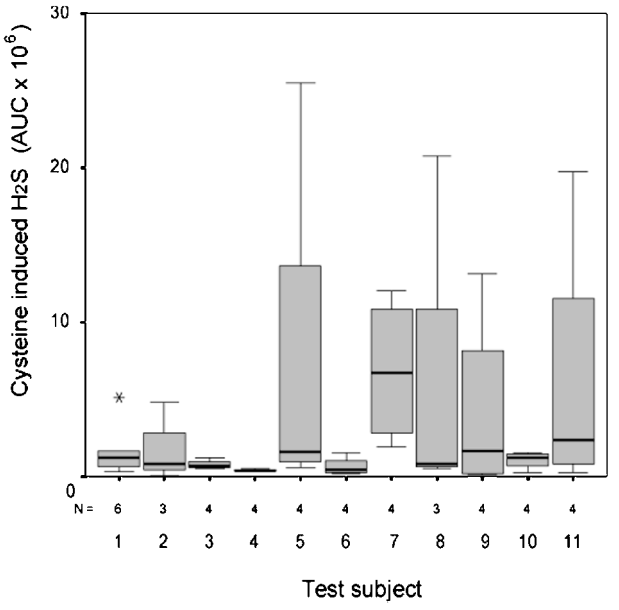

Fig. 2. Results in the form of box plots of gas chromatographic analyses for 11 graduate dental students during a period of 3–6 weeks, showing production of hydrogen sulphide (H_2S) immediately after oral rinsing with an aqueous cysteine solution (5 ml, 6 mmol for 30 s). VSC is shown in units as measured by the area under the curve (AUC). Median, 25% and 75% levels, and whiskers showing maximum values and minimum values are represented. The total number of rinses for each subject (*n*) are shown. One extreme value (>3 box lengths from the upper or lower edge of the box) is shown.

Discussion

The first part of the study (Fig. 1) was performed using a Halimeter. An instrument of this type was used in the original studies by Kleinberg & Codipilly (14), where the use of oral VSC in predicting development of periodontitis was suggested. Mouth rinses with solutions of cysteine were included in the methodology in the present study in order to standardise the amount of substrate available for VSC formation, as mentioned previously. The second part of the study (Fig. 2) involved gas chromatography, the method of choice for VSC measurements. The intra-individual variations in oral VSC levels were thus monitored using two independent methods. The present study demonstrated, using both methods, that individual, repeated measurements of oral VSC in young, healthy individuals are not consistent over time. The study therefore did not support the hypothesis that formation of VSC is a stable, individual characteristic. It appears that such variations over time may reduce the possibility of using this criterion for longitudinal studies. As the individual oral VSC levels vary over time, the necessary, reliable control values with which subsequent data can be compared are not available. This problem should also be kept in mind in relation to longitudinal measurements of oral VSC levels in connection with monitoring treatment of oral malodour. However, Kleinberg & Codipilly (15) have suggested a method for testing inhibitors, where this problem can be avoided. The use of cysteine-containing mouth rinses to enhance oral malodour in a test individual allows the use of one-day studies, which are particularly well suited to testing oral malodour inhibitors. With this method, the individual's response to cysteine has no influence on the results, since the individual functions as his/her own control on the test day.

Cysteine rinses were used in the present study to examine whether the presence or absence of sufficient substrate affects VSC formation. This procedure caused an immediate rise in VSC formation, as shown previously (15), though surprisingly an increase in the intra-individual variation in VSC formation was demonstrated in part 1 of the study. It was also interesting to note that cysteine rinses, without exception, enhanced the VSC levels in test subjects in some cases as much as five times or more. This result indicates that all subjects harboured a microflora that could catabolize cysteine, and thus 'acquire' oral malodour, if exposed to abundant amounts of substrate. A natural example of this phenomenon could be dairy products that are known to induce oral malodour and which contain much cysteine (17). However, there are also individual modifying factors that can result in some subjects being more or less liable to acquire oral malodour. It is known that the volatile compounds causing malodour are highly soluble in water. This most likely explains why

people who suffer from xerostomia often exhibit oral malodour. On the contrary, an abundant production of saliva protects against oral malodour because VSCs dissolve in saliva and are largely eliminated during swallowing.

The immediate formation of VSCs upon rinsing with cysteine indicates that the enzymes involved (probably desulphydrases from many sources) must be constitutive rather than induced. It appears that all subjects had these enzymes available in the oral cavity. Previous studies indicate that the enzyme is mostly located on the tongue, but is present also to some degree at other locations. Whole saliva has only a limited enzyme content (15).

In conclusion: marked variations in longitudinal intra-individual levels of oral VSC were observed in mouth air in young healthy individuals. Cysteine rinses as a means of standardizing substrate availability resulted in larger intra-individual variations in VSC formation. Some consequences of these observations have been discussed.

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Received for publication 25 January 2002
Accepted 29 May 2002