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ORIGINAL ARTICLE

Comparison of ninhydrin method of detecting amine compounds with other methods of halitosis detection

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OBJECTIVES: Halitosis is a result of overactivity of Gram-negative bacteria for which protein amino acids are the major source of energy. Therefore, statistical correlation between concentrations of volatile sulphur compounds (VSCs) and low-molecular-weight amines should be expected.

MATERIALS AND METHODS: Eighty-four patients suffering from halitosis and 40 healthy volunteers aged 20–62 years (average 39.7) participated in the study. In all subjects low-molecular-weight amines were evaluated by the ninhydrin method. Patients with halitosis were randomized into treatment groups. Zinc tablets, tablets and mouthwash containing chlorhexidine or lyophilized lactic acid-forming bacteria were used.

RESULTS: Analysis showed that the level of amines was highest in subjects with halitosis (0.39, s.d. ± 0.06, $P < 0.001$) and correlated significantly with results of VSC measurement and organoleptic scores ($P < 0.001$). Reduction of amine levels after treatment was statistically significant ($P < 0.001$). Three months following treatment the amine levels began to increase (0.37, s.d. ± 0.05). The amine levels in healthy controls were lower (0.29, s.d. ± 0.07) and remained at a stable level.

CONCLUSION: The salivary amine levels significantly correlated with VSC levels and organoleptic scores. Therefore, the ninhydrin method of detecting salivary amines may be an alternative or additional method of diagnosing halitosis. This method may also be used to evaluate treatment efficacy.

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Introduction

Patients presenting with halitosis expect effective diagnosis and treatment from dental practitioners. Therefore rapid, simple and relatively inexpensive methods to diagnose halitosis and control its treatment are necessary. Such a possibility is given by using a portable sulphide monitor to measure levels of volatile sulphur compounds (VSC) (Rosenberg *et al*, 1991). Unfortunately some of the components of oral malodour, e.g. low-molecular-weight amines cannot be measured using this method. Gases which are components of oral malodour originate from the breakdown of peptides and glycopeptides by bacterial putrefaction in the oral cavity. During this process peptides are hydrolysed to amino acids which further are metabolized to amines or polyamines (lysine, arginine and ornithine) (Patocka and Kuehn, 2000). Correlations between malodour levels and diamines in saliva have been found previously (Goldberg *et al*, 1994) and Klineberg and Codipilly (1995) have shown elevated odour levels after addition of lysine to cultures of oral anaerobes. The ninhydrin colorimetric reaction is a simple, rapid and inexpensive method which may be used for examination of amino acids and low-molecular-weight amines. Detection of low-molecular-weight amines will give information about the level of substrates used during bacterial putrefaction. In theory, the level of the bacterial putrefaction substrates should be important as an aetiologic factor in halitosis.

The aim of the study was to evaluate statistical correlation between the concentration of volatile sulphur-containing compounds, organoleptic measurement and low-molecular-weight amines.

Materials and methods

The study was performed on patients who visited the Department of Conservative Dentistry in Warsaw, Poland. Eighty-four subjects suffering from oral halitosis diagnosed at the beginning of the study (mean age 41.2 ± 11.94, range from 20 to 62; 38.1% males, 61.9%

females) and 40 healthy subjects (mean age 36.8 ± 11.74, range from 22 to 57; 27.5% males, 72.5% females) were recruited for participation in the study. Halitosis was diagnosed if the average level of VSCs was ≥125 ppb and organoleptic measurement was ≥2. Healthy subjects' average VSC level was ≤75 ppb and organoleptic measurement was close to zero. Subjects did not report any systemic or periodontal disorders. No one had a history of periodontal treatment or antibiotic medications during the previous 3 months. Patients suffering from halitosis were randomized into four test groups: A, 25 persons; B, 25 persons; C, 20 persons; D, 14 persons. Group A was treated three times a day with 7 mg zinc tablets for 21 days. Group B was given 0.1% chlorhexidine digluconate mouthwash in 30 s rinses three times a day and group C 2.0 mg chlorhexidine digluconate tablets four times a day for 2 weeks. Group D received three tablets containing lyophilized lactic acid-forming bacteria once a day after breakfast for 7 days – powder was applied under the tongue. Examination of VSC, organoleptic measurement and saliva analysis was performed for subjects from the test groups on day 0 (before treatment), 7, 14 and 21 and after 3 months and for controls on day 0, 21 and after 3 months. Examinations were performed by three independent examiners. Organoleptic measurement was always performed in the morning before breakfast. Subjects exhaled into a single-use 10 cm plastic tube. The level of oral malodour was evaluated on a 0 to 5 scale as follows: 0 = absence of odour, 1 = questionable odour, 2 = slight malodour, 3 = moderate malodour, 4 = strong malodour, 5 = severe malodour (Rosenberg *et al*, 1991). Volatile sulphide measurements were performed using a portable hydrogen sulphide monitor (Halimeter RH-17K, Interscan Corp., Chatsworth, CA, USA). Subjects were examined in the morning, before organoleptic measurement. The single-use plastic tube was connected to the air inlet of the monitor. Patients were asked to put the other side of this tube in to the oral cavity and to breathe through the nose during the examination. Maximum and average values from three measurements of VSC were obtained in parts per billion (ppb). Data were collected and visualized on the computer using special software (HaliSoft v.1.0e, Ansyco GmbH, Ostring, Germany). Total amine level in saliva was determined with ninhydrin colorimetric reaction (Moore and Stein, 1948; Amato and Ladd, 1994) with some modification. Briefly, sample of saliva (100 μl) and isopropanol (100 μl) was mixed and centrifuged. The supernatant (100 μl) was diluted with isopropanol (400 μl), buffer solution pH 5 (1.5 ml) and ninhydrin reagent (2 ml). The mixture was refluxed in a water bath for 30 min, cooling to 21°C and diluted with isopropanol to a total volume of 10 ml. Light absorbance readings were determined at 570 nm using a MK6/6 Carl Zeiss (Jena, Germany) spectrometer. Resting saliva was taken for re-examination and archival storage at −20°C. Statistical analysis was performed using Wilcoxon's test. Spearman rank correlation was used to determine the relationship between organoleptic scores, VSC and amine levels.

Results

Analysis showed that the levels of amines were highest in subjects with halitosis (0.39, s.d. ± 0.06, $P < 0.001$) (Table 1) and correlated significantly with results of VSC measurement and organoleptic scores ($P < 0.001$) (Table 2). Reduction of salivary amine levels after treatment were statistically significant in all test groups except those using 0.1% chlorhexidine digluconate mouthwash (Figure 1). After 3 months the amine levels began to increase (0.37, s.d. ± 0.05). The amine levels in healthy controls were lower (0.29, s.d. ± 0.07) and remained at a stable level. Table 3 shows organoleptic improvement after treatment in all test groups and changes in VSC levels. Although the levels of VSC were lower in all groups, changes after treatment with tablets containing lyophilized lactic acid-forming bacteria were not statistically significant.

Discussion

A method of diagnosing halitosis using a combination of organoleptic method and measurement of VSC levels using a halimeter has been shown to be effective and confirmed by many workers. These diagnostic methods are fast, simple and inexpensive. Correlations between VSC levels and organoleptic scores in the present study were comparable with those previously obtained by Rosenberg *et al* (1991). The examination of polyamines is complicated; therefore most often analyses are limited to detection of putrescine and cadaverine. Usually high-performance liquid chromatography is used for detection of polyamines. Because this is relatively expensive and time consuming it cannot be used for examination of a large subject population (Goldberg *et al*, 1994). The ninhydrin colorimetric reaction is widely used for detecting amino acids and low-molecular-weight amines using a classical amine analyser, e.g. for checking amine levels in the blood. It was therefore decided to use this simple method in this study to determine amine levels in the saliva. Amine levels were higher in the saliva of subjects suffering from halitosis and lower in healthy controls. As well, amine levels correlated significantly

Table 1 Mean amine levels of halitosis and healthy subjects measured in extinction (E)

	Amine levels ± s.d.				
	Day 0	*Day 7*	*Day 14*	*Day 21*	*3 months*
Halitosis	0.389 ± 0.06	0.361 ± 0.07	0.362 ± 0.06	0.366 ± 0.06	0.371 ± 0.05
Healthy	0.285 ± 0.05			0.289 ± 0.06	0.292 ± 0.06

Table 2 Correlations between volatile sulphur compound (VSC) levels, organoleptic measurements and amine levels

Examination	*VSC*	*Organoleptic*
Amine levels	$r = 0.5389$; $P < 0.001$	$r = 0.5982$; $P < 0.001$
VSC		$r = 0.7820$; $P < 0.001$

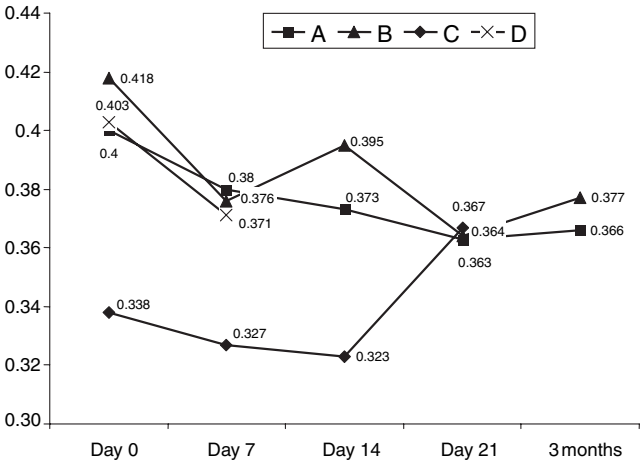

Figure 1 Mean amine levels in treatment groups – 3-month observation. A = three times a day 7 mg zinc tablets for 21 days. B = 0.1% chlorhexidine digluconate mouthwash in 30 s rinses two times a day. C = 2.0 mg chlorhexidine digluconate tablets four times a day for 2 weeks. D = three tablets containing lyophilized lactic acid-forming bacteria once a day after breakfast for 7 days – powder was applied under the tongue

Table 3 Organoleptic and volatile sulphur compound (VSC) changes after treatment in all test groups

	Mean organoleptic score ± s.d.		*Mean VSC ± s.d.*	
	Day 0	*After treatment*	*Day 0*	*After treatment*
A	3.40 ± 0.65	1.79 ± 0.88	277.40 ± 215.17	123.04 ± 62.14
B	3.00 ± 0.76	1.87 ± 0.92	255.08 ± 185.52	202.25 ± 111.71
C	3.70 ± 1.30	2.30 ± 0.86	319.75 ± 219.08	166.55 ± 92.25
D	3.79 ± 0.89	2.50 ± 1.65	295.29 ± 191.77	210.71 ± 97.76
K	0	0	60.08 ± 12.90	57.05 ± 12.86

A = three times a day 7 mg zinc tablets for 21 days.
B = 0.1% chlorhexidine digluconate mouthwash in 30 s rinses two times a day.
C = 2.0 mg chlorhexidine digluconate tablets four times a day for 2 weeks.
D = three tablets containing lyophilized lactic acid-forming bacteria once a day after breakfast for 7 days – powder was applied under the tongue.
K = healthy controls – no treatment.

with results of VSC measurement and organoleptic scores before treatment. Moreover, amine, VSC levels and organoleptic scores were lower after treatment of halitosis in all groups. Changes in VSC levels and organoleptic measurements were similar to those observed in other studies (Rolla *et al*, 2002; Rosing *et al*, 2002; Young *et al*, 2003). This suggests that in the oral cavity of subjects with halitosis the level of substrates used to produce oral malodour is higher and may be controlled. The ninhydrin colorimetric reaction was helpful in the indication of malodour substrate quantity in general, as well as concentrations of amines in particular.

In conclusion, the salivary amine levels significantly correlated with VSC levels measured by halimeter and organoleptic scores. Therefore, the ninhydrin method of detecting salivary amines may be an alternative method of diagnosing halitosis. This method may also be used to evaluate treatment efficacy.

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