Correlation between the BANA Test and Oral Malodor Parameters

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Abstract. The purpose of the present investigation was to test the association between the BANA test (Perioscan®, Oral-B), and oral malodor parameters. The subject population consisted of 52 Israeli adults, 43 of whom complained of oral malodor. Oral malodor measurements consisted of peak and steady-state volatile sulphide measurement by a portable sulphide monitor (Interscan Corp., model 1170), as well as organoleptic measurements of malodor from whole mouth, tongue, and saliva. Samples for the BANA test were obtained from four loci (shallow pocket, deep pocket, tongue dorsum, saliva); results were scored as negative (0), weak (1), or strong (2). BANA scores were significantly associated with odor-judge ratings, with the highest association obtained when BANA saliva scores and odor-judge saliva assessment were compared (r = 0.500; p < 0.001). BANA tests from the different loci were not significantly associated with sulphide monitor levels. Step-wise multiple-regression analysis of odor-judge measurements in terms of sulphide levels and average BANA scores showed that both log peak sulphide levels as well as BANA scores were significantly factored into the equations, yielding, in all cases, highly significant correlations (multiple r = 0.57, 0.50, and 0.59, respectively, with significance levels of 0.0001, 0.001, and < 0.0001, for whole mouth, tongue, and saliva malodor, respectively). The results suggest that the BANA scores are associated with a component of oral malodor which is independent of volatile sulphide measurements and suggest its use as an adjunct test to volatile sulphide measurement.

Introduction

Oral malodor (also known as bad breath, halitosis, *fetor ex ore*) usually originates when the halitus emitted through the mouth combines with malodorous volatile compounds originating in the oral cavity (Tonzetich, 1977). Tonzetich and Richter (1964) were the first to report that oral malodor is primarily associated with volatile sulphur compounds (VSC), rather than with amines and ammonia. VSC are produced in the oral cavity through the putrefactive action of micro-organisms on exogenous and endogenous proteinaceous substrates, including exfoliated cells, leukocytes, saliva, blood, and food debris (Tonzetich and Kestenbaum, 1969; Tonzetich, 1977). The most popular method for instrumental analysis of VSC has been gas chromatography (GC) (Tonzetich, 1971). More recently, a portable sulphide monitor has been proposed for the rapid chairside measurement of overall sulphide levels (Rosenberg et al., 1991a,b). Quantitative measurement is often accompanied by hedonic (organoleptic) measurement by one or more odor judges (Rosenberg and McCulloch, 1992). Both quantitative techniques of gas analysis have been shown to be significantly correlated to hedonic measurements (Schmidt et al., 1978; Rosenberg et al., 1991a,b). Subjects with periodontal disease frequently suffer from oral malodor (Tonzetich, 1973; Tonzetich and Ng, 1976), and positive correlations have been demonstrated between the severity of periodontitis and VSC levels in mouth air (Tonzetich and McBride, 1981; Yaegaki and Sanada, 1992) as well as in pockets (Rizzo, 1967; Coil and Tonzetich, 1985; Mardinger et al., 1992). Many oral bacteria, including those isolated from deep periodontal pockets, are capable of producing VSC, primarily hydrogen sulfide and methyl mercaptan, from serum and from L-cysteine and L-methionine (Persson et al., 1990).

Three of the bacterial species associated with periodontitis—*Porphyromonas gingivalis* (Loesche *et al.*, 1985), *Treponema denticola* (Moore *et al.*, 1985; Simonson *et al.*, 1988), and *Bacteroides forsythus* (Dzink *et al.*, 1988)—are among the most active hydrogen sulphide producers *in vitro* (Persson *et al.*, 1990). The presence of these organisms in dental plaque can be demonstrated based on their ability to hydrolyze the synthetic trypsin substrate N-benzoyl-DL-arginine-2-naphthylamine (BANA; Laughon *et al.*, 1982; Tanner *et al.*, 1985; Loesche, 1986; Loesche *et al.*, 1987).

The purpose of the present investigation was to examine the relationship between the presence of periodontopathic micro-organisms in dental plaque, tongue scrapings, and saliva, as measured by the BANA assay, with instrumental and organoleptic measurements related to oral malodor.

Materials and methods

Patient population

The study consisted of 52 patients (mean age, 37.9 ± 11.9 years; 35 females), ranging from 11 to 63 years old. Among the subjects, 43 complained of bad breath, having responded to a newspaper article on the subject. Exclusion criteria included smokers, subjects suffering from systemic disease or taking antibiotics, and denture wearers. The study included parameters not reported here, including salivary cadaverine levels (Goldberg *et al.*, 1994), as well as tests of self-estimation of oral malodor, and psychological data (in preparation).

On the day of the examination, the participants were instructed to refrain from using scented personal products. Subjects were also requested to refrain from oral activities, including drinking, eating, chewing gum, and mouthrinsing two hours prior to appointment. Measurements were obtained on three dates within over a 12-day period.

Clinical examination

All the participants were scored for plaque accumulation and gingival health of all tooth surfaces (including wisdom teeth) by the Plaque Index (PI; Silness and Löe, 1964) and Gingival Index (GI; Löe and Silness, 1963). Probing depth measurements were recorded on days 2 and 3 of the study (N = 30) with a Michigan O probe, at six reference loci on the buccal and lingual sides of each tooth, including wisdom teeth. All participants were subjected to whole-mouth probing so that shallow and deep pockets could be identified for BANA sampling.

BANA assay

The BANA reagent card (Perioscan®, Oral-B Laboratories, Redwood City, CA; Loesche *et al.*, 1990a,b) was used according to manufacturer's instructions. Results were recorded as either: strong, dark blue spots (score = 2); weak, light blue spots (score = 1); or no color change (score = 0).

Subgingival plaque was removed by curette from a tooth presenting a shallow pocket (< 4 mm) and one presenting a deep pocket (≥4 mm), following local supragingival plaque removal. Subgingival plaque samples were then deposited directly onto the reagent card. Tongue samples were obtained by scrapings from the posterior tongue dorsum, obtained by means of a disposable plastic blade, and applied directly onto the reagent card. Saliva samples were taken following the dental examination by asking the subjects to expectorate into a Petri dish. Aliquots (*ca.* 10 μL) of expectorated saliva were immediately applied to the reagent card. The same sample expectorates were subsequently used for odor-judge measurements (below).

Volatile Sulphide Concentrations

VSC measurements were made by use of a portable industrial sulphide monitor (model 1170, 0.5 ppm full-scale, Interscan Corp., Chatsworth, CA), zeroed on ambient air before each measurement. A disposable 6.5-mm-circumference plastic straw was inserted into the air inlet of the monitor. Each patient was instructed to bring his/her slightly opened mouth over the straw so that it extended approximately 4 cm into the oral cavity, and to breathe through the nose during the measurement. Both peak (maximum) and steady-state levels attained were determined in parts *per* billion (ppb) sulphide equivalents.

Odor-judge assessment

Organoleptic assessment was made by an odor judge (M.R.) whose organoleptic scores have been shown to correlate with those of other judges, as well as volatile sulphide levels, in previous studies (Rosenberg *et al.*, 1991a,b). Organoleptic malodor scores were recorded on a continuous unmarked 10-cm scale, marked at both ends as no odor and maximal odor, respectively. Results were scored independently for (i) whole mouth malodor, (ii) tongue malodor, and (iii) saliva malodor. For measurement of whole-mouth malodor, subjects were requested to breathe out from the mouth at *ca.* 10 cm from the nose of the judge. For measurement of tongue malodor, the subjects were instructed to extend their tongue and lick their wrist in a perpendicular fashion. The odor judge assessed the odor from a distance of *ca.* 3 cm, following 5 s. For measurement of saliva malodor, the subjects were asked to expectorate into Petri dishes, which were incubated for approximately 5 min at 37°C, and then assessed at a distance of *ca.* 4 cm from the nose of the odor judge. Expectoration was carried out post-probing in order to potentiate saliva odor levels. VSC and malodor measurements were carried out following the dental examination.

Statistical analyses

Pearson and Spearman correlation coefficients were used as indicated. ANOVA was used to test for differences in log VSC scores over the three days of the study. To test for

Table 1. Frequency distribution of BANA scores

| Site	Negative	Weak-positive	Strong-positive
Shallow Pocket	3[a]	29	20
Deep Pocket	2	20	30
Tongue	27	22	3
Saliva	33	15	4

[a]   Number of samples.

differences between males and females, the unpaired *t* test was used.

Results

Table 1 presents the distribution of BANA scores from the four loci examined. Shallow and deep pockets yielded high scores, with 30 and 20 strongly positive results, respectively, as opposed to only 3 and 2 negative scores, respectively. In contrast, 27 and 33 samples taken from tongue dorsa and from saliva, respectively, were negative. In all instances, significant correlations ($p \leq 0.005$; Spearman) were found when BANA scores were compared among subjects. The highest association was between BANA tongue *vs.* saliva scores ($r = 0.589$; $p < 0.001$). Shallow pocket levels appeared to be more highly related to tongue scores ($r = 0.548$; $p < 0.001$), whereas deep pocket scores were more highly associated with saliva results ($r = 0.561$; $p < 0.001$). In order to obtain a wider overall distribution, the average score of the four BANA tests was calculated for each subject (Fig.).

Table 2 presents the mean ± standard deviation for the dental parameters and malodor measurements. Transformation of peak and steady-state VSC measurements to natural logarithms was carried out in order to obtain near-normal distributions. Among the three
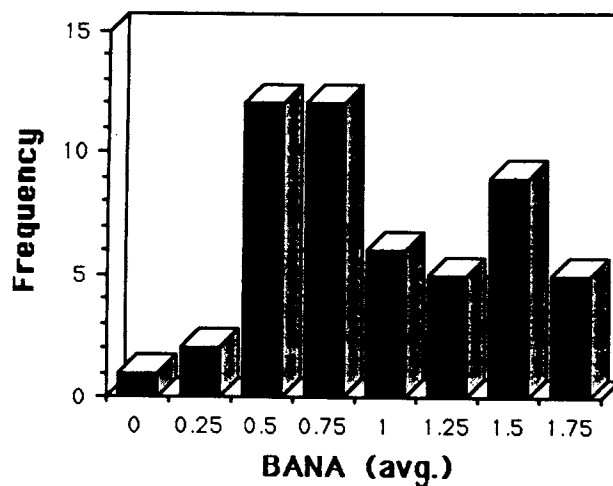
Figure. Frequency distribution of mean BANA scores.

organoleptic measurements, whole mouth odor scores were generally higher than saliva and tongue odor assessments (Table 2). Among the various measurements, the only significant difference between males (N = 17) and females (N = 35) was in mean BANA saliva scores (0.772 ± 0.187 for males, as opposed to 0.530 ± 0.090 for females, $p = 0.037$). No significant decrease was observed in mean peak of steady-state VSC measurements over the three days of the study ($p > 0.05$, ANOVA). Similarly, the time of day of measurement was not significantly related to any of the parameters.

The comparison between BANA scores and both oral malodor and dental parameters is summarized in Table 3. Associations with peak and steady-state VSC measurements were, for the most part, not significant. Only in the comparison of average BANA scores with steady-state

Table 2. Dental parameters and malodor measurements

Measurement	Mean ± Standard Deviation			No. of Observations
Plaque Index (PI)	1.2	±	0.5	52
Gingival Index (GI)	1.1	±	0.4	52
Mean Probing Depth	2.9	±	0.7 mm	30
BANA (shallow pocket)	1.3	±	0.6	52
BANA (deep pocket)	1.5	±	0.6	52
BANA (tongue dorsum)	0.5	±	0.6	52
BANA (saliva)	0.4	±	0.6	52
BANA (average)	1.0	±	0.5	52
Peak VSC	155	±	62 ppb	51
Steady-state VSC	103	±	38 ppb	51
Log Peak VSC	2.2	±	0.14	51
Log Steady-state VSC	2.0	±	0.14	51
Whole mouth odor (odor judge)	4.3	±	2.0	52
Tongue odor (odor judge)	2.5	±	1.3	52
Saliva odor (odor judge)	3.4	±	2.5	52

Table 3. Spearman Correlation Coefficients comparing BANA results with oral malodor and dental parameters

	BANA (Shallow Pocket)	BANA (Deep Pocket)	BANA (Tongue)	BANA (Saliva)	BANA (Avg.)
Log Peak VSC	r = -0.019	0.151	0.134	0.059	0.098
	p = 0.894	0.290	0.349	0.679	0.493
Log Steady-state VSC	0.209	0.263	0.255	0.245	0.304
	0.142	0.063	0.071	0.083	0.030
Whole mouth	0.332	0.259	0.364	0.359	0.399
(odor judge)	0.016	0.034	0.008	0.009	0.003
Tongue	0.260	0.331	0.271	0.372	0.373
(odor judge)	0.063	0.017	0.052	0.007	0.006
Saliva	0.399	0.312	0.338	0.500	0.455
(odor judge)	0.003	0.025	0.014	< 0.001	0.001
PI	0.325	0.101	0.293	0.329	0.306
	0.019	0.476	0.035	0.017	0.028
GI	0.452	0.282	0.481	0.406	0.472
	0.001	0.043	< 0.001	0.003	< 0.001
Mean Probing Depth	0.491	0.284	0.326	0.551	0.514
	0.006	0.129	0.079	0.002	0.004

measurements was a significant association (r = 0.304, p = 0.030) obtained. In contrast, organoleptic ratings of whole mouth, tongue, and saliva malodor were generally associated with BANA results. The highest association was found for BANA saliva scores vs. saliva malodor (r = 0.500; p < 0.001). Average BANA scores were highly associated (p ≤ 0.006) with all three organoleptic ratings. When BANA scores were compared with GI, highly significant correlations (p ≤ 0.001) were found for BANA average, BANA shallow pocket, and BANA tongue. Probing depth was highly associated with BANA shallow pocket (p = 0.006), BANA average (p = 0.004), and BANA saliva (p = 0.002) scores. Deep pocket BANA scores were not significantly associated with PI or probing depth.

Table 4 presents Pearson correlation coefficients comparing the oral-malodor-associated results as well as dental parameters. Among the dental parameters, GI scores provided the highest correlations with all odor-judge measurements (p ≤ 0.001). PI values were highly associated with GI, probing depth, and odor-judge saliva scores (p ≤ 0.001), but not with sulphide measurements. Correlations between GI and peak sulphide levels were higher than between GI and steady-state sulphide values (p = 0.017 and p = 0.053, respectively). Probing depth scores were more highly associated with steady-state than with peak sulphide scores (p = 0.008 and p = 0.050, respectively). All odor-judge parameters were highly associated with one another (p < 0.001). Correlations between both peak and steady-state sulphide scores vs. odor-judge ratings of whole mouth and saliva were highly associated (p < 0.001). Correlations between peak and steady-state sulphide scores and odor-judge tongue malodor scores were slightly lower (p = 0.003 and p = 0.013, respectively).

In an attempt to relate each of the three odor-judge measurements to both VSC levels and BANA (avg.) scores, step-wise forward multiple-regression analysis was carried out (Table 5). In all three cases (whole mouth, tongue, and saliva), both log peak monitor levels and BANA (avg.) scores factored into the equations, yielding, in all cases, highly significant correlations (multiple r = 0.568, 0.501, and 0.586, respectively, with significance levels of p = 0.0001, p = 0.001, and p < 0.0001, respectively). Following entry of log peak monitor levels into the regression equation, steady-state levels did not contribute significantly and were not entered.

Discussion

The Perioscan® BANA assay is a user-friendly chairside technique which has previously been shown to correlate with the levels of three specific oral microbial species whose presence reflects periodontal disease activity (Loesche, 1986; Loesche et al., 1990a,b; Schmidt et al., 1988). The results presented here provide further support for previously demonstrated associations between BANA test results and periodontal parameters (e.g., GI, probing depth). Among the sites tested here, deep pocket BANA samples generally yielded less significant associations with dental parameters than BANA samples obtained from the other three sites. This may be associated with the low number (n = 2) of deep pocket samples which gave negative results, in agreement with previous data suggesting relatively low specificity (Loesche et al., 1990b) of the BANA assay when plaque samples were compared with dental parameters. Results suggest that the BANA status of a single deep pocket may not be representative of parameters involving overall status.

The present study provides initial data comparing BANA scores with oral malodor parameters. Of considerable

Table 4. Pearson Correlation Coefficients comparing oral malodor-associated results and dental parameters

	PI	GI	Mean Probing Depth	Whole Mouth (odor judge)	Tongue (odor judge)	Saliva (odor judge)	Log Peak VSC	Log Steady-state VSC
PI	r = 1.00							
	p = 0.000							
GI	0.813	1.00						
	<0.001							
Mean probing	0.577	0.668	1.00					
depth	<0.001	<0.001						
Whole mouth	0.383	0.536	0.581	1.00				
(odor judge)	0.003	<0.001	<0.001					
Tongue	0.383	0.468	0.466	0.725	1.00			
(odor judge)	0.003	<0.001	0.005	<0.001				
Saliva	0.433	0.583	0.513	0.730	0.599	1.00		
(odor judge)	0.001	<0.001	0.002	<0.001	<0.001			
Log peak VSC	0.188	0.298	0.305	0.472	0.381	0.439	1.00	
	0.093	0.017	0.050	<0.001	0.003	0.001		
Log steady-state	0.092	0.230	0.436	0.404	0.313	0.413	0.718	1.00
VSC	0.261	0.053	0.008	0.002	0.013	0.001	<0.001	

interest is the finding that BANA results, although significantly associated in most cases with odor-judge ratings, were generally poorly associated with VSC levels. When multiple-regression analysis was performed with odor-judge measurements as the dependent variable, both peak VSC levels and BANA scores factored into the regression, yielding highly significant associations. These findings suggest that BANA scores are associated with some other factor(s), which helps account for odor-judge assessment of oral malodor, exclusive of VSC data. Possibly, micro-organisms associated with BANA assay contribute additional non-sulphide odorants, such as cadaverine (Goldberg *et al., 1994*), to the oral halitus.

In the present study, highly significant correlations were found for odor-judge scores as compared with dental parameters. Correlations between dental parameters and malodor levels have been shown in most previous studies (Rizzo, 1967; Tonzetich, 1973, 1977; Tonzetich and McBride,

1981; Coil and Tonzetich, 1985; Rosenberg *et al.*, 1991a; Mardinger *et al.*, 1992; Yaegaki and Sanada, 1992). The most striking associations obtained here were: (i) whole mouth odor assessment, as compared with mean probing depth (r = 0.581; p < 0.001); and (ii) saliva odor assessment, as compared with GI (r = 0.583; p < 0.001). It should be emphasized that, in the present study, saliva samples were collected following probing (even in those cases in which average probing depth was not recorded, probing was performed to assess deep *vs.* shallow pockets). The introduction of subgingival components into the saliva samples may have contributed to the highly significant correlations found with parameters involving saliva.

Quantification of oral malodor is a problem which has delayed scientific investigations into its causes and treatments. Oral malodor measurement by multiple judges is cumbersome and introduces problems of subjectivity and reproducibility. Agreement among judges, while highly significant, is usually

Table 5. Stepwise multiple-regression analysis of odor-judge assessments in terms of VSC and BANA (Avg.) scores

Dependent Variable	Intercept	Coefficient for			Multiple r
		Log Peak VSC	Log Steady-state VSC	BANA (avg.)	
Whole mouth	-6.845	5.820	N.E.[a]	0.333	0.568
	0.016[b]	0.001	0.684	0.010	0.0001
Tongue	-3.24	2.90	N.E.	0.217	0.501
	0.082	0.010	0.460	0.012	0.001
Saliva	-9.648	6.533	N.S.	0.507	0.585
	0.006	0.002	0.796	0.002	<0.0001

[a]   Not entered into the equation due to lack of significance.

[b]   Numbers in italics represent levels of significance.

moderate (Rosenberg *et al.*, 1991a,b; Rosenberg and McCulloch, 1992). One major difficulty is that once a subject has expelled breath for estimation by one judge, the halitus emitted subsequently for other judge(s) may differ in intensity and composition (Rosenberg and McCulloch, 1992). Intra-oral measurements with the sulphide monitor provide rapid quantitative data which are reproducible and sensitive, easy-to-use, and relatively inexpensive. Peak and steady-state sulphide monitor levels have been previously shown to provide highly significant correlations with odor-judge measurements (Rosenberg *et al.*, 1991a,b, 1992) but cannot be inferred to be a "gold standard" for bad breath measurement and thus to replace organoleptic assessment (Rosenberg and McCulloch, 1992). We have previously proposed that clinical measurement of oral malodor be conducted by organoleptic examination of oral malodor deriving from several loci, in conjunction with sulphide monitor measurements, as well as dental assessment (Rosenberg *et al.*, 1991a). The results of the present study suggest that the BANA test may be a simple, adjunct assay together with volatile sulphide determination in order to provide additional quantitative data which contribute to the overall association with odor-judge estimation. These techniques may prove advantageous in facilitating comparison of oral malodor investigations conducted at different locations by various investigators.

Acknowledgments

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