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Association between oral malodour and periodontal disease-related parameters in the general population

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Abstract

Aim. To determine the association between halitosis detection and periodontal status in systemically healthy non-smokers and to assess whether halitosis was related to quantities of *Fusobacterium nucleatum* and *Porphyromonas gingivalis* on the tongue dorsum. ***Methods.*** Periodontal examinations, tongue coating determination, Halimeter® readings and organoleptic assessments of mouth odour were performed in 28 chronic periodontitis patients, 23 chronic gingivitis patients and 27 healthy individuals. The quantities of *P. gingivalis* and *F. nucleatum* were determined in tongue specimens by real-time PCR. ***Results.*** Halitosis was more likely to be detected in patients with periodontitis (OR = 9.2) and gingivitis (OR = 4.6) than in healthy subjects. The posterior tongue odour was similar for all groups; had the highest score of all organoleptic assessments and was significantly correlated with Halimeter® scores and the odour of the whole mouth air. Periodontitis patients harboured significantly greater amounts of *P. gingivalis* on their tongue, yet similar quantities of *F. nucleatum* compared to gingivitis patients and healthy subjects. The amount of *P. gingivalis* residing on the tongue dorsum of periodontitis patients was significantly associated with halitosis recordings, while the amount of *F. nucleatum* was related to tongue coating in healthy controls, which corroborates its role in biofilm formation. ***Conclusions.*** Patients with periodontal disease were at higher risk for halitosis detection than healthy individuals. The posterior portion of the tongue dorsum seems to be an important source of odourous compounds, regardless of periodontal condition. *P. gingivalis* residing on the tongue of periodontitis patients may play a key role in oral malodour production.

Key Words: *halitosis, organoleptic, VSC, periodontal disease, real-time PCR*

Introduction

Volatile sulphur compounds (VSC; hydrogen sulphide, methyl-mercaptan, dimethyl-sulphide and dimethyl-disulphide) that result from the microbial putrefaction of food debris, cells, saliva and blood in the oral cavity constitute the main source of oral malodour [1]. A positive correlation has been observed between the concentration of VSC in gingival crevicular sites and the severity of periodontitis [2], while a number of putative periodontal pathogens are implicated in VSC production [3,4]. However, individuals with healthy periodontium have also been identified with oral halitosis [5,6]. It has been shown that tongue coating constitutes the main cause of oral malodour in young individuals, while both tongue coating and periodontal disease seem to be the main sources in older individuals [7]. The tongue rather than the periodontium is deemed to be the major source of bad breath, as the periodontal pocket is considered a 'closed' environment and only a small fraction of malodourous gases escape into the mouth air [8]. Tongue coating played the most significant role in VSC production, followed by periodontal status in a Chinese and European population [9,10].

The dorsal surface of the tongue is colonized by large amounts of bacteria and the development of an anaerobic microbiota has been considered a significant microbial niche for the production of malodorous products. *Fusobacterium nucleatum* is a prominent micro-organism in gingivitis and periodontitis [11]

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that is also found in large numbers in healthy sites, since it acts as a bridge between early and late colonisers [12]. *Porphyromonas gingivalis* is a putative periodontal pathogen that is also implicated in oral malodour production [4]. *F. nucleatum* is generally non-proteolytic, but the co-existence with *P. gingivalis* can change its metabolic properties [13]. A synergistic effect of *F. nucleatum* and *P. gingivalis* on biofilm formation has been demonstrated [14,15].

The current study aimed to determine the association between halitosis detection and periodontal status in non-smoking subjects and to assess whether halitosis is related to quantities of *F. nucleatum* and *P. gingivalis* on the tongue dorsum.

Materials and methods

Study population

Seventy-eight systemically healthy non-smokers, all of Caucasian origin, were screened for oral halitosis in the Department of Periodontology, Aristotle University of Thessaloniki, Greece. These were non-halitosis complaining subjects who either attended the Department for periodontal treatment or were staff members of the Faculty. Ethical approval was obtained from the Dental School Ethics Committee and a consent form was signed prior to enrolment.

The clinical inclusion criteria were (i) chronic periodontitis patients having at least one site *per* quadrant with clinical probing depth (CPD) ≥5 mm and radiographic evidence of bone loss, (ii) generalized chronic gingivitis patients presenting with bleeding on probing (BOP) at >30% of sites, CPDs <4 mm and no evidence of bone loss and (iii) healthy subjects having <10% sites with BOP and no sites with CPD >3 mm. Exclusion criteria included less than 20 teeth, smoking (during the previous year), presence of systemic disease including ENT complications, prescribed medication that can cause xerostomia, full and/or partial dentures, heavy restorations, large carious cavities, pathology of the oral tissues (pericoronitis, dental/periodontal abscess, etc), antibiotic therapy within 3 months of recruitment, periodontal treatment/scaling within the last year and pregnancy or lactation.

Periodontal charting

A full-mouth periodontal charting was recorded including plaque index (PI), CPD, clinical attachment levels (CAL) and BOP at six sites *per* tooth using a manual periodontal probe (Hu-Friedy XP-23/QW). The subjects that fulfilled the inclusion criteria were recruited in the study and were further clinically and microbiologically monitored. The demographic details and the periodontal status of the study participants are presented in Table I.

Assessment of tongue coating

The Winkel Tongue Coating Index was visually determined by dividing the dorsum of the tongue into sextants [16]. A score between 0–2 was given to each sextant according to the amount of deposits, and these scores were added, giving a total ranging from 0–12.

Tongue specimen collection

A specimen was collected from the rear of the tongue dorsum, while the area was isolated from saliva by placing cotton rolls around the tongue and using a saliva ejector. The dorsal tongue surface was then gently air-dried in an antero-posterior direction and the specimen was collected with repeated strokes under relative pressure, using a sterile plastic microbiology loop of 10 μl capacity from the terminal sulcus to the apex of the tongue. Specimens were placed in sterile tubes containing 0.4 ml distilled water, vortexed for 30 s and stored at −70°C until use.

In order to avoid interference of BOP and tongue sampling with the organoleptic assessments and primarily with the evaluation of the tongue odour, subjects were recalled on a second visit at the same time of the day, 2–4 days later for oral malodour assessment. They were asked not to change their routine dietary and oral hygiene habits, except for 48 h prior to halitosis assessment, according to written instructions regarding food and drink consumption and oral hygiene habits [17].

Table I. Demographic details and the periodontal status of the participants.

Clinical group	Gender	Age (years)	No. of teeth	BOP	PI	CPD	sites ≥5 mm (%)	CAL
Healthy (27)	15 M, 12 F	31 (5)	30.0 (2.0)	0.07 (0.04)	0.15 (0.15)	—	—	—
Gingivitis (23)	10 M, 13 F	37 (14)	27.0 (4.0)	0.41 (0.18)	0.40 (0.24)	—	—	—
Periodontitis (28)	17 M, 11 F	54 (12)	23.0 (4.0)	0.58 (0.20)	0.71 (0.21)	3.56 (0.53)	22.0 (3.0)	4.2 (0.9)

Mean (SD).
In total, 54% of the participants were males; BOP, bleeding on probing; PI, plaque index; CPD, clinical pocket depth; CAL, clinical attachment levels.

Halitosis recordings

The Halimeter® (Halimeter® RH-17, Interscan Corporation, Chatsworth, CA) was used to measure the concentration of volatile sulphur compounds (VSC) in parts *per* billion (p.p.b.) [18] between 10.00–12.00 at the same dental chair in the clinic to minimize daily variability in the room's temperature and humidity [9]. Organoleptic assessments ranging from 0–5 [18] assessed the odour of: whole mouth air (ORG_1)—subjects were asked to exhale gently; anterior region of the tongue (ORG_2)—subjects were asked to lick their wrists and wait a couple of seconds to allow the area to dry out before odour assessment; posterior region of tongue (ORG_3)—material was harvested from the rear of the lingual dorsum in a standardized manner using a plastic spoon and the odour was then assessed from this material; nose air—subjects were asked to exhale through one nostril after capping the opposite nostril with their finger to exclude extra-oral causes that may be related to bad breath.

A calibrated single 'examiner' scored the periodontal indices and Halimeter® readings, whereas one calibrated 'judge' carried out the organoleptic assessments of halitosis, without being aware of the examiner's recordings. Prior to and during this investigation the odour judge and an additional odour 'evaluator' were repeatedly standardized against a wide range of n-butanol solutions with intensities from 25–6075 parts *per* million [19,20] and also on the mouth odour of patients attending the periodontal clinic. In order to assess the reliability of the judge's ratings, the judge and the evaluator examined the mouth odour of a number of study participants without being aware of one another's score. A statistically significant correlation was found between the scores (Spearman's rho = 0.64 for the odour of whole mouth air, $p < 0.001$) [21]. It should be noted that only the judge's assessments were used in data analysis. Subjects were designated as halitosis positive (+) when either the organoleptic score of the whole mouth air was 2 or more and/or the readings of the Halimeter® exceeded 140 p.p.b.

Real-time polymerase chain reaction

DNA was extracted from specimens by boiling. For the quantitative analysis of the 16S rRNA of *F. nucleatum* and *P. gingivalis* in tongue specimens, two real-time PCR assays were performed at a total volume of 20 μl using the Light CyclerTM Fast Start DNA Master Hybridization Probes kit (Roche Diagnostics, Manheim, Germany), 500 nM each of sense and antisense primers, 200 nM probe and 5 μl of lysed cells. Primers' and probes' sequences for the detection of *P. gingivalis* and *F. nucleatum* have been reported in previous studies [22,23]. Amplification and detection were performed with the LightCycler Sequence Detection System (Roche Diagnostics, Manheim, Germany) with the following cycle conditions, identical for both assays: 95°C for 10 min and then 45 cycles of 95°C for 10 s, 58°C for 25 s and a final elongation at 40°C for 30 s. Standard curves were plotted for each assay by using serial dilutions of known quantities of purified genomic DNA of *P. gingivalis* strain W50 and *F. nucleatum* strain ATCC 10953, which was kindly donated by Dr Lappin (University of Glasgow).

Statistical methods

The association between halitosis detection (organoleptic score of the whole mouth air ≥2 and/or Halimeter® score >140 p.p.b) and periodontal status was examined by logistic regression. Since the size of each clinical group was above 20 and the minimum number of halitosis-positive subjects *per* clinical group exceeded 10, with the exception of the healthy group (nine halitosis-positive subjects), the sample size was deemed adequate for the application of this statistical model [24]. Logistic regression was also used to determine the association between halitosis detection and quantities of test bacteria. Spearman's rho correlation coefficient estimated the association between scale variables. The assumption of normality for scale variables was tested by the Shapiro Wilk test. Comparisons between groups were performed using the Kruskal-Wallis test, while pair-wise comparisons were conducted by the Mann-Whitney test with Bonferroni's adjustment of type I error. Fischer's exact test with Bonferroni adjustment compared the detection frequencies of the test bacteria between groups. SPSS version 16.0 software was used and the level of statistical significance was set at $p < 0.05$.

Results

Halitosis was detected in 9/27 (33%) healthy individuals; 16/23 (70%) subjects with gingivitis; and 23/28 (82%) periodontitis patients. Gingivitis and periodontitis patients were at higher risk for halitosis detection compared with the healthy controls (logistic regression: OR = 4.6 (95% CI: 1.4–15.1) for gingivitis and OR = 9.2 (95% CI: 2.6–32.3) for periodontitis patients). A large inter-group variability was noted in Halimeter® readings with no significant differences between groups (Figure 1). Organoleptic assessments of the odours of whole mouth air and anterior part of the tongue were significantly higher for periodontitis patients, compared with the other two groups, but this was not the case for the posterior tongue odour, which was similar for all clinical groups (Figure 2). The assessment of the nose air was zero in all cases except for one (score: 1) and, therefore, this parameter was

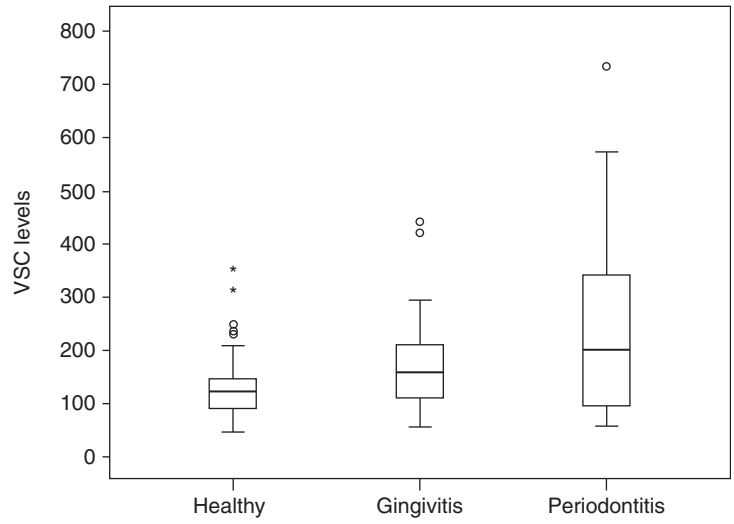

Figure 1. Halimeter® readings (parts *per* billion). Mean values (SD): 137.6 (76.5) for healthy; 174.8 (101.4) for gingivitis; 239.4 (180.2) for periodontitis subjects; Mann Whitney pair-wise comparisons with Bonferroni adjustment: $p > 0.05$.

not statistically analysed. In all individuals Halimeter® readings were significantly correlated with organoleptic assessments and the odour of the whole mouth air was significantly correlated with the odour of the anterior and posterior part of the tongue (Table II). Tongue coating determined by WTCI was similar among the participants, albeit slightly elevated in the groups with periodontal disease [Mann Whitney pair-wise comparisons with Bonferroni adjustment: $p > 0.05$; mean values (SD): 2.9 (1.9) for healthy; 4.1 (2.1) for gingivitis; 3.2 (2.1) for periodontitis subjects].

The three groups presented with similar quantities and detection frequencies of *F. nucleatum* in tongue specimens (Table III). In contrast, *P. gingivalis* was detected more frequently in patients with periodontal disease than in healthy subjects. More specifically, periodontitis patients harboured significantly greater amounts of *P. gingivalis* on their tongue, compared to gingivitis patients and healthy subjects. No significant associations were found between halitosis detection and the amount of *F. nucleatum* in any of the clinical groups (logistic regression analysis, $p > 0.05$). A similar pattern was followed by *P. gingivalis* in gingivitis and healthy subjects with the exception of periodontitis patients. When the amount of *P. gingivalis* in the tongue specimen of a patient with periodontitis increased by three times, this individual was twice as likely to exhibit oral malodour (logistic regression analysis: OR = 2.2; 95%

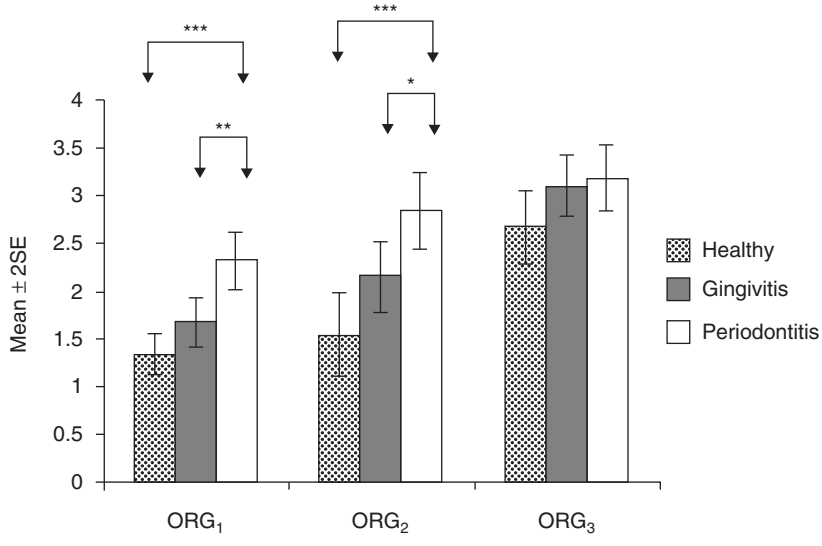

Figure 2. Organoleptic assessments (range 0–5) of the odour of the whole mouth air (ORG_1), the tip of the tongue (ORG_2), and posterior region of the tongue (ORG_3). Mean values (SD) for healthy, gingivitis and periodontitis subjects, respectively: ORG_1: 1.3 (0.6); 1.7 (0.6); 2.3 (0.8); ORG_2: 1.5 (1.2); 2.2 (0.9); 2.8 (1.1); ORG_3: 2.7 (1.0); 3.1 (0.8); 3.2 (0.9); Mann Whitney pair-wise comparisons with Bonferroni adjustment: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table II. Correlations between halitosis recordings (Halimeter® and organoleptic scores).

$n = 78$	ORG_1	ORG_2	ORG_3
Halimeter®	$r = 0.48$***	$r = 0.49$***	$r = 0.50$***
ORG_1		$r = 0.57$***	$r = 0.48$***
ORG_2			$r = 0.39$***

r, Spearman's rho correlation coefficient; ORG_1, whole mouth air odour; ORG_2, anterior tongue odour; ORG_3, posterior tongue odour; *** $p < 0.001$.

CI = 1.06–4.47; $p = 0.03$). In this category of patients *P. gingivalis* was significantly associated with the posterior tongue odour ($r = 0.41$; $p < 0.05$) and with the Halimeter® scores ($r = 0.48$; $p < 0.05$), whereas no significant associations were noted between the amounts of the test bacteria and the organoleptic or Halimeter® readings in healthy and gingivitis subjects. Although no significant association was found between halitosis recordings and WTCI in any of the clinical groups, WTCI was significantly correlated with *F. nucleatum* in healthy controls ($r = 0.39$, $p < 0.05$).

Discussion

Based on the threshold for oral malodour detection patients with periodontal disease were at higher risk for halitosis detection than healthy controls. This finding is in line with previous reports [25,26] and in partial agreement with Bosy et al. [5], who failed to associate oral malodour with periodontitis, despite the fact that the intensity of malodour was greater in periodontitis patients than in healthy subjects. Although Halimeter® readings tended to increase from periodontal health to periodontal disease, this finding failed to reach statistical significance. On the contrary, organoleptic scores of the whole mouth air and the anterior part of the tongue were significantly greater for periodontitis patients than subjects with gingivitis or with healthy periodontium. The organoleptic examination evaluates a wide range of odours and discloses evidence of oral halitosis, which simulates conditions in everyday life. On the other hand, the Halimeter® measures the concentration of VSCs without being able to discriminate between the sulphur gases and shows lower sensitivity for methylmercaptan, which is prominent in periodontal disease over hydrogen sulphide, which is the main constituent in periodontally healthy individuals [27,28]. Although there is evidence to support the efficacy of the Halimeter® in measuring VSC [18,29], the organoleptic assessment is still considered the 'gold standard' for defining subjects with bad breath in industry and academia [28-30]. In the present study the olfactory system and an industrial monitor were used in a complementary manner for halitosis detection. Although the pre-training of judges improves objectivity and reproducibility within and between examiners, such standardization may simultaneously restrict personal judgement and introduce bias [18]. In the current study n-butanol was used for standardization [31], but more recent data do not recommend this compound as a training odourant because of its irritant nature [20].

The organoleptic assessments revealed that the anterior part of the tongue appeared to smell, but the odour originating from the posterior region had the highest rating of all organoleptic assessments in all groups. Posterior tongue odour was similar among the three clinical groups and was significantly correlated with the organoleptic assessment of the whole mouth air and VSC levels, indicating that it may play a key role in oral malodour production, regardless of periodontal status.

The significance of tongue coating in oral malodour formation has been reported by several investigations [7,10,28,32]. However, there is a large heterogeneity in the literature regarding the methodology employed to determine tongue coating (i.e. distribution area, thickness and extent, amount and extent). Current findings did not associate tongue coating evaluated by WTCI with halitosis recordings. The mere presence of tongue coating does not necessarily lead to oral malodour and reduction of malodorous gases can be achieved in presence of tongue coating [16]. It has been suggested that the composition of tongue coating rather than its thickness or the extension has an impact on oral malodour production [16]. This study focused on the quantitative rather than

Table III. DNA concentration and detection frequencies of putative periodontal pathogens in tongue specimens, determined by real-time PCR.

Clinical group	*P. gingivalis* (μg/100 μl)	*F. nucleatum* (μg/100 μl)	*P. gingivalis* (% detection)	*F. nucleatum* (% detection)
Healthy	0.014 (0.050)[a]	0.34 (0.54)	55.6%[c,d]	100.0%
Gingivitis	0.001 (0.003)[b]	0.17 (0.26)	95.7%[c]	100.0%
Periodontitis	0.130 (0.350)[a,b]	0.51 (1.60)	85.7%[d]	92.9%

Mean values (SD).
Mann Whitney pair-wise comparisons with Bonferroni adjustment: [a] $p < 0.001$; [b] $p < 0.01$.
Fischer's exact test with Bonferroni adjustment: [c] $p < 0.01$; [d] $p = 0.05$.

the qualitative analysis of the pathogens for the accurate evaluation of bacteria related to oral malodour [22,23]. Of the highly variable and diverse tongue microflora, the current study examined the quantities of two putative periodontal pathogens and demonstrated that in periodontitis patients the amount of *P. gingivalis* seemed to play a key role in oral malodour production, whereas in healthy controls the quantity of *F. nucleatum* was related to the amount of tongue coating, which corroborates its role in biofilm formation.

It should be borne in mind that the participants of the current study had no obvious complaints of oral malodour and the impact of several factors upon oral malodour detection may not be as pronounced for the general population as it is for subjects with halitosis complaints, albeit sometimes imaginary.

Conclusions

Patients with periodontal disease were at higher risk for halitosis detection than individuals with a healthy periodontium.

Tongue coating and the odour of the posterior tongue were similar for all groups. The posterior tongue odour was the highest of all organoleptic assessments and was significantly correlated with VSC levels and the odour of the whole mouth air, suggesting that the posterior portion of the tongue dorsum may be an important source of odourous compounds, regardless of periodontal condition.

The amount of *P. gingivalis* residing on the tongue dorsum of periodontitis patients was significantly associated with halitosis, while the amount of *F. nucleatum* was related to tongue coating in healthy controls, which corroborates its role in biofilm formation.

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