

THE ROLE OF ORAL ANAEROBIC BACTERIA, AND INFLUENCE OF SOCIAL AND HEALTH FACTORS IN HALITOSIS AETIOLOGY

Dagnija Rostoka, Juta Kroiča, Aigars Reinis, and Valentīna Kuzņecova

Department of Biology and Microbiology, Rīga Stradiņš University, Dzirciema iela 16, Rīga LV-1007, LATVIA
dagnija.rostoka@apollo.lv

Communicated by Māra Pilmane

*The aim of this work was to identify the bacteria associated with halitosis, and by questionnaire to test whether diet, oral hygiene habits and illness factors were associated with bacterial amounts in the oral cavity and the II-1 α /II-1 β polymorphism. Bad breath is a frequent problem in Latvia and for many patients may cause important emotional and psychological distress. As there are different causes of halitosis, this might be also reflected in the bacterial community of the oral cavity. The concentration of bacteria in the oral cavity was significantly higher in halitosis patients than in the studied control group, who did not complain about halitosis. The PCR results corresponded with halimetric values. The main cause of halitosis was found to be oral pathology — increased amounts of oral anaerobic bacteria *Porphyromonas gingivalis*, *Tannerella forsythi*, *Treponema denticola*, and *Prevotella intermedia*.*

Key words: *halitosis, obligate anaerobic bacteria, volatile sulphur compounds, II-1 α /II-1 β polymorphism.*

INTRODUCTION

Halitosis, commonly referred to as bad breath, may be due to physiological and/or pathological causes of oral (about 90%), gastrointestinal, nasopharyngeal or other origin (Rosenberg, 1996; Delanghe *et al.*, 1997; Seemann *et al.*, 2004). The most frequent complaint of dental patients is bad breath or halitosis. Oral anaerobic bacteria hydrolyse proteins and further degrade the amino acids, which leads to halitosis. Gram-negative anaerobes, such as *Porphyromonas*, *Prevotella* and *Treponema* species produce volatile sulphur compounds (VSC) from amino acids that contain sulphur (Tonzetich and Kestenbaum, 1969; McNamara *et al.*, 1972; Loesche *et al.*, 2002). *Porphyromonas gingivalis*, an obligate anaerobic that cannot utilise carbohydrates, produces malodour via six amino acids: cystine, cysteine, methionine, thryptophan, ornithine and arginine. *Prevotella* can utilise carbohydrates; however, it also participates in the formation of VSC. The normal salivary pH of 6.5 suppresses the growth of gram-negative bacteria that activate certain enzymes and cause malodour. An acidic pH favours the growth of gram-positive bacteria that produce little odour, and alkaline pH (7.2) encourages growth of gram-negative organisms. Besides VSC, there are many other metabolic products that produce malodour, such as indole, skatole, putrescine, and cadaverine. Cadaverine and putrescine are malodorous diamines, commonly encountered as bacterial degradation products. Cadaverine may be produced in saliva as the result of decarboxylation of lysine. Putrescine may be produced by decarboxylation of ornithine,

decarboxylation of arginine, or direct conversion of arginine to ornithine, followed by decarboxylation of ornithine to putrescine (Goldberg *et al.*, 1994). Many recent studies indicate the surface of the tongue as the main source of halitosis. However, for patients with periodontal disease, halitosis depends on severity of gingivitis (De Boever and Loesche, 1995; Miyazaki *et al.*, 1995). In advanced cases, significant amounts of the malodorous components result in high levels of VSC, especially hydrogen sulphide, methyl mercaptan, dimethyl sulphide, disulphide cysteine, cysteine, methionine, indole, and lactic acid, which are mostly derived from the affected sites (Tonzetich and McBride, 1981; Kleinberg and Codipilly, 1999).

Bad breath is a frequent problem in Latvia and for many patients may cause important emotional and psychological distress. As there are different causes of halitosis, this might be also reflected in the bacterial community of the oral cavity. Bacteriological tests have been available to clinicians since the end of the 1980s. Biological examinations can help clinicians in a number of ways: aid diagnosis and prognosis, verify adequacy of the applied treatment, choice of the correct antibiotic therapy and choice of the most appropriate molecules. A number of microorganisms participate in the aetiology of periodontal diseases. The bacterial aetiology of periodontal disease is complex, as there are many organisms responsible for the initiation and progression of disease. Periodontal destruction is likely caused by various toxic products produced by specific pathogenic subgingival plaque bacteria, and also from the host responses to plaque

bacteria and their products (Nonnenmacher *et al.*, 2001). The choice of the appropriate treatment and prognosis need to consider the pathogenic bacteria identified. Bacterial samples for determination of the microbiota can be taken from one subgingival pocket of a patient, or for better evaluation samples from a series of sites. In the case of halitosis it is advisable to take as many samples of microbiological material from as many sites in the oral cavity, as possible. The presence of certain pathogens, such as *Porphyromonas gingivalis* can provide information on the potential progression of periodontal lesions.

Cross-reactivity of some bacterial colonies can increase their virulence and destruction of periodontal tissue, as in *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*. Inflammatory reactions are characterized by an increased secretion of proinflammatory and catabolic cytokines, mainly the activator interleukine 1 (Il-1). High levels of interleukine production due to Il-1 α /Il-1 β gene polymorphism have been associated with periodontitis (Lee *et al.*, 2003). However, other reports are contradictory, and show an interaction of the Il-1 α /Il-1 β genotype with age, smoking and *Porphyromonas gingivalis*. Volatile sulphur components, such as methylmercaptan, enhance the production of interstitial collagenase, the production of Il-1 by mononuclear cells and the production of cathepsin B, and thus mediate connective tissue breakdown. Il-1 has both local and systemic effects on cell metabolism and on immune and inflammatory reactions. Il-1 is considered to be an important mediator of inflammation, based on its presence at inflammatory sites and its ability to induce many of the hallmarks of the inflammatory response. Microbial products, such as endotoxins and exotoxins induce Il-1 production by monocytes (Lancero *et al.*, 1996; Ratkay *et al.*, 1996).

The aim of this work was to identify the bacteria associated with halitosis, and by questionnaire to test whether diet, oral hygiene habits and illness factors are associated with bacterial amounts in the oral cavity and the Il-1 α /Il-1 β polymorphism.

MATERIALS AND METHODS

Questionnaire. A questionnaire on social and health factors was given to 258 untreated halitosis patients (age 9–74 years), of which 117 (45.3%) were male and 141 (54.7%) were female. Also, a control group consisting of 40 restorative dentistry patients, who did not complain about halitosis, was asked to answer the questionnaire. This questionnaire was approved by the Committee of Ethics of the Ministry of Welfare of the Republic of Latvia and covered questions about the use of antibiotics and other medicine, especially which affect the quality and quantity of saliva, as well as questions concerning smoking, the use of alcohol, diet, and presence of systemic diseases. The questionnaire was based on the authors experience in the University of British Columbia, Vancouver, Canada (Yaegaki, 1999) and then adapted for use in Latvia.

Laboratory analysis. Oral odour or bad breath was confirmed by measurements made by the portable sulphide monitor or halimeter (Interscan Corporation, Model RH-17E). The halimeter quantifies breath measurements in parts-per-billion (ppb) of VSC. All 258 untreated halitosis patients and control group were examined. Halitosis patients were divided into three age groups (group 1 — till 40 years, group 2 — 41–60 years, group 3 — 61 years and older). For the distribution of bacterial concentrations each age group was divided into non-smokers and smokers. Bacterial material was taken from periodontal pockets (Noiri *et al.*, 2001). Microbiota was analysed by quantitative PCR (micro-IDent®, Hain Lifescience) for amounts of oral anaerobic bacteria: *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythi*, *Treponema denticola*, and *Prevotella intermedia*. The PCR protocol was conducted using Hain Lifescience PCR set-up for the microDent and Taq polymerase from Eppendorf (www.hain-lifescience.de). Bacterial amounts were log-transformed for statistical analysis. Polymorphism of the Il-1A–889 and Il-1B+3953 gene cluster was checked (GenoType®PST, Hain Lifescience). The PCR material for polymorphism analysis was buccal cells. This protocol describes the PCR set-up for the DNA strip tests using HotStarTaq DNA polymerase from Qiagen.

Statistical analysis. Pearson correlation coefficients (Excel data analysis pack) were determined between bacterial amounts in the oral samples. Forward selection in stepwise regression (using the Canoco programme) was used to determine the best model for bacterial amounts explaining halimeter measurements. Differences between male and female patients on distribution of response on questions were tested using the Pearson χ^2 test with a significance level of $P = 0.05$. For each question in the questionnaire, the patients were divided into response groups based on their answers. Multiple response permutation procedure (MRPP) was chosen as a multivariate statistical method as it avoids distribution and uneven sample size problems that are inherent in this type of data. MRPP on a calculated Sorenson distance matrix was used to test for differences in the bacterial community between response groups (McCune and Mefford, 1999). MRPP is a robust nonparametric multivariate technique based on a distance matrix and does not require distributional assumptions. Significance is estimated by the probability of obtaining a weighted mean within-group distance (expected) that is smaller than or just as small as that observed. Equal chance of groupings is assumed when calculating the expected distance. When significance between groups was shown, Indicator species analysis was conducted to test for significant differences in bacterial amounts and Il-1 α /Il-1 β polymorphisms between response groups. A generalized linear model with a binomial distribution was used to test for the relationship between halimeter measurements and the presence of Il-1 α /Il-1 β polymorphism, with patient age and smoking as interaction variables.

RESULTS

Bacterial concentrations in young persons (age group 1) of halitosis patients are presented in Figure 1. Data showed that both non-smokers and smokers had very high bacterial concentrations and there was no difference between smokers and non-smokers. Only two classes of concentrations (10^3 and 10^6) of *Actinobacillus actinomycetemcomitans* were higher in the smoker group. Only 21% of young non-smokers had acceptable *Actinobacillus actinomycetemcomitans* concentration ($<10^3$) in periodontal pockets. The most frequent *Porphyromonas gingivalis* concentration was 10^5 . 55 per cent of smokers had this high bacterial amount in their periodontal pockets. Also, 44% of non-smokers had 10^5 *Porphyromonas gingivalis* in periodontal pockets. Concentrations of 10^6 and 10^7 of *Tannerella forsythi* occurred in 36% and 27% of persons in the smoker group, which was much higher than in the non-smoker groups — 10^6 in 23%, and 10^7 in 17%. Only 8% of young non-smokers and 9% of young smokers had acceptable *Tannerella forsythi* concentration ($<10^4$) in periodontal pockets. Only 6% of young non-smokers and 18% of young smokers had acceptable *Treponema denticola* concentration ($<10^4$) in periodontal pockets. The most frequent *Treponema denticola* concentration was 10^5 . 50 per cent of smokers had

this high bacterial amount in their periodontal pockets. Also 41% of non-smokers had 10^5 *Treponema denticola* in periodontal pockets. The counts of *Prevotella intermedia* did not differ between smokers and non-smokers. 34% of young non-smokers and 45% of young smokers had acceptable *Prevotella intermedia* concentration ($<10^4$) in periodontal pockets.

Bacterial concentrations in mid-aged persons (age group 2) of halitosis patients are presented in Figure 2. Data showed that both non-smokers and smokers had very high bacterial concentrations but there was no significant difference between middle-aged smokers and non-smokers. Only three concentration classes (10^3 , 10^4 and 10^6) of *Actinobacillus actinomycetemcomitans* were higher in the smoker group. Only 18% of middle-aged non-smokers had acceptable *Actinobacillus actinomycetemcomitans* concentration ($<10^3$) in periodontal pockets. The most frequent *Porphyromonas gingivalis* concentration was 10^5 . 35 per cent of smokers had this very high bacterial amount in their periodontal pockets. Also, 32% of non-smokers had 10^5 *Porphyromonas gingivalis* in periodontal pockets. Concentrations 10^5 and 10^6 of *Tannerella forsythi* occurred in 26% and 39% of persons in the smokers group, which was higher than in the non-smoker group — 10^5 had 25%,

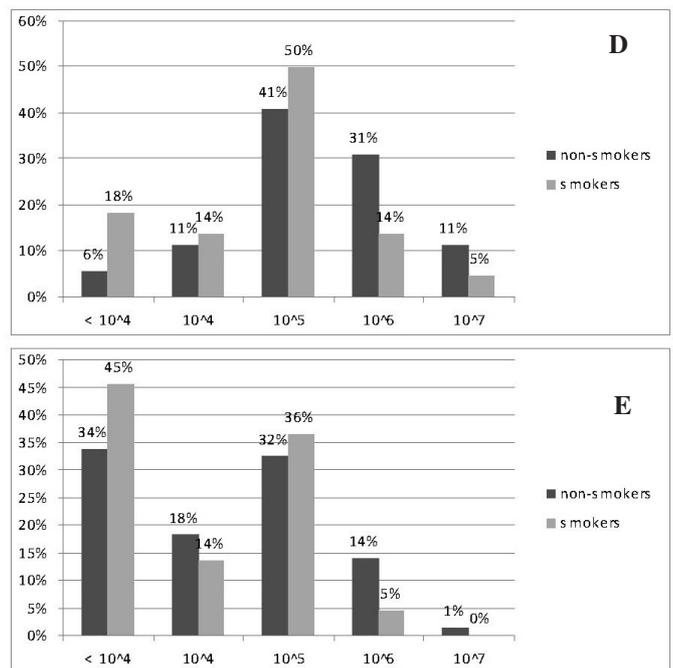
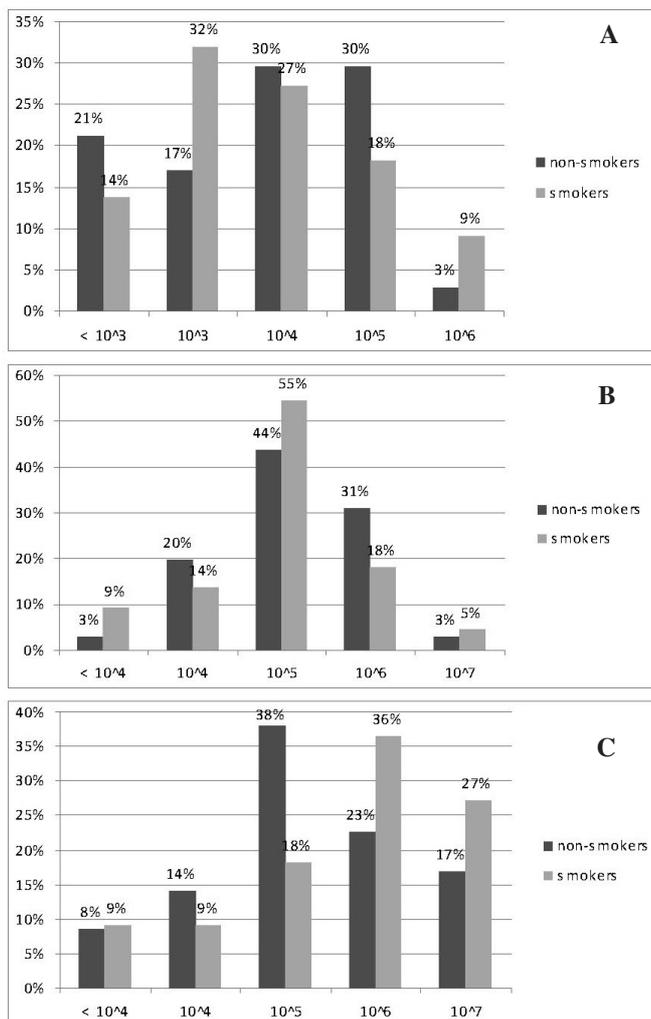


Fig. 1. Distribution of bacterial concentrations in age group 1 of halitosis patients of bacteria *Actinobacillus actinomycetemcomitans* (A), *Porphyromonas gingivalis* (B), *Tannerella forsythi* (C), *Treponema denticola* (D), *Prevotella intermedia* (E).

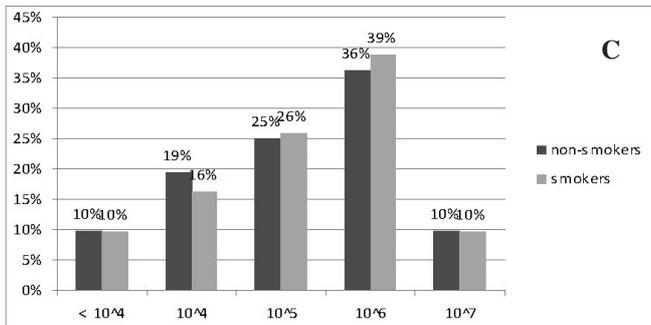
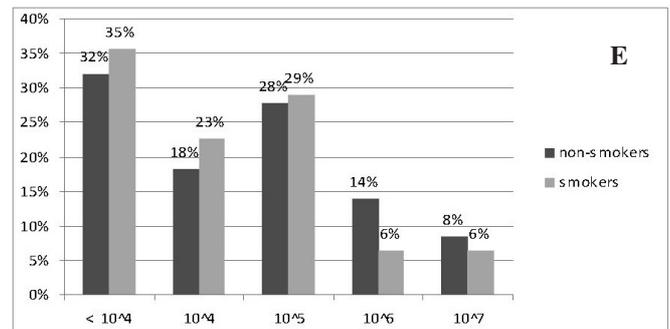
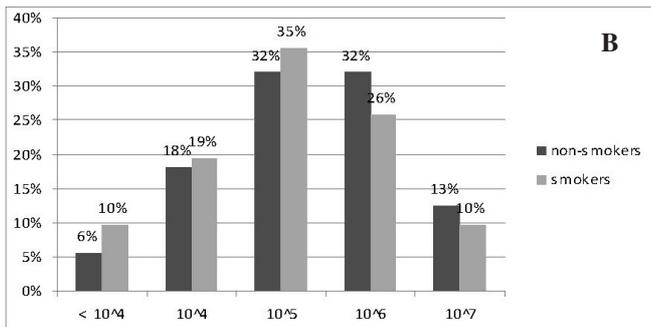
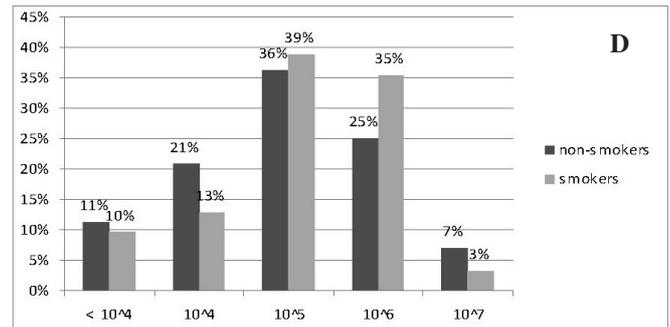
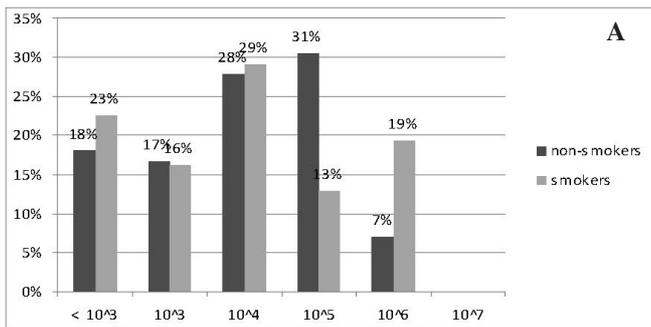


Fig. 2. Distribution of bacterial concentrations in age group 2 of halitosis patients of bacteria *Actinobacillus actinomycetemcomitans* (A), *Porphyromonas gingivalis* (B), *Tannerella forsythienseis* (C), *Treponema denticola* (D), *Prevotella intermedia* (E).

and 10^6 had 36%. Only 10% middle-aged non-smokers and smokers had acceptable *Tannerella forsythienseis* concentration ($<10^4$) in periodontal pockets. Only 11% of middle-aged non-smokers and 10% middle-aged smokers had acceptable *Treponema denticola* concentration ($<10^4$) in periodontal pockets. The most frequent *Treponema denticola* concentration was 10^5 . 39 per cent of smokers had this high bacterial amount in their periodontal pockets. Also, 36% of non-smokers had 10^5 *Treponema denticola* in periodontal pockets. The concentration of *Prevotella intermedia* did not differ between smokers and non-smokers. 32 per cent of middle-aged non-smokers and 35% of middle-aged smokers had acceptable *Prevotella intermedia* concentration ($<10^4$) in periodontal pockets.

Bacterial concentrations in senior persons (age group 3) of halitosis patients are presented in Figure 3. Data showed that both non-smokers and smokers had very high bacterial concentrations, although there was no significant difference between smokers and non-smokers. Only two concentrations ($<10^3$ and 10^5) of *Actinobacillus actinomycetemcomitans* were higher in smokers groups. Only 25% of senior non-smokers had acceptable *Actinobacillus actinomycetemcomitans* concentration ($<10^3$) in periodontal pockets. The most frequent *Porphyromonas gingivalis* concentration was 10^5 and 10^6 . 29% of smokers had 10^5 bacterial concentra-

tions in their periodontal pockets. Also 36% of non-smokers had 10^5 *Porphyromonas gingivalis* in periodontal pockets. Concentrations of 10^5 and 10^6 of *Tannerella forsythienseis* were observed in 43% of persons in each smokers group, which was much higher than in the non-smoker group — 10^5 had 32%, and 10^6 had 34%. Only 13% of senior non-smokers and 14% of senior smokers had acceptable *Tannerella forsythienseis* concentration ($<10^4$) in periodontal pockets. Only 13% of senior non-smokers and 14% of seniors smoker had acceptable *Treponema denticola* concentration ($<10^4$) in periodontal pockets. The most frequent *Treponema denticola* concentration was 10^5 . 57 per cent of smokers had this high bacterial concentration in their periodontal pockets. Also, 38% of non-smokers had 10^5 *Treponema denticola* in periodontal pockets. The concentration of *Prevotella intermedia* did not differ between smokers and non-smokers. Only 42% of senior non-smokers and 57% of senior smokers had acceptable *Prevotella intermedia* concentration ($<10^4$) in periodontal pockets.

Correlation coefficients among concentrations of all tested bacteria as well among among amounts of bacteria and to halimeter measurements were correlated (Table 1). The highest correlation with halimeter measurements was shown for *Porphyromonas gingivalis*, followed by *Tannerella forsythienseis*, *Treponema denticola*, *Prevotella intermedia*

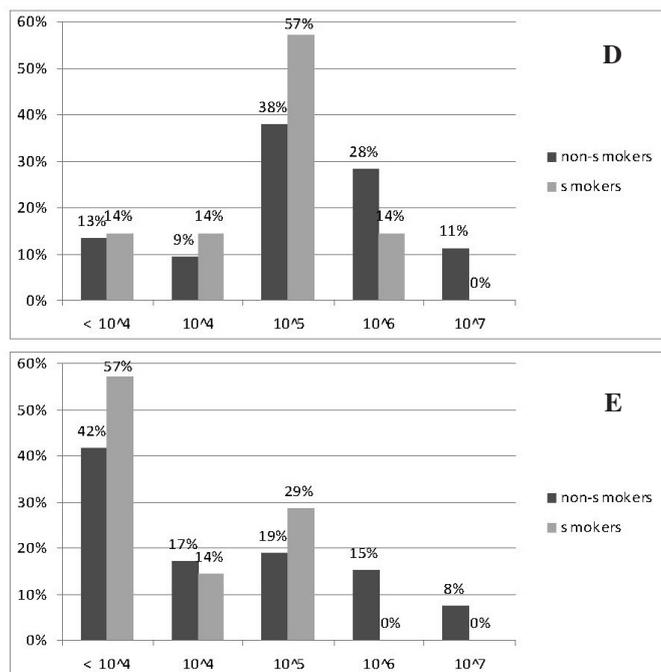
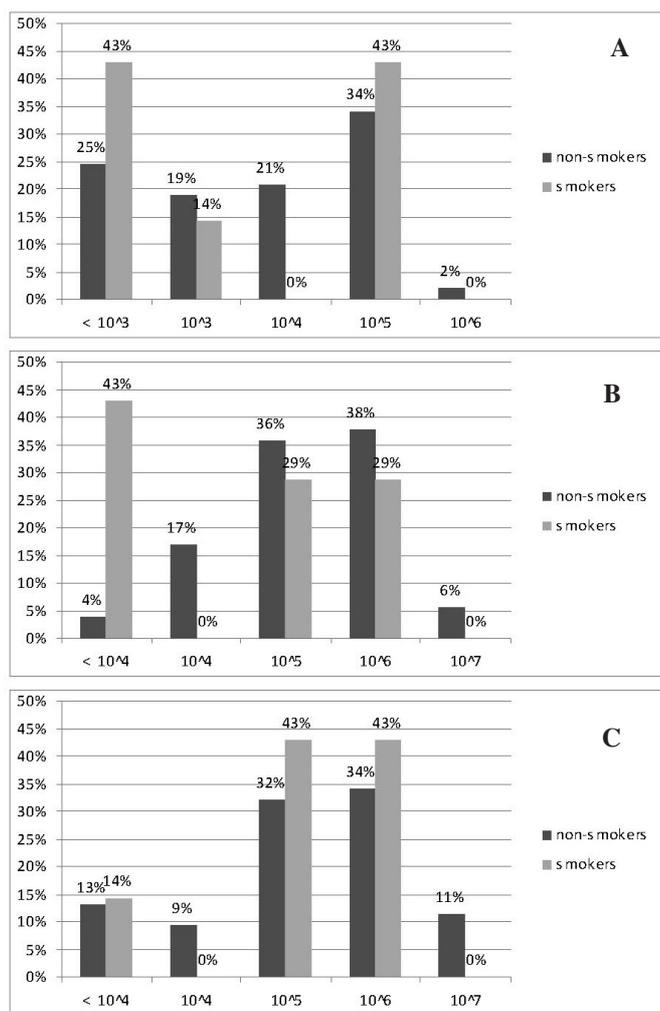


Fig. 3. Distribution of bacterial concentrations in age group 3 of halitosis patients of bacteria *Actinobacillus actinomycetemcomitans* (A), *Porphyromonas gingivalis* (B), *Tannerella forsythienseis* (C), *Treponema denticola* (D), *Prevotella intermedia* (E).

Table 1

CORRELATION COEFFICIENTS BETWEEN BACTERIAL AMOUNTS AND HALIMETER MEASUREMENTS

| | <i>Actinobacillus actinomycetemcomitans</i> | <i>Porphyromonas gingivalis</i> | <i>Tannerella forsythienseis</i> | <i>Treponema denticola</i> | <i>Prevotella intermedia</i> |
|---|---|---------------------------------|----------------------------------|----------------------------|------------------------------|
| <i>Actinobacillus actinomycetemcomitans</i> | | | | | |
| <i>Porphyromonas gingivalis</i> | 0.14 | | | | |
| <i>Tannerella forsythienseis</i> | 0.02 | 0.31 | | | |
| <i>Treponema denticola</i> | -0.13 | 0.14 | 0.20 | | |
| <i>Prevotella intermedia</i> | 0.07 | 0.14 | 0.12 | 0.36 | |
| Halimeter measurements | 0.15 | 0.72 | 0.56 | 0.38 | 0.26 |

and *Actinobacillus actinomycetemcomitans* (all significant, $P < 0.05$). The best model explaining halimeter measurements was *Porphyromonas gingivalis* + *Tannerella forsythienseis*, + *Treponema denticola*, which explained 71% of the variability. Addition of *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* did not improve the explanatory power of the model. No significant relation was shown between the presence of the II-1 α /II-1 β polymorphism and halimeter measurements, with or without patient age and smoking habit as interaction terms. Mean age of patients was 42.5 years, which did not differ significantly between male and female patients. Significant differences (Table 2) between male and female response on the ques-

tions occurred only for association with other diseases ($\chi^2 = 16.21$, $P < 0.05$), and smoking ($\chi^2 = 19.17$, $P = 0.001$). Female patients recognised past ear, nose, throat, as well as gastric illnesses than male patients. On the other hand, more male patients were smokers. The smoker group included 60 (23.3%) halitosis patients: 42 (35.9%) males and 18 (12.8%) females. Since other questionnaire responses did not differ between sexes, the answers were pooled.

Answers to questioner showed (Table 3) that patients with halitosis have mostly recognised this problem for a number of years (70.9%), but have taken no previous action (72.5%). They generally brush their teeth twice a day

Table 2

CLINICAL ANSWERS OF HALITOSIS PATIENTS TO A QUESTIONNAIRE CONCERNING ASSOCIATION WITH OTHER DISEASES

| Illnesses | Sex | | | | Total | |
|------------------------------------|--------|------|---------|------|--------|------|
| | males | | females | | | |
| | number | % | number | % | number | % |
| Non | 75 | 64.1 | 71 | 50.4 | 146 | 56.6 |
| Sinusitis or other nasal condition | 7 | 6.0 | 14 | 9.9 | 21 | 8.1 |
| Lung and bronchial diseases | 2 | 1.7 | 1 | 0.7 | 3 | 1.2 |
| Stomach dysfunction | 11 | 9.4 | 19 | 13.5 | 30 | 11.6 |
| Diabetes | 6 | 5.1 | 7 | 5.0 | 13 | 5.0 |
| Liver dysfunction | 6 | 5.1 | 1 | 0.7 | 7 | 2.7 |
| Anaemia | 1 | 0.9 | 6 | 4.3 | 7 | 2.7 |
| Emotional | 0 | 0 | 2 | 1.4 | 2 | 0.8 |
| Other illness | 9 | 7.7 | 20 | 14.2 | 29 | 11.2 |
| Total | 117 | 100 | 141 | 100 | 258 | 100 |

(91.5%), but do not use dental floss (15.3%), while about half (46.1%) use rinsing solutions. Bleeding gums (62%) and extracted teeth (72.1%) and coated tongue (41.7%) are typical associated characteristics. Bad breath is often self-recognised to be an all day problem (55.4%), for others on waking (33.7%), and this generally causes some kind of communication problem (66%). Almost all respondents drink liquids at least four times a day. The larger part of the patients does not know the cause of halitosis (57.4%), while the majority of others believe that the problem arises from teeth. For the 30 questions given, only a few showed differences in the bacterial community between the response groups. The identified response groups to the question "When do you notice malodour" were shown to significantly differ in the bacterial community (MRPP $P < 0.05$). However, as only 4 of 21 pair-wise comparisons were significant, we accepted the null hypothesis of no difference between groups to avoid Type I statistical error. MRPP showed a significant difference ($P < 0.05$) between groups that acknowledged different illnesses, and pair-wise comparison showed that the only illness groups that significantly differed from the no-illness group were the diabetic and anaemia groups. Interestingly, both of these groups showed significantly lower (Indicator species analysis) bacterial amounts of *Tannerella forsythietensis* ($P < 0.05$) and *Treponema denticola* (slightly non-significant $P > 0.05$) in the illness groups. The bacterial community of patients on a special diet significantly differed (MRPP $P < 0.05$) in having a lower amount of *Prevotella intermedia* (Indicator species analysis: $P < 0.01$) from the non special diet group.

DISCUSSION

The responses to the questionnaire given by halitosis patients clearly indicates that most patients who are aware of the problem have taken no action, even though they know that communication with others has become difficult. This suggests that patients do not regularly visit a dentist, perhaps only when a visit cannot be avoided, which is also sug-

Table 3

CLINICAL ANSWERS OF HALITOSIS PATIENTS TO A QUESTIONNAIRE CONCERNING DIAGNOSIS OF HALITOSIS

| Breath testing questions | Variations | Total number | % |
|--------------------------------------|------------------------------|--------------|-------|
| First perception of malodour | Before many years | 183 | 70.9 |
| | Before many months | 10 | 3.9 |
| | Before a few weeks | 3 | 1.2 |
| | Do not remember | 62 | 24 |
| Source of information | Himself/herself | 170 | 65.9 |
| | Someone else | 74 | 28.7 |
| | Other | 14 | 5.4 |
| Action taken | Nothing | 187 | 72.5 |
| | Self treatment | 50 | 19.4 |
| | Turned to specialist | 21 | 8.1 |
| | Asked for advice | 30 | 11.6 |
| | Talked to family and friends | 24 | 9.3 |
| Number of times you brush your teeth | Never | 7 | 2.7 |
| | 1 times daily | 15 | 5.8 |
| Use of | 2 times daily | 236 | 91.5 |
| | Dental floss | 39 | 15.31 |
| | Rinsing solutions | 119 | 46.1 |
| Symptoms of | Bleeding gums | 160 | 62 |
| | Extracted teeth | 186 | 72.1 |
| | Dry mouth | 61 | 23.6 |
| | Dry eyes | 3 | 1.2 |
| | Canker sores | 30 | 11.6 |
| | Bad taste in mouth | 71 | 27.5 |
| | Coated tongue | 107 | 41.7 |
| Time of day of bad malodour | On waking | 87 | 33.7 |
| | When hungry | 1 | 0.4 |
| | When tired | 2 | 0.8 |
| | When thirsty | 4 | 1.6 |
| | At work | 2 | 0.8 |
| | While talking | 3 | 1.2 |
| | Morning | 16 | 6.2 |
| | All day | 143 | 55.4 |
| Stress factors | Work or social problems | 117 | 45.3 |
| | Personal problems | 117 | 45.3 |
| Communication problems | None | 62 | 24 |
| | Unable to talk with others | 34 | 13.2 |
| | Feel uneasy with others | 85 | 32.9 |
| | Do not like to meet others | 27 | 10.5 |
| | Others avoid contact | 9 | 3.5 |
| | Other problems | 41 | 15.9 |
| Use of medicine | None | 105 | 40.7 |
| | Vitamins | 51 | 19.8 |
| | Antacid | 8 | 3.1 |
| | Other | 94 | 36.4 |
| Intake of liquids (times daily) | 2 | 2 | 0.8 |
| | 3 | 7 | 2.7 |
| | 4 | 61 | 23.6 |
| | 5 | 55 | 21.3 |
| | 6 | 67 | 26 |
| | 7 | 28 | 10.9 |
| | 8 | 38 | 14.7 |
| | Dieting | 43 | 16.7 |
| Allergy | 25 | 9.7 | |
| Perceived reason for malodour | Do not know | 148 | 57.4 |
| | Teeth | 83 | 32.2 |
| | Stomach | 23 | 8.9 |
| | Dentures | 4 | 1.6 |

gested by association of bad breath with other dental problems (bleeding gums and extracted teeth). Most of the patients do not know the reason for malodour, and have not taken any actions. Probably, if a person regularly visits a dentist, then the practitioner would recognise the problem and give advice. Thus, malodour in the community could easily be avoided by regular visits to the dentist (Brunette, 2002). It can be argued that the questionnaire survey was biased, since it did not include a control group, but the results do characterize the patient group with malodour. Several literature sources acknowledge contradictions in patient answers, which testify to the fact that respondents tend to give what are believed by them to be the right answers (Yaegaki, 1999; Murata *et al.*, 2002).

The level of halitosis, estimated by halimeter readings, was clearly associated with bacterial amounts. The main bacteria contributing to malodour was *Porphyromonas gingivalis* which explained 52% of the variability in halimeter readings ($r = 0.72$). A further 20% of the residual variation was explained by *Tannerella forsythiitensis* and *Treponema denticola*. The results show that bacterial testing should focus on *Porphyromonas gingivalis*, but noncovariable variation in malodour was also explained by *Tannerella forsythiitensis* and *Treponema denticola*. That corresponds with experimental study where VSC were produced by oral anaerobic bacteria. Hydrogen sulphide producers were: *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella loescheii*, *Treponema denticola*, *Porphyromonas endodontalis*. Methyl mercaptan producers were: *Treponema denticola*, *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Fusobacterium nucleatum*, *Eubacterium spp.* *Fusobacterium periodonticum* (Donaldson *et al.*, 2005). *Porphyromonas gingivalis* is mostly restricted to the growth conditions in the subgingival region, characterised by a protein-rich environment in the gingival pockets and a low red-ox potential in the subgingival region (Paster *et al.*, 2001; Quirynen *et al.*, 2002). *Porphyromonas gingivalis* is also associated with several virulence factors that are not affected by the human immune system: 1) inhibition of PMN activity, 2) complement resistance to inter-products, 3) production of capsules resistant to phagocytosis, and 4) entry to epithelial and connective epithelial cells. These species are also associated with periodontal diseases (Morita and Wang, 2001; Loesche and Kazor, 2002). However, the bacterial community did not differ much among the patient response groups classified according to answers to the questionnaire. The study showed that bacterial amounts of *Tannerella forsythiitensis* and *Treponema denticola* were lower in diabetic and anaemia patients, as well as in patients who were on a special diet. This might suggest that the medication used and the special diet of diabetic and/or anaemic patients may influence the amount of oral microbiota (Kamaraj *et al.*, 2011). There were few differences between genders with regard to questionnaire answers. Women more frequently seemed to recognise illnesses, but this may be attributed to social differences, women being more concerned about their health. This is also confirmed by the greater proportion of smokers among men, which suggests that halito-

sis also is a more common problem among males, but this cannot be tested by the data obtained in this study.

The main cause of halitosis is oral pathology — increased amounts of oral anaerobic bacteria: *Porphyromonas gingivalis*, *Tannerella forsythiitensis*, *Treponema denticola*, and *Prevotella intermedia*. There were few differences between genders, risk factors, and age with regard to bacterial amounts in periodontal pockets. The concentration of bacteria in the oral cavity is significantly higher in halitosis patients than in the studied control group, who do not complain about halitosis. The PCR examinations correspond with halimetric examinations.

REFERENCES

- Brunette, D. (2002). Introduction to the proceedings of the fifth international conference on breath odour research. *Int. Dent. J.*, **52**, 177–180.
- DeBoever, E., Loesche, W. (1995). Assessing the contribution of anaerobic microflora of the tongue to oral malodor. *J. Amer. Dent. Associat.*, **126**(10), 1384–1393.
- Delanghe, G., Ghyselen, J., Van Steenberghe, D., Feenstra, L. (1997). Multidisciplinary breath-odor clinic. *Lancet*, **350**, 187–188.
- Donaldson, A., McKenzie, D., Riggio, M., Rolph, H., Flanagan, A., Bagg, J. (2005). Microbiological culture analysis of the tongue anaerobic microflora in subjects with and without halitosis. *Oral Diss.*, **11** Suppl. 1, 61–63.
- Furne, J., Majerus, G., Lenton, P., Springfield, J., Levitt, D., Levitt, M. (2002). Comparison of volatile sulphur compound concentrations measured with a sulfide detector vs. Gas chromatography. *J. Dent. Res.*, **81**, 140–143.
- Goldberg, S., Kozlovsky, A., Gordon, D., Gelernter, I., Sintov, A., Rosenberg, M. (1994). Cadaverine as a putative component of oral malodor. *J. Dent. Res.*, **73**(6), 1168–1172.
- Kamaraj, D.R., Bhushan, K.S., Laxman, V.K., Mathew J. (2011). Detection of odoriferous subgingival and tongue microbiota in diabetic and nondiabetic patients with oral malodour using polymerase chain reaction. *Indian J. Dent. Res.*, **22**(2), 260–265.
- Kleinberg, I., Codipilly, M. (1999). Modeling of the oral malodor system and methods of analysis. *Quintessence Int.*, **30**, 357–369.
- Lancero, H., Niu, J., Johnson, P. W. (1996). Thiols modulate metabolism of gingival fibroblasts and periodontal ligament cells (pp. 63–78). In: van Steenberghe, D., Rosenberg, M., *Bad Breath: A Multidisciplinary Approach*. Leuven University Press.
- Lee, C., Kho, H., Chung, S., Lee, S., Kim, Y. (2003). The relationship between volatile sulphur compounds and major halitosis-inducing factors. *J. Periodontol.*, **74**, 32–37.
- Loesche, W., Kazor, C. (2002). Microbiology and treatment of halitosis. *Periodontology 2000*, **28**, 256–279.
- McCune, B., Mefford, M. J. (1999). PC-ORD. Multivariate Analysis of Ecological Data, Version 4.0. MjM Software Design, Gleneden Beach, Oregon. 237 pp.
- McNamara, T., Alexander, J., Lee, M (1972) The role of microorganisms in the production of oral malodor. *Oral Surg. Oral Med. Oral Pathol.*, **34**, 41–48.
- Miyazaki, H., Sakao, S., Katol, Y., Takehara, T. (1995). Correlation between volatile sulphur compounds and certain oral health measurements in the general population *J. Periodontol.*, **66**(8), 679–684.
- Morita, M., Wang, H. (2001). Relationship between sulcular sulphide level and oral malodour in subjects with periodontal disease. *J. Periodontol.*, **72**, 79–84.
- Murata, T., Yamaga, T., Lida, T., Miyazaki, H., Yaegaki, K. (2002). Classification and examination of halitosis. *Int. Dent. J.*, **52**, 181–186.

- Noiri, Y., Ebisu, L. L., Ebisu, S. (2001) The localization of periodontal-disease-associated bacteria in human periodontal pockets. *J. Dent. Res.*, **80**, 1930–1934.
- Nonnenmacher, C., Mutters, R., Flores, L. de Jacoby (2001). Microbiological characteristics of subgingival microbiota in adult periodontitis, localized juvenile periodontitis and rapidly progressive periodontitis subjects. *Clinical Microbiology and Infection*, **7**(4), 213–217.
- Paster, B., Falkler, W., Enwonwu, C. (2001). Predominant bacterial species and novel phylotypes in advanced noma lesions. *J. Clin. Microbiol.*, **40**, 2187–2191.
- Quiryren, M., Zhao, H., Van Steenberghe, D. (2002) Review of the treatment strategies for oral malodour. *Clin. Oral Invest.* **6**, 1–10.
- Ratkay, L. G., Tonzetich, J., Waterfield, J. D. (1996). The effect of methyl mercaptan on the enzymatic and immunological activity leading to periodontal tissue destruction (pp. 35–46). In: van Steenberghe, D., Rosenberg, M. *Bad Breath: A multidisciplinary approach*. Leuven University Press.
- Rosenberg, M. (1996). Clinical assessment of bad breath: Current concepts. *J. Amer. Dent. Assoc.* **127**, 475–485.
- Rupf, S., Merte, K., Brader, I., Vonderlind, D. et al. (2003). Clinical-microbiological trial of four scaling and root planning methods. *J. Clin. Periodontol.*, **30**, 5 (Abstract).
- Seemann, R., Bizhang, M., Höfer, U., Djamchidi, C., Kage, A., Jahn, K. (2004) Ergebnisse der Arbeit einer interdisziplinären Mundgeruchsstunde. *Deutsch. Zahnärztl. Z.* **59**, 514–517.
- Tonzetich, J., Kestenbaum, R. (1969). Odour production by human salivary fractions and plaque. *Arch. Oral Biol.*, **14**, 815–827.
- Tonzetich, J., McBride, B. (1981). Characterization of volatile sulphur production by pathogenic and non-pathogenic strains of oral Bacteroides. *Arch. Oral Biol.*, **26**, 963–969.
- Yaegaki, K. (1999). Clinical application of a questionnaire for diagnosis and treatment of halitosis. *Quintessence International*, **30**(5), 302–306.

Received 21 October 2011

MUTES DOBUMA ANAEROBO BAKTĒRIJU LOMA, KĀ ARĪ SOCIĀLO UN VESELĪBAS FAKTORU IETEKME UZ HALITOZES ETIOLOĢIJU

Pētījuma mērķis bija noteikt mutes baktēriju lomu nepatīkamas elpas izcelsmē, un, izmantojot aptaujas karti, meklēt saistību starp dažādiem diētas, mutes dobuma higiēnas, slimību, smēķēšanas, dzimuma, interleikīnu $1\alpha/1\beta$ polimorfisma faktoriem, kuri ietekmē halitozes esamību. Latvijā bieži sastopamās sūdzības par nepatīkamu elpu rada emocionālu un psiholoģisku diskomfortu. Ir vairāki faktori un dažādas slimības, kuras var veicināt nepatīkamu elpu, kā rezultātā rodas izmaiņas mutes dobuma mikrofloras sastāvā un mikroorganismu skaitliskajā daudzumā. Baktēriju koncentrācija mutes dobumā halitozes slimniekiem bija ievērojami augstāka nekā kontroles grupai. Ar polimerāzes ķēdes reakcijas palīdzību noteiktie baktēriju daudzumi ir tiešā korelācijā ar halimetriskajiem mērījumiem. Visbiežākais nepatīkamas elpas iemesls ir saistīts ar palielinātu anaerobo mutes dobuma baktēriju *Porphyromonas gingivalis*, *Tannerella forsythietensis*, *Treponema denticola*, un *Prevotella intermedia* daudzumu.