doi:10.25143/prom-rsu_2013-21_dts

RĪGAS STRADIŅA UNIVERSITĀTE

Dagnija Rostoka

DIAGNOSIS AND TREATMENT OF ORAL HALITOSIS

Summary of the Doctoral Thesis
Speciality – Oral Pathology

Riga, 2013

RĪGAS STRADIŅA UNIVERSITĀTE

Dagnija Rostoka

Diagnosis and Treatment of Oral Halitosis

Summary of Doctoral Thesis

Speciality – Oral Pathology

Rīga, 2013

Promotion work has been worked out at: Rīga Stradiņš University Department of Biology and Microbiology and private dental practice in Rīga, 45-2A Baznīcas Street

Scientific supervisor:

Dr. med., Professor **Juta Kroiča**, Rīga Stradiņš University

Official reviewers:

Dr. med., Professor **Jānis Vētra**, Rīga Stradiņš University

Dr. med., Professor **Aija Žilēvica**, University of Latvia

Dr. med., Associated Professor **Nomeda Baseviciene**, Lithuanian University of Health Sciences

Defence of the promotion work will take place on 26th of August, 2013 at 15.00 of Rīga Stradiņš University Fundamental Science open meeting of Promotion Council in Riga, 16 Dzirciema Street, Hippocrate auditorium.

Promotion work is available at RSU library and RSU home page: www.rsu.lv

Secretary of Promotion Council:

Dr. habil. med., Professor **Līga Aberberga-Augškalne**

TABLE OF CONTENTS

1. ABBREVIATIONS

Anaerobic bacteria – bacteria, which need an environment with decreased levels of oxygen for normal metabolism processes

DNA – Deoxyribonucleic acid

DNA strip tests – commercial kits for DNA determination

HotStarTaq DNA polymerase – enzyme

MRPP – multiple response permutation procedure

PCR – polymerase chain reaction

PMN – polymorphonuclear leykocytes

ppb – parts per billions

RSU – Rīga Stradiņš University

SD – standard deviation

Spearman correlation (r) – Spearman correlation coefficient

Taq polymerase – enzyme

VSC – volatile sulphur compounds

2. INTRODUCTION

Scientific literature uses a variety of terms to describe bad breath: "Halitosis", "*Foetor ex ore/ Foetor oris*", "Bad breath", "Oral malodour/Oral malodour", "Mouth odour", "Breath odour", "Unpleasant oral odour", "Breath malodour" or "Offensive breath" and "Foul smells" (Geist *et al.*, 1956; DeBoever and Loesche, 1995; Scully, 1994; Furne *et al.*,, 2002). The most popular terms currently used are "*Foetor ex ore*" (lat. *foetor*: odour) and "Halitosis" (lat. *halitus*: breath). Halitosis [hal'i – tō'sis] is bad breath that stems as a result of local changes and metabolism processes (e.g., bad oral hygiene, gum diseases, sinusitis, tonsillitis, bronchopulmonary diseases, acidosis and uraemia) (Mosby's Dental Dictionary, 2004).

Many scientists have acquired extensive interest in this oftentimes concealed subject in the last century. Up until the end of the 20th century, halitosis and bad breath was an uncommon subject not only in Latvia, but also in Europe. Bad breath studies carried out over several centuries indicate that halitosis has oral origins (90%) (McNamara *et al.*, 1972; Nonnenmacher *et al.*, 2001; Lee *et al.*, 2003). There is a lack of extensive epidemiological studies about the incidence of halitosis in various countries. No known bad breath epidemiological studies have been carried out in Latvia. Additionally, studies carried out in various countries show differing results, as they are based on patients' self-evaluation of their breath rather than an objective halitosis evaluation. It is possible that these self-evaluation results, which to a great extent are carried out by anti-halitosis product manufacturers do not correspond with the objective reality of halitosis diagnosis (Miyazaki et al., 1995; Lancero et al., 1996; Kleinberg and Codipilly, 1999). For example, in a telephone study carried out in 1996, 60% of women and 50% or men said they regularly used breath fresheners (Fukushima, 1986). In order to evaluate and determine the

spread of bad breath complaints, which would be based on examinations, an epidemiological study was carried out in Japan. This study showed that 6% to 23% of the population have a bad breath (Miyazaki et al., 1995). Halitosis complaints in Latvia tend to have not only medical, but also social aspects, since there is no systematic medical help for halitosis prevention. Patients as well as many doctors and dentists to this day continue to be poorly informed about the reasons for halitosis and its treatment. (Brinkmane and Selga, 2003). The 58th Scientific conference of RSU medical students investigation aim was to estimate prevalence of bad breath in patients with oral candidosis, to measure strength of breath malodour organoleptically and with usage of halimeter, to compare halimeter data with organoleptical examination (Brinkmane and Čēma, 2009). Scientific studies regarding oral halitosis origins and most common causes have not been carried out in Latvia, thus there is also no scientifically based proof, which hinders the existence of a unified action plan. The only available sources are RSU scientific conference thesis, which show that untreated oral cavity problems, poor oral hygiene, and low quality dentures are the most common causes of bad breath. By using oral cavity hygiene products it is possible to decrease halitosis for a short term (Selga *et al.*, 2004). Diagnosis and treatment results are negatively affected by diagnosis methods, which are steeped in doctor's individual clinical experience and conventional methods, which have no scientific proof. Dentists have an important role in the early diagnosis and treatment of halitosis (Brinkmane and Selga, 2003). Journal *Zobārstniecības raksti*, which is available to the dentists of Latvia mentions that removable partial dentures are halitosis promoting factors (Vidžis and Brinkmane, 2004). The research of this study is important, because it affects a wide array of population and various age groups.

2.1. Aim of study

To study the diagnosis and treatment opportunities of oral halitosis.

2.2. Objectives of study

1. Study halitosis patients, and evaluate risk factors and their importance in halitosis development.
2. Evaluate optimal halitosis diagnosis options in Latvia.
3. Determine qualitative and quantitative amounts of anaerobic proto-lithic bacteria in halitosis patients. Study the amount of anaerobic bacteria in biofilm and its connections with halimetric measurements.
4. Compare anaerobic bacteria quantity on tongue and periodontal pocket biofilms.
5. Update halitosis treatment problems in Latvia.
6. Develop action algorithm for halitosis diagnosis and treatment.

2.3. Novelty of study

1. For the first time in Latvia, as a result of diagnosis and treatment, GSS amount in halitosis patients was determined using haligrams.
   Quantitative amount of anaerobic bacteria was determined in halitosis patients, using PCR.
2. It was determined that halitosis patient identification in Latvia is insufficient for systematic action plan objective diagnosis and treatment of halitosis.
3. A clinical map was developed for the examination of patients with bad breath.
4. An algorithm was developed for halitosis diagnosis and treatment.

3. STRUCTURE AND VOLUME OF STUDY

The promotion thesis is written in the Latvian language. It consists of annotation, introduction, literature survey, materials and methods, work results, discussion, conclusions, supplements, practical recommendations and the literature sources used. The total volume of scientific work is 132 pages, analytically illustrated material depicted in 18 tables, 85 figures. The list of literature contains 103 references.

4. STUDY MATERIAL AND METHODS

Objective bad breath complaint diagnosis or halimetric measurement analysis (VSC determination) was carried out in total on 618 people. All 618 study participants were informed about bad breath diagnosis study. From 1997 until 2008, of all the people who complained about bad breath in the dental clinic, 578 were randomly chosen for the study. A control group was created for the study (n=40), in which patients that complained of other issues (i.e., not bad breath issues) were also included in the study. Patients for the control group were sampled on a random basis, ensuring that people of all age groups and genders are represented. Potential study participants were informed about the process of the study, and they were requested to provide a written consent for their participation in the study "Bad breath diagnosis". For doing the promotion study the confirmation was received from the Committee of Ehtics of the Ministry of Welfare of the Republic of Latvia (No. A-19, 10.08.2000.).

The study consisted of three stages:

A All 578 respondents were initially requested to reply to questionnaire questions, which were asked by the author of the study; their answers were noted in the questionnaire. Results were summarized, published (Rostoka D., 2003; Clinical Microbiology and Infection **9**:1) and stored.

B Microbiological examination of halitosis patients (n=258) and control group (n=40).

C The individual treatment plan of halitosis patients (n=215) included treatment with etheric oils and polyphenol containing rinsing solutions.

4.1. Inclusion criteria of patient group

1. Subjective complaints for bad breath
2. VSC$\geq$ 100 *ppb*

4.2. Exclusion criteria of patient group

1. No subjective complaints for bad breath
2. VSC < 100 *ppb*
3. Antibacterial therapy in the previous month

4.3. 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. Participants of a control group, which consisted of 40 restorative dentistry patients who did not complain about halitosis, were 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 medication, especially those that affect the quality and quantity of saliva. The questionnaire also included questions concerning smoking, the use of alcohol, and diet, presence of systemic diseases (questions are presented in table 2). The questionnaire was based on the authors experience in University of British Columbia, Vancouver, Canada (Yaegaki K., 1999) and then adapted for use in Latvia.

4.4. Halimetric and laboratory analysis

The oral odour or bad breath was confirmed by the 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 the control group were examined. Halitosis patients were divided into three age groups (group 1 – 1 to 40; group 2 – 41 to 60; 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 Y. *et al.*, 2001). Microbiota was analysed by quantitative PCR (micro-IDent®, Hain Lifescience) for amounts of oral *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythietensis, Treponema denticola,* and *Prevotella intermedia*. The PCR protocol was done using Hain Lifescience PCR set-up for the microDent and Taq polymerase from Eppendorf (www.hain-lifescience.de).

4.5. 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.

5. STUDY RESULTS

5.1. Microbiological diagnostics of anaerobic bacteria by PCR in oral halitosis patients

The amounts of all bacteria tested were correlated to halimeter measurements, and showed differing degrees of covariability (Table 5.1).

Table 5.1

Correlation coefficients (r) between bacterial amounts and halimeter measurements (ppb)

	Aggregati-bacter actinomycet emcomitans	*Porphyro-monas gingivalis*	*Tannerella forsythensis*	*Treponema denticola*	*Prevotella inter-media*
Aggregatibacter actinomycetemco mitans	1				
Porphyromonas gingivalis	0.14	1			
Tannerella forsythensis	0.02	0.31	1		
Treponema denticola	-0.13	0.14	0.20	1	
Prevotella intermedia	0.07	0.14	0.12	0.36	1
Halimeter reading	0.15	0.72	0.56	0.38	0.26

The best correlation with halimeter measurements was shown for *Porphyromonas gingivalis*, followed by *Tannerella forsythensis*, *Treponema denticola*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* (all significant, $p<0.05$). The best model explaining halimeter measurements was *Porphyromonas gingivalis* + *Tannerella forsythensis*, + *Treponema denticola*, which explained 71% of the variability. Addition of *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* did not improve the

explanatory power of the model. Mean age of patients was 42.5 years, which did not differ significantly between male and female patients. Significant differences (Table 5.2) between male and female response on the questions occurred only for association with other diseases (χ^2=16.21, p=0.04), and smoking (χ^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. In total 60 (23.3%) halitosis patients were smokers, including 42 (35.9%) males and 18 (12.8%) females. As other questionnaire responses did not differ between sexes, the answers were pooled.

Table 5.2

Halitosis patient clinical answers in the questionnaire for 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

Results show (Table 5.3) that patients with halitosis in most cases indicate that they have noted this problem for a number of years (70.9%), but have taken no actions (72.5%).

Table 5.3

Clinical answers in the questionnaire for diagnosis of halitosis

Breath testing questions	Variations	Total Number	%
First perception of malodour	Many years ago	183	70.9
	Many months ago	10	3.9
	A few weeks ago	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
	Approached a 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
	Once a day	15	5.8
	Twice a day	236	91.5
Use of	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

Continue Table 5.3

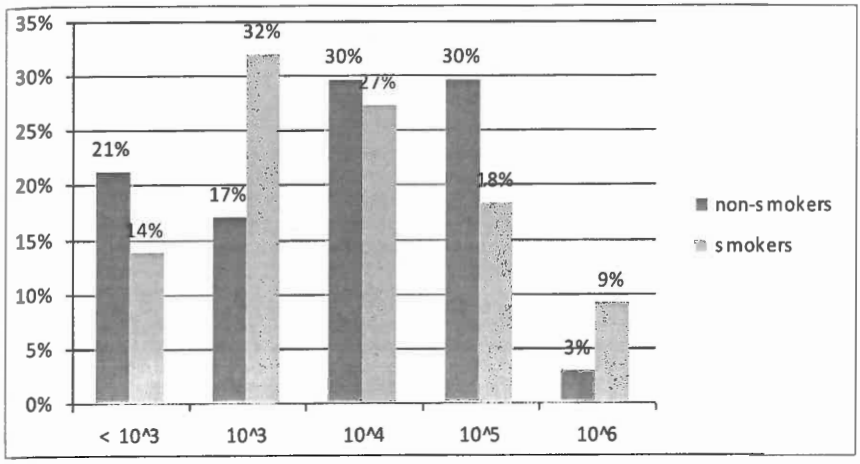
Breath testing questions	Variations	Total Number	%
Stress factors	Work or social problems	117	45.3
	Personal problems	117	45.3
Communication problems	None	62	24
	Unable to talk with others	34	13.2
	Do not like to meet others	27	10.5
	Others avoid contact	9	3.5
	Other problems	41	15.9
Use of medicine	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
	Feel uneasy with others	85	32.9

Respondents indicated that generally they brush their teeth twice a day (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%),

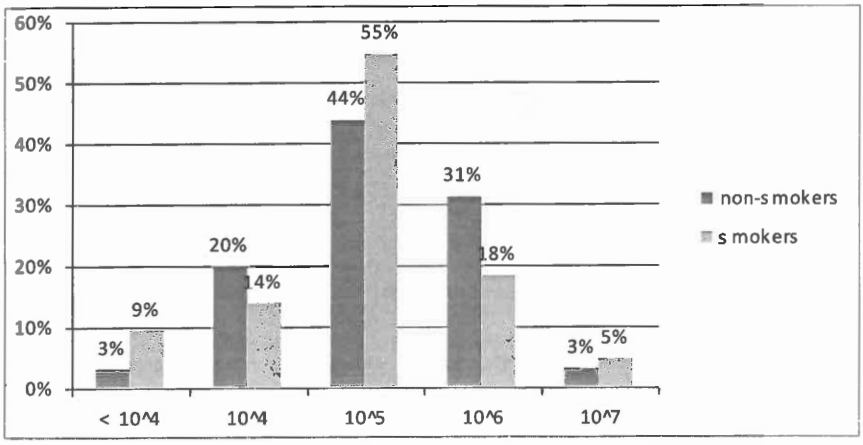
and this generally causes some kind of communication problem (66%). Almost all respondents drink liquids at least 4 times a day. The larger part of the patients does not know the cause of halitosis (57.4%), while the majority of others believe that the problem arises from teeth. For the 30 questions 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=0.03). 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=0.039) 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 forsythensis* (p=0.02) and *Treponema denticola* (slightly non-significant p=0.06) in the illness groups. The bacterial community of patients on a special diet significantly differed (MRPP p=0.36) in having a lower amount of *Prevotella intermedia* (Indicator species analysis: p=0.008) from the no-diet group. Differences in the bacterial concentrations between halitosis patient non-smokers and smokers in young person group are presented in Fig. 5.1.

Fig. 5.1. Distribution of bacterial concentrations in age group 1 of halitosis patients

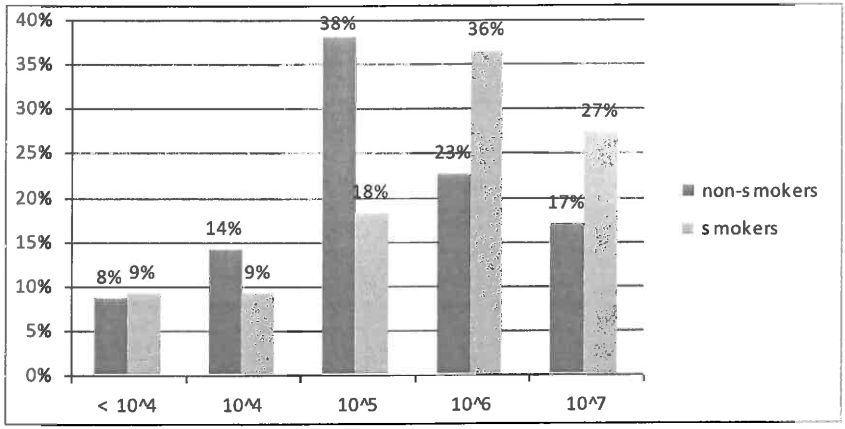
5.1.1. Concentrations (DNA copy number/ml) of *Aggregatibacter actinomycetemcomitans*



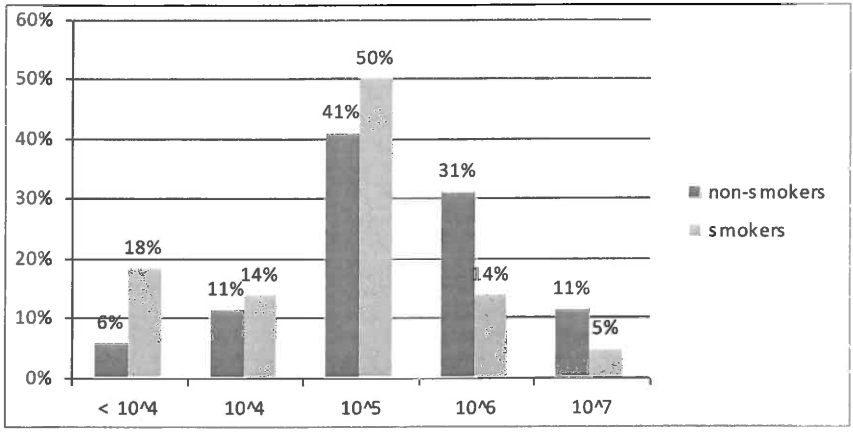
5.1.2. Concentrations (DNA copy number/ml) of *Porphyromonas gingivalis*



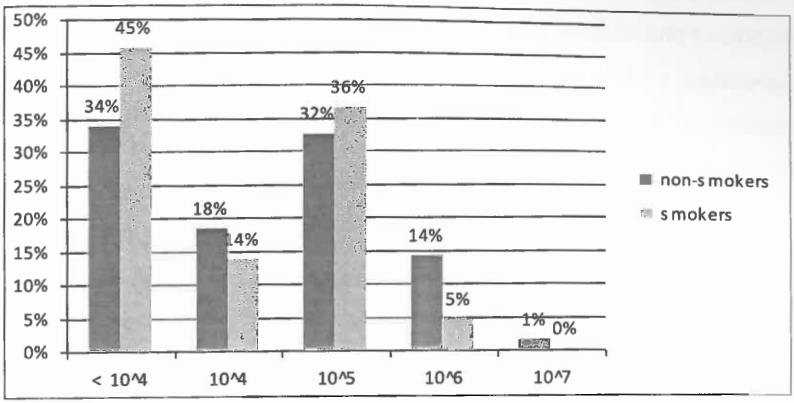
5.1.3. Concentrations (DNA copy number/ml) of *Tanerella forsythensis*



5.1.4. Concentrations (DNA copy number/ml) of *Treponema denticola*



5.1.5. Concentrations (DNA copy number/ml) of *Prevotella intermedia*

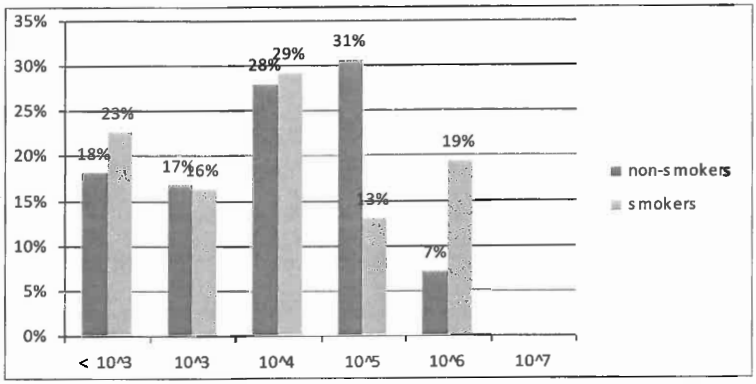


Data showed that both non-smokers and smokers had very high bacterial concentrations, and there were no considerable differences between smokers and non-smokers. Only two concentrations (10^3 and 10^6) of *Aggregatibacter actinomycetemcomitans* were higher in smokers groups. Only 21% of young non-smokers had acceptable *Aggregatibacter actinomycetemcomitans* concentration ($<10^3$) in periodontal pockets. The most frequent *Porphyromonas gingivalis* concentration was 10^5. 55% smokers had high bacterial amount of this in their periodontal pockets. Also 44% of non-smokers had 10^5 *Porphyromonas gingivalis* in periodontal pockets. Concentrations 10^6 and 10^7 of *Tannerella forsythensis* had 36% and 27% persons in smoker groups that were much higher than in non-smoker groups – 10^6 had 23%, and 10^7 had 17%. Only 8% of young non-smokers and 9% young smokers had acceptable *Tannerella forsythensis* 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% of smokers had high bacterial amount of this in their periodontal pockets. Also 41% of non-smokers had 10^5 *Treponema denticola* in periodontal pockets. The amount of *Prevotella*

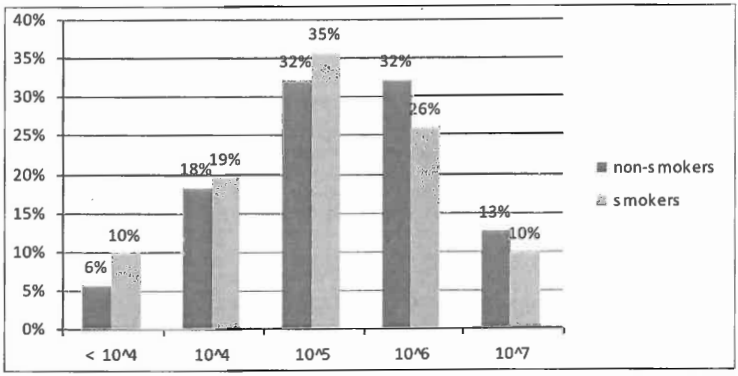
intermedia did no differ between smokers and non-smokers. 34% of young non-smokers and 45% of young smokers had acceptable *Prevotella intermedia* concentration ($<10^4$) in periodontal pockets. Differences in the bacterial concentrations between halitosis patient non-smokers and smokers in the middle-aged group are presented in Fig. 5.2.

Fig. 5.2. Distribution of bacterial concentrations in age group 2 halitosis patients

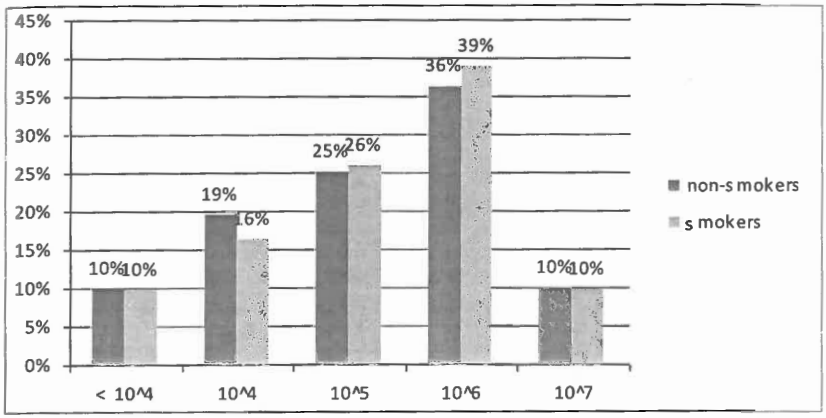
5.2.1. Concentrations (DNA copy number/ml) of *Aggregatibacter actinomycetemcomitans*



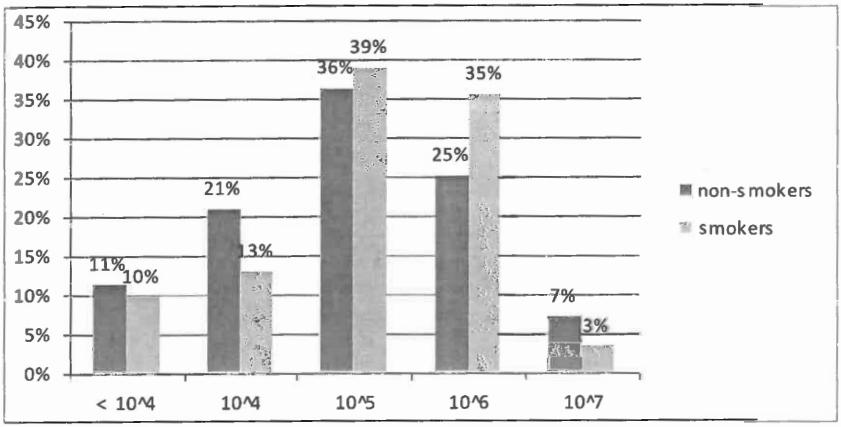
5.2.2. Concentrations (DNA copy number/ml) of *Porphyromonas gingivalis*



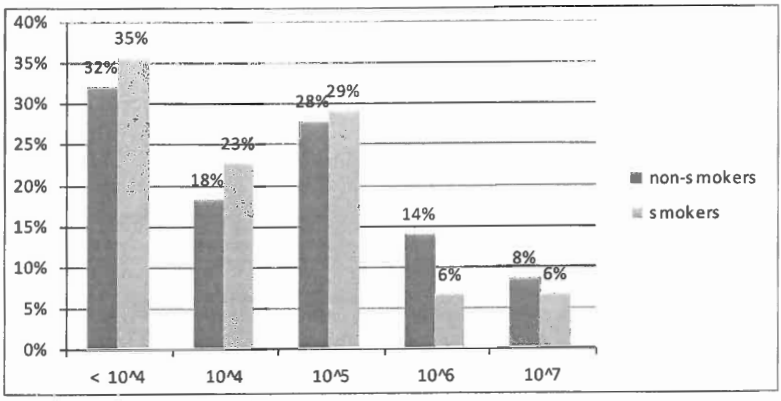
5.2.3. Concentrations (DNA copy number/ml) of *Tanerella forsythensis*



5.2.4. Concentrations (DNA copy number/ml) of *Treponema denticola*



5.2.5. Concentrations (DNA copy number/ml) of *Prevotella intermedia*

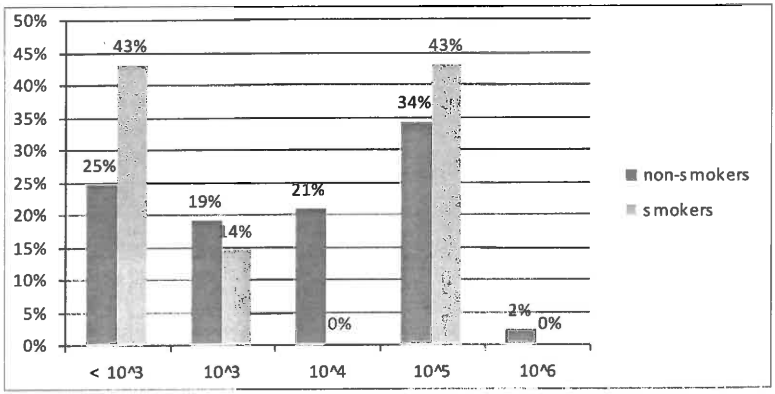


Data showed that both non-smokers and smokers had very high bacterial concentrations, and they did not differ between middle-aged smokers and non-smokers. Only three concentrations (10^3, 10^4 and 10^6) of *Aggregatibacter actinomycetemcomitans* were higher in smoker group. Only 18% of middle-aged non-smokers had acceptable *Aggregatibacter actinomycetemcomitans* concentration ($<10^3$) in periodontal pockets. The most frequent *Porphyromonas gingivalis* concentration was 10^5. 35% of smokers had very high bacterial amount of this 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 forsythensis* had 26% and 39% persons in smoker groups that were higher than in non-smoker group – 10^5 had 25%, and 10^6 had 36%. Only 10% middle-aged non-smokers and smokers had acceptable *Tannerella forsythensis* concentration ($<10^4$) in periodontal pockets. Only 11% 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% smokers had high bacterial amount of this in their periodontal pockets. Also 36% of non-smokers had 10^5 *Treponema denticola* in periodontal pockets. The amount of *Prevotella*

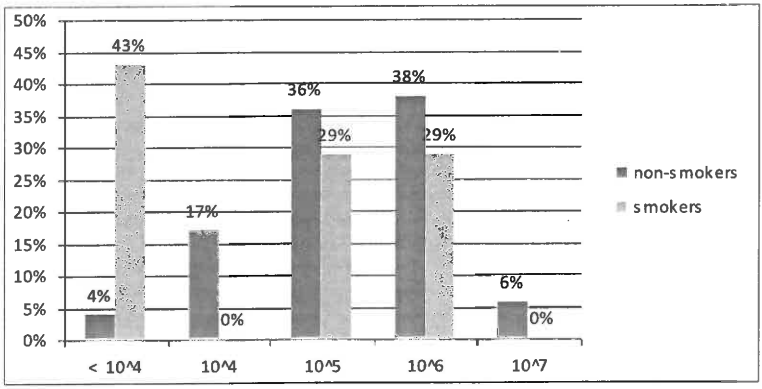
intermedia did not differ between smokers and non-smokers. 32% of middle-aged non-smokers and 35% of middle-aged smokers had acceptable *Prevotella intermedia* concentration ($<10^4$) in periodontal pockets. Differences in the bacterial concentrations between halitosis patient non-smokers and smokers in senior group are presented in Fig. 5.3.

Fig. 5.3. Distribution of bacterial concentrations in age group 3 halitosis patients

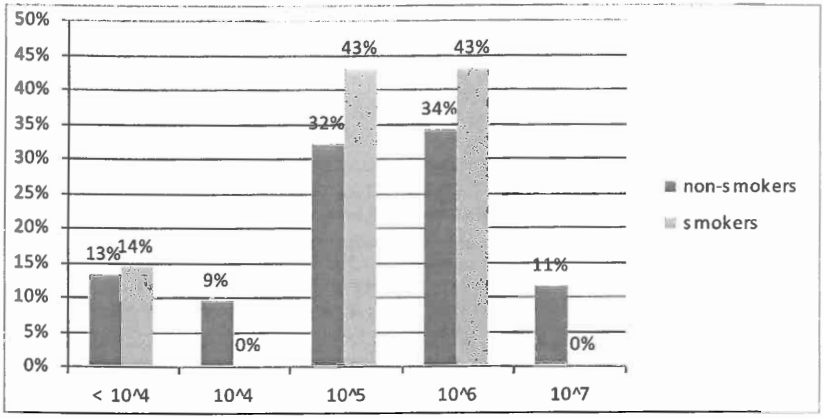
5.3.1. Concentrations (DNA copy number/ml) of *Aggregatibacter actinomycetemcomitans*



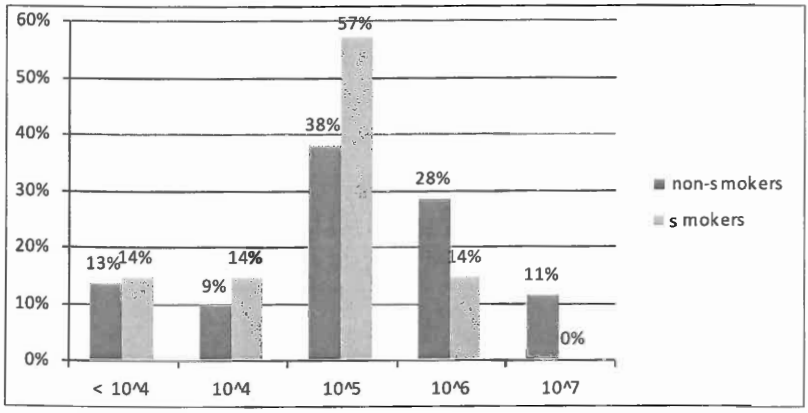
5.3.2. Concentrations (DNA copy number/ml) of *Porphyromonas gingivalis*



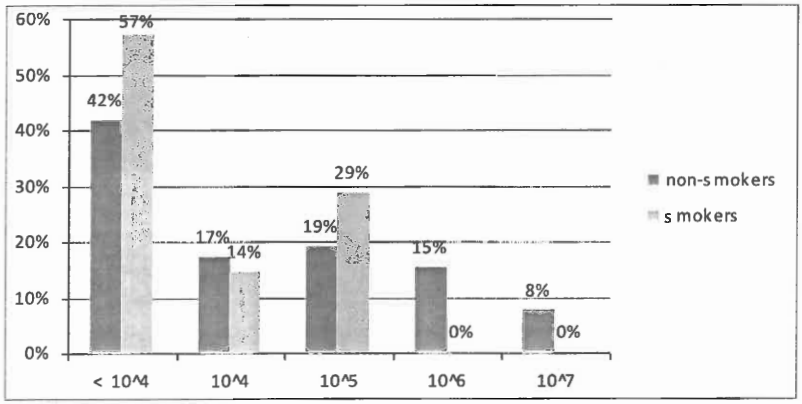
5.3.3. Concentrations (DNA copy number/ml) of *Tanerella forsythensis*



5.3.4. Concentrations (DNA copy number/ml) of *Treponema denticola*



5.3.5. Concentrations (DNA copy number/ml) of *Prevotella intermedia*



Data showed that both non-smokers and smokers had very high bacterial concentrations that did not differ between smokers and non-smokers. Only two concentrations ($<10^3$ and 10^5) of *Aggregatibacter actinomycetemcomitans* were higher in smokers groups. Only 25% of senior non-smokers had acceptable *Aggregatibacter 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 of bacterial amount in their periodontal pockets. Also 36% of non-smokers had 10^5 *Porphyromonas gingivalis* in periodontal pockets. Concentrations 10^5 and 10^6 of *Tannerella forsythensis* had 43% persons in each smoker group that were much higher than in non-smoker groups – 10^5 had 32%, and 10^6 had 34%. Only 13% of seniors non-smokers and 14% of senior smokers had acceptable *Tannerella forsythensis* concentration ($<10^4$) in periodontal pockets. Only 13% of senior non-smokers and 14% senior smokers had acceptable *Treponema denticola* concentration ($<10^4$) in periodontal pockets. The most frequent *Treponema denticola* concentration was 10^5. 57% of smokers had high bacterial amount of this in their periodontal pockets. Also 38% of non-smokers had 10^5 *Treponema denticola* in periodontal

pockets. The amount 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.

5.2. Halitosis patient treatment, including essential oils and polyphenol containing rinsing solutions in the individual treatment plan

Halitosis treatment was carried out on 215 persons. Their average age was 42.78 (SD ±13.09). Initial VSC measurements showed that the average halimetric VSC measurement in examined persons was 317.53 *ppb* (SD ± 126.54). An individual treatment plan was developed for the treatment of halitosis, in which various oral cavity rinsing solutions were included – both oral cavity rinsing solution Listerine, and solution made of home remedies that can be prepared from medical herb infusions. The majority of study participants (in total 190 persons) chose oral cavity rinsing solution Listerine, and only 25 participants chose to prepare self-made medical herb oral cavity rinsing infusions. Medical herb infusions were mostly used by older people. The average age of the numerically largest and youngest group who chose Listerine was 40.3 years. Figure 5.4. shows the graphic of halimetric measurement decrease after oral cavity rinsing with Listerine. The graph shows rather notable dispersion of results, however many results for patients until the age of 50 show notable halimetric measurement decrease after therapy – 200 *ppb*. In patients who used Listerine for oral cavity rinsing results show therapeutically significant decrease in halimetric measurements, especially for initially high halimetric measurement values.

Fig. 5.4. Halimetric measurement changes (ppb) after oral cavity rinsing with Listerine

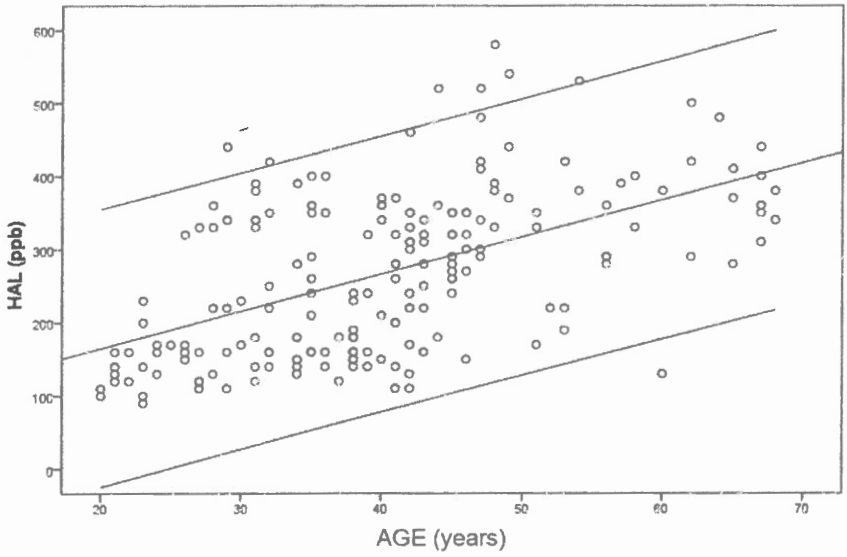
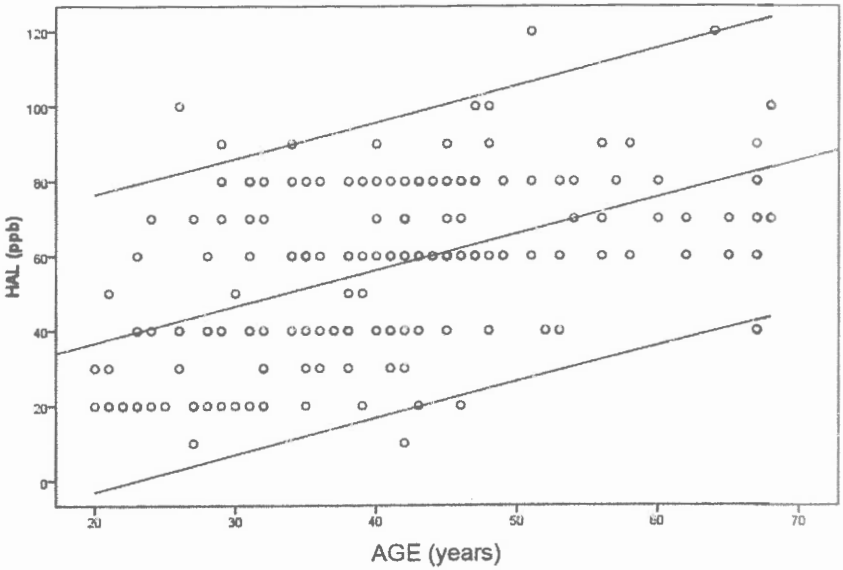


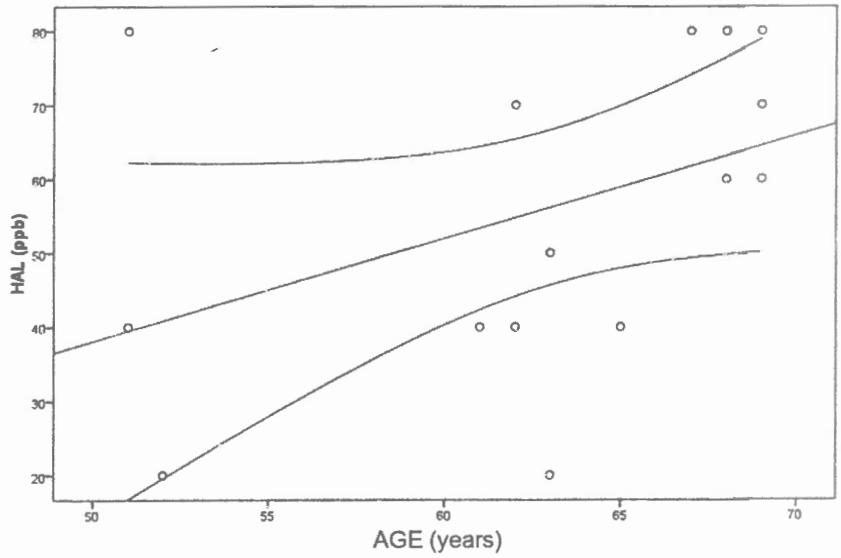
Fig. 5.5. shows the graphic of halimetric measurement decrease of the examined group of people who rinsed with camomile flower infusion. A notable halimetric measurement decrease (– 40 ppb) can also be identified after oral cavity rinsing with camomile infusion. This may be considered a considerable halimetric measurement decrease, because in the specified patient group it shows an improvement by more than half of the initial halimetric measurements as recorded prior to commencement of therapy. This shows that camomile flower infusion is also an effective oral cavity rinsing solution for the reduction of halimetric measurements.

Fig. 5.5. Halimetric measurement changes (ppb) after oral cavity rinsing with camomile flower infusion



A comparatively small number of patients chose a combined infusion of camomile flowers and thyme herbs for the prevention of halitosis. Its graphic results are shown in Fig. 5.6. That is why such a notable dispersion can be observed. The halimetric measurement improvement after therapy is approximately 40 – 60 ppb in this group.

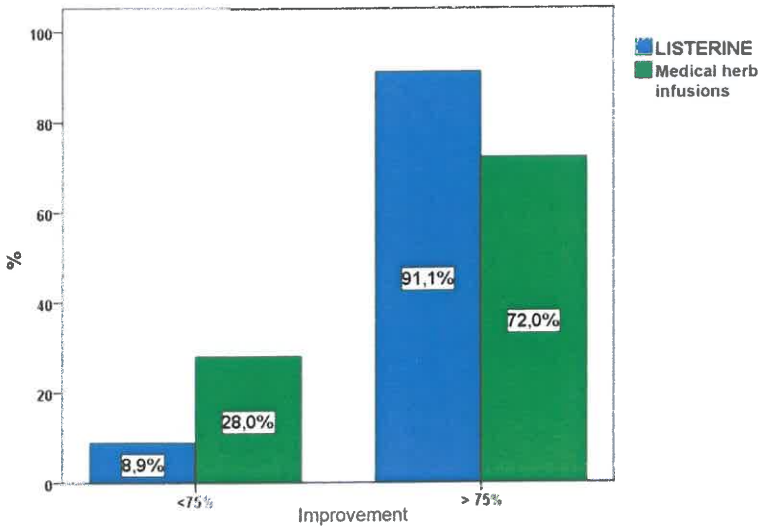
Fig. 5.6. Halimetric measurement changes (ppb) after oral cavity rinsing with camomile flower and thyme herbs infusion



75% halimetric measurement improvement after treatment was chosen to compare the rinsing efficacy of various oral solutions. Percentile halimetric measuring improvement is shown in Fig. 5.7. In order to demonstrate the efficacy of a variety of mouth rinsing solutions, the group that used chamomile flower infusion was added to the groups that used thyme and chamomile flower infusion and medical herb infusion. A comparatively small percentage of patients (8.9%) who were treated with Listerine mouth rinse product after therapy showed less than 75% improvement of treatment halimetric measurements. 28% of the people in the group that used medical herb infusions showed less than 75% improvement of treatment halimetric measurements. Considering that the initial halimetric measurements were smaller in this group, it may be concluded that approximately one quarter or 28% of patients who used medical herb infusion experienced sufficient improvement of as much as 50% reduction of halimetric measurement. Highly significant reduction was

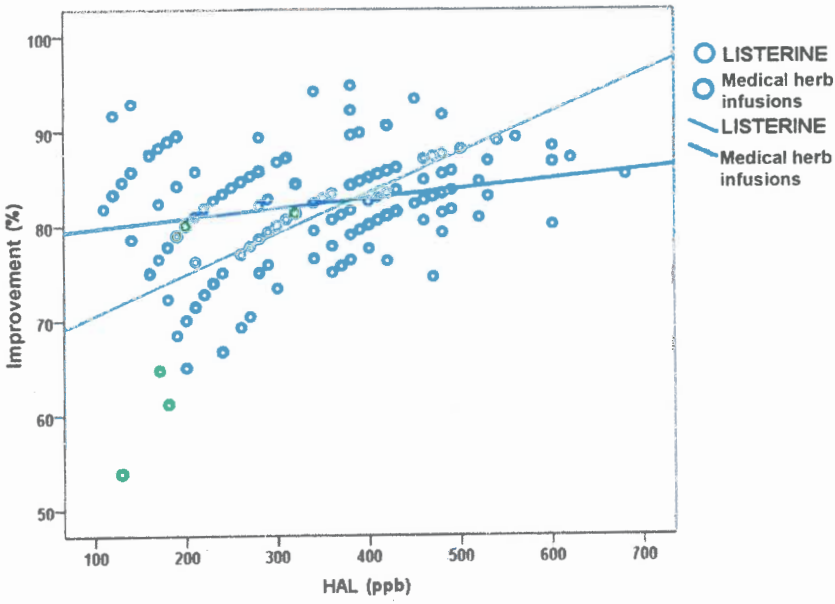
measured in the patient group that used Listerine. For 91.1% of the study participants the improvement of halimetric measurements was ≥ 75% after oral rinsing with Listerine. This shows that Listerine is an effective oral rinsing agent in the treatment of halitosis with initial high halimetric measurement values. 72% of the people in the group that used medical herb infusions showed an improvement of ≥ 75%, which may be deemed a positive therapeutic effect.

Fig. 5.7. Halimetric measurement improvement (≥or < than 75%) after halitosis therapy



In order to demonstrate the percentile improvement of halimetric measurements at different initial values, a full percentage halimetric measurement improvement was created, and it is shown in Fig. 5.8. Listerine features a robust, steady improvement in 80% of the initial halimetric different numerical values of the measurements.

Fig. 5.8. Percentile halimetric measurement improvement after oral cavity rinsing with herb infusions and Listerine



6. DISCUSSION

The responses to the questionnaire given by halitosis patients clearly indicate 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 suggested 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. There is a high probability that if a person regularly visited a dentist, the practitioner would be able to recognise the problem and offer advice. Thus, malodour in the community could easily be avoided by regular visits to the dentist (Brunette D., 2002). It can be argued that the questionnaire survey was biased, since it include a control group, 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). Further 20% of the residual variation was explained by *Tannerella forsythensis* and *Treponema denticola*. The results show that bacterial testing should focus on *Porphyromonas gingivalis*, but noncovariable variation in malodour was also explained by *Tannerella forsythensis* and *Treponema denticola.* That corresponds with experimental studies, 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, characterized 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 shows that bacterial amounts of *Tannerella forsythensis* and *Treponema denticola* were lower in diabetic and anaemia patients, as well as in patients who are 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 halitosis 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 forsythensis, 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.

7. CONCLUSIONS

1. Qualitative and quantitative microbiological diagnostics of anaerobic bacteria concentration in halitosis patients directly correlates with halimetric measurements.
2. Microbiological comparative qualitative and quantitative anaerobic bacteria diagnosis in smoker and non-smoker halitosis patients by age group show insignificant levels in bacterial concentration.
3. Microbiological comparative qualitative and quantitative anaerobic bacteria diagnosis of various biofilm localisations (especially tongue biofilm) in halitosis patients show high levels ($<10^5$, $< 10^6$, $> 10^7$ bacterial DNA copy number/ml) of bacterial concentration.
4. The study proves chronic inflammation regulating mediator Il-1 gene polymorphism ties with halitosis.
5. A distinctive reduction of halitosis measurements was observed in patient group that used Listerine for oral cavity rinsing. 91.1% of the study participants showed ≥75 % improvement of halitosis measurements after rinsing their mouth with Listerine.
6. 72.0% of participants showed a positive therapeutic effect of ≥75 % improvement in halitosis measurements after rinsing oral cavity with medical herb infusion.
7. Use of Listerine shows a robust, steady 80% improvement of various initial halimetric measurement values.

8. LIST OF PUBLICATIONS AND REPORTS ON THE STUDY THEME

1. Rostoka D., Kroiča J., Reinis A., Skadins I. Microbiological *real-time* PCR examination of a clinical material in halitosis patients.// Proceedings of the Latvian Academy of Sciences: Section B (publication in 2013.)
2. Rostoka D., Kroiča J., Reinis A., Kuzņecova V. The role of anaerobic bacteria, social, and health factors in halitosis aethiology.// Proceedings of the Latvian Academy of Sciences: Section B, 2011; 3/4(65):102-109.
3. Д.Ростока, Ю.Кройча, В. Ириете, А.Рейнис, В.Кузнецова, У.Тейбе. лечение галитоза средствами, содержащими жирные масла.// Стоматология, 2012; 3: 27.-34. стр.
4. Rostoka D., Kroiča J., Īriste V., Reinis A., Kuzņecova V., Teibe U. Dažādu ēteriskās eļļas saturošo līdzekļu efektivitāte halitozes ārstēšanā.// RSU Zinātniskie raksti, 2009; 504-517.
5. Д. Ростока, Ю. Кройча, В. Кузнецова, А. Рейнис, Р. Треймане, Уиковская А. Ванка. Адгезия Candida albicans к корригирующим пластмассам, используемым при ортопедическом лечении съемными протезами.// Стоматология, 2004; 5:стр.
6. Kroiča J., Rostoka D., Reinis A., Kuzņecova V., Treimane R., Strautmane N., Žīgurs G. (2004) Candida albicans adherence zobu protezēšanā izmantojamiem materiāliem. // RSU Zinātniskie raksti, 2003; 452-455.
7. D. Rostoka, A. Reinis, J. Kroiča, D. Brāzma, V. Kuzņecova. Halitosis: Diagnosis and Relationship with oral and non-oral diseases.// Proceedings of the Latvian Academy of Sciences: Section B, 2002; 6(56):221-226.
8. D. Rostoka, J. Kroiča, V. Kuzņecova, A. Reinis. Non-Oral and Oral Aspects of Halitosis.// RSU Zinātniskie raksti, 2002; 336-341.

9. Rostoka D., Brāzma D., Kroiča J., Kuzņecova V., Reinis A., Rozenblats A. (2001) Epidemiological study of caries and cariogenic microorganisms in young adult population in Latvia.// LU Zinātniskie raksti, IV Acta Universitatis Latviensis, Medicine, II 643: 153-163.
10. Ростока Д., Кройча Ю., Кузнецова В., Бразма Д., Рейнис А. Слюна и кариес зубов: диагностические тесты в зубоврачебной практике.// Стоматология, 2001; 80(5): 7-10.
11. Rostoka D., Kroiča J., Kuzņecova V., Reinis A. Risk of dental caries: diagnostic tests using saliva.// Proceeding of the Latvian Academy of Sciences: Section B, 2001; 55(1): 33-35.

Theses and poster reports in congresses and conferences

1. Dagnija Rostoka, Juta Kroiča, Aigars Reinis. Microbiological PCR Examination of a Clinical Material in Halitosis Patients. 11th Baltic Congress of Laboratory Medicine, Lithuania, Vilnius, May 10-12, 2012. P.50.
2. Dagnija Rostoka, Juta Kroiča, Aigars Reinis, Ingus Skadiņš, Natālija Bērza. Mikrobioloģiska klīniskā materiāla izmeklēšana ar PĶR halitozes slimniekiem. RSU Zinātniskā konference, 2012.gada 29.-30.marts. P.185.
3. Rostoka D., Kroica J., Reinis A., Kuznecova V. Periodontal pathogenic bacteria diagnostic methods for halitosis patients in Latvia. Clinical Microbiology and Infectious diseases 2009, R2193.
4. Rostoka D., Kroiča J., Kuzņecova V., Treimane R., Reinis A. Etiology and incidence of halitosis in Latvia, Clinical Microbiology and Infection, 2003, Vol. 9, Suppl. 1, p123.
5. Rostoka D., Kroiča J., Īriste V., Reinis A., Kuzņecova V. Dažādu ēteriskās eļļas saturošu līdzekļu efektivitāte halitozes ārstēšanā. RSU 2009.gada Medicīnas nozares zinātniskās konferences tēzes. 72. lpp.

6. Kroiča J., Rostoka D., Reinis A., Kuzņecova V.,Treimane R., Strautmane N., Žīgurs G. Candida albicans adherence zobu protezēšanā izmantojamiem materiāliem. RSU 2003.gada Medicīnas nozares zinātniskās konferences tēzes.
7. Rostoka D., Kroiča J., Reinis A., Kuzņecova V. Zobu kariess un siekalu diagnostiskie testi. RSU 2002.gada Medicīnas nozares zinātniskās konferences tēzes. 143.lpp.
8. Kroiča J., Reinis A., Kuzņecova V., Treimane R., Rostoka D., Strautmane N., Žīgurs G. Experimental study of *Candida albicans* adherence to denture soft lining materials 5th Nordic-Baltic Congress on Infectious Diseases "Towards optimal diagnostics and management". 2002. Abstract 51.pp.
9. Rostoka D., Kroiča J., Kuzņecova V., Reinis A. Halitoze, tās etioloģija un diagnostika. RSU 2002.gada Medicīnas nozares zinātniskās konferences tēzes. 142.lpp.
10. Rostoka D., Rozenblats A., Kroiča J., Kuzņecova V., Reinis A. Zobu kariess izplatība jauniem cilvēkiem Latvijā, kāriess izraisošo mikroorganismu diagnostika siekalās. Pasaules latviešu ārstu 4. kongress. 2001. Tēzes 153.lpp.
11. Rostoka D., Rozenblats A., Kroiča J., Kuzņecova, Reinis A. Halitozes sastopamība Latvijā, tās diagnostika un saistība ar citām organisma un mutes dobuma patoloģijām. Pasaules latviešu ārstu 4. kongress. 2001. Tēzes 153.lpp.
12. Rostoka D., Reinis A., Rozenblats A. Siekalas un zobu kariess, diagnostiskie testi zobārstniecības praksē. Otrais Latvijas Zobārstniecības kongress. 2000. Tēzes 68.lpp.
13. Rostoka D. Halitozes diagnostika un ārstēšana. Pirmais Latvijas Zobārstniecības kongress. 1998. Tēzes

9. REFERENCES

1. Brinkmane, A., Čēma, I. (2009) Halitozes saistība ar sēnītes infekciju mutes dobumā. RSU 58. Medicīnas nozares student zinātniskās konferences tēzes, 32.-33.
2. Brinkmane, A., Selga, G. (2003) Slikta elpa – halitoze. Zobārstniecības raksti, 1:32.-34.
3. Brunette, D. (2002) Introduction to the proceedings of the fifth international conference on breath odour research. Int. Dent. J., 52:177-180.
4. DeBoever, E., Loesche, W. (1995) Assessing the contribution of anaerobic microflora of the tongue to oral malodor. J. Am. Dent. Associat., 126 (10):1384-1393.
5. Delanghe, G., Ghyselen, J., Van Steenberghe, D., Feenstra, L. (1997) Multi-disciplinary breath-odor clinic. Lancet, 350:187-188.
6. 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.
7. 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.
8. 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.
9. Kamaraj, D. R., Bhushan, K. S., Laxman, V. K., Mathew J. (2011) Detection of odoriferous subgingival and tongue microbiata in diabetic and nondiabetic patients with oral malodour using polymerase chain reaction. Indian J. Dent. Res., 22(2): 260-265.
10. Kleinberg, I., Codipilly, M. (1999) Modeling of the oral malodor system and methods of analysis. Quintessence Int., 30:357-369.
11. Lancero, H., Niu, J., Johnson, P. W. (1996) Thiols modulate metabolism of gingival fibroblasts and periodontal ligament cells. In: van Steenberghe, D.,

Rosenberg, M., Bad Breath:a multidisciplinary approach. Leuven University Press, pp. 63-78.

12. 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.
13. Loesche, W., Kazor, C. (2002) Microbiology and treatment of halitosis. Periodontology 2000., 28:256-279.
14. McCune, B., Mefford, M. J. (1999) PC-ORD. Multivariate Analysis of Ecological Data, Version 4.0. MjM Software Design, Gleneden Beach, Oregon. 237 p.p.
15. 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.
16. Miyazaki, H., Sakao, S., Katol, Y., Takehara, T. (1995) Correlation between volatile sulphur compounds and certain oral health measurements in the general population J.Periodont., 66(8):679-684.
17. Morita, M., Wang, H. (2001) Relationship between sulcular sulphide level and oral malodour in subjects with periodontal disease. J. Periodontol., 72:79-84.
18. Murata, T., Yamaga, T., Lida, T., Miyazaki, H., Yaegaki, K. (2002) Classisication and examination of halitosis. Int. Dent. J., 52:181-186.
19. 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.
20. 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.
21. Paster, B., Falkler, W., Enwonwu, C. (2001) Predominant bacterial species and novel phylotypes in advanced noma lesions. J. Clin. Microbiol. 40:2187-2191.
22. Quirynen, M., Zhao, H., Van Steenberghte, D. (2002) Review of the treatment strategies for oral malodour. Clin. Oral Invest. 6:1-10.
23. Yaegaki, K. (1999) Clinical application of a questionnaire for diagnosis and treatment of halitosis. Quintessence International, 30(5):302-306.

24. Fukushima, K. (1986) A fundamental and clinical study on the ingredients of bad breath in various oral conditions. Jpn. J. Oral Maxillofac. Surg., 32: 84-104.
25. Geist, H. (1956) Vom Foeter ex ore in der Antike. Zahnärztliche Praxis, 7: 12-13.
26. Miyazaki, H., Hanada, N., Andoh, M. (1989) Periodontal disease prevalence in different age groups in Japan as assessed according to the CPITN. Community Dent. Oral Epidemiol., 17: 71-74.
27. Scully, C., Porter, S., Greenman, J. (1994) What to do about halitosis. Br. Med. J., 308: 217-218.
28. Selga, G., Brinkmane, A., Vidžis, A. (2004) Halitozes izpausmes stomatoloģiskajiem pacientiem, tās novēršanas iespējas. RSU Medicīnas nozares zinātniskā konference, tēzes, 134.
29. Vidžis, A., Brinkmane, A. (2004) Izņemamās parciālās zobu protēzes – halitozi veicinošs factors. Zobārstniecības raksti 2:18.-21.