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Tobacco Smoking and Periodontal Health in a Saudi Arabian Population

By
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ABSTRACT

Background & Aim: Tobacco smoking exerts a harmful effect on the periodontal tissues manifested by periodontal pockets, attachment loss and periodontal bone loss. Current evidences on the effects of tobacco on periodontal health mainly concern cigarette smoking. In view of the increasing popularity of water pipe smoking in Arabian countries and reports confirming that water pipe smoking has health effects similar to those of cigarette smoking, there is a need for a better understanding of the potential harm of this smoking habit. The present thesis was carried out in order to explore whether water pipe smoking is associated with periodontal health in a manner similar to cigarette smoking.

Material & Methods: Residents in Jeddah City, Saudi Arabia, were invited to participate in the study by means of announcements in two daily newspapers. 355 individuals, 100 women and 255 men (17-60 years) responded to a standardized questionnaire and digital panoramic dental radiographs were taken. The questionnaire included information about oral hygiene practices, dental care and smoking habits. Of these subjects, 262 (73%) also volunteered for clinical examination, including assessments of oral hygiene, gingival inflammation and probing depth. Subgingival microbial test was carried out in 198 individuals for the detection of 12 different bacterial species most commonly associated with periodontal disease using the checkerboard DNA-DNA hybridization technique. Participants were stratified into water pipe smokers 33%, cigarette smokers 20%, smokers of both water pipe and cigarettes (mixed smokers 19%) and non-smokers 28%.

Results: Tobacco smoking is associated with a suppression of the gingival bleeding response to plaque accumulation. A suppressive effect was observed in both cigarette and water pipe smokers compared to non-smokers (Study I). Both cigarette and water pipe smoking were associated with the presence of more than 10 pockets of ≥5 mm probing depth. The relative risk for periodontal disease was 5.1-fold and 3.8-fold increased in water pipe and cigarette smokers, respectively, compared to non-smokers ($p < 0.01$). The relative risk associated with heavy smoking was about 8-fold elevated in water pipe smokers and 5-fold elevated in cigarette smokers, suggesting an exposure-response effect (Study II). Tobacco smoking was associated with a reduction of the periodontal bone height. The reduction was of similar magnitude in water pipe smokers and cigarette smokers. The relative risk of periodontal bone loss of more than 30% of the root length was 3.5-fold and 4.3-fold elevated in water pipe and cigarette smokers, respectively, compared to non-smokers ($p < 0.01$). The relative risk associated with heavy smoking was 7.5-fold elevated in water pipe smokers and 6.3-fold elevated cigarette smokers (Study III). Further more, cigarette smokers, water pipe smokers and non-smokers exhibited similar periodontal microflora (Study IV).

Conclusion: Tobacco smoking is associated with inferior periodontal health. The impact of water pipe smoking is of largely the same magnitude as that of cigarette smoking. The association between tobacco smoking and an inferior periodontal health seems to be independent of the subgingival microflora. Water pipe smoking habit should be considered in periodontal health.

Key words: Bone height, bone loss, cigarette smoking, gingival bleeding, periodontal disease, periodontal microflora, plaque index, probing depth, Saudi Arabia, smoking, tobacco, water pipe.

TABLE OF CONTENTS

PREFACE

The thesis consists of an introductory text with original research articles appended. These are listed below and will be referred to in the text by their Roman numerals I-IV:

I. **Natto S**, Baljoon M, Abanmy A, Bergström J. Tobacco smoking and gingival health in a Saudi Arabian population. *Oral Health Prev Dent* 2004; 2: 351-357.

II. **Natto S**, Baljoon M, Bergström J. Tobacco smoking and periodontal health in a Saudi Arabian population. *J Periodontol* 2005; Accepted.

III. **Natto S**, Baljoon M, Bergström J. Tobacco smoking and periodontal bone height in a Saudi Arabian population. *J Clin Periodontol* 2005; 32: 1000-1006.

IV. **Natto S**, Baljoon M, Dahlén G, Bergström J. Tobacco smoking and periodontal microflora in a Saudi Arabian population. *J Clin Periodontol* 2005; 32: 549-555.

GLOSSARY

ANOVA	A statistical test for heterogeneity of means by analysis of group variances.
Confidence intervals	The range of values that provides information about the precision of the investigation.
Correlation coefficient	A quantity that gives the quality of a least squares fitting to the original data.
Multiple regression	A regression giving conditional expectation values of a given variable in terms of two or more other variable.
Multivariate analysis	The simultaneous statistical consideration of relationships among many measured properties of a given system.
Odds ratio	A method used to assess the risk of a particular outcome (or disease) if a certain factor (or exposure) is present.
Precision	Precision in measurement and estimation corresponds to the reduction of random error.
Prevalence	The proportion of a population that has disease at a specific point in time.
R^2	Test of regression is a test of the hypothesis that all regression coefficients are zero.
Regression	A method for fitting a curve through a set of points using some goodness-of-fit criterion.
Relative risk	A measure of how much a particular risk factor influences the risk of a specified outcome.
Replicate	One out of a set of identical observations in a given experiment under identical conditions.
Scheffé Test	A comparison test used to evaluate a subset of the possible contrasts with unequal sample sizes.
Standard deviation	A measure of the spread of a set of values from the mean value.

INTRODUCTION

Tobacco smoking

Mankind has probably been using tobacco since the dawn of history. *Nicotiana tabacum* and *Nicotiana rustica* are native to South America, where they were sniffed, chewed, eaten and drunk. The most popular method in ancient times to smoke tobacco was in a pipe. Ready-made cigarettes were first marketed during the First World War in Europe and since then cigarette smoking has been taken up world-wide. Smoking has been described as 'a tragic accident of history' (Musk & Klerk 2003).

The prevalence of cigarette smoking according to the WHO report is estimated to be 53% in China, 47% in Japan, 35% in Germany, 34% in Kuwait, 30% in Bahrain, 28% in Denmark, 24% in Italy, and 19% in Sweden (WHO 2005).

Other cultures have other tobacco smoking habits such as cigar, pipe and water pipe smoking. Even though cigar and pipe smoking has declined since 1965, they are still observed, with a prevalence of 5% and 3% respectively (Gilpin & Pierce 2001, Henley et al. 2004).

Counter to this general trend, water pipe smoking is increasing and becoming fashionable. This centuries-old tobacco habit is known under different names (e.g., sheesha, hookah, arghile), with the term water pipe implying a unifying feature of all these forms: *the passage of smoke through water before inhalation by the smoker* (Rastam et al. 2004).

The prevalence of water pipe smoking is estimated to be 43% in Egypt, 25% in Syria, 28% in Lebanon, and 23% in Kuwait (Memon et al. 2000, Israel et al. 2003, Maziak et al. 2004, Chaaya et al. 2004). There are no epidemiological studies regarding the prevalence of water pipe smoking in Saudi Arabia, but it appears to be overtaking cigarette smoking in popularity, and public tolerance of this habit is becoming greater.

A special device is needed for water pipe smoking, where the tobacco is burnt using a piece of charcoal. The smoke produced passes through a long tube and a water trap at the base of the device that is meant to act as a filter and reduce nicotine inhalation. However, water pipe smoke includes the same harmful compounds as cigarette smoke, such as carbon monoxide, tar, and nicotine (Shihadeh & Saleh 2005). A cigarette can be smoked in about 5 minutes whereas a single water pipe run takes 20-30 minutes. Thus, it is estimated that a single water pipe run corresponds to 4-6 cigarettes.

Until the 1980s, most water pipe smokers were old men who quit smoking cigarettes and shifted to smoking unflavored tobacco in café houses. The main rise in water pipe popularity during the 1990s was probably due to the introduction of a specially prepared tobacco with sweetened fruit flavors and mild aromatic smoke called *Moassel* (Maziak et al. 2005). The sweetened taste and fruity smell of the burning tobacco attract young people to start water pipe smoking. The general belief is that because the smoke passes through water before it is inhaled, it is cleaner and thus less harmful than the cigarette smoke. Counter to popular belief, untoward health effects of water pipe smoking have been reported, such as increased blood carboxyhemoglobin levels, impaired pulmonary function and increased heart rate and blood pressure (Zahran et al. 1985, Kiter et al. 2000, Shafagoj & Mohammed 2002).

Periodontal disease

Periodontal disease includes all disorders of the supporting structures of teeth, the gingival, periodontal ligament, and periodontal bone. This may vary from inflammation of the gingival, termed gingivitis, to the destruction of periodontal bone and eventual tooth loss, termed periodontitis. It is characterized clinically by gingival bleeding on probing, loss of attachment, pocket formation and periodontal bone loss. Periodontal pocket formation is usually a sequel of the disease process unless gingival recession accompanies attachment loss, in which case the pocket depth may remain shallow even in the presence of ongoing loss of attachment and bone loss. The pattern of periodontal bone loss observed in periodontal disease may be vertical, when the loss of attachment and bone loss on one tooth surface is greater than that occurring on an adjacent surface, or horizontal, when attachment and bone loss proceed at a uniform rate on the majority of tooth surfaces.

Epidemiological studies have assessed periodontal disease by means of clinical examination of periodontal tissues, radiographic assessment of periodontal bone, or a combination of clinical and radiographic means (Papapanou et al. 1988, Hougoson et al. 1998, Albandar et al. 1999). Even though the prevalence of the disease varies among different populations, and even though oral hygiene conditions differed considerably, only a small proportion of individuals had severe periodontal destruction (Baelum et al. 1996, Ronderos et al. 2001, Papapanou et al. 2002).

The initiation and progression of periodontal disease are modified by local and systemic factors. *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, Treponema denticola,* and *Actinobacillus actinomycetemcomitans* are necessary pathogens

but not sufficient for disease activity to occur. The environment of the periodontal pocket is an important local factor in the regulation of virulence factors produced by the pathogenic species (Socransky & Haffajee 1992).

Systemic factors that have been linked to disease activity include susceptibility of the individual host that facilitate periodontal disease progression (Genco 1992). Diabetes mellitus – especially in individuals in whom metabolic control is poor – and tobacco smoking are the major systemic risk factors for periodontal disease (Taylor et al. 1996, Bergström 2004). Furthermore, ageing is commonly associated with periodontal disease, although this relationship is thought to be due to cumulative periodontal breakdown over time rather than physiological ageing (Papapanou et al. 1991).

Tobacco smoking and periodontal health

A substantial body of evidence in various populations has demonstrated that tobacco smokers have increased prevalence of periodontal disease, and that the disease is more severe than in non-smokers. A positive association has been observed between smoking and measures of periodontal disease. Greater probing depth, clinical attachment loss and periodontal bone loss have been shown to be both more prevalent and more severe among smokers as compared to non-smokers. The observed effects have been confirmed in different studies after correction for a variety of potential cofounders such as socio-economic status, education, and oral hygiene. Despite the existence of these potential confounding factors, the impact of smoking is not obscured when all factors accounted for (Feldman et al. 1983, Bergström & Eliasson 1987a,b, Feldman et al. 1987, Haber & Kent 1992, Horning et al. 1992, Bolin et al. 1993, Martinez-Canut et al. 1995, Bergström et al. 2000a,b, Calsina et al. 2002, Khader et al. 2003, Teng et al. 2003, Razali et al. 2005, Apatzidou et al. 2005).

Tobacco smoking and gingival inflammation

Gingival bleeding after probing is widely used as a clinical marker of gingival inflammation. Observational and experimental studies have shown that cigarette smoking suppresses the gingival inflammatory response to a given amount of plaque as measured by bleeding on probing (Bergström & Flodérus-Myrhed 1983, Preber & Bergström 1985, Bergström & Preber 1986, Bergström 1990, Danielsen et al. 1990, Lie et al. 1998, Bergström & Boström 2001, Giannopoulou et al. 2003a, Dietrich et al. 2004). However, once cigarette smokers quit smoking, bleeding on probing that was not previously

apparent can be found within weeks, even if plaque control has improved, suggesting a causal relationship (Nair et al. 2003, Morozumi et al. 2004).

Tobacco smoking and periodontal destruction

Tobacco smoking is associated with periodontal morbidity as diagnosed by an increase in periodontal probing depth, an elevated reduction rate of periodontal bone height and a higher rate of tooth loss (Preber & Bergström 1986, Bergström 1989, MacFarlane et al. 1992, Holm 1994). The noxious effect of cigarette smoking on periodontal health has been shown to be positively related to the amount of tobacco smoked (Haber et al. 1993, Baljoon et al. 2004, Susin et al. 2004, 2005, Razali et al. 2005). This dose-response relationship is confirmed in longitudinal studies comparing smokers and non-smokers, where long-term tobacco exposure was significally associated with disease severity (Norderyd et al. 1999, Bergström et al. 2000a, Baljoon et al. 2005a).

Tobacco smoking and periodontal microflora

The inferior periodontal health in smokers has been correlated to the presence of greater numbers of specific periodontal microorganisms in smokers than in non-smokers (Zambon et al. 1996, Umeda et al. 1998, Kamma et al. 1999, Haffajee & Socransky 2001). On the other hand, other studies have not shown a difference between smokers and non-smokers with established periodontal disease (Preber et al. 1992, Boström et al. 2001, Eggert et al. 2001, Salvi et al. 2005, Buduneli et al. 2005).

Most studies that have investigated the relation between smoking and periodontal health have dealt with cigarette smoking. Although cigarette smoking is the most common tobacco habit, and therefore most widely studied, other tobacco smoking habits such as cigar and pipe smoking have a negative impact on the periodontal health similar to that of cigarette smoking (Krall et al. 1997, 1999, Albandar et al. 2000).

A review of the literature shows that the relationship between water pipe smoking and periodontal health is little investigated (Ashri & Al-Sulamani 2003, Baljoon et al. 2005b). In view of the increasing popularity of water pipe smoking in Arabian countries and reports that have confirmed health effects similar to those of cigarette smoking, there is a need for a better understanding of the potential harm of this smoking habit to the periodontal tissues. The present thesis was carried out in order to explore whether or not water pipe smoking affects the periodontal health in a manner similar to cigarette smoking.

AIMS

General aim

The general aim of this thesis was to investigate the association between tobacco smoking and periodontal health with a special focus on water pipe smoking in a Saudi Arabian population.

Specific aims

- To study the association between tobacco smoking and gingival health in terms of gingival index and gingival bleeding (Study I).
- To study the association between tobacco smoking and periodontal health in terms of pocket probing depth (Study II).
- To study the association between tobacco smoking and periodontal health in terms of radiographic bone height (Study III).
- To study the association between tobacco smoking and subgingival periodontal microflora (Study IV).

MATERIAL AND METHODS

Study population

Residents in Jeddah City, Saudi Arabia, were invited to participate in the study by means of announcements in two daily newspapers. Out of 375 individuals who appeared for screening, 355, 100 women and 255 men, in the age range 17 to 60 years responded to a standardized questionnaire and digital panoramic dental radiographs were taken. Out of these subjects, 262 (73%) also volunteered for a clinical examination, which included a subgingival microbial test in 198 individuals. The characteristics of the sub-samples of the study population are presented in **Table 1**. Slight differences were observed between the overall study population and the sub-samples of individuals participating in the different studies. Twenty individuals were excluded: former smokers who had given up smoking more than 1 year ago (n = 4), pregnant women (n = 3), edentulous individuals (n = 2), and individuals below 17 years of age (n = 11).

The radiographic and clinical examinations were carried out at King Faisal Specialty Hospital and Research Center, Jeddah, Saudi Arabia. Participants were informed individually, verbally and in writing, about the purpose of the research and signed an informed consent form.

The Institutional Review Board at King Faisal Specialist Hospital and Research Centre, Jeddah, Saudi Arabia has approved all studies (I-IV).

Interview questionnaire (Study I)

Each participant was interviewed prior to the clinical examination in accordance with a predetermined questionnaire with fixed response alternatives. Information about oral hygiene and dental care habits was obtained from a questionnaire that included questions about the frequency of tooth brushing and the regularity of visits to dental clinics **(Appendix)**.

Table 1. Characteristics of the sub-samples of the study population

	Study I	Study II	Study III	Study IV
Individuals (*n*)	244	262	355	198
Age (95% CI)	37.4 (36.2; 38.6)	36.5 (35.3; 37.7)	36.9 (35.8; 37.9)	37.4 (35.9; 38.9)
Male (%)	70	65	72	63
Female (%)	30	35	28	37
Retained teeth (95% CI)	26.4 (26.0; 26.8)	26.5 (26.2; 26.9)	25.9 (25.6; 26.3)	26.6 (26.0; 26.9)
Plaque index (95% CI)	1.2 (1.1; 1.3)	1.2 (1.1; 1.3)	1.2 (1.1; 1.3)	1.2 (1.1; 1.3)
Gingival index (95% CI)	0.9 (0.8; 0.9)	0.9 (0.8; 1.0)	0.9 (0.8; 0.9)	0.8 (0.7; 0.8)
Smokers (%)	71	70	72	60
Water pipe smokers (%)	31	31	33	29
Cigarette smokers (%)	20	19	20	18
Mixed smokers (%)	20	20	19	13
Non-smokers (%)	29	30	28	40

Based on the school system in Saudi Arabia, the education status was classified on a five-point scale according to maximum length of formal education: no education, primary education (6 years), intermediate school (9 years), secondary school (12 years), and university (more than 12 years).

The history of tobacco smoking was obtained by asking the subject to state type of smoking habit, consumption and duration. The life-time smoking exposure as found by the product of daily consumption (cigarettes per day or water pipe runs per day) and duration (years of smoking) was expressed in terms of cigarette-years or run-years, respectively. The mean (95% CI) life-time exposure for cigarette smokers and water pipe smokers was 230.4 (193.4; 267.5) cigarette-years and 56.8 (48.0; 65.6) run-years, respectively. The mean (95% CI) life-time exposure for mixed smokers was 174.0 (141.0; 206.9) cigarette-years and 23.8 (17.9; 29.5) run-years.

Radiographic examinations (Study III)

All 355 individuals had extraoral digital panoramic radiographs taken **(Fig. 1)**. The periodontal bone height was measured from the radiographs using the Image Tool 3.0

program for digital radiographic measurements in pixels (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, USA).

The periodontal bone height was measured mesially and distally to each tooth and expressed as a percentage of the root length. For single rooted teeth, the length of the root was defined as the mean of the mesial and distal distances from the cemento-enamel junction (CEJ) to the root apex. In molars, the root length was defined as the distance from the CEJ to the root apex and was determined on the mesial aspect of the mesial root and the distal aspect of the distal root. The height of the periodontal bone was determined as the distance from the root apex to a point where the lamina dura became continuous with the marginal bone of the interdental septum. If a vertical bone defect was evident, the bone level was defined as the most apical point of the defect. A tooth was judged non-measurable, if the CEJ or the bone crest could not be properly identified due to overlapping, caries or restorations. In cases where any one of the dental or bony landmarks could not be identified on one aspect (mesial or distal), the tooth was excluded. A total of 205 teeth (2%), most often maxillary premolars, were excluded. The bone height of the individual was given by the mean across all measured interdental bone height values. All teeth except third molars were assessed. However, if a first or second molar was missing, the third molar of the same quadrant if normally erupted was included. Thus, a maximum of 28 teeth of the individual was considered for radiographic assessment.

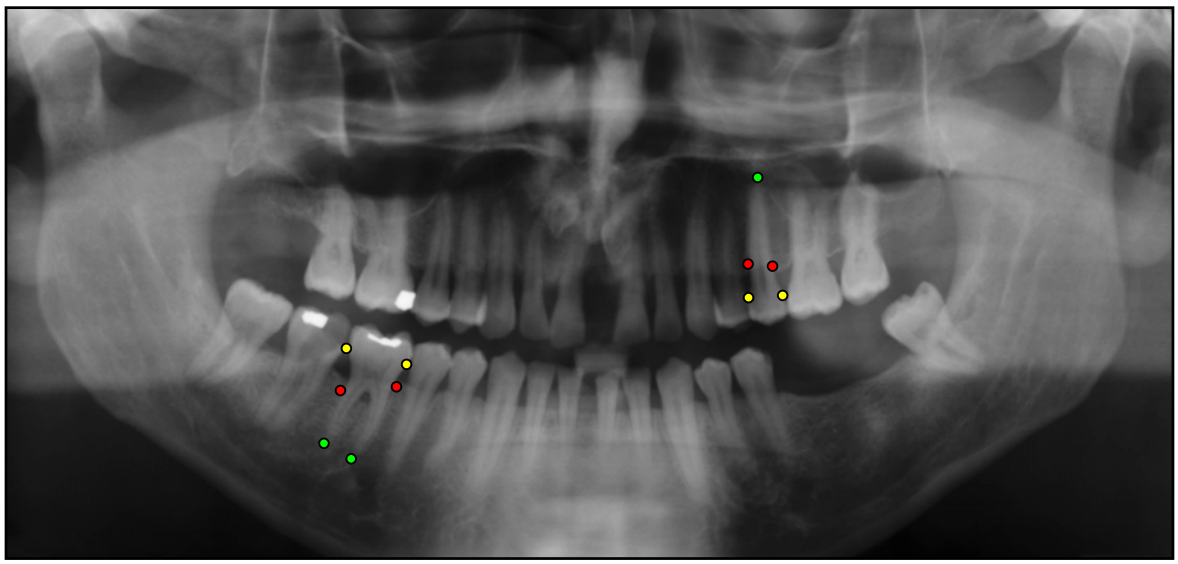

Fig. 1. Example of the digital panoramic radiograph with the land marks.
Cemento-enamel junction Marginal bone Root apex

Clinical examinations (Studies I-IV)

Clinical data were available for 262 individuals and included recording of supragingival plaque (Silness & Löe 1964), inflammatory condition of the gingiva (Löe & Silness 1963) and probing depth. The relative frequency of surfaces with a plaque score of 1 or more was given as a percentage for each individual (plaque %) and the relative frequency of gingival sites with score 2 or 3, denoting gingival bleeding on probing, was calculated for each individual and given as a percentage (gingival bleeding %).

The periodontal probing depth was recorded at 4 sites (mesial, buccal, distal, lingual) around each tooth using a Hillming probe. Probing depths of 4 mm or more were recorded in mm whereas sites with a probing depth below 4 mm were set to 2 mm. For each individual a mean probing depth value was computed based on all available sites.

Microbiological sampling and analysis (Study IV)

After the probing depth of all available teeth was recorded, the site with the greatest probing depth in each quadrant was selected for subgingival plaque sampling. A paper point (ISO 50) was inserted into the gingival sulcus or the periodontal pocket and left in place for 30 sec. After removal the 4 paper points from each individual were placed in an Eppendorf tube and the samples were transported to the laboratory of Oral Microbiology, Göteborg University, Göteborg, Sweden for the evaluation of 12 different bacterial species most commonly associated with periodontal disease: *P. gingivalis, P. intermedia, Prevotella nigrescens, T. forsythensis* (*Bacteroides forsythus*), *A. actinomycetemcomitans, Fusobacterium nucleatum, T. denticola, Peptostreptococcus micros, Campylobacter rectus, Eikenella corrodens, Selenomonas noxia,* and *Streptococcus intermedius.* The checkerboard DNA-DNA hybridization technique was used (Socransky et al. 1994, Papapanou et al. 1997).

The number of bacteria in the samples was evaluated comparing the chemiluminescence signals with the ones generated by pooled standard samples containing 10^5 cells and 10^6 cells of each microorganism **(Fig. 2).** The signals were transformed into a scale from 0 to 5, where 0 indicates no signal; 1, a signal weaker than the one of the low standard ($<10^5$ bacterial cells); 2, a signal equal to the low standard ($=10^5$ bacterial cells); 3, a signal stronger than that of the low standard but weaker than that of the high standard ($>10^5$ but $<10^6$ bacterial cells); 4, a signal equal to that of the high standard ($=10^6$ bacterial cells); 5,

a signal stronger than that of the high standard (>10^6 bacterial cells). In all analyses, the individual was the computational unit.

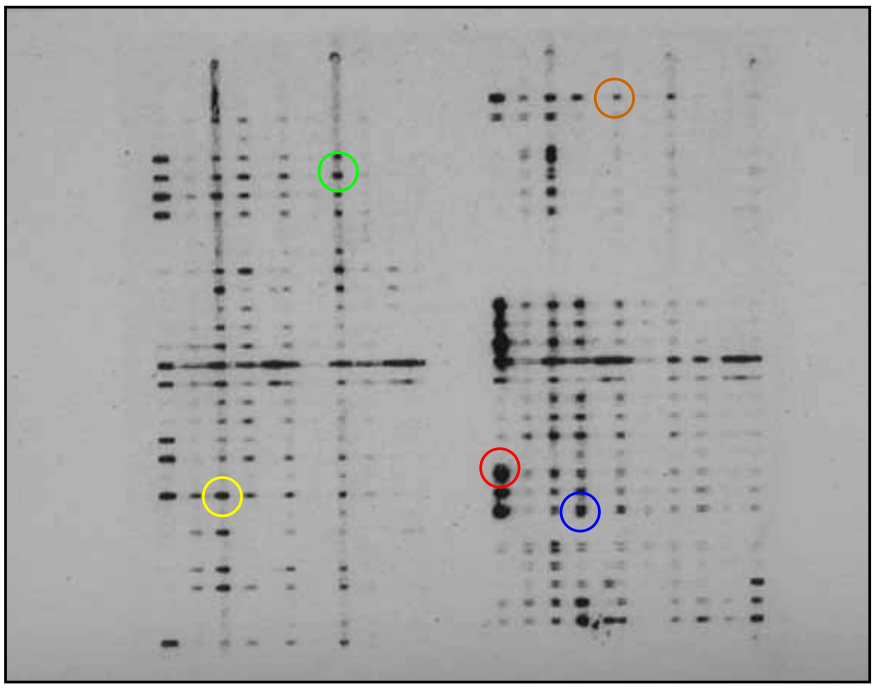

Fig. 2. Example of the checkerboard DNA-DNA hybridization technique being used to detect the 12 microorganisms.

- Signal 1 (<10^5 bacterial cells)
- Signal 2 (=10^5 bacterial cells)
- Signal 3 (>10^5 but <10^6 bacterial cells)
- Signal 4 (=10^6 bacterial cells)
- Signal 5 (>10^6 bacterial cells)

STATISTICAL ANALYSIS

Data are presented as means and 95% confidence intervals (95% CI). The distributions of the variables plaque index, gingival index, mean probing depth, and mean periodontal bone height followed normality (Kolmogorov-Smirnov test). Statistical significance was tested with 1- or 2-factor ANOVA (analysis of variance), including *post hoc* multiple comparisons testing according to Scheffé. Ordinal data were tested with the Chi-square distribution. Furthermore, pairwise correlations were carried out by means of Pearson's product moment method (Study I). Multiple linear regression analysis was run with the mean probing depth and the mean bone height in Studies II and III, respectively, as the dependent variable. Smoking was transformed into a dummy variable including water pipe smokers, cigarette smokers, and mixed smokers versus non-smokers. Logistic regression was used to estimate the relative risk expressed as odds ratio and 95% confidence interval (OR; 95% CI). The number of sites with a probing depth of 5 mm or more was used as the dependent variable, dichotomized ($\geq$ 10 sites = 1, else = 0) in Study II. The bone height was used as the dependent variable dichotomized ($\leq$ 70% = 1, else = 0) in Study III. In Study IV, the microorganisms transformed into a 0/1 variable were the response variable. At score 1 cutoff, 0 denoted score 0 and 1 scores 1-5. At score 3 cutoff, 0 denoted scores 0-2 and 1 scores 3-5.

In the logistic regression analyses, gender was stratified into (1) male and (2) female; education into (1) low (no and primary education, n = 48), (2) medium (intermediate and secondary education, n = 129), and (3) high (university, n = 178); gingival index into (1) low (0-0.58, n = 86), (2) medium (0.59-1.11, n = 89), and (3) high (1.12-3.0, n = 87); and plaque index into (1) low (0-0.69, n = 83), (2) medium (0.70-1.30, n = 86), and (3) high (1.31-3.0, n = 93);

The individual was the statistical unit in the analyses and statistical significance was accepted at $p < 0.05$ (Studies I-III). In Study IV, the null-hypothesis was rejected at $p < 0.01$ because of multiple comparisons and p-values $0.01 < p < 0.05$ were considered to indicate a trend. The data were analyzed using the STATISTICA (6.1) program.

RESULTS

I. Socio-demographic characteristics (Study I)

A summary of the socio-demographic characteristics obtained from the interview questionnaire is presented in **Table 2**. Most participants (95%) had a formal education of at least 6 years and 50% had a university education (more than 12 years). Individuals with a high education level were more often regular dental attenders and had better oral hygiene habits ($p < 0.001$). Education level, however, decreased with age ($p < 0.01$).

The majority of the individuals (72%) described themselves as irregular dental attenders who visited a dental clinic in case of pain only, while 18% were regular dental attenders. The frequency of individuals claiming daily tooth brushing was 82%. Regular dental attenders brushed their teeth more frequently than irregular dental attenders ($p < 0.001$).

According to smoking habit, 33% of the participants were water pipe smokers, 20% cigarette smokers, 19% smokers of both water pipe and cigarettes (labelled mixed smokers), and 28% non-smokers. The overall mean (95% CI) age was 36.9 (35.8; 37.9) years. The age of mixed smokers was significantly lower than that of water pipe smokers, cigarette smokers, and non-smokers, respectively ($p < 0.05$). Among all smoking groups, men were predominant ($p < 0.001$).

The relationship between smoking and dental care habit was statistically significant ($p < 0.01$) suggesting that water pipe smokers were more frequent among irregular dental care attenders (irregular or never) than were cigarette smokers and non-smokers. In addition, mixed smokers were more common among regular dental care attenders than cigarette smokers and non-smokers. There was no significant relationship between smoking and oral hygiene habit or between smoking and educational standard ($p > 0.05$).

Table 2. Demographic and behavioral characteristics of the study population

Variables	Category	*n* (%)
Education level	No education	15 (5%)
	Primary	33 (9%)
	Intermediate	45 (13%)
	Secondary	84 (23%)
	University	178 (50%)
Visit to the dentist	Regularly	63 (18%)
	Irregularly	257 (72%)
	Never	35 (10%)
Tooth brushing	More than twice daily	37 (10%)
	Twice daily	141 (39%)
	Once daily	121 (34%)
	Never	56 (18%)
Smoking	Yes	
	Water pipe	117 (33%)
	Cigarette	72 (20%)
	Both	67 (19%)
	No	99 (28%)

II. Gingival health (Study I)

The mean (95% CI) plaque index was 1.6 (1.4; 1.8) for water pipe smokers, 1.1 (0.9; 1.3) for cigarette smokers, 1.3 (1.1; 1.5) for mixed smokers, and 0.7 (0.6.4; 0.8) for non-smokers. The association between smoking and plaque index was statistically significant ($p < 0.001$). *Post hoc* comparisons testing revealed that all active smoking groups exhibited significantly higher plaque levels than non-smokers. Furthermore, the differences between water pipe smokers and cigarette smokers, and between water pipe smokers and mixed smokers were statistically significant ($p < 0.001$ and $p < 0.01$, respectively). The association remained significant when controlled for age or dental care habit.

The mean (95% CI) gingival index was 1.0 (0.8; 1.1) for water pipe smokers, 0.9 (0.7; 1.0) for cigarette smokers, 1.0 (0.7; 1.2) for mixed smokers, and 0.6 (0.5; 0.8) for non-smokers. There was an overall significant association between smoking and gingival index ($p <$

0.001). *Post hoc* comparisons showed that all active smoking groups exhibited significantly higher gingival index than non-smokers. There were, however, no significant differences between water pipe smokers, cigarette smokers and mixed smokers. The association remained significant when controlled for age and dental care habit, respectively. When controlled for plaque index, however, the significant association between smoking and gingival index disappeared ($p > 0.05$). The same results were obtained when gingival bleeding % was used as measure of the gingival health condition.

The relationship between oral hygiene and gingival bleeding was studied by Pearson's product moment correlation coefficient (r). The correlation between plaque % and gingival bleeding % in cigarette smokers (r = 0.23) was significantly weaker ($p < 0.01$) than that in non-smokers (r = 0.57). The correlation between plaque % and gingival bleeding % in water pipe smokers was also weaker (r = 0.37) than that in non-smokers, but the difference was not statistically significant (**Fig. 3**).

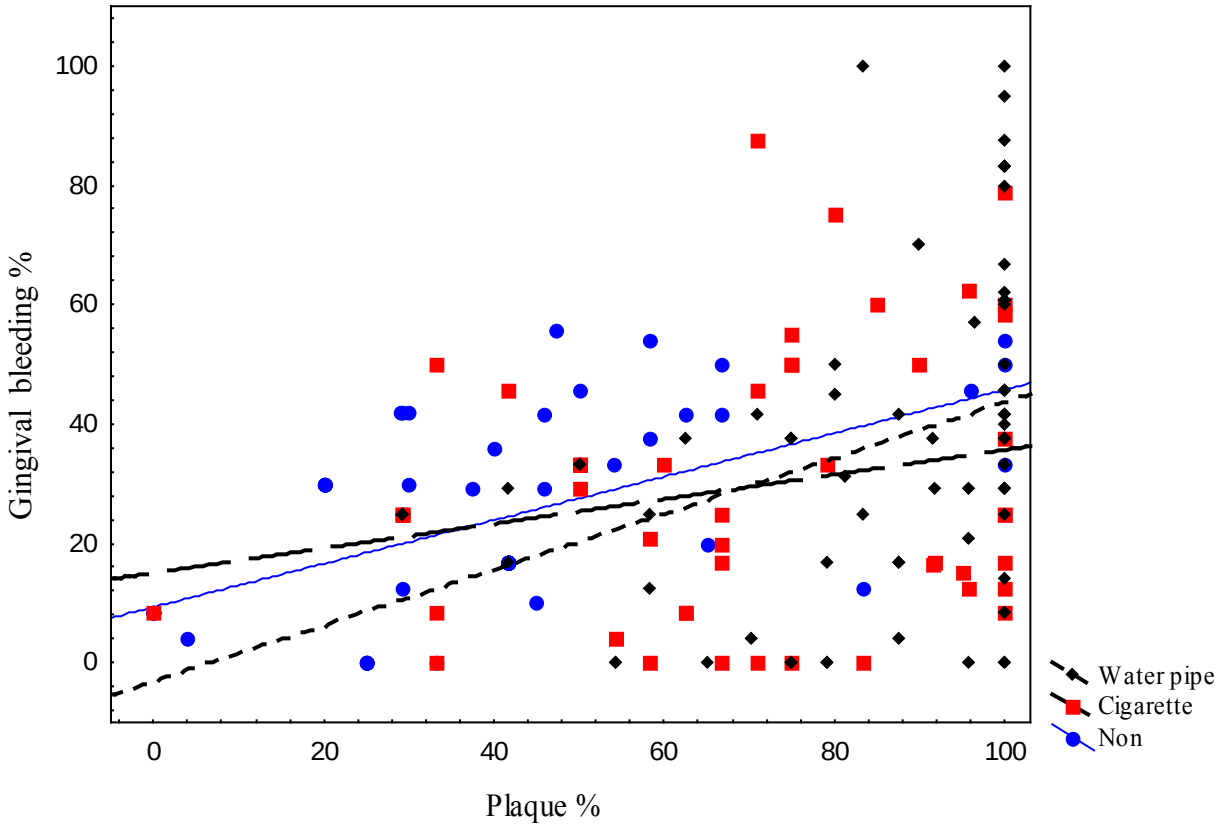

Fig. 3. The correlation between plaque % and gingival bleeding % in cigarette (r = 0.23, $p > 0.05$), water pipe (r = 0.37, $p < 0.001$) and non-smokers (r = 0.57, $p < 0.001$).

III. Periodontal health (Study II)

The mean (95% CI) probing depth per person was 3.1 mm (2.9; 3.2) for water pipe smokers, 3.0 mm (2.9; 3.2) for cigarette smokers, 2.8 mm (2.6; 2.9) for mixed smokers, and 2.3 mm (2.1; 2.5) for non-smokers **(Fig. 4)**. The association between smoking and mean probing depth was statistically significant ($p < 0.001$). The association remained significant after controlling for age ($p < 0.001$), plaque ($p < 0.001$), or gender ($p < 0.001$). *Post hoc* comparisons testing (Scheffé) revealed statistically significant differences in the mean probing depth between cigarette smokers, water pipe smokers, and mixed smokers, respectively, and non-smokers ($p < 0.001$), and between cigarette and water pipe smokers, respectively, and mixed smokers ($p < 0.01$ and $p < 0.05$, respectively).

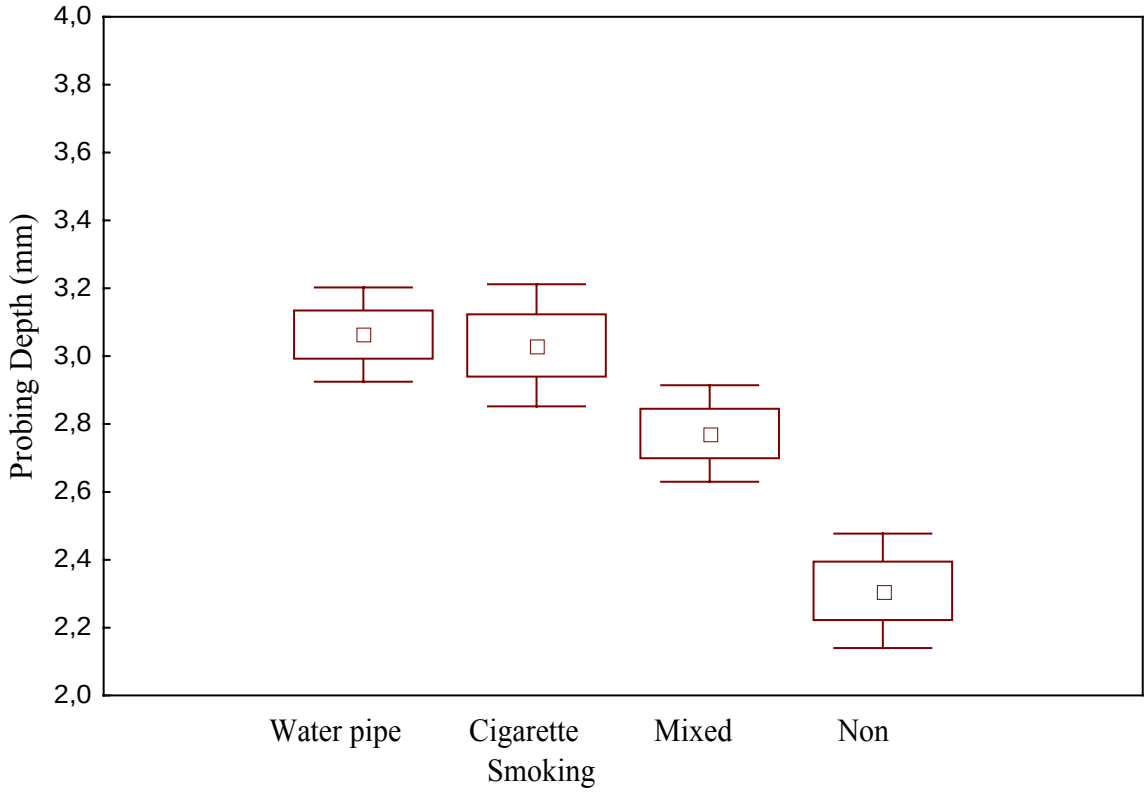

Fig. 4. Probing depth according to smoking habits. Mean and 95% CI.

The association between life-time smoking exposure and mean probing depth was statistically significant in water pipe as well as cigarette smokers after controlling for age ($p < 0.001$) or gender ($p < 0.001$). *Post hoc* comparisons testing (Scheffé) indicated that the differences between heavy and light exposure smokers and between light exposure

smokers and non-smokers were statistically significant among water pipe as well as cigarette smokers **(Table 3)**.

The mean probing depth as the dependent variable could be predicted by means of multiple linear regression from the variables age, smoking (yes/no), gingival index, plaque index, gender, and number of teeth as predictors entered in one block. These predictors explained 35% of the variance in the dependent variable (R^2 *(adj)* = 0.35, $p < 0.001$). The strongest predictors were smoking, plaque index, and gingival index **(Table 4)**. Virtually the same result was obtained when education, and dental care habits were also included as predictors in the analysis.

Table 3. Two-factor ANOVA with pocket probing depth as the dependent variable and smoking exposure as independent (PD) variable together with age as co-factor. *Post hoc* Scheffé tests between exposure groups in water pipe and cigarette smokers. Mean and 95% confidence intervals (CI)

Exposure	Water pipe smokers			Cigarette smokers		
	Mean PD	95% CI		Mean PD	95% CI	
No	2.3	2.1; 2.5	†, ‡	2.3	2.1; 2.5	*, ‡
Light	2.8	2.6; 3.0	*, ‡	2.8	2.5; 3.0	*, ‡
Heavy	3.3	3.1; 3.4	*, ‡	3.2	3.0; 3.5	*, ‡

* $p < 0.05$ † $p < 0.01$ ‡ $p < 0.001$

Table 4. Multiple regression analysis with mean probing depth per person as the dependent variable. (R^2 (*adj*) = 0.35)

Variable	Parameter	Standard error	t	*p*
Age	0.007	0.004	1.78	0.075
Gingival index	0.244	0.081	2.98	0.003
Plaque index	0.266	0.063	4.19	0.000
Smoking	0.522	0.096	5.39	0.000
Gender	-0.061	0.083	-0.73	0.463
Teeth (*n*)	0.014	0.014	0.98	0.325

IV. Periodontal bone height (Study III)

The mean (95% CI) periodontal bone height per person was 76.2% (74.8; 77.6) for water pipe smokers, 75.8% (74.2; 77.6) for cigarette smokers, 80.2% (78.7; 81.7) for mixed smokers, and 80.9% (79.2; 82.6) for non-smokers **(Fig. 5)**. The association between smoking and mean bone height was statistically significant after controlling for age ($p < 0.001$), or gender ($p < 0.001$). *Post hoc* comparisons testing according to Scheffé revealed statistically significant differences between cigarette smokers and water pipe smokers, respectively, and non-smokers ($p < 0.001$) or mixed smokers ($p < 0.01$).

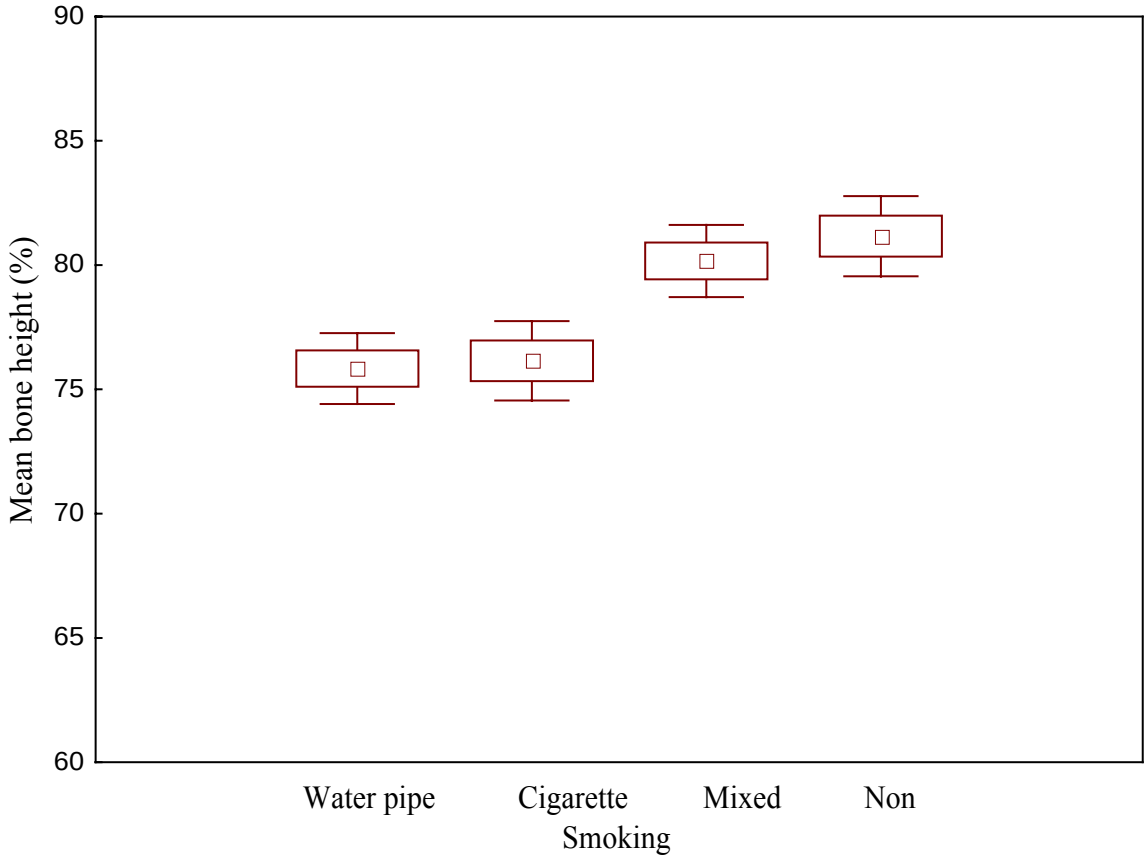

Fig. 5. Mean bone height per person according to smoking habits. Mean and 95% CI.

The association between life-time smoking exposure and mean periodontal bone height was statistically significant in water pipe as well as cigarette smokers after controlling for age ($p < 0.01$) or education ($p < 0.001$ and $p < 0.01$, respectively). *Post hoc* comparisons testing indicated that the difference between heavy and light exposure smokers was statistically significant among water pipe as well as cigarette smokers **(Table 5).**

Table 5. Two-factor ANOVA with mean periodontal bone height (BH) as the dependent variable and smoking exposure as independent variable together with age as co-factor. *Post hoc* Scheffé tests between exposure groups in water pipe and cigarette smokers. Mean and 95% confidence intervals (CI)

Exposure	Water pipe smokers			Cigarette smokers		
	Mean BH	95% CI		Mean BH	95% CI	
No	80.8	79.4; 82.1	†	80.8	79.4; 82.1	†
Light	79.2	77.5; 80.8	†	78.3	75.8; 80.8	†
Heavy	75.8	72.9; 78.7		74.0	71.4; 76.5	

† $p < 0.001$

The relation between mean periodontal bone height as the dependent variable and the variables age, gender, gingival index, plaque index, mean probing depth, number of retained teeth, education level, dental care habit, and smoking (yes/no) as predictors, entered in one block, was analyzed by means of multiple linear regression in a subset of the population (n = 262). Age, gingival index, education level, and smoking were statistically significant predictors, explaining 49% of the variance in the dependent variable **(Table 6**).

Table 6. Multiple regression analysis with mean periodontal bone height per person as dependent variable. (R^2 (*adj*) = 0.49, F (9, 252) = 28.7)

Variable	Parameter	Standard error	t	*p*
Age	-0.324	0.041	-7.9	0.000
Gingival index	-2.225	0.800	-2.7	0.006
Plaque index	-1.154	0.615	-1.8	0.062
Smoking	-3.626	0.988	-3.6	0.000
Gender	1.363	0.809	1.7	0.093
Teeth (*n*)	0.222	0.142	1.6	0.119
Probing depth	-0.956	0.593	-1.6	0.108
Education level	3.668	1.079	3.4	0.001
Dental care habit	1.026	0.727	1.4	0.159

V. Disease prevalence estimates and risk assessment

Periodontal pocketing (Study II)

By an arbitrary definition of periodontal disease as the occurrence of 10 or more sites with a probing depth of 5 mm or more per individual, the overall prevalence was 19.5%. The prevalence was 30% in water pipe smokers, 24% in cigarette smokers, 17% in mixed smokers, and 8% in non-smokers. The prevalence was significantly dependent on smoking habit ($p < 0.01$). Throughout all age strata, the prevalence was greater in all categories of active smokers than in non-smokers **(Fig. 6).**

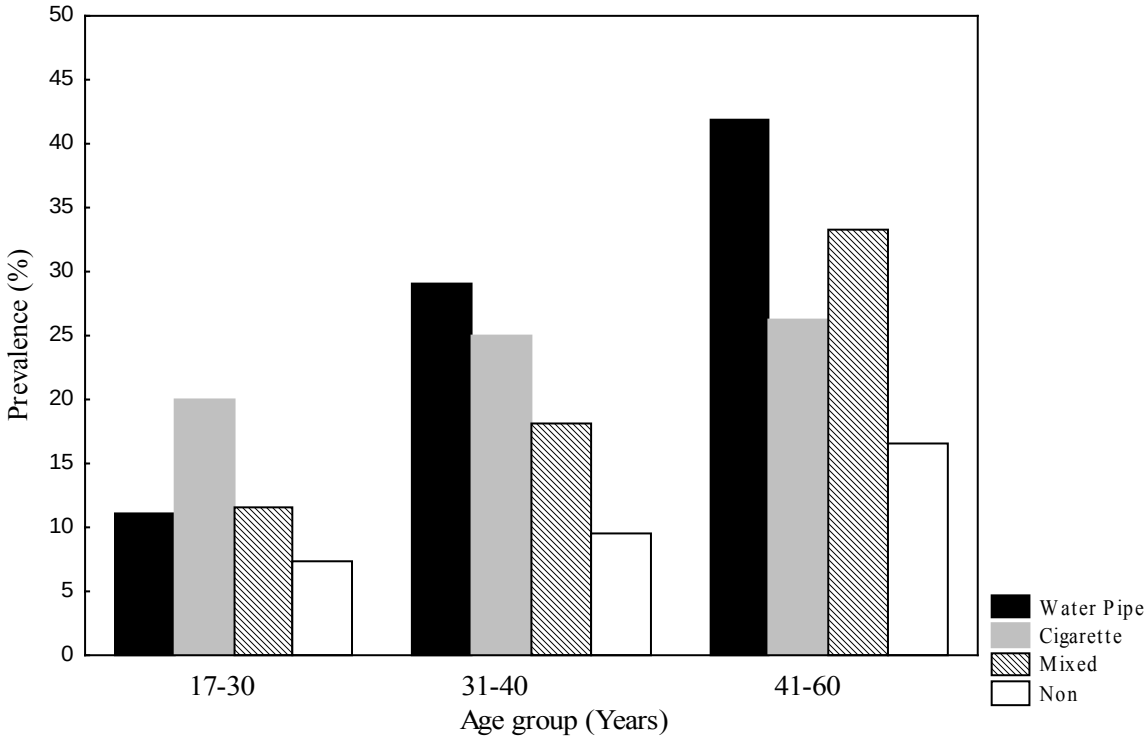

Fig. 6. Prevalence of periodontal disease using a cut-off of 10 sites with a probing depth of 5 mm or more according to smoking and age.

Multivariate logistic regression including age, gingival index, plaque index, and smoking (yes/no) as independent factors was run to estimate the relative risk for disease using the number of sites with a probing depth of 5 mm or more as the dependent variable, dichotomized (≥ 10 sites = 1, else = 0). Smoking, gingival index, and plaque index were significantly associated with increased risk **(Table 7)**. The relative risk associated with smoking was 3.6-fold increased compared to non-smoking ($p < 0.05$). The relative risk run

by water pipe smokers and cigarette smokers was 5.1-fold elevated ($p < 0.001$) and 3.8-fold elevated ($p < 0.05$) compared to non-smokers after adjustment for age. The relative risk of light and heavy water pipe smokers was 2.9-fold and 8.2-fold elevated, respectively, compared to non-smokers ($p < 0.001$) after adjustment for age. The relative risk of light and heavy cigarette smokers was 1.5-fold and 5.0-fold elevated, respectively, compared to non-smokers ($p < 0.001$) after adjustment for age.

Table 7. Multivariate logistic regression analysis with number of sites with a probing depth of 5 mm or more (< 10 vs ≥ 10) as dependent variable and smoking, age, plaque index, and gingival index as independent variables. Odds ratio (OR) and 95% confidence intervals (CI)

Variable	OR	95% CI	*p*
Smoking			
No	1.0		
Yes	3.6	1.2; 10.6	0.021
Age			
17-30 yr	1.0		
31-40 yr	1.2	0.8; 1.9	
41-60 yr	1.4	0.6; 3.5	0.435
Plaque index			
Low	1.0		
Medium	1.9	1.1; 3.3	
High	3.6	1.2; 11.0	0.025
Gingival index			
Low	1.0		
Medium	2.8	1.6; 4.7	
High	7.6	2.6; 22.2	0.000

Periodontal bone loss (Study III)

By an arbitrary definition of periodontal disease as a bone height level of 70 % or less, the overall prevalence was 17.5%. The prevalence was 27% in water pipe smokers, 24% in cigarette smokers, 9% in mixed smokers, and 6% in non-smokers ($p < 0.001$). Throughout all age strata, the prevalence was comparably greater in all categories of active smokers than in non-smokers (**Fig. 7)**.

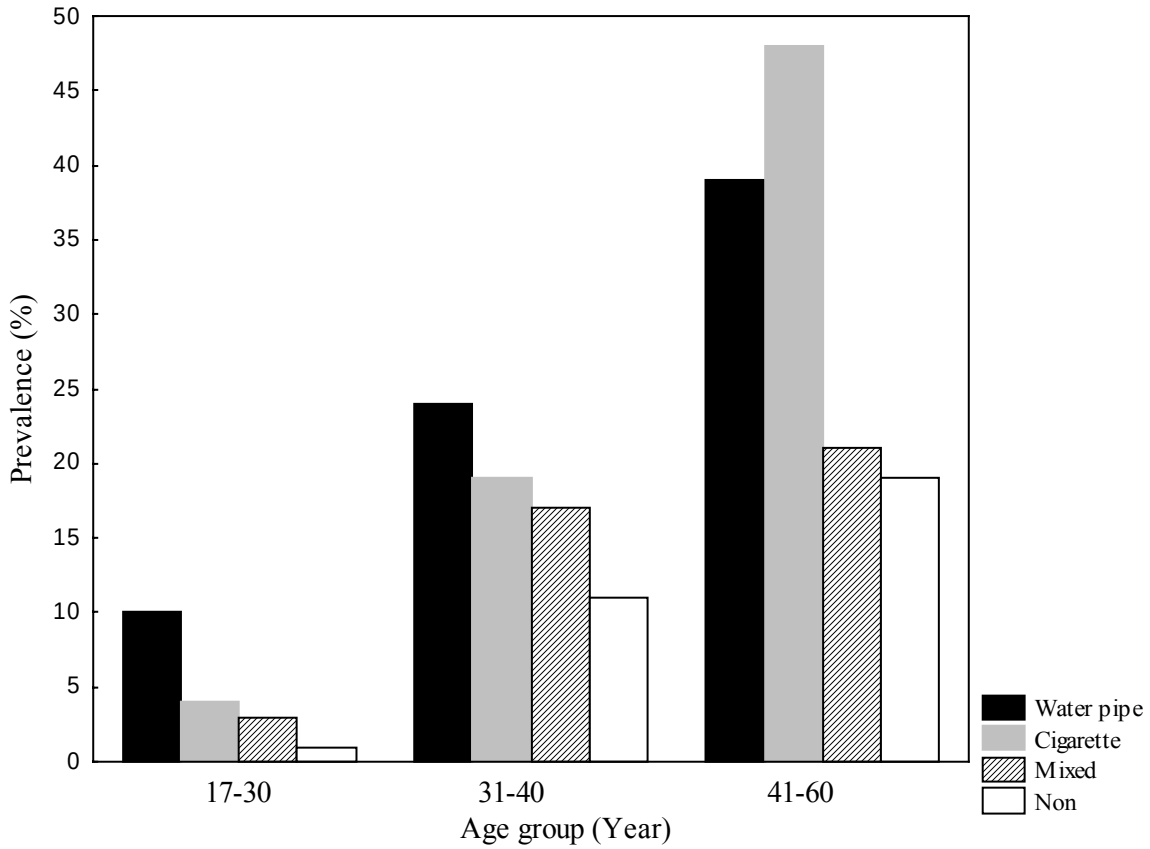

Fig. 7. Prevalence of periodontal disease defined as a bone height of 70% or less according to smoking and age.

Logistic regression analysis including age, gingival index, plaque index, number of teeth, and smoking (yes/no) as independent factors was run to estimate the relative risk for the disease using bone height as the dependent variable dichotomously transformed (≤ 70% =1, else = 0). The relative risk associated with smoking was 4.3-fold increased compared to non-smoking ($p < 0.01$, **Table 8)**. The risk run by water pipe smokers and cigarette smokers was 3.5-fold elevated ($p < 0.01$) and 4.3-fold elevated ($p < 0.01$) compared to non-smokers after adjustment of age. The relative risk of light and heavy water pipe smokers was 1.0-fold and 7.5-fold elevated, respectively, compared to non-smokers after adjustment for age ($p < 0.01$). The relative risk of light and heavy cigarette smokers was 1.9-fold and 6.3-fold elevated, respectively, compared to non-smokers after adjustment for age ($p < 0.01$).

Table 8. Multivariate logistic regression analysis with bone height as the dependent variable dichotomously transformed (≤ 70% = 1, else = 0). as dependent variable and smoking, age, plaque index, gingival index, and number of retained teeth as independent variables. Odds ratio (OR) and 95% confidence intervals (CI)

Variable	OR	95% CI	*p*
Smoking			
No	1.0		
Yes	4.3	1.7; 13.4	0.003
Age			
17-30 yr	1.0		
31-40 yr	7.5	3.3; 17.4	
41-60 yr	16.8	10.6; 30.4	0.000
Plaque index			
Low	1.0		
Medium	2.2	1.1; 4.6	
High	4.9	1.2; 21.3	0.033
Gingival index			
Low	1.0		
Medium	2.3	1.3; 4.2	
High	5.5	1.7; 17.7	0.004
Teeth (*n*)	0.9	0.8; 1.0	0.236

VI. Periodontal microflora (Study IV)

Using a score 1 cutoff (i.e. less than 10^5 bacterial cells), all studied microorganisms were detected in water pipe smokers, cigarette smokers and in non-smokers. *P. micros, T. denticola, P. nigrescens, P. intermedia, S. intermedius, T. forsythensis, A. actinomycetemcomitans,* and *F. nucleatum* were detected in 40% or more of the individuals **(Fig. 8)**. The detection rate of *T. denticola* was significantly higher in water pipe smokers ($0.01 < p < 0.05$) and tended to be higher in cigarette smokers ($p < 0.05$) compared to non-smokers. Regarding the other microorganisms, there were no significant differences between water pipe smokers, cigarette smokers, and non-smokers.

Using score 3 cut-off (i.e. more than 10^5 but less than 10^6 bacterial cells), the prevalence decreased throughout and *S. noxia* was not detected at all **(Fig. 9)**. *T. denticola* tended to be more prevalent among cigarette smokers ($0.01 < p < 0.05$) whereas *T. forsythensis, P. nigrescens,* and *P. intermedia* were more frequently detected in non-smokers than in cigarette smokers or water pipe smokers, but the differences were not statistically significant ($p > 0.05$).

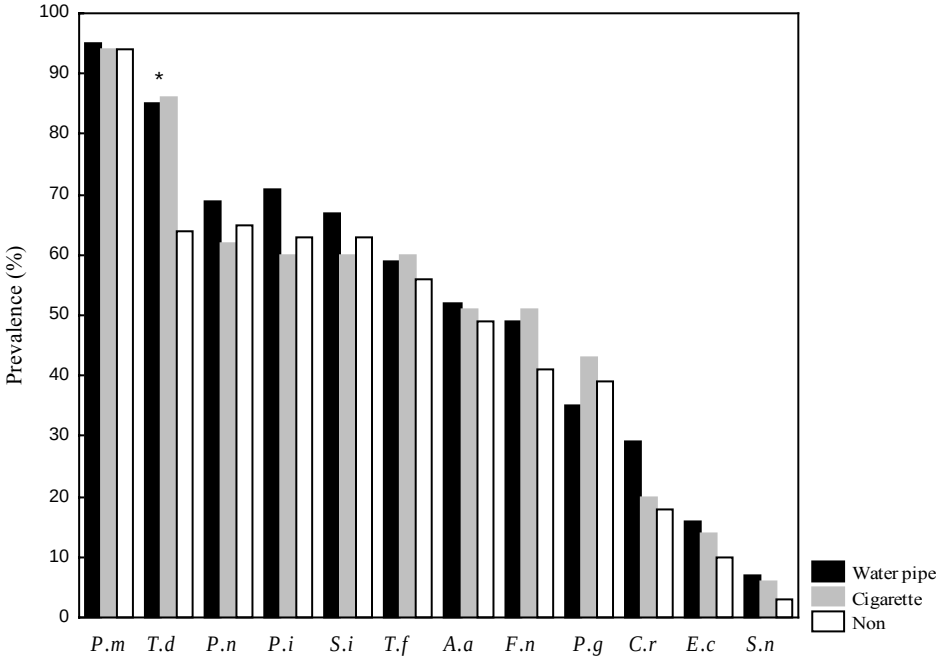

Fig.8. Prevalence of individuals positive for the 12 studied periodontal microorganisms according to smoking. Score 1 cut-off. * $p < 0.01$

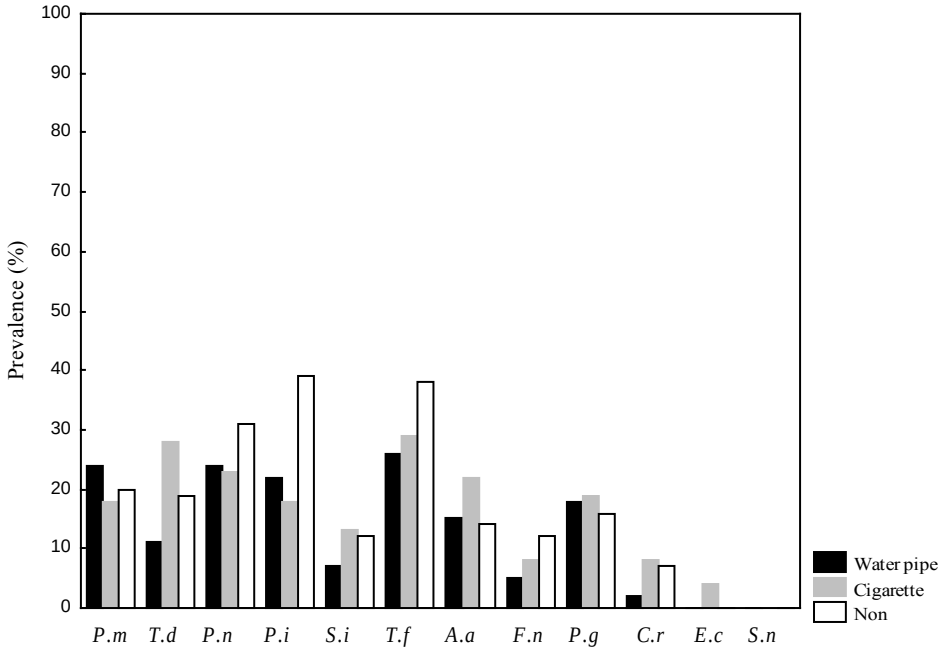

Fig. 9. Prevalence of individuals positive for the 12 studied periodontal microorganisms according to smoking habits. Score 3 cut-off.

The prevalence of individuals positive for the various microorganisms in relation to smoking habits and depth of sample site using score 1 cut-off is shown in **Table 9**. Among individuals with a sample site probing depth less than 6 mm, 73-100% of smokers and non-smokers were positive for *P. micros, T. denticola, P. nigrescens, P. intermedia,* and *S. intermedius.* The detection rates for *A. actinomycetemcomitans* and *C. rectus* were lower in cigarette smokers compared to water pipe smokers and non-smokers. However, there were no statistically significant differences between smoking groups (including non-smokers) for any one of the microorganisms studied.

Among individuals with a sample site probing depth of 6 mm or more, the detection rate of *P. micros* was almost 100% for all smoking categories (including non-smokers), while *C. rectus* and *E. corrodens,* were not found in non-smokers. In addition, *S. noxia* was not found in cigarette smokers or non-smokers. There was a trend towards a higher detection rate for *T. denticola* in water pipe smokers and cigarette smokers compared to non-smokers ($0.01 < p < 0.05$), whereas *P. nigrescens* tended to be more frequently detected in non-smokers compared to water pipe smokers or cigarette smokers ($0.01< p < 0.05$).

At score 3 cut-off and a sample site probing depth less than 6 mm the prevalence of *P. intermedia* and *T. forsythensis* was high in non-smokers ($p < 0.01$). Furthermore, non-smokers tended to have higher scores for *T. denticola* and *P. gingivalis* compared to water pipe smokers and cigarette smokers ($0.01 < p < 0.05$). Among individuals with a sample site probing depth of 6 mm or more the detection rate for *T. denticola* was high in cigarette smokers compared to water pipe smokers and non-smokers. In addition, fewer non-smokers were positive for *P. nigrescens* compared to water pipe and cigarette smokers. For the other microorganisms there were no statistically significant differences between water pipe smokers, cigarette smokers and non-smokers **(Table 10)**.

Table 9. The prevalence of individuals positive for the 12 studied periodontal microorganisms according to smoking habit and depth of sample site at score 1 cut-off

Microorganism	Depth of sample site < 6 mm					Depth of sample site ≥ 6 mm				
	WPS *n* = 24 %	CS *n* = 15 %	NS *n* = 70 %	χ^2	*p*	WPS *n* = 34 %	CS *n* = 20 %	NS *n* = 10 %	χ^2	*p*
P. micros	92	100	93	1.2	ns	97	90	100	2.0	ns
T. denticola	75	73	64	1.2	ns	91	95	60	8.3	0.015
P. nigrescens	67	93	63	5.3	ns	71	40	80	6.6	0.037
P. intermedia	79	80	61	3.7	ns	65	45	70	2.6	ns
S. intermedius	79	80	61	3.7	ns	59	45	70	1.9	ns
T. forsythensis	42	53	53	1.0	ns	71	65	80	0.7	ns
A. actinomycetemcomitans	42	20	50	4.6	ns	59	75	40	3.6	ns
F. nucleatum	42	53	44	0.5	ns	47	50	20	2.7	ns
P. gingivalis	17	40	39	4.1	ns	47	45	40	0.2	ns
C. rectus	17	6	20	3.6	ns	38	35	0	5.5	ns
E. corrodens	29	20	11	4.2	ns	6	10	0	1.2	ns
S. noxia	0	13	3	5.0	ns	12	0	0	3.8	ns

Table 10. The prevalence of individuals positive for the 12 studied periodontal microorganisms according to smoking habit and depth of sample site at score 3 cut-off

Microorganism	Depth of sample site < 6 mm					Depth of sample site ≥ 6 mm				
	WPS *n* = 24 %	CS *n* = 15 %	NS *n* = 70 %	χ^2	*p*	WPS *n* = 34 %	CS *n* = 20 %	NS *n* = 10 %	χ^2	*p*
P. micros	17	7	20	1.5	ns	27	25	10	1.2	ns
T. denticola	0	0	19	8.2	0.016	18	50	10	8.4	0.014
P. nigrescens	8	13	33	7.0	0.030	35	30	20	0.9	ns
P. intermedia	8	7	37	11.1	0.004	29	25	40	0.7	ns
S. intermedius	0	0	7	2.9	ns	18	25	30	2.4	ns
T. forsythensis	4	7	40	15.3	0.001	41	45	30	0.6	ns
A. actinomycetemcomitans	13	0	13	2.2	ns	15	35	10	4.0	ns
F. nucleatum	8	7	14	1.1	ns	0	10	0	4.5	ns
P. gingivalis	0	0	14	6.1	0.047	29	30	20	0.4	ns
C. rectus	0	0	6	2.3	ns	3	10	0	2.0	ns
E. corrodens	-	-	-	-	-	-	-	-	-	-
S. noxia	-	-	-	-	-	-	-	-	-	-

n = number of individuals, WPS = Water pipe smokers, CS = Cigarette smokers, NS = Non-smokers, ns = Not significant

Smoking associated risk

The smoking associated risk of harboring the periodontal microorganisms studied was estimated by means of multivariate logistic regression analysis. The periodontal microorganisms, one at time, served as the dependent variable, dichotomized, and smoking (yes / no) and depth of sample site served as independent variables. The analysis at score 1 cut-off indicated that the risk of harboring the microorganisms was not associated with smoking in general ($p > 0.05$, **Table 11**). However, only a trend towards a higher detection rate of *T. denticola* and *E. corrodens* was observed (OR = 2.3 and OR= 2.9 respectively, $0.01 < p < 0.05$). On the otherhand, deep sample sites was associated with a higher risk of detection of *T. forsythensis* (OR = 1.7, $p < 0.01$) and a trend towards a lower rate of detection for *A. actinomycetemcomitans* and *E. corrodens* (OR = 1.5 and OR = 0.5, respectively, $0.01 < p < 0.05$).

At score 3 cut-off, the risk of being positive for *T. forsythensis* and *P. intermedia* was significantly decreased in smokers (OR = 0.3, $p < 0.01$, **Table 12**). For the other microorganisms, however, smoking was not associated with an increased risk ($p > 0.05$). The risk of harboring *T. forsythensis, T. denticola,* and *P. gingivalis* was increased in deep sample sites (OR = 1.9, OR = 2.0 and OR = 2.4 respectively, $p < 0.01$).

Table 11. Multiple logistic regression with the 12 studied microorganisms, one at a time, as the dependent variable and smoking and depth of sample site as independent variables. Score 1 cut-off. OR= odds ratio, 95% CI = 95% confidence interval

Dependent variable	Smoking			Depth of sample sites		
	OR	95% CI	*p*	OR	95% CI	*p*
P. micros	1.02	0.24; 4.30	ns	1.18	0.54; 2.57	ns
T. denticola	2.33	1.04; 5.25	0.039	1.49	0.93; 2.38	ns
P. nigrescens	1.27	0.61; 2.63	ns	0.83	0.58; 1.21	ns
P. intermedia	1.60	0.77; 3.34	ns	0.74	0.51; 1.08	ns
S. intermedius	1.53	0.73; 3.19	ns	0.70	0.48; 1.02	ns
T. forsythensis	0.72	0.35; 1.45	ns	1.66	1.14; 2.41	0.008
A. actinomycetemcomitans	0.77	0.39; 1.55	ns	1.50	1.04; 2.16	0.027
F. nucleatum	1.41	0.71; 2.82	ns	0.90	0.63; 1.28	ns
P. gingivalis	0.70	0.34; 1.44	ns	1.39	0.96; 2.01	ns
C. rectus	1.14	0.48; 2.69	ns	1.47	0.97; 2.24	ns
E. corrodens	2.87	1.05; 7.85	0.039	0.46	0.25; 0.84	0.015
S. noxia	2.49	0.41; 15.1	ns	1.09	0.50; 2.39	ns

Table 12. Multiple logistic regression with 7 studied microorganisms, one at a time, as the dependent variable and smoking and depth of sample site as independent variables. Score 3 cut-off. OR= odds ratio, 95% CI = 95% confidence interval

Dependent variable	Smoking			Depth of sample sites		
	OR	95% CI	*p*	OR	95% CI	*p*
T. forsythensis	0.30	0.13; 0.70	0.005	1.86	1.21; 2.84	0.004
P. nigrescens	0.49	0.22; 1.09	ns	1.41	0.94; 2.13	ns
P. intermedia	0.25	0.11; 0.60	0.002	1.56	1.00; 2.42	0.046
P. micros	0.92	0.38; 2.19	ns	1.23	0.79; 1.91	ns
T. denticola	0.49	0.18; 1.31	ns	1.97	1.21; 3.23	0.007
P. gingivalis	0.47	0.17; 1.35	ns	2.40	1.42; 4.06	0.001
A. actinomycetemcomitans	0.93	0.34; 2.56	ns	1.46	0.89; 2.40	ns

ns = Not significant

DISCUSSION

The present thesis has focused on the association between tobacco smoking – in particular water pipe smoking – and some clinical, radiographic and microbiologic aspects of periodontal health/disease. The main finding is that water pipe smoking is negatively associated with periodontal health and that the effect is similar in quality and strength to that of cigarette smoking. Both water pipe smokers and cigarette smokers exhibited an inferior gingival health (Study I), a greater frequency of periodontal pockets (Study II), and a reduced periodontal bone height (Study III) when compared to non-smokers. In spite of these findings suggesting an inferior periodontal health condition in tobacco smokers, smoking was not associated with the composition of the periodontal microflora (Study IV).

Gingival health

For the study population as a whole, the gingival index was 0.9, a value corresponding to the initial stages of clinical inflammation. On the average all categories of smokers had inferior gingival health condition compared to non-smokers, as was evident from higher gingival index and gingival bleeding (Study I). The inferior gingival health of smokers was due to the fact that their oral hygiene was, on average, inferior. However, when the oral hygiene level was taken into account they did not show increased levels of gingival bleeding. Rather, the correlation between plaque and gingival bleeding was weaker in smokers than in non-smokers. This held particularly true for cigarette smokers, but there was a trend in the same direction also for water pipe smokers. Thus, it appears that tobacco smoking is associated with a reduced inflammatory response in terms of gingival bleeding. This finding is in agreement with those of numerous epidemiological and clinical studies reported previously with regard to cigarette smoking (Bergström & Flodérus-Myrhed 1983, Preber & Bergström 1985, Bergström & Preber 1986, Bergström 1990, Danielsen et al. 1990, Lie et al. 1998, Darby et al. 2000, Dietrich et al. 2004).

Periodontal pockets

Periodontal probing depth is a diagnostic criterion that is widely used to estimate periodontal disease prevalence (Armitage 2004). Studies that used periodontal probing depth as a diagnostic criterion for periodontal disease have reported that cigarette smokers had both a significantly greater pocket frequency and deeper probing depth compared to non-smokers (Horning et al. 1992, Bergström 1989, Faddy et al. 2000, Van der Weijden et

al. 2001, Susin et al. 2005). In general agreement with these reports a significant association between tobacco smoking and periodontal probing depth was observed (Study II). The strength of the association with water pipe smoking was similar to that with cigarette smoking, suggesting a negative impact of water pipe smoking on periodontal health. Nevertheless, with the reduced inflammatory response as measured by gingival bleeding, and the increase in tissue fibrosis in smokers as suggested by Biddle et al. (2001) the severity of the periodontal disease in the present smokers might have been underestimated.

Periodontal bone height

The relationship between water pipe smoking and periodontal health was further explored by means of radiographic assessment of the interdental bone height. The results indicated that the bone height was reduced in water pipe smokers as well as cigarette smokers compared to in non-smokers, suggesting that both smoking habits were associated with excess periodontal bone loss (Study III). This finding provided convincing evidence to support the role of tobacco smoking as a factor associated with periodontal bone height reduction and is in agreement with previous studies concerning cigarette smoking and periodontal bone loss of horizontal and vertical patterns (Bergström et al. 1991, Grossi et al. 1995, Norderyd & Hugoson 1998, Persson et al. 1998, Bergström et al. 2000b, Baljoon et al. 2004).

Periodontal disease prevalence

The occurrence of periodontal pockets and the radiographically measured level of the periodontal bone height were, additionally, used as descriptors (surrogate end-points) for the examination of periodontal disease prevalence.

When periodontal disease was defined as the occurrence of 10 or more sites with a probing depth of 5 mm or more in an individual, the overall prevalence was about 20% in the population studied (Study II). This overall prevalence is high compared to the overall prevalence of about 9%, reported in a large-scale survey in the USA, where 2 teeth per individual with 5 mm or more probing depth was used as the disease criterion (Albandar et al. 1999).

When we used periodontal bone height level of 70% or less to define periodontal disease, the overall prevalence of the periodontal disease was about 18% in the present study population (Study III). This prevalence is higher than that reported in a Swedish

population (13%) selecting a minimum of bone loss of 1/3 of the root length as the disease criterion (Hugoson et al. 1998). However, neither the study of Albander et al. nor that of Hugoson et al. considered tobacco smoking in the analysis. As the prevalence of periodontal disease is dependent of how it is defined and on the selection of a disease-specific criterion (Bergström & Eliasson 1989), comparisons between studies may be difficult. The major reason for the comparatively high prevalence of disease currently observed is considered to be the large proportion of smokers in the present study population, since the disease prevalence was significantly dependent of smoking habit.

Periodontal microflora

In the present study, no major differences were observed between smokers and non-smokers regarding the microorganisms investigated, particularly not when the depth of the sampled site was accounted for. Although both cigarette smokers and water pipe smokers were more frequently positive for *T. denticola* than non-smokers when a low cut-off level was used for detection of microorganisms, the difference disappeared in the logistic regression when probing depth was included in the analysis. The microorganism detection rate was higher in samples from deep sites than in samples from shallow sites irrespective of the individual's smoking habits. These results support the view that smoking has a limited influence on the subgingival microflora (Preber et al. 1992, Stoltenberg et al. 1993, Renvert et al. 1998, Boström et al. 2001, Eggert et al. 2001, Salvi et al. 2005, Buduneli et al. 2005). However, others have linked specific periodontal microorganisms to the elevated disease severity in cigarette smokers (Zambon et al. 1996, Umeda et al. 1998, Kamma et al. 1999, Van Winkelhoff et al. 2001, Haffajee & Socransky 2001).

The conflicting results might be explained by differences in microbial analysis, periodontal status of the sampled sites or the statistical expression of the data as the rate of detection of certain bacteria in individuals, or proportion and counts of the microorganisms (Haffajee & Socransky 2001).

Water pipe smoking

In water pipe smoking, despite the fact the smoke is filtered through water, inhalation of toxic substances is similar to or even greater than that during cigarette smoking (Shihadeh & Saleh 2005). It is likely, therefore, that water pipe smoking would affect the periodontal tissues in the same way as cigarette smoking. This assumption was supported by the present results.

It might be speculated that water pipe smoking would be associated with a comparatively low socioeconomic standard, and that this factor might confound the influence of smoking on periodontal health. However, in the multiple regression analyses of the present results, when socioeconomic standard was controlled for by means of individual's education level in addition to other variables, the association between water pipe smoking and periodontal pockets or periodontal bone height reduction remained statistically significant.

Tobacco smoking exposure

A gradient of increasing periodontal morbidity with increasing smoking exposure was evident in water pipe as well as cigarette smokers. The association between smoking exposure and the frequency of periodontal pockets or periodontal bone height suggests a dose-response relationship. The dose-response relationship was also suggested by the observation that the relative risk increased with increasing exposure. This observation confirms the evidence of previous studies concerning cigarette smoking (Bergström et al. 1991, Haber & Kent 1992, Haber et al. 1993, Grossi et al. 1995, Norderyd & Hugoson 1998, Bergström et al. 2000a,b, Tomar & Asma 2000, Teng et al. 2003, Baljoon et al. 2004, 2005a, Bergström 2004, Razali et al. 2005). This strength the association found in this cross sectional study.

Limitations of the study

Participation in the present study was limited to individuals who responded to newspaper announcements that were designed to attract individuals with various smoking habits. This resulted in a higher smoking prevalence (70%) than that in the Saudi population at large (25-35%, Saeed 1991, Saeed et al. 1996). Regarding the prevalence of cigarette smoking, however, the present population was similar to the Saudi population (Siddiqui et al. 2001, Siddiqui & Ogbeide 2001). The prevalence of water pipe smoking in Saudi Arabia is not well known since the above cited surveys on smoking habits in Saudi Arabia have not considered water pipe smoking.

Assignment of individuals to different smoking groups was based on interview data, and this may have caused some misclassification. In addition, smoking exposure was measured by the number of cigarettes or the number of water pipe runs smoked per day, which may have been underestimated by smokers. Other methods that could more accurately estimate smoking exposure are expired carbon monoxide levels and cotinine measurements in body fluids such as saliva, serum and urine (Dolcini et al. 2003). The relationships between self-

reported smoking data and cotinine levels in some body fluids have been shown to be highly correlated (Binnie et al. 2004).

In order to avoid misclassification bias with regard to smoking habit, we separated smokers of water pipe alone from smokers of both water pipe and cigarettes, placing individuals who smoked both water pipe and cigarettes in a category labeled mixed smokers. By doing so, we aimed to increase the validity of our observations so that any impaired periodontal health observed in water pipe smokers could be attributed to water pipe smoking *per se* without a confounding effect of concomitant cigarette smoking.

The levels of education used in the present study roughly correspond to the International Standard Classification of Education (ISCED 1997). Using this classification, 50% of participants were at university level or above, which is more advanced than in the population at large.

A further limitation is the gender bias towards a male predominance among the present participants, which was particularly strong among smokers. This gender bias arises because the large majority of women in Saudi Arabia do not smoke, whereas smoking is common among men. Even though the periodontal microflora investigated did not differ between men and women, women had a higher periodontal bone level and a lower mean probing depth than men. This gender effect disappeared in the multiple regression analyses, suggesting that other determinants - particularly smoking - were responsible for the periodontal manifestations observed in the present study.

The use of panoramic radiography in the present study to evaluate periodontal bone level is another limitation. The main disadvantage of panoramic radiography is that the images do not display the fine anatomic details. A break in the lamina dura at the mesial or distal aspect of the interdental septum, which is considered as the earliest radiographic changes in periodontal disease, is best determined from intraoral radiographs (White & Pharoah 2000). In addition, overlapping of the proximal teeth surfaces and unequal magnification across the image might have underestimated the overall bone loss. However, for the purpose of the present study and because it has been shown earlier that panoramic radiography is well suited for epidemiological studies, the use of this fast and reliable diagnostic method is considered sufficient (Ahlqwist et al. 1986). Furthermore, the frequency of periodontal pockets and the assessment of periodontal bone height were based on full-mouth examinations that were derived from a large number of measurements in each individual. This, together with the fact that the number of retained teeth was high,

assured a high precision of the clinical and radiographic measures with a minimum influence of measurement error.

In the present study, we used the checkerboard DNA-DNA hybridization technique, which is considered an efficient method; moreover, it does not require bacterial viability and is particularly applicable in epidemiological research (Ali et al. 1997, Papapanou et al. 2000, 2002, Craig et al. 2001, Dowsett et al. 2002). However, this technique, based on whole genomic probes, may give cross-hybridization of bacteria with closely related species (Wong et al. 1996). The specificity was checked for the species in the test panel of 12 strains and cross-reactions were noted between *P. intermedia* and *P. nigrescens*. It is still possible that cross-reactions were occurring between the probes and other microorganisms not included in the panel, however, this disturbing factor would have similar effect on all samples and might not interfere with the overall result. A further limitation is that the DNA-DNA hybridization technique detected only species for which DNA probes have been prepared. Thus, novel pathogens, which might be detected in culture or by other molecular techniques, would not be detected by this method (Socransky et al. 2004).

Presently, the microbiological sampling was based on only four sites to represent an individual's periodontal microflora. Even though other studies have sampled each tooth in each individual (Haffajee & Socransky 2001, Socransky et al. 2004), four sites per individual have been suggested to be a valid representation for *A. actinomycetemcomitans* and *P. gingivalis* (Mombelli et al. 1991, 1994).

Possible mechanisms for tobacco's harmful effects

Although the correlation between tobacco smoking and periodontal disease is strong, the precise mechanisms of action of smoking in the pathogenesis of periodontal disease are not well understood. The inhibition of inflammatory signs in tobacco smokers - even in the presence of extensive periodontal destruction - is mainly attributed to the presence of gingival vasoconstriction (Mavropoulos et al. 2003). The smoking-induced vasoconstriction could contribute to impaired gingival blood flow, and decrease the amount of oxygen and other blood constituents that reach the gingiva. The capacity to remove tissue waste products might also be reduced, leading to periodontal tissue destruction and compromising the immune response (Palmer et al. 1999).

Another hypothesis suggests that tobacco smoke components restrict the periodontal angiogenic responsiveness to plaque bacteria (Scott & Singer 2004). Hanioka et al.

(2000a,b) have reported that smokers exhibit a lower gingival oxygen sufficiency in healthy gingival sites compared to non-smokers. This suggests that smoking might lead to functional impairments in the gingival microcirculation. In addition, the lowered oxygen supply in smokers' periodontal pockets might be associated with alteration of the subgingival microflora. However, no major qualitative differences in the subgingival microflora between smokers and non-smokers have been observed, and no specific microorganism has been detected in smokers that could not be found in non-smokers or vice versa. The quantitative differences that have been observed might be explained by the deeper periodontal pockets - that are usually exhibited in smokers - act as a habitat for selected microorganisms. It seems more likely, therefore, that the possible differences in the prevalence of the microorganisms are related to differences in the probing depth between smokers and non-smokers and not to the smoking effect itself.

Furthermore, nicotine as well as cigarette smoke have detrimental effects on bone cells and osteoprogenitor cells (Gullihorn et al. 2005). The osteoclastic effects of nicotine are marked by the elevated levels of the inflammatory mediators of bone resorption in the gingival cervicular fluid of smokers such as TNF-α, interleukin-4, interleukin-6, and interleukin-8 (Boström et al. 1998, Giannopoulou et al. 2003b).

Finally, the inhibition effect of nicotine on the fibroblast cells proliferation and their collagen production is dose-dependent. The impairment of these functions by nicotine may limit the host defense system and may explain the increased severity of periodontal disease in smokers (Giannopoulou et al. 2001).

In summary, the present thesis has added to the literature that beside cigarette, cigar and pipe smoking, water pipe smoking is a smoking habit that should be considered in periodontal health. The present observations have provided convincing evidence to support the role of tobacco smoking as a factor associated with periodontal disease. Future studies are needed to explore the role of water pipe smoking in periodontal health and the mechanisms involved in the periodontal breakdown. Prospective longitudinal studies should help to confirm the present findings.

CONCLUSIONS

On the basis of the results obtained, it is concluded that:

- Tobacco smoking is associated with a suppression of the gingival bleeding response to plaque accumulation. A suppressive effect was observed in both cigarette and water pipe smokers (Study I).
- Tobacco smoking is negatively associated with periodontal health in terms of mean probing depth and number of diseased sites. Both cigarette and water pipe smokers exhibited an elevated probing depth (Study II).
- Tobacco smoking is associated with a reduction of the periodontal bone height. The association was of similar magnitude in cigarette and water pipe smokers (Study III).
- Tobacco smoking is not associated with a particular periodontal microflora. The prevalence of the microorganisms investigated was largely the same in cigarette smokers, water pipe smokers and non-smokers (Study IV).

GENERAL CONCLUSION

Tobacco smoking is associated with inferior periodontal health. The association with water pipe smoking is of largely the same magnitude as that with cigarette smoking. The association between tobacco smoking and an inferior periodontal health seems to be independent of the subgingival microflora.

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Appendix. **The questionnaire used during the interviews**

Name: **Tel. No:** **Code:**

Gender: ❒ M **❒ F** **Age:** **years**

1. What is your educational level?
   ❒ No education ❒ Primary school ❒ Intermediate school
   ❒Secondary school ❒ University level

2. How often do you visit the dental clinic?
   ❒Regular check up ❒Pain only ❒No visits

3. Frequency of tooth brushing/ day
   ❒Once ❒Twice ❒More than twice ❒Never

4. Do you smoke?
   ❒No, never smoke .
   ❒Yes, but I stopped smoking …….. Years / Months ago.
   ❒Yes, ❒Cigarette ❒Water pipe ❒Both

5. Duration of smoking?
   - ❒ 1-5 years
   - ❒ 6-10 years
   - ❒ 11-15 years
   - ❒ 16-20 years
   - ❒ More than 20 years

6. Number of cigarettes / day?
   - ❒ 1-5 cigarettes
   - ❒ 6-10 cigarettes
   - ❒ 11-15 cigarettes
   - ❒ 16-20 cigarettes
   - ❒ More than 20 cigarettes

6. Number of water pipe runs/ day?
   - ❒ 1 run
   - ❒ 2 runs
   - ❒ 3 runs
   - ❒ 4 runs
   - ❒ 5 runs
   - ❒ More than 5 runs