

Network of Academic Health Centers, Department of General Practice and Primary Health Care, Institute of Clinical Medicine, Unit of General Practice, Helsinki University Central Hospital, Institute of Dentistry, and Department of Public Health, Hjelt Institute, University of Helsinki, Helsinki, Finland

ORAL HEALTH, SMOKING AND ADOLESCENCE

Anna Maria Heikkinen

ACADEMIC DISSERTATION

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Supervised by
Professor Jukka H. Meurman, MD, DDS, PhD
Institute of Dentistry, University of Helsinki, and Department of Oral and
Maxillofacial Diseases, Helsinki University Central Hospital, Helsinki, Finland.
and
Professor Markku Koskenvuo, MD, PhD
Department of Public Health, Hjelt Institute, University of Helsinki, Helsinki, Finland

Reviewed by
Professor Anders Gustafsson, DDS, PhD
Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden
and
Docent Kristiina Patja, MD, PhD
Department of Public Health, University of Eastern Finland,
Department of Lifestyle and Participation, National Institute for Health and Welfare,
Helsinki, Finland

Faculty Opponent
Professor Matti Knuuttila, DDS, PhD
Institute of Dentistry, University of Oulu, Oulu, Finland

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ABBREVIATIONS

<i>A.a.</i>	<i>Aggregatibacter actinomycetemcomitans</i>
AL	Attachment loss
BMI	Body Mass Index
BOP	Bleeding on probing, gingival bleeding
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders
DT	Decayed tooth
FTND	Fagerström test for nicotine dependence
GCF	Gingival crevicular fluid
GEE	Generalized estimating equations
ICD-10	International Classification of Disease
IFMA	Immunofluorometric assay
MMPs	Matrix metalloproteinases
MMP-8	Matrix metalloproteinase-8
NRTs	Nicotine replacement therapies
OD	Optical density units
PCR	Polymerase chain reaction
PD	Pocket depth
<i>P.g.</i>	<i>Porphyromonas gingivalis</i>
<i>P.i.</i>	<i>Prevotella intermedia</i>
PMN elastase	Polymorphonuclear leukocyte elastase
<i>P.n.</i>	<i>Prevotella nigrescens</i>
PR	Prevalence Ratio
RC	Root calculus
RR	Relative risk
SAAVNA	Succinyl-alanyl-alanyl-valine-p-nitroanilide
SRA	Self-rated addiction
<i>T.f.</i>	<i>Tannerella forsythia</i>
<i>T.p.</i>	<i>Treponema denticola</i>
Δ OD405/h	The difference in the optical density units (OD) detected by spectrophotometer at 405 nm before and after 1 hour

ABSTRACT

The present cross-sectional study examined the effect of smoking on oral health in a birth cohort of 15 to 16-year-old Finnish adolescents. The hypothesis was that oral health parameters were poorer among smoking than non-smoking subjects. Furthermore, it was expected that a tobacco intervention program could be effective among the adolescents.

The study was conducted in the Kotka Health Center, Kotka, Finland. Altogether 501 out of 545 subjects (15- to 16-year-old boys [n = 258] and girls [n = 243]) were clinically examined in 2004 and 2005. The sample frame was a birth cohort of all subjects in 1989 and 1990, living in Kotka. A structured questionnaire was also filled in by the participants to record their general health and health habits, such as smoking, tooth brushing, and medication used. The participants were classified into nonsmokers, current smokers, and former smokers. Subgingival pooled plaque samples were taken from the teeth with ≥ 3 mm pockets. Stimulated salivary samples were also collected following the examination.

The subjects were asked from which of seven professional groups (doctors, school nurses, dental nurses, general nurses, dentists, teachers and media professionals) they would prefer to receive information about tobacco. The two most popular groups they picked up were dentists and school nurses. Current smokers (n=127) were then randomly assigned into three groups: the dentist group (n =44), the school-nurse group (n =42), and the control group (n =39). The intervention was based on a national recommendation of evidence based guidelines by The Finnish Medical Society Duodecim ("5A" counseling system). Two months after the intervention, a second questionnaire was sent to the smokers in the intervention groups. Smoking cessation, smoking quantity per week, and self-rated addiction for smoking (SRA) were recorded. The results were analyzed using the R-statistical program.

The results showed that 15% of the subjects had periodontitis. Smokers (25%) had more periodontitis than non-smokers (66%) ($p < 0.001$). Smoking boys (24%) also had more caries lesions than non-smokers (69%) ($p < 0.001$), and they brushed their teeth less frequently than non-smokers. Smoking significantly impaired periodontal health of the subjects, even when the confounding effects of plaque and tooth brushing were adjusted. Smoking duration and load, as calculated in pack-years, intensified the effects of smoking, but these did not affect the periodontal attachment loss. Periodontal bacteria *Prevotella nigrescens*, *Prevotella intermedia*, *Tannerella forsythia* and *Treponema denticola* were more frequently detected among the smokers than non-smokers, especially among smoking girls. Smoking significantly decreased the values of both the salivary periodontal biomarkers MMP-8 ($p = 0.04$) and PMN elastase ($p = 0.02$) in boys. The effect was strengthened by pack years of smoking (MMP-8 $p = 0.04$; elastase $p = 0.01$).

Of those who participated in the intervention, 19 % quit smoking. The key factors associated with smoking cessation were best friend's influence, nicotine dependence and diurnal type. When the best friend was not a smoker, the risk ratio (RR) of quit smoking after the intervention was 7.0 (CI 95% 4.6–10.7). Of the diurnal types, the morning people seemed to be more likely to quit (RR 2.2 [CI 95% 1.4–3.6]). Nicotine dependence also elicited an opposite effect: those who scored between 3 and 5 dependence scores were less likely to quit.

In conclusion, smoking appears to be a major etiological risk factor for oral health, regarding both the clinical effects and when assessed using the periodontal inflammatory markers. However, the early signs of periodontal disease were mild in the subjects studied. Based on the opinions of the adolescent's, dental professionals may have a key position in their smoking cessation. The harmful effects of smoking on oral health could be used in counselling. Best friend's influence, nicotine dependence and diurnal type, all factors associated with smoking cessation, should be taken more carefully into account in the prevention programs for adolescents.

LIST OF ORIGINAL PUBLICATIONS

Study I: Heikkinen AM, Pajukanta R, Pitkäniemi J, Broms U, Sorsa T, Koskenvuo M, Meurman JH. The effect of smoking on periodontal health of 15- to 16-year-old adolescents. *J Periodontol* 2008;79:2042-7.

Study II: Heikkinen AM, Broms U, Pitkäniemi J, Koskenvuo M, Meurman J. Key factors in smoking cessation intervention among 15-16-year-olds. *Behav Med* 2009;1;35:93-9.

Study III: Heikkinen AM, Sorsa T, Pitkäniemi J, Tervahartiala T, Kari K, Broms U, Koskenvuo M, Meurman JH. Smoking affects diagnostic salivary periodontal disease biomarker levels in adolescents. *J Periodontol* 2010;81:1299-307.

Study IV: Heikkinen AM, Pitkäniemi J, Kari K, Pajukanta R, Elonheimo O, Koskenvuo M, Meurman JH. Effect of teenage smoking on the prevalence of periodontal bacteria. *Clin Oral Investig* 2011 Feb 22. [Epub ahead of print]

1. INTRODUCTION

Smoking is not only a leading cause of preventable diseases and premature death (Doll et al. 2005), but cigarette smoke contains at least 500 potentially toxic substances. Thus, health professionals have good reason to encourage smoking cessation, especially in the case of adolescents. In Finland, as reported in most Western countries, smoking has been slowly decreasing both in men and women. However, the habit of smoking seems to be associated with low socio-economic status. Although smoking among teenagers has also decreased slightly in Finland during the past ten years, smoking is associated with other types of unhealthy behaviors, such as alcohol consumption (Paavola et al. 2004). Subsequently all efforts to control smoking are worthwhile; in particular, by supporting teenagers to quit smoking one might be able to prevent them adopting worse health habits. Some recent studies support that alcohol abuse is associated with especially tobacco use and nicotine dependence (Li et al. 2007, Bierut et al. 2000).

People usually initiate smoking at age 13 to 15, and smoking behaviour is typically influenced by a social environment as a predisposing factor and nicotine dependence. Smoking cessation is difficult for many adults and adolescents alike. According to Broms et al. (2004), Kemppainen et al. (2006) and Rogacheva et al. (2008), the factors predicting cessation include social environment; especially peer influence, age of initiation and the nicotine dependence. Alcohol use and use of other tobacco products have shown to be associated with smoking initiation (O'Loughlin et al. 2009).

Health professionals in Finland, including school nurses and dental professionals, meet adolescents regularly, almost annually. Nevertheless, only a few studies in the field of preventive dentistry have highlighted the importance of counseling in smoking cessation, albeit the results from these studies have been encouraging (Cohen et al. 1987, Stevens et al. 1995, Kentala et al. 1999, Albert et al. 2006).

Smoking is a major health risk contributing to many diseases, such as heart disease, and malignancies of the lungs and other organs (Doll et al. 2005). Smoking has also been showed to be a major risk factor for dental health, including oral cancer and precancer, periodontal disease, caries and tooth loss, gingival recession, benign mucosal disorders, and implant failure (Warnakulasuriya et al. 2010). The effects of smoking are both local and systemic (Baharin et al. 2006, Meyer et al. 2008).

Periodontal disease is a chronic, destructive condition affecting a large portion of the adult population of Finland, and is one of the major causes of tooth loss in adults in general (Papanou et al. 1996). It is characterized by chronic oral bacterial infection which results in inflammation of the gums with gradual destruction of periodontal tissues and loss of alveolar bone support (Irfan et al. 2001, Michaud et al. 2007). According to the Finnish Health 2000 Survey, 64% of the dentate population had periodontitis (at least one tooth with a pocket of ≥ 4 mm), ranging from 48% in the youngest age group (from 30 to 34 year-olds) to

70% in the oldest of more than 65 years of age. In this regard, periodontitis is a major health problem in the population of Finland. Compared to non-smokers, smokers present more deep pockets, loss of bone height and loss of attachment (Machuca et al. 2000, Shimazaki et al. 2006, Bergström J 2004a, Bergström J 2004b, Martinez-Canut et al. 1995). The magnitude of the problem is emphasized because periodontal disease may also contribute to the pathogenesis of cardiovascular disease (Meurman et al. 2004). However, although the detrimental effect of smoking on periodontal health is evident in adults, there are not many studies on adolescents in this regard, which was the background for the series of studies reported here.

The principal focus of this investigation was the effects of smoking on oral health of adolescents, and the factors contributing to smoking cessation among teenagers. One particular interest was to investigate how smoking cessation programs could be developed in the future and what might be the role of dental health care personnel in such programs. The outcome of this study may also help to identify adolescents who are at risk for developing periodontal disease. The focus was only on cigarette smoking as smokeless tobacco is still rare among adolescents in eastern Finland, Kotka.

2. REVIEW OF THE LITERATURE

2.1 Smoking and oral health

Smoking is a risk factor for periodontal disease in adults and its detrimental effects increase with age (Bergström & Boström 2001, Haffajee & Socransky 2001, Baljoon et al. 2005). Pindborg et al. (1949) already found an association between acute necrotizing ulcerative gingivitis and smoking.

In general, smoking stains the teeth markedly, which is more often observed in men than women (Ness et al. 1977). Bad breath is commonly caused by smoking and both the sense of smell and taste are affected by tobacco (Pasquali 1997). Smoking has been shown to be a risk factor for oral cancer and leukoplakia (Winn 2001). According to the studies of Blot et al. (1988) and Hayes et al. (1999) cigarette smokers have two to five times increased risk for oral cancer than non-smokers, however, Shanks & Burns 1998 reported that cigar smokers could have seven to ten times more at risk to develop oral cancer than non-smokers and the risk is elevated by the depth of inhalation and the number of cigarettes smoked daily. In Finland oral cancer is developing quite slowly and the incidence is low, of men 1.3% and of women 0.8% are reported to have oral cancer according to Duodecim. Although former smokers have a lower risk for oral cancer than current smokers, they still have a three times increased risk for oral cancer in the ten years following cessation of smoking when compared to non-smokers (Schlecht et al. 1999). Cancer risk is highest in those smokers who abuse alcohol (Blot et al. 1988, Adewole et al. 2002 and Cruz et al. 2002). Cigarette smoke contains many carcinogens such as N-nitrosamines, aromatic amines, and polycyclic aromatic hydrocarbons, which are classified to be the most harmful in the development of oral cancer (IARC 2004)

Smoking is associated with oral mucosal conditions such as nicotinic stomatitis and hairy black tongue but also with oral leukoplakia (Meraw et al. 1998). Leukoplakia changes may develop to oral cancer (Gupta et al. 1995). Leukoplakias at the lateral borders of the tongue are more common among non-smokers than non-smokers and oral precancer changes in the floor of the mouth are also linked with smoking (Schepman et al. 2001). Smokeless tobacco is a strong risk factor for both leukoplakia and snuff-induced lesions (IARC 2007).

2.1.1 Smoking and caries

Smoking is a known risk factor for dental caries, although the mechanisms involved are not known (Vellappally et al. 2007). Locker (1992) and Jette et al. (1993) have shown an association between smoking and a higher rate of dental caries in older age. Axelsson et al. (1998) observed that 35-year-old non-smokers had less decayed, filled and missing surfaces than smokers. Smoking might be associated with poor oral health behavior according to Sgan-Cohen et al. (2000) who found smoking to be linked with untreated caries among young adults. Bruno-Ambrosius et al. (2005) showed in their study that young smokers (eight grade students) exhibited significantly higher mean decayed, missed and filled surfaces index (DMFS) increment than non-smokers. Albandar et al. (1995) observed an association between caries lesions and the progression of periodontal diseases in adolescents.

2.1.2 Smoking and periodontal disease

Both local and systemic factors affect periodontal health (Baharin et al. 2006). In this regard, cigarette smoking is a major environmental risk factor for periodontal disease (Haber et al. 1993). The focus of this section is on the effects of smoking on periodontal health, especially in teenagers.

2.1.3 Studies of smoking and periodontal diseases

The risk effect of smoking on periodontal health has been established by several cross-sectional (Grossi et al. 1995, Dolan et al. 1997) and longitudinal studies (Bergström et al. 2000b, Bergström 2004b, Beck et al. 1997). Further, there are longitudinal studies that correlate with the cross-sectional studies by comparing the risks of periodontal disease progression between smokers and non-smokers (Airila-Månsson et al. 2005, Baljoon et al. 2005). According to one of the largest epidemiological studies, the National Health and Nutrition Examination Survey (NHANES III), which involved 12329 adults 20 years or older, smokers were 4 times more likely to have periodontitis when compared with non-smokers; heavy smokers (> 31 cigarettes or more a day) had higher risk than light smokers (Tomar & Asma 2000).

Young smoking adults were 3 times more likely than non-smokers to get at least one site with 4 mm or more attachment loss in the study conducted by Hashim et al. (2001). The risk for aggressive periodontitis in 14- to 29-year-olds was 3-times higher in moderate or heavy smokers than in non-smokers (Susin & Albandar

2005). It seems that smoking has been more strongly linked with the generalized form of aggressive periodontitis than localized one (Schenkein et al. 1995, Mullally et al. 1999).

2.1.4 Influences of smoking on periodontal health

Smokers have more loss of periodontal bone height than non-smokers (Bergström et al. 1991, Bergström 2004a, Bergström 2004b, Hashim et al. 2001). Smokers have deeper probing depths (Machuca et al. 2000, Shimazaki et al. 2006) and are reported to have more supragingival calculus than non-smokers (Kerdvongbundit et al. 2000). According to Martinez-Canut et al. (1995) smoking one cigarette or up to 10, and up to 20 cigarettes daily probing attachment level was increased by 0.5%, 5% and 10%, respectively. However, Shimazaki et al. (2006) have demonstrated that past and current smoking can reduce gingival bleeding. Tobacco is a vasoconstrictor causing ischemia (Balaji 2008). Smokers indeed have less gingival bleeding on probing than non-smokers eventually because smoking masks the effects of inflammation (Bergstrom & Boström 2001, Shimazaki et al. 2006). There are studies suggesting that the effect of smoking on alveolar bone loss is strongest in the maxillary arch (Mullally et al. 1999), especially on the palatal side (Kamma et al. 1999). The local effect of smoking on palatal areas is possibly strongest when smoke is being inhaled. The effect of smoking is stronger in men than in women (Calsina et al. 2002). Young smokers with aggressive periodontitis seem to have more affected teeth and a higher mean loss of periodontal attachment than non-smokers (Schenkein et al. 1995, Mullally et al. 1999).

2.1.5 Dose-effect of smoking and periodontal health

Cigarette consumption and duration of smoking are associated with the severity of periodontal disease. The more tobacco is smoked the more periodontal attachment loss has been observed (Martinez-Canut et al. 1995). Smoking is associated with a 2 to 8-fold increased risk for periodontal attachment and /or bone loss, depending on the definition of disease severity and smoking dose (Bergström et al. 2000a, Calsina et al. 2002, Martinez –Canut et al. 1995). In a study by Calsina et al. (2002), Spanish adults over the age of 20 were discovered to have a 2.7 times probability to gain periodontitis, which increased to 3.7 times when they had smoked for more than 10 years. More attachment loss was observed in young male heavy smokers (Machuca et al. 2000). In another study among 12-21 year-old students, subjects with the highest smoking exposure had the highest odds for clinical attachment loss (Lopez et al. 2001). Mullally et al. (2000) reported in their study on 612 subjects aged 14 to 29 years that those displaying generalized early onset periodontitis smoked more than those with a localized form of periodontitis.

2.1.6 Periodontal bacteria

Aggregatibacter actinomycetemcomitans (A.a.), *Porphyromonas gingivalis* (P.g.), *Tannerella forsythia* (T.f.), *Prevotella intermedia* (P.i.), *Prevotella nigrescens* (P.n.), and *Treponema denticola* (T.d.) have been shown to be involved in the aetiology of periodontitis. These strains are considered periodontal disease indicator bacteria (Zambon et al. 1996). Haffajee et al. (1998) described T.f, P.g. and T.d. to constitute the “red

complex” since they were most significantly increased in the periodontitis subjects expressing progressive disease, while *A.a.* belongs to the “green complex” and hence less virulent bacteria. According to Chen et al. (2005) these species are found less frequently in shallow than in deep pockets. Although, red complex species are also found in a small proportion of sites in healthy subjects, higher levels are detected in diseased sites (Ximenez-Fyvie et al. 2000). “Orange complex” bacteria of Haffajee et al. (1998) such as *P.i.* and *P.n.* are also found in periodontitis subjects (Meyer et al. 2008). The *Prevotella* species are often detected in patients with gingival inflammation (Tanaka et al. 2008).

There are only a few published studies on periodontal bacteria flora in adolescents. The mechanism how these bacteria primary colonize the oral cavity is unknown. In the study of Kimura et al. (2002) *P.g.* and *T.d.* were not detected in periodontally healthy children between 2-13 years of age. Timmerman et al. (1998) reported in Indonesian adolescents with untreated periodontal disease that no significant association was observed between clinical periodontal parameters and the prevalence of the certain bacteria, but both *P.g.* and spirochetes were more prevalent in the sites with attachment loss. Umeda et al. (2004) reported in their study among Japanese children that plaque has shown to promote the colonization of periodontal pathogens such as *T.f.*, *P.i.*, *P.n.* and *T.d.* in the oral cavities of children.

The role of certain periodontal bacteria in the pathogenesis of gingivitis which further develops to periodontitis with attachment loss has been investigated previously (Albandar 2002). *A.a.* has been identified in young persons with rapid disease progression (Slots & Ting 1999), however Albandar et al. (1997) only found low prevalence of this micro-organism in periodontitis patients. Furthermore, Albandar et al. (1997) reported that *P.g.*, *T.d.* and *P.n.* are significantly associated with the generalized and/or rapidly progressing forms of aggressive periodontitis in young adults; it was reported that those subjects with generalized periodontitis had a 16-fold increase in *P.g.*, 5-fold increase in *T.d.* and 2.5-fold increase in *P.i.* compared with those without progressive disease. Mombelli et al. (1995) reported very low levels of *A.a.* and *P.g.* in adolescents in puberty, but Ellwood et al. (1997) observed that *P.g.* was frequently associated with deeper pockets and bleeding sites in 11- to 13-year-old children. However, Tanner et al. (2006) proposed that *T.f.* collected from subgingival samples is associated with early adult periodontitis. Narayanan et al. (2005) showed in their study of an adolescent population (N=228 aged 11-13 years) that 25% carried *T.f.* Shimomura-Kuroki et al. (2009) reported that in 11 to 16 years old subjects, *T.f.* was detected in the deepest periodontal pockets and associated with periodontal disease, and the bacterium was also related to localized aggressive periodontitis.

2.1.7 Smoking effects on oral microbiota

In addition to periodontal bacteria specifically, smoking may cause changes in the bacteria of plaque in general and affect the host response to the plaque (Hilgers & Kinane 2004). Nonetheless, there are studies published suggesting that not only does smoking not affect subgingival plaque (Lie et al. 1998, Darby et al.

2000, Boström et al. 2001), but also that no statistically significant difference is found in the prevalence of any of the bacteria between smokers and non-smokers (Stoltenberg et al. 1993). In contrast, Mager et al. (2003) showed that experimental gingivitis induced changes in the supra- and subgingival plaque in both smokers and nonsmokers, but almost no changes were found in the microbiota of the oral mucous membranes.

Subgingival bacteria does differ between smokers and non-smokers (Kamma et al. 1999, Eggert et al. 2001, van Winkelhoff et al. 2001). Zambon et al. (1996) reported that smokers harboured subgingivally significantly higher levels of *B. forsythus*, whilst Haffajee and Socransky (2001) suggested that the more severe periodontitis in smokers may account for the differences in subgingival bacterial profiles.

In an earlier study of the same group (Haffajee et al. 1998) the prevalence (percentage of sites colonized) of *P.i.* and *P.n.* and the species *P.g.*, *T.f.* and *T.d.* was significantly higher in smokers than in past smokers or non-smokers. Naryanan et al. (2005) reported that *T.f.* positive male smokers showed increased disease severity compared with *T.f.* negative subjects. Könönen et al. (2007) reported that daily smokers had not only *T.d.* but also *P.g.* and *P.i.* in saliva more frequently than non-smokers, although they investigated an adult population. The risk of having *T.d.* in saliva was 5-fold higher among current adult smokers than in non-smokers (Umeda et al. 2004). However, Cortelli et al. (2008) found no correlation between smokers and non-smokers in the prevalence of *A.a.*, *P.g.*, *T.f.* or *P.i.* Herrera et al. (2008) found differences in the microbiology depending on the disease severity and smoking status of the subjects. As mentioned however, the effect of smoking on periodontal pathogens in adolescents has not been thoroughly investigated.

2.2 Smoking and effects on oral host response

Smoking affects the immune system and impairs host defenses by inhibiting granulocyte function (Söder et al. 2002). Subsequent nicotine metabolites cause vasoconstriction and impair the function of polymorphonuclear cells (PMN) and macrophages and decrease the number of lymphocytes which may also affect B-cell and antibody production (Barbour et al. 1997). Smoking increases the number of neutrophils in peripheral blood but their ability to migrate through capillary walls is dampened because of the paralysis of the cell membrane (Hind et al. 1991). PMNs elastase proteinases are released during phagocytosis from the neutrophils (Lindhe et al. 2003). Gingival crevicular fluid levels of functional elastase have been shown to be lower in smokers than non-smokers (Alavi et al. 1995). Pauletto et al. (2000) reported that smoker levels of salivary elastase are lower than those in non-smokers. This might be due to the impaired migration of neutrophils through the gingival crevice to the saliva which, in turn, might cause accumulation of elastase in the periodontium and finally cause tissue destruction. Pauletto et al. (2000) further observed that smoking contributes to the activation of monocytes which, unlike PMNs, direct an antigen response to lipopolysaccharides leading to the secretion of cytokines. Of these, prostaglandin PGE₂, for example, is linked with aggressive or early onset periodontitis (Offenbacher 1996). Nicotine also affects the lifespan and

activation of neutrophils reducing their ability to react against bacterial invasion of periodontium. Tobacco and smoking appear to modify the immune system by exposing B- and T-lymphocytes and thus reducing the production of protective immunoglobulins against oral pathogens (Barbour et al. 1997). The effects of tobacco on periodontium have recently reviewed by Laxman et al. (2008).

2.2.1 MMP-8 and PMN elastase as periodontal inflammatory biomarkers

Matrix metalloproteinases (MMPs) are structurally related but genetically distinct endopeptidases with important roles in the regulation of host response to inflammation (Sorsa et al. 2004). They are involved in the degradation of extracellular matrix and basement membranes and play a role in the key pathogenic mechanisms of periodontal disease (Sorsa et al. 2004). MMP-8 is mainly secreted by neutrophils, but can also be expressed by non PMN cells such as fibroblasts, endothelial cells, epithelial cells, plasma cells, macrophages and bone cells (Sorsa et al. 2004, 2006; Hanemaaijer et al. 1997). MMP-8 is the main collagenase in inflamed gingival tissue and can be analyzed from gingival crevicular fluid (GCF) and saliva (Sorsa et al. 2004, 2006, 1988; Tervahartiala 2000). For example, salivary MMP-8 may reflect the severity of periodontitis in adults (Uitto et al. 1990, Ingman et al. 1993).

PMN elastase is released during phagocytosis from degranulating neutrophils and it cleaves natural substrates such as collagen and proteoglycans (Froeschle et al. 1983). It has shown to be increased in inflammation, such as gingivitis (Giannopoulou et al. 1992) and in periodontitis (Giannopoulou et al. 1992, Eley et al. 1992a, Ingman et al. 1994, Meyer et a. 1997, Jin et al. 2002). It has also been demonstrated that GCF elastase levels are significantly higher in sites with progressive periodontal attachment (Palcanis et al. 1992).

2.2.2 The effect of smoking and MMP-8 and PMN elastase

Smoking appears to affect the periodontal inflammatory biomarkers of saliva, possibly impairing salivary levels of cytokines and enzymes, however controversial reports have been published in the literature. From GCF analyses Mäntylä et al. (2006) reported that the mean MMP-8 concentrations in adult smokers were lower than in non-smokers. However, they discovered that the sites with progressive periodontal disease expressed similar MMP-8 concentrations irrespective of smoking status. Raitio et al. (2005) did not identify any differences in MMP-8 levels between smokers and non-smokers. The finding confirmed earlier results by Liede et al. (1999), who observed lower salivary MMP-8 levels in adult smokers than non-smokers. Correspondingly, Pauletto et al. (2000) observed that in salivary samples of adult patients with chronic periodontitis, smokers had lower elastase levels than former smokers or non-smokers. No studies on MMP-8 or PMN elastase have, however, been published in young smokers.

2.2.3 Body Mass Index (BMI) and periodontal inflammatory biomarkers MMP-8 and PMN elastase

Many epidemiological studies have observed an association between obesity and periodontitis (Saito et al. 2001, Linden et al.2007, Al-Zahrani et al.2003) also observed this link among young subjects. Ylöstalo et al. (2008) observed, using the Finnish National Health 2000 Examination survey, an association between body weight and periodontal infection among the non-diabetic, non-smoking population aged 30–49. However, the relationship between tobacco smoking and salivary inflammatory biomarkers, such as MMP-8 and PMN elastase in adolescents is an unknown.

2.3 Smoking and adolescence

This section reviews the prevalence of teenage smoking in 14 to 16-year-old girls and boys. Smoking initiation and behavior is affected by specific factors such as the number of cigarettes smoked daily, starting age of smoking, influence of the best friend and parents, nicotine dependence, gender, stress and diurnal type.

2.3.1 Prevalence of smoking in Finland

Smoking has become more rare in Finland but population-based surveys still estimate that the prevalence of daily smoking is about one-fifth among the adult population (22% of men, 16% of women) (Helakorpi et al. 2010). According to the latest national report (Rainio et al. 2009, NNTT 2009) daily use of cigarettes among 14 to 16 year-old boys has, however, recently increased (from 2007 to 2009), having decreased earlier in the beginning of 1990 until 2007. The trend for 14-year-old girls, on the other hand, shows that the daily smoking increased from the beginning of 1980 until 2001, then decreased till 2007. From 2007 to 2009 smoking prevalence again increased among the girls. The trend for 16 to 18-year-old girls shows that the decrease in daily smoking has been slowed down. A recent study shows that the number of smoking adolescents has slightly decreased, but future research will reveal whether the trend observed is true or not (Raisamo et al. 2011, NNTT 2011).

The national report from the year 2005 on youth in Finland shows that 15% of boys and 13% of girls smoked at the age of 14 to 15 and that the respective figures at the age of 15 to 16 were 22% and 18%, respectively (Rimpelä et al. 2005, NNTT 2005). In the Kymenlaakso Regional Hospital of Kotka in Finland, where the present study was made, however, smoking rates among 14- to 16- year-olds were 11% for boys and 13% for girls (Kymenlaakso Regional Hospital School Health Report, 2005).

The latest national report on youth in Finland shows that 8% of girls and boys smoked daily at the age of 14 years and that the respective figure at the age of 16 years was 23% (NNTT 2009). At the age of 18 years 29% of boys and 26% of girls smoked daily (Rainio et al.2009). Experiments with smokeless tobacco are another issue and it seems to be increased lately, especially among boys. In 2009 of 12-year-old boys 12% had tried snuff; at the age of 16 the percentage was 21%, and at the age of 18 snuff had been used by 41%, respectively, according to national report (NNTT 2009). Using snuff is rare among girls.

2.3.2 Factors associated with smoking in adolescence

Multiple factors influence the initiation and maintenance of smoking behaviour. There are physiological, psychological, and social factors influencing both smoking initiation and cessation. Adolescent smoking status predicts smoking in the adulthood (Paavola et al. 1996, Krainuwat 2005). Therefore, it is essential to know the factors associated with smoking in adolescence. The following section focuses particularly on starting age, gender, best friend and parental influence, the number of cigarettes smoked, nicotine dependence, smoking and diurnal type, and smoking and stress. Socio-economic factors are also important but are here left out from discussion.

2.3.2.1 Starting age and gender

Smoking initiation in Finland is between the ages of 12 and 15 (Rimpelä et al. 2005). Those with parents of higher level of education typically start smoking later (< 17 years of age) (Palombini et al. 2001). Further, it has been shown that if a teenager has not experienced smoking by the age of 14 he or she does not become a smoker so easily (Pulkkinen et al. 1988). Few studies in adolescents have shown that the age of smoking initiation is significantly related to daily smoking (Everett et al. 1999). It is reported that early initiation of smoking behavior in childhood is a strong predictor of regular smoking in later adolescence and the odds of becoming a smoker are increased four to six times compared to those who never try as adolescents (Krainuwat 2005). Students of USA high schools, who began smoking at the age 12 years or younger, were shown to be more likely to be regular or heavy smokers than the older students (Escobedo et al. 1993). Chassin et al. (1990) have reported that even an infrequent experimenting in adolescence significantly raises the risk for adult smoking. According to the study of Khuder et al. (1999) men who started smoking earlier than the age of 16 years were less likely to quit smoking, with odds of 2:1, compared with those who started later. Nordström et al. (2000) reported that younger smokers were those continuing to smoke, although this study concerned only men. The studies of Broms et al. (2004) and Ellickson et al. (2001) showed that those who start smoking at an early age are less likely to quit. Chen & Millar (1998) suggested that physical dependence on nicotine is greater if the person starts smoking at a younger age and an early age of smoking initiation could also mean that the psychological and/or social factors that contribute to dependency are stronger.

The studies of gender effect on smoking cessation are inconsistent. According to Perkins et al. (1999) the smoking behavior of women may be influenced more by non-nicotine stimuli associated behaviour than that in men. According to meta-analysis study by Cepeda-Benito et al. (2004) nicotine replacement therapies (NRTs) indicated to be less effective in female smokers. Patton et al. (1998) reported in their study of teenagers, aged 14 to 15 years, that female daily smokers were half as likely as males to cease smoking. Wetter et al. (1999) observed that men had higher cessation rates than women at follow-up. However, Chen et al. (2001) found no gender differences, while Weden et al. (2006) reported that joblessness was more strongly associated with persistent daily smoking in women than in men. There are many studies suggesting

no gender differences were found in the rates of smoking cessation (Puente et al. 2011, Gritz et al. 1998, Chatkin et al. 2006).

2.3.2.2 The influence of the best friend and parents

Tobacco use in households, especially by parents or siblings, has showed to be a strong predictor for smoking in adolescence (Chandola et al. 2004, de Vries et al. 2003, Kemppainen et al. 2006). It is also associated with smoking cessation (Kemppainen et al. 2006). Parental smoking (Stramari et al. 2009) or smoking of the mother (Zhu et al. 1999) has been associated with not quitting smoking in adolescence. Smoking during adolescence is strongly related to the imitation of peer smoking and, according to study of West et al. (1999), the best-friend effect has shown to be the most significant factor, even stronger than parental smoking, on smoking cessation. Those never-smokers whose peers smoke are likely to initiate smoking over the next year or two (Conrad et al. 1992). Thus the social environment of young people has an important influence on smoking onset. Johnson et al. (2002) reported that the strongest correlate of smoking in eighth grade was having a best friend who smoked and intention not to smoke in fifth grade predicted non-smoking in eighth grade. According to Sasco et al. (2003), having a best friend who smokes, and having a brother and/or sister who smokes, is associated with regular smoking in adolescence. Smoking adolescents with non-smoking friends were more likely to quit by 23 years of age (Ellickson et al. 2001). In the study of Paavola et al. (2001) and Kemppainen et al. (2006), if the best friend smoked then quitting smoking was less probable.

2.3.2.3 Nicotine dependence

The number of cigarettes per day has an influence on smoking cessation (Broms et al. 2004). Sussman et al. (1998) reported that heavy smokers are less likely to quit.. According to Sargent et al. (1998) occasional teenage smokers (smoking less than 1 cigarette per day within the last 30 days) are more likely to quit smoking (OR=6:7) than daily smokers (smoked 1 or more cigarettes per day during the last 30 days). However, many light and occasional smokers could have a high level of tobacco addiction with different pharmacodynamics compared to heavy smokers (Benowitz 2010). Successful cessation among adolescents is linked to both the social environment and nicotine dependence. Indeed, Broms et al. (2004) reported that the nicotine content per cigarette predicts quitting smoking. Colby et al. (2000) suggested that between 20% and 68% of adolescents who smoke could suffer from nicotine dependence, and smoking cessation is predicted by the degree of nicotine dependence (Chandola et al. 2004). DiFranza (2007a) has shown that even adolescents who smoke only one or two cigarettes a week have the same kind of withdrawal symptoms as adults. Furthermore, DiFranza et al. (2007b) suggested that the process of dependence is initiated by the first dose of nicotine. Studies of adolescent smokers show that symptoms of addiction, such as withdrawal, craving for cigarettes and failed attempts at quitting, could occur even within the first weeks of smoking with low exposure such as 1-2 cigarettes per week. Nicotine dependence (ND) diagnosis is defined by DSM-IV (American Psychiatric Association 1994, Diagnostic and Statistical Manual of Mental Disorders) and ICD-

10 (International Classification of Diseases). ND is an impaired control of one's smoking, having withdrawal effects when trying to stop smoking, such a powerful drive to smoke that it overwhelms the strong desire to resist, and adaptation to repeated drug exposure. This syndrome includes a heterogeneous collection of symptoms that cluster to produce a physiological, behavioral and cognitive phenomenon. Nicotine dependence could be measured using the Fagerström test for Nicotine Dependence (FTND) (Heatherton et al. 1989,1991).

2.3.2.4 Other factors

Ishihara et al. (1985) showed in their study of university students that for evening types it was more common to be smokers than for morning types. Diurnal type has been associated with smoking in earlier study of 14 to 94 year olds in German and Austria (Wittmann et al. 2006) too. Recently, Bross et al. (2011) showed that those adults who belong to the group of evening types are more likely to be current smokers and nicotine addicts.

Jones & Parrott (1997) suggested that smokers seemed to be more stressed than non-smokers. A recent study by Park (2009) showed that smoking initiation was linked with loneliness at school, self-control, delinquent behaviour, depressive symptoms, and stress. Those smokers who have had depression period during life time tend to relapse after an attempt to quit (Consequently, Hrubá & Zaloudiková (2010) reported that in children aged 9 and 11 years, about 40% considered smoking an effective way to cope with stress and about 20% of them declared smoking for mood improvement.

Notably, Saarni et al. (2009) observed that adolescent smoking significantly increased the risk of becoming overweight among women when they smoked at least 10 cigarettes daily; smoking at the age of 16 to 18 years increased the risk of adult abdominal obesity with an odds ratio (OR) of 1:77 (95% confidence interval [CI]=1.39, 2.26).

2.4 Smoking prevention and cessation intervention in adolescents in health care

The intervention programmers have not given much attention to smoking cessation among adolescents. Various reasons for this lack of interest could be assumed. First, it is thought that adolescents are not nicotine dependent and could quit smoking anytime. In fact the origin of tobacco addiction among adolescents has not been studied. The tobacco addiction measurements developed for adults might not be suitable for young smokers whose brains are still developing (Ollila et al. 2010). Amos et al. (2006) reported in their study with 99 16- to 19-year olds that only few adolescent were interested in nicotine replacement therapy or cessation services and that they felt addiction to belong to the world of older addicted smokers. Furthermore, adolescents are assumed to not be willing to stop smoking. Also, according to the study of Albert et al. (2006), cessation programs designed for adults are thought to be effective for adolescents too.

The focus of this section is on smoking cessation intervention in adolescents and counseling in health care, the role of dental care in this regard, and also in the guidelines for smoking cessation.

2.4.1 Smoking cessation and counseling in health care

A three-minute discussion with a doctor has shown to be effective in terms of encouraging tobacco abstinence (Silagy & Stead 2001) and a short advice intervention can increase quitting from 1 to 3% when assumed quit rate is from 2 to 3% (Stead et al. 2008). In health care the counseling by a physician has been found to be the most effective (Fiore et al. 2000, Gorin & Heck 2004). However, interventions by other health care professionals, such as nurses and dentists, have also been reported to be effective in smoking cessation (Rice & Stead 2006, Gorin & Heck 2004). However, smoking cessation is only one of their tasks among many health promoting tasks.

According to the study conducted by Solberg et al. (2007), only 2% and 13% of young adults who smoke receive cessation assistance or follow-up advice from physicians. Notably, in the study of An et al. (2008), smokers who were asked about smoking by two or more types of professionals increased the odds of recent quitting (OR=2.37; 95% CI=1.15-4.88). Other earlier studies had shown physicians' advice to quit smoking to be valuable (Lichtenstein et al. 1996, Ockene 1987). Rice & Stead (2008) pointed in their review that the effect on smoking cessation was weaker when counsellings were short and given nurses who did not have a clear attitude in health promotion.

A pediatric practice-based intervention can be effective in both discouraging the initiation of smoking among nonsmoking adolescents, even for 1 year, and also in increasing the abstinence rates among smokers for 6 months. This randomized, controlled trial was conducted by Pbert et al. (2008) with intervention based on the 5As intervention model (see **Table 1.**) Pbert et al. (2006) reported earlier that a four-session smoking cessation intervention based on the 5As can be effectively delivered also by school nurses and could increase the self-reported short-term abstinence rates among smoking students. Their results showed that students in the intervention schools had 6-week odds of quitting 8 times greater than those in the control schools while at 3 months the odds was still 6 times greater in the intervention group. Finally, according to Cochrane review by Lancaster et al. (2005) there is no evidence of benefit from more intensive counseling compared to brief counseling.

2.4.2 Role of dental care in adolescence

Dental professionals are in a key position to advise patients to quit smoking and a few studies have highlighted the importance of counseling in order to encourage smoking cessation in the field of preventive dentistry (Davis et al. 2005, Albert et al. 2006). A dentist sees the patients regularly and thus even the initial harmful effects of tobacco could be noted. Smoking history could be easily recorded and followed up in dental care and oral examinations. Dental professionals should not just provide health care limited to the oral cavity. However, only 48% of dentists recorded smoking history and less than 27% discussed with their smoking patients routinely according to the study by John et al. (2003). For example, patients in a 12-month tobacco intervention group were more likely to quit (OR 4.85 after three and 5.25 after six months) than those in a control group (Gordon et al. 2005). Only a mere request to stop smoking could be positive in

dental health care (Carr & Ebbert 2007). Similarly for non-smoking adolescents, all that was needed for them to refrain from smoking was a suggestion of not to smoke (Garg et al. (2006).

Smoking counseling was relatively poor in Texas, according to a survey by Hu et al. (2006), which was conducted among 783 dentists. Of them less than 20 % spent 3 or more minutes on smoking cessation per consultation. An et al. (2008) observed that compared to physicians dentists rarely asked their patients about smoking (83% vs. 39%) and offered help in quit smoking much less often (3.4% vs. 32%). However, in a study by Carr and Ebbert (2007) interventions by oral health professionals increased tobacco abstinence rates for 12 months or longer (OR 1.44; 95% CI: 1.16-1.78).

Teenagers regularly visit their dentists or dental hygienists in Finland, however there are few studies on the role of the dentist in the prevention of tobacco use among adolescents. Kentala et al. (1999) showed in their 2-year follow-up study that a mini-intervention by dentists among 13-year-olds resulted in a 3% reduction in smoking.

Recently published review by Nasser (2011) reported that smoking cessation interventions provided by dental settings is an effective method of reducing tobacco use in smokers and users of smokeless tobacco; and in preventing starting smoking of non-smokers. Furthermore, as reported in the review by Needleman et al. (2010), the two RTCs published showed no difference in quit rates of smoking between counseling in dental offices and smoking cessation specialist units.

The National Institutes of Health and the National Cancer Institute recommend using the “5A” counseling system in smoking cessation in dental care. These “5As” contains Ask, Advice, Assess, Assist and Arrange (**Table 1**).

Table 1. Description of the “5A” counseling system according to Lozier et al. 2009.

“5 A”	Description
Ask	All patients should be asked about their tobacco use as frequently as possible
Advice	Patients who identify themselves as tobacco users should be directly advised to quit. Advice should be made personal by noting oral implications of tobacco use that the patient may be experiencing.
Assess	Based on the conversation, the patient’s willingness to quit should be assessed.
Assist	Assistance to quit smoking can be provided by offering informational pamphlets, further coaching on the quitting process, writing prescriptions for NRT, or referral to a quitting program or help line.
Arrange	Arrange for follow-up contact.

3. HYPOTHESES AND AIMS OF THE STUDY

The purpose of the present study was to examine the effect of smoking on oral health in a birth cohort of 15-16-year-old Finnish adolescents. It was anticipated that oral health parameters were poorer among smoking than non-smoking subjects. Furthermore, it was expected that a tobacco intervention and smoking cessation program would be effective among the adolescents. The specific aims were:

1. to study the effect of duration and quantity of smoking on periodontal health of the adolescents taking into account eventual gender differences;
2. to study early signs and differences in periodontitis in the smoking and non-smoking adolescents;
3. to study the prevalence of periodontal bacteria in the subjects and the influence of smoking on the oral microbial profile taking into account the clinical oral health status;
4. to study associations between salivary MMP-8 and PMN elastase values with periodontal health indices and whether a high BMI affects these salivary biomarkers; and
5. to study key factors associated with smoking cessation among the adolescents in a tobacco-intervention program.

4. SUBJECTS AND METHODS

4.1 Study cohort

This cross-sectional study was carried out at the Kotka Health Center, Kotka, Finland. Altogether 501 out of 545 subjects living in Kotka were examined while 44 refused to participate for reasons that remained unknown. On two occasions in 2004 and 2005, 15- to 16-year-old boys (n = 258) and girls (n = 243), volunteered to participate in the study. The sample frame was a birth cohort of all subjects born in 1989 and 1990 and living in Kotka. Of the 501 participants, 66% did not smoke, 25% (n = 127) were current smokers, and 9% were former smokers.

The study protocol was approved by the ethics committee of the Kymenlaakso Central Hospital, Kotka, Finland. The study was conducted according to the principles of the Declaration of Helsinki.

4.2 Methods

4.2.1 Questionnaire I (baseline)

First, the participants filled out a structured questionnaire to record their general health and health habits, such as smoking, tooth brushing, and medication used. The examination was carried out by only one researcher, and the smoking status of the participant was unknown before the clinical examination. Most subjects were healthy. General diseases were rare: allergies (n = 18), respiratory diseases (n = 12), and skin diseases (n = 10).

How many cigarettes were smoked daily or weekly was asked in the questionnaire, including an item concerning the number cigarettes of at the ages of 9, 10, 11, 12, 13, 14, 15 and 16 years. For smokers, the quantity and duration of smoking in pack-years (years x cigarettes smoked/20) was recorded. Pack-years were classified into four categories: non-smokers, low (0.03 to 0.5), medium (0.51 to 1.25), and high (1.26 to 4.75) smokers. Tooth brushing weekly (the number of tooth brushings per week) was recorded.

There were also questions about nicotine dependence (Heatherton et al. 1989, 1991) life satisfaction (Koivumaa-Honkanen et al. 2000) and stress (Reeder et al. 1973), and the responses were rated on based on these widely used validated psychometric scales. Furthermore, the age at which the participants started to smoke, whether their best friend and/or parents smoked, and the potential effect of education and diurnal type of smoking behavior were recorded. Diurnal type was measured according to self-reported feelings of being a morning or an evening person by a question in the 1981 survey based on the Diurnal Type Scale (Torsvall & Åkerstedt 1980). Life satisfaction was assessed on a 4-item scale (Koivumaa-Honkanen et al. 2000). This was used as a proxy for pre-existing depression (Korhonen et al. 2007). Assessment of stress was measured using the 4-item scale developed by Reeder et al (1973). Nicotine dependence was measured using the Fagerström test for Nicotine Dependence (FTND) (Heatherton et al. 1989, 1991). FTND is not a diagnostic measure, but rather a symptom scale usually assessed by questionnaire, and it is widely used in both clinical and research work. FTND comprises six questions, and the score ranges from 0 to 10 (Radzius

et al. 2001). FTND is used as a dichotomous variable, with the cut-off point varying from 2 to 8 depending on the study (Moolchan et al. 2002). The highest dependence rating is acquired by a smoker who smokes large quantities of cigarettes and who smokes prominently in the morning. **Table 2** gives details of the FTND test. In this study FTND was also used as a dichotomous variable so that nicotine dependence was defined if the score was 4 or more (Berrettini et al. 2008, Bierut et al. 2007). Details of all the measures used in questionnaire I are explained in more detail in **Study II**.

Table 2. The six questions of the Fagerström Test for Nicotine Dependence (Heatherton et al. 1989,1991).

1. How soon after you wake up do you smoke your first cigarette?		
5 minutes		-> 3 points
6-30 minutes		-> 2 points
31-60 minutes		-> 1 point
After 60 minutes		-> 0 points
2. Do you find it difficult to refrain from smoking in places where it is forbidden?		
Yes		-> 1 point
No		-> 0 points
3. Which cigarette would hate most to give up?		
First cigarette in the morning		-> 1 point
Some another cigarette		-> 0 points
4. How many cigarettes/day do you smoke?		
1-10 cigarettes		-> 0 points
11-20 cigarettes		-> 1 point
21-30 cigarettes		-> 2 points
31 cigarettes or more		-> 3 points
5. Do you smoke more frequently during the first hours after waking than during the rest of the day?		
Yes		-> 1 point
No		-> 0 points
6. Do you smoke when you are so ill that you are in bed most of the day?		
Yes		-> 1 point
No		-> 0 points

4.2.2 Clinical Examination

After completing the questionnaire, the oral health status was recorded according to the World Health Organization (WHO) criteria in a normally equipped dental clinic (World Health Organization 1980, 1987). There was no pre-study calibration conducted but the examiner was a specially trained dentist measuring the following indexes: Visible Plaque Index (VPI), Bleeding on Probing (BOP) (Ainamo & Bay 1975), Root Calculus (RC), Pocket Depth (PD), and Attachment Loss (AL; considered normal at values below 2 mm (Davidovich et al. 2005, Aass et al. 1994, Nieminen et al. 1995). VPI and RC were recorded from the WHO index teeth and BOP and PD values were recorded for all teeth and at four sites. PD was measured at every tooth and site, but was recorded in the database only if the values were ≥ 3 mm. Bilateral bite-wing x-rays were taken in order to assess bone loss (BL) by measuring the distance from the cemento-enamel junction (CEJ) to the alveolar bone margin mesial and distal from the second molar to the first premolar in each jaw quadrant. The distal site of the second molars and the mesial site of the first premolars were excluded, however. Body Mass Index (BMI) (Cole et al. 2000) was calculated based on anthropometric measurements. A written statement by a radiologist was available. The examiner was a priori unaware of the smoking status of the subjects.

4.2.3 Plaque samples

The inclusion of subjects was based on a pre-study power calculation showing that at least 260 subjects were needed to observe an anticipated difference of approximately 20% between smokers and non-smokers. Subgingival samples were taken from 264 participants, of whom 166 were non-smokers, 15 were former smokers and 83 were current smokers. Of the current smokers 44 were boys and 39 were girls, which is in line with the gender distribution of smokers in Finland. Subgingival pooled plaque samples were taken from the teeth with ≥ 3 mm pockets using a sterile paper point after drying and isolating the tooth with cotton rolls. If the subject did not have any deep periodontal pockets then a sample was taken from shallow sites. The subgingival pooled plaque samples were placed in 100 μ l of sterile water and stored at -75°C . Polymerase chain reaction (PCR) analysis was used to detect the putative periodontal pathogens *A.a.*, *P.g.*, *T.f.*, *P.i.*, *P.n.*, and *T.d.* with specific primers as given by Wahlfors et al. (1995) and Meurman et al. (1997), with slight modifications. Briefly, the thawed samples were centrifuged at 2100 x g for one minute, and 5 μ l aliquots of the supernatants were added to the PCR reaction mixture, final volume 50 μ l. The enzyme used was Dynazyme II Hot Start DNA Polymerase (Finnzymes, Espoo, Finland). The GeneAmp® PCR System (Perkin-Elmer Corporation, Norwalk, CT, USA) was used for the PCR amplification. The PCR products were visualized by UV light after electrophoresis on agarose gel containing ethidium bromide.

4.2.4 Saliva samples

After clinical examination, salivary samples were collected. Stimulated saliva (about 5 ml) was collected between 8 a.m. and 3 p.m. First the subjects rinsed their mouths with water and were then given a 1 g piece of paraffin wax to chew. The samples were centrifuged at 1000 x g for 5 minutes immediately after

collection and the supernatants were used for the enzyme studies. The samples were immediately frozen and kept at -20°C until assayed (Uitto et al. 1990).

For assessment of MMP-8 levels ($\mu\text{g/l}$) the salivary samples were analyzed by time-resolved immunofluorometric assay (IFMA) (Hanemaaijer et al. 1997, Mäntylä et al. 2006, Sorsa et al. 2010). The increase in optical density units (OD) was detected by spectrophotometer at 405 nm before and after 1 hour incubation (Nieminen et al. 1993). The difference in the OD values was used as the measure of elastase activity ($\Delta\text{OD}_{405}/\text{h}$). The details are given in **Study III**.

4.2.5 Intervention study

The participants were classified into 3 groups: nonsmokers, current smokers, and former smokers. The subjects were also asked from which of seven professional groups (doctors, school nurses, dental nurses, general nurses, dentists, teachers and media professionals) they would prefer to receive information about tobacco. The 2 most popular groups were dentists and school nurses. The participants were then randomized accordingly, as discussed later and shown in **Figure 1**.

Of the current smokers, 61 were boys and 66 were girls ($n = 127$) and 44 reported having stopped previously. These respondents were randomly assigned into 3 groups, the dentist group ($n = 44$), the school-nurse group ($n = 42$), and the control group ($n = 39$). The information was based mainly on a national recommendation of evidence-based guidelines by The Finnish Medical Society: “ask about the patient’s tobacco use, assess the patient’s willingness to quit smoking, keep an account of tobacco use (record the amount and duration of smoking), advise the patient to quit (commence treatment when necessary), assist the patient in quitting (give positive feedback and refer for further treatment when appropriate), and arrange monitoring in ensuing visits”. The willingness to change smoking behavior was assessed, applying the Stages of Change Model (Prochaska & DiClemente 1983). Those who were interested in stopping were shown an animation picturing the effect of nicotine-molecules on the brain (www.paihdelinkki.fi). Both the dentist and school nurses used the same intervention material, although the school nurses spent more time than the dentist because of scheduling constraints (the mean-values were 49 minutes vs. 24 minutes, $p < 0.001$). The participants in the control group were sent a leaflet about the harmful effects of smoking.

4.2.6 Questionnaire II, key factors in smoking cessation (follow-up study)

After three months, questionnaire II was sent to the smokers in the intervention group. Smoking cessation, smoking quantity per week, and self-rated addiction to smoking (SRA) were noted (Rubinstein et al. 2007).

The study design is shown in **Figure 1**.

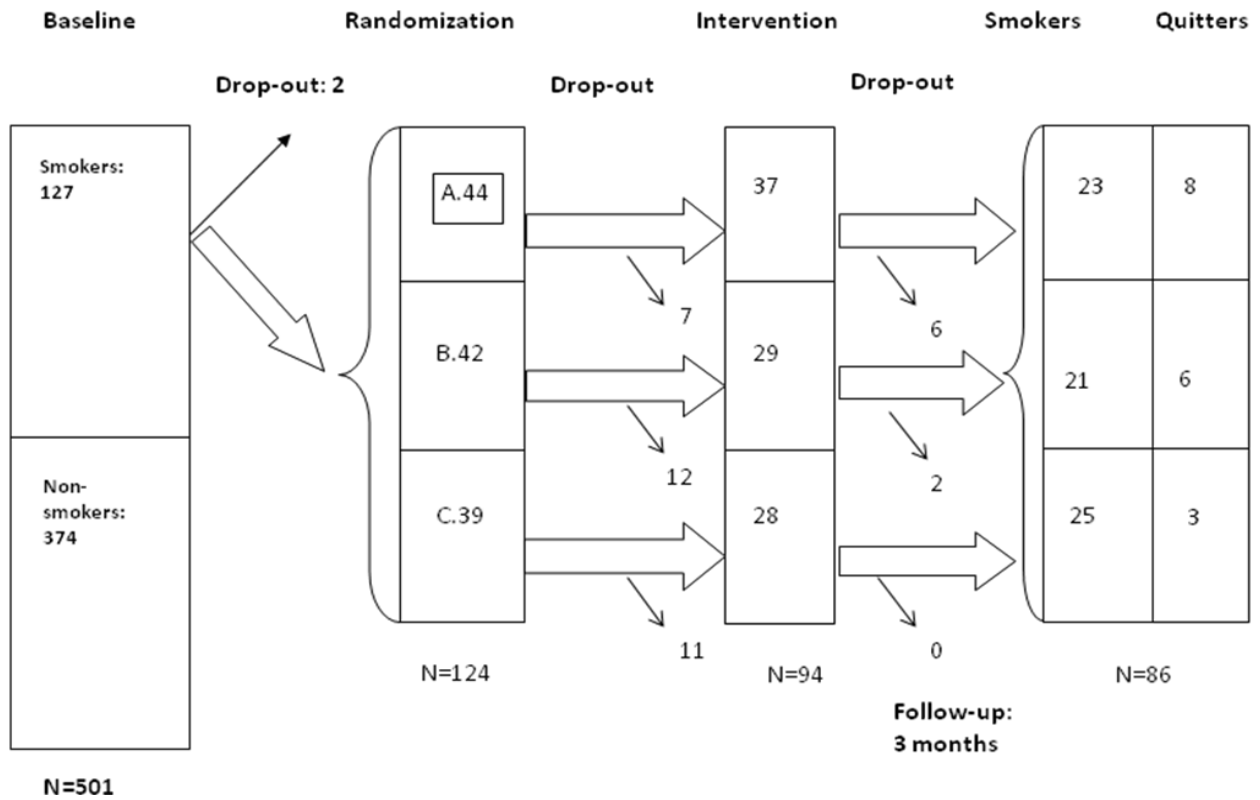


Figure 1. Randomization, intervention and follow-up design of the study.

4.2.7 Statistical Methods

Defining of variables

Binary variables were created in order to compare the periodontal health index and periodontal bacteria, and caries positivity values between the non-smokers and the smokers. For the smokers the quantity and duration of smoking in pack-years (years x the cigarettes smoked / 20) were also recorded. Based on the calculated tertiles of the pack-year figures, the subjects were further classified into non-smokers, and low (0.03-0.50), mediate 0.51-1.25) and high (1.25-4.75) smoker groups. Tooth brushing (the number of times the teeth were brushed per week) was treated as a continuous variable in the statistical analyses. Subjects were considered to be VPI, BOP and AL positive if percentage of measured sites were more than corresponding median value of all subjects. A person was considered to be RC and PD positive if at least one measured site was positive and caries positive if at least one caries lesion was reported (DT=decayed tooth).

Dental health variables

These methods were used in Paper I. Measurements were made of the dental health variables at four sites (mesial, distal, buccal, and palatal/lingual) and at each tooth. We modeled the binary dental health variables with logistic regression and applied generalized estimating equations (GEE) in order to account correlation in dental health variables (Horton et Lipsitz 1999). For other periodontal health indexes, the number of positive sites and the number of examined sites per mouth were recorded in the data. Because all site-specific information for periodontal health indexes was not available, it was assumed to be an independent working correlation matrix. Reported confidence intervals and p values were calculated using robust standard errors of parameter estimates from GEE-based analyses. The analyses were performed using a statistical program (R Foundation for Statistical Computing 2008). Proportions of periodontal health indices adjusted for tooth brushing are reported in this regard. These were obtained by assuming subjects brushed their teeth 14 times per week. The dose-response between smoking and periodontal health indexes was tested using the Wald test for linear hypothesis of the regression coefficients.

Factors associated with smoking cessation

These methods were used in Paper II. We used the Bayesian logistic regression model to analyze the univariate prospective associations between smoking cessation and several explanatory variables such as gender, diurnal type, parental smoking, nicotine dependency, stress, life satisfaction, pack-years, age of starting smoking, school attended, and feelings of nicotine dependency. We report the posterior medians of the relative risks (RR) and 95% credible intervals (CI) together with the Bayesian p-values (RR >1.0 or RR < 1.0).

Estimation of the prevalence of periodontal bacteria

These methods were used in Paper III. The exact binomial-method-based confidence limits (CI 95%) are shown for the proportion of the putative periodontal pathogen positives together with the prevalence ratios (PR) comparing pathogen positivity between the smokers and non-smokers. Because the **Prevalence Ratio (PR)** has been shown to be the best statistical choice in terms of measuring the association between exposure and disease in cross-sectional studies, we chose to use it (Thompson et al. 1998); PRs were calculated separately for all the dental-health variables (DHVs). In order to assess the statistical significance of the PRs, we used a generalized linear regression model with binomially distributed response and a log-link function (Horton & Lipsitz 1999), then fitted a series of models as described in detail in paper III.

The reported p-values are for the null hypothesis, where the prevalence ratio is one, and are based on the Wald test of the corresponding regression model coefficients. The glm package in the R-statistical program (version 2.7.0) was used for the analysis (R Foundation For Statistical Computing 2008). It was desired to avoid falsely rejecting the null hypothesis, thus the false discovery rate (FDR) for each of the four hypotheses was calculated separately.

Periodontal biomarkers and periodontal health variables

These methods were used in Paper IV. Because neither the MMP-8 nor the PMN elastase or their log-transformed values were normally distributed (Shapiro-wilk test for normality, $P < 0.05$), medians and 95% confidence intervals of MMP-8 and PMN elastase were reported as the measure of central tendency. Next, the quantile regression analysis was applied in order to explore the association between median (50% quantile) of elastase/MMP-8 (response) and oral health indices (VPI, BOP, RC, PD, AL) (Koenker 2005). Quantile regression allowed us to perform a non-parametric regression, which is more valid method than linear regression, because error terms cannot be assumed to be normally distributed. Therefore p-values obtained from quantile regression are more reliable than those based on a linear regression model. In addition, quantile regression allows simultaneous modeling of several explanatory variables.

In the univariate quantile regression analysis, the medians of MMP-8 and PMN elastase values were compared with respect to the periodontal index values in order to assess the possible effects of BMI and orthodontic treatment on the association between the salivary biomarkers and the periodontal health indices. A series of quantile regression models were fitted in the analyses where the periodontal variables were included as an explanatory variable one at a time together with the BMI values or with yes/no of orthodontic treatment. All models were fitted separately for boys and girls as well as for non-smokers and smokers. Wald-based confidence limits for the medians are reported together with p-values from the quantile regression. In Tables 2a, 2b, 3a, and 3b of paper IV, the reported p-values are based on the Wald-test, obtained from quantile regression, when testing the medians between the non-smokers and smokers and adjusted for multiple comparisons using the false-discovery rate based correction. To illustrate our findings a

box-plot of RC negative and positive subjects was plotted. All results were obtained using the R-statistical program version 2.9.2 and package quantile regression.

4.2.8 Study variables

Methods, variables and measurements used in this study.

Methods		<i>N = 501 subjects, one birth cohort</i>
<i>I Clinical variables</i>	<i>Measurements</i>	<i>N=toothsites</i>
Plaque (VPI)	Index teeth dd.16,11,24,36,41,44; 4 sites	12024
Gingival bleeding (BOP)	All teeth; 4 sites	56112
Root calculus (RC)	Index teeth dd.16,11,24,36,41,44; 4 sites	12024
Pocket depth (PD)	All teeth, recorded when ≥ 3 mm	56112
Attachment loss (AL)	All teeth, recorded from bite-wing x-rays, when ≥ 2 mm	56112
Dose-effect of smoking	Pack-years from low (0.03 to 0.05), medium (0.51 to 1.25), high (1.26 to 4.75)	127 subjects
Prevalence of periodontitis	More than one ≥ 4 mm pocket	501 subjects <i>prevalence percent (%)</i>
Prevalence of caries, decayed tooth (DT)	At least one	n=483, n=18 missing; n=126 smokers, n=1 missing; n=313 non-smokers, n=15 missing; n= 44 former smokers, n=2 missing
		<i>N=subjects</i>
<i>II Saliva samples and saliva periodontal biomarkers</i>	Paraffin stimulated saliva samples were collected	497 subjects, n=4 missing
PMNs elastase MMP-8 MMP-8 and clinical markers Saliva periodontal biomarkers, MMP-8 and elastase and dose-effect		
<i>III Plaque samples</i>	Pooled two samples, PCR	264 subjects; n=181 non-smokers, n=83 smokers
<i>Aggregatibacter actinomycetemcomitans</i> <i>Porphyromonas gingivalis</i> <i>Prevotella intermedia</i> <i>Prevotella nigrescens</i> <i>Tannerella forsythia</i> <i>Treponema denticola</i>		
<i>IV Questionnaire I + II</i>		127 (all smokers)
FTND	Fagerström test for Nicotine Dependence (see Methods section)	94
SRA (asked only in Q II)	Self-rated addiction for smoking (not at all, to some extent, to a great extent)	13
Life satisfaction	4-item scale of interest in life, feelings of happiness, ease of living and loneliness	109
Stress	4-item scale of feelings of tension and nervous, stress,	108
Diurnal type	4-item-scale of being morning or evening type	107
Best friend's smoking	Yes or no	108

5. RESULTS

The detailed results from this series of studies are given in the original communications, **Studies I – IV**. In the following section, the main results are briefly summarized.

Of the 501 participants, 66% (n=330) did not smoke, 25% (n = 127) were smokers, and 9% (n=44) were former smokers. Among boys, 24% (n= 61) were smokers, 7% (n = 19) were former smokers, and 69% (n = 178) were non-smokers. In girls, the respective figures were 27% (n = 66), 10% (n = 25), and 63% (n = 152). Of all the subjects 25 subjects (5%) had tried smokeless tobacco (21 boys (4.2%) and 4 girls (0.8%)). Of the smokers 29% started to smoke at 13-years of age, 24% at 12-years and 20% at 14-years. Most of the girls and boys, started to smoke at 13-years of age, where corresponding values were 18% and 11%, respectively, as shown in **Figure 2**. A number of the smokers, boys 41% and girls 42%, had tried to quit smoking at least once. Of former smokers, 63% of boys and 48% of girls had tried to quit at least once as shown in **Figure 3**.

The mean values of BMI for non-smoking boys was 21.5 (CI 95% 20.64-22.34) and for smoking boys 21.5 (CI 95% 20.94- 22.03). The corresponding values of BMI for non-smoking girls were 21.1 (CI 95% 20.25 - 21.87) and for smoking girls 21.0 (CI 95% 20.34-21.65).

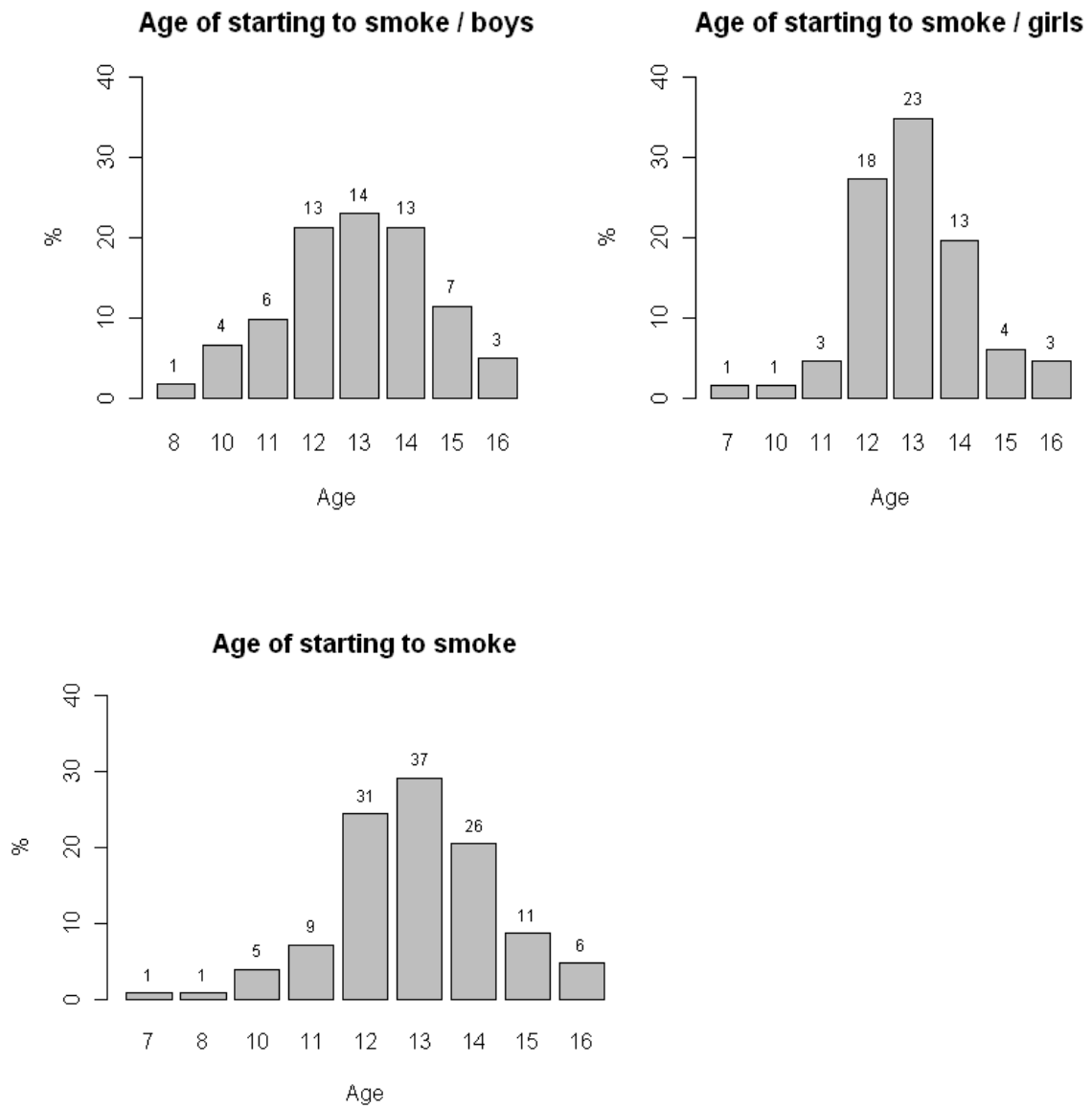
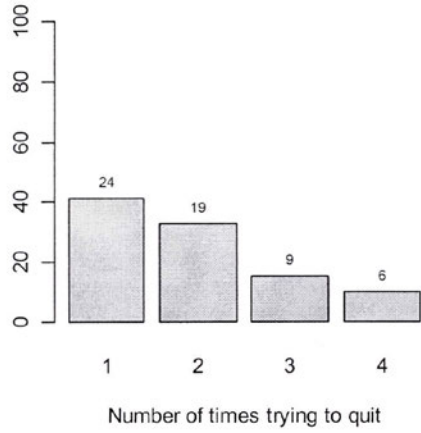
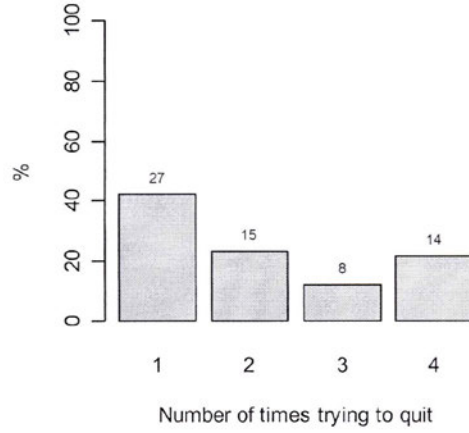


Figure 2. Age of starting to smoke for boys, girls, and all subjects combined.

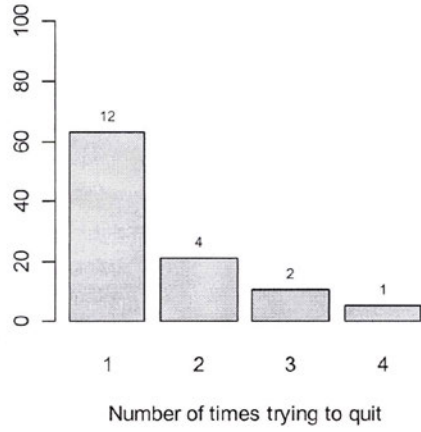
Times trying to quit / smoking boys



Times trying to quit / smoking girls



Times trying to quit / former smoker boys



Times trying to quit / former smoker girls

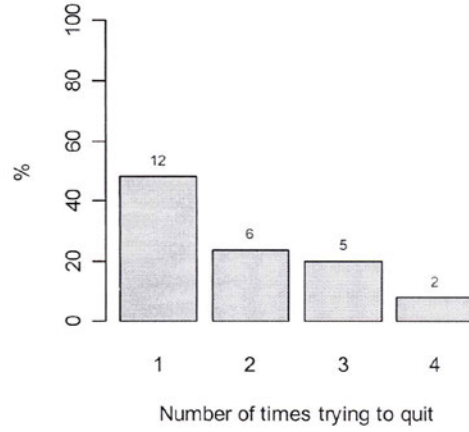


Figure 3. Number of times trying to quit smoking among the smoking, and formerly smoking, boys and girls.

5.1 Tooth brushing

Smoking boys brushed their teeth less frequently than non-smokers; 39.3% of the smokers brushed at least once a day compared to 54.4% of the non-smokers, and respectively 13.1% and 22.3% of subjects brushed at least twice daily. The difference was statistically significant ($p < 0.001$). Approximately 42.9% of non-smoker girls, and 40.9% of smoking girls, brushed their teeth at least once a day, and respectively 48.5% and 48.6% brushed at least twice a day as shown in **Figure 4**. Thus, no difference in tooth brushing frequency was recorded between smoking and nonsmoking girls.

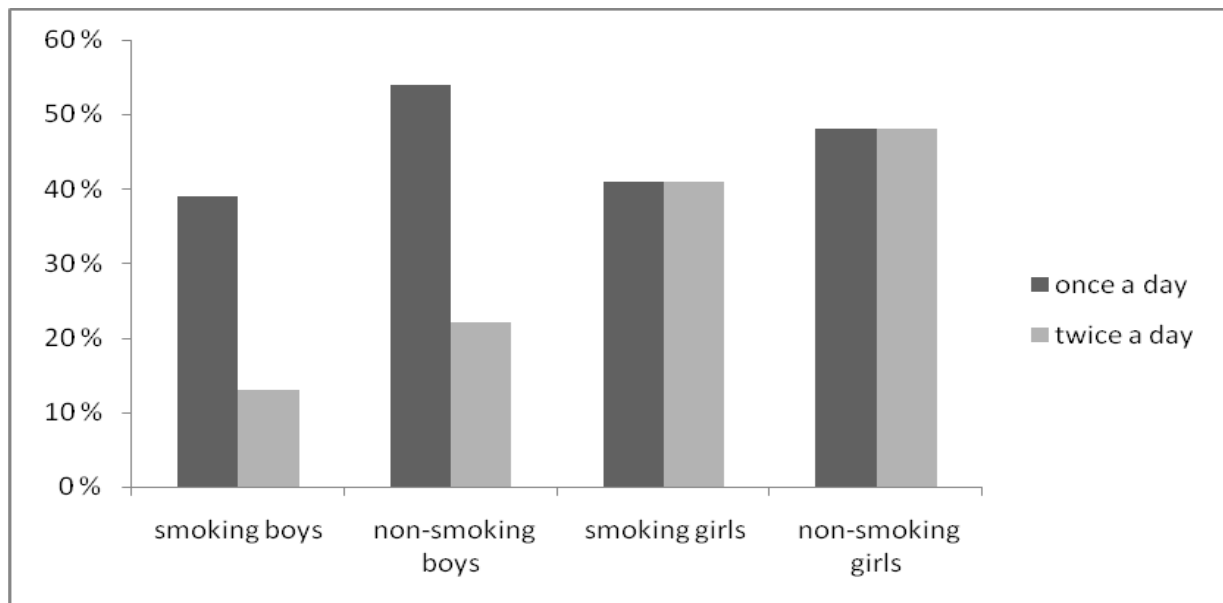


Figure 4. Tooth brushing frequency of the smokers and non-smokers.

5.2 Early signs of periodontitis

We found that of the participants, 56.1% (CI 51.1-60.5%) had more than one ≥ 4 mm pocket of this Finnish birth cohort. Boys had significantly more pockets than girls, with values of 63.2 % (CI 57.0-69.1%) versus 48.6 % (CI 42.1-55.0%) ($p=0.001$). Smokers had more pockets than non-smokers ($p 0.001$). The corresponding values for smokers were 76.5% (CI 68.0-83.5%) and for non-smokers 47.8 % (CI 42.1-53.1%).

5.3 Periodontal health and smoking in adolescents

In general, boys had more plaque (higher VPI scores), bleeding on probing (higher BOP scores) and deeper pockets (higher PD scores) than girls. The differences were statistically significant ($p<0.001$). Boys had more sites with attachment loss ≥ 2 mm than girls ($p<0.05$).

Smoking significantly impaired periodontal health in adolescents, even when the confounding effects of plaque and tooth brushing were adjusted. Smoking boys and girls had consistently higher periodontal indexes than non-smokers (i.e., VPI, RC index, and PD ≥ 4 mm). The statistical significance also remained the same when the plaque effect (VPI) was adjusted for smokers who had root calculus and ≥ 4 mm periodontal pockets. Overall, tooth brushing reduced the values of the periodontal indexes (**Table 3**). The smoking duration and load, as calculated in pack-years, intensified the effects of smoking, but they did not affect the attachment loss (**Table 4**). No statistically significant difference was observed for the interaction between smoking and tooth brushing with any of the periodontal indexes (VPI, RC, BOP, PD, AL).

Overall, Finnish adolescent smokers had more periodontitis than non-smokers ($p< 0.001$), although non-smokers having more gingivitis than smokers ($p< 0.001$) as shown in **Figure 5**. However, if more stringently restricted, summarizing only AL and PD positive subjects, 10% of these adolescents had periodontitis.

Table 3. Smoking-specific and tooth brushing-adjusted prevalence (%) of periodontal health indexes among the boys and girls (n=501).

		Non-smokers		Smokers	
	Indexes, percentage of affected sites	Adjusted for Toothbrushing % [#]	95% CI	Adjusted for Toothbrushing % ^{&}	95% CI
Boys	Visible plaque	39.5	34.6-44.7	47.1	38.4-56.0
	Bleeding on Probing	46.2	42.7-49.7	49.7	44.6-54.8
	Root Calculus	10.8	8.6-13.6	17.9***	13.3-23.5
	Pocket Depth	2.9	2.1-3.8	5.3***	3.9-7.3
	Attachment Loss	5.3	4.7-6.0	5.3	4.4-6.2
Girls	Visible Plaque	37.5	33.0-42.2	48.0**	41.9-54.0
	Bleeding on Probing	44.1	41.1-47.3	47.3	43.3-51.3
	Root Calculus	9.5	8.0-11.1	16.3***	12.6-20.8
	Pocket Depth	1.6	1.2-2.1	4.0***	3.0-5.2
	Attachment Loss	4.6	4.3-5.0	4.9	4.4-5.4

& p-value based on the Wald-test between non-smokers and smokers using logistic regression model with binary smoking variable (0=non-smoker, 1=smoker) ; * 0.05<p≤0.01; ** 0.01<p≤0.001; ***p<0.001

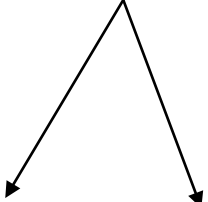
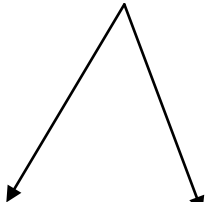
[#] Adjusted estimates of periodontal health indexes (PHI) prevalence corresponding to tooth brushing twice a/day for 14/weeks.

[§] Confidence intervals obtained using generalized linear model with logit link function using GEE and assuming independent working correlation matrix between site specific PHI measurements (within study subject)

Table 4. Pack-year effect on the periodontal health indexes among the boys and girls.

Indexes, percentage of affected sites	Pack-years [#]				Dose response trend test p-values
	Non-smokers	Low= 0.03-0.50	Medium= 0.51-1.25	High= 1.26-4.75	
	%	%	%	%	
Boys					
Visible Plaque [§]	50.0	53.0	61.6	63.1	0.004**
Bleeding on Probing	52.1	55.2	53.9	60.3	0.01*
Root Calculus	11.4	11.9	18.1	22.8	<0.001***
Pocket Depth	3.2	4.1	4.8	6.9	<0.001***
Attachment Loss	5.3	5.1	6.1	4.8	n.s
Girls					
Visible Plaque	41.3	49.2	44.0	59.4	0.0008***
Bleeding on Probing	47.4	53.3	47.8	51.0	n.s
Root Calculus	10.9	12.5	12.7	26.3	<0.001***
Pocket Depth	1.7	3.3	3.7	4.9	<0.001***
Attachment Loss	4.7	5.1	4.4	5.4	n.s

[§] Wald-test for trend in the proportion of PHI positives according to the amount of smoking using generalized estimating equations. [#] In the logistic regression pack-years were treated as a continuous variable (0=non-smoker, 1=0-0.5 pack-years, 2=0.51-1.25 pack-years, 3=1.26-4.75 pack-years. * 0.05<p≤0.01; ** 0.01<p≤0.001; ***p<0.001, n.s.=non significant

Smoking status					
Smokers (n=127)			Non Smokers (n=330)		
Healthy / Gingivitis		Periodontitis	Healthy / Gingivitis		Periodontitis
AL negative PD negative → n=20		AL positive PD positive → n=17	AL negative PD negative → n=115		AL positive PD positive → n=28
		AL negative PD positive → n=87			AL negative PD positive → n=169
		AL positive PD negative → n=3			AL positive PD negative → n=18
Healthy BOP negative (n=1) 0.8 %	Gingivitis BOP positive (n=19) 15.0 %	Periodontitis (total n=107) 84.2 %	Healthy BOP negative (n=8) 2.4 %	Gingivitis BOP positive (n=107) 32.4 %	Periodontitis (total n=215) 65.2 %
p=0.49*	p=0.0003*	p<0.0001*			

AL positive: $\geq 2\text{mm}$, AL negative: $< 2\text{mm}$. PD positive: $\geq 4\text{mm}$, PD negative $< 4\text{mm}$. BOP positive: $> 20\%$ of all the sites, BOP negative $\leq 20\%$ of all the sites. Total n=457 (subjects), former smokers n=44 are excluded.

*All comparisons were made between non-smokers vs. smokers.

Figure 5. Classification of the study subjects according to smoking (smokers, non-smokers) and periodontal disease (healthy, gingivitis, periodontitis) in Finnish adolescents.

5.4 Prevalence of periodontal pathogens and smoking

In general, no differences were found between the genders in harboring any of the periodontal indicator bacteria analyzed (*A.a.*, *P.g.*, *P.i.*, *P.n.*, *T.d.*, *T.f.*). However, *P.n.*, *P.i.*, *T.f.* and *T.d.* were more frequently detected among the smokers than non-smokers, especially in smoking girls. In general, *A.a* and *P.g* were rarely detected in this study (**Table 5**). The frequency of positive findings of *P.n.*, *P.i.*, *T.f.* and *T.d.* seemed to slightly increase with the increasing number of pack-years. A significant association was found between pack-years and the prevalence of *P.n.* among the girls ($p < 0.007$), while no such result was seen in boys. **Table 6** gives the results.

A significant association between the median values of bleeding on probing (BOP) and *T.f.* and *T.d.* was seen. Smokers had more positive sites with *T.f.* and *T.d.* associating with BOP than non-smokers. For root calculus (RC) a significant association was also found between the frequencies of *T.f.* when comparing smokers to non-smokers ($p = 0.004$), and in *T.d.* and smoking. Smokers had more root calculus than non-smokers. Only with *T.d.* did smoking seem to be associated with positive sites with deep pockets (PD). The results are given in detail in **Table 7**.

Table 5. Prevalence percentages and prevalence ratios (RR) of the periodontal bacteria in smokers and non-smokers by gender.

Bacteria Analyzed [§]	Smokers (Total Boys n=44 and Girls n=39)	Non-Smokers (Total Boys n=94 and Girls n=72)	Prevalence Ratio (PR) [†] (95% CI)	
	Prevalence (95% CI)	Prevalence (95% CI)		
Boys	<i>Aggregatibacter actinomycetemcomitans</i>	0.0(0.0-8.0)	1.1(0.03-5.8)	
	<i>Porphyromonas gingivalis</i>	0.0 (0.0-8.0)	0.0 (0.0-3.8)	-
	<i>Prevotella intermedia</i>	11.4 (3.8-24.6)	4.3 (11.7-10.5)	2.7 (0.8-9.5)
	<i>Prevotella nigrescens</i>	68.2(52.4-81.4)	64.9 (54.4-74.5)	1.1 (0.8-1.4)
	<i>Tannerella forsythia</i>	22.7 (11.5-37.8)	11.7 (6.0-20.0)	1.9 (0.9-4.2)
	<i>Treponema denticola</i>	18.2 (8.2-32.7)	8.5 (3.7-16.1)	2.1 (0.9-5.3)
Girls	<i>Aggregatibacter actinomycetemcomitans</i>	0.0 (0.0-9.0)	2.8 (0.3-9.7)	-
	<i>Porphyromonas gingivalis</i>	0.0 (0.0-9.0)	2.8 (0.3-9.7)	-
	<i>Prevotella intermedia</i>	20.5 (9.3-36.5)	4.2 (0.9-11.7)	4.9* (1.4-17.5)
	<i>Prevotella nigrescens</i>	82.1 (66.5-92.5)	61.1 (48.9-72.4)	1.3* (1.1-1.7)
	<i>Tannerella forsythia</i>	23.1 (11.1-39.3)	8.3 (3.1-17.3)	2.8* (1.1-7.2)
All	<i>Aggregatibacter actinomycetemcomitans</i>	0 (0.0-4.3)	1.8 (0.4-5.2)	-
	<i>Porphyromonas gingivalis</i>	0 (0.0-4.3)	1.2 (0.1-4.3)	-
	<i>Prevotella intermedia</i>	15.7 (8.6-25.3)	4.2 (1.7-8.5)	3.7*** (0.8-9.5)
	<i>Prevotella nigrescens</i>	74.7 (63.9-83.6)	63.3 (55.4-70.6)	1.2*(1.0-1.4)
	<i>Tannerella forsythia</i>	22.9 (14.4-33.4)	10.2 (6.1-15.9)	2.2*** (1.2-4.1)
	<i>Treponema denticola.</i>	20.4 (12.4-30.8)	8.4 (4.7-13.7)	2.4*** (1.3-4.7)

The *p* values are adjusted for multiple comparisons (based on false discovery rate (FDR)). Univariate *p*-values obtained using Wald tests. All comparisons were between non-smokers vs. smokers; *0.10<*p*≤0.05, **0.05<*p*≤0.01; ***0.01<*p*≤0.001; *****p*<0.001

[§]For further explanation, see the material and methods section. [†] The Prevalence Ratio (PR) is given between the prevalence of smokers and non-smokers.

Table 6. Prevalence of periodontal bacteria according to pack-years stratified by gender.

		Pack-years				
Bacteria Analyzed§		Non-Smokers (95% CI)	Low (0;0.5) (95% CI)	Mediate [0.5;1.25) (95% CI)	High [1.25;4.75) (95% CI)	p value of the Test for Trend
Boys	<i>Prevotella intermedia</i>	4.3 (11.7-10.5)	4.8 (0.1-23.8)	23.1 (5.0-53.8)	5.3 (0.1-26.0)	n.s.
	<i>Prevotella nigrescens</i>	64.9 (54.4-74.5)	71.4 (47.8-88.7)	69.2 (38.6-91.0)	68.4 (43.4-87.4)	n.s.
	<i>Tannerella forsythia</i>	11.7 (6.0-20.0)	14.3 (3.0-36.3)	30.8 (9.1-61.4)	26.3 (9.1-51.2)	n.s.
	<i>Treponema denticola.</i>	8.5 (3.7-16.1)	23.8 (8.3-47.2)	7.7 (0.2-36.0)	15.8 (3.4-39.6)	n.s.
Girls	<i>Prevotella intermedia</i>	4.2 (0.9-11.7)	20.0 (4.3-48.1)	18.8 (4.1-45.6)	14.3 (1.8-42.8)	n.s.
	<i>Prevotella nigrescens</i>	61.1 (48.9-72.4)	73.3 (44.9-92.2)	75.0 (47.6-92.7)	92.9 (66.1-99.8)	0.007**
	<i>Tannerella forsythia</i>	8.3 (3.1-17.3)	20.0 (4.3-48.1)	25.0 (7.3-52.4)	14.3 (1.8-42.8)	n.s.
	<i>Treponema denticola.</i>	8.3 (3.1-17.3)	20.0 (4.3-48.1)	12.5 (1.6-38.3)	28.6 (8.4-58.1)	n.s.
All	<i>Prevotella intermedia</i>	4.2 (1.7-8.5)	11.1 (3.1-26.1)	20.7 (8.0-39.7)	9.1 (1.9-24.3)	0.03*
	<i>Prevotella nigrescens</i>	63.3 (55.4-70.6)	72.2 (54.8-85.8)	72.4 (52.8-87.3)	78.8 (61.1-91.0)	0.03*
	<i>Tannerella forsythia</i>	10.2 (6.1-15.9)	16.7 (6.4-32.8)	27.6 (12.7-47.2)	21.2 (9.0-38.9)	0.01*
	<i>Treponema denticola.</i>	8.4 (4.7-13.7)	22.2 (10.1-39.2)	10.3 (2.2-27.4)	21.2 (9.0-38.9)	0.04*

p values adjusted for multiple comparisons (based on false discovery rate (FDR)). Univariate p-values obtained using the Wald-based trend test.

All comparisons made between low, mediate, high pack-year categories vs. non-smokers; * 0.05<p≤0.01; ** 0.01<p≤0.001; ***p<0.001, n.s. = non significant

§For further explanation, see the material and methods section.

5.5 Caries and smoking

Smoking boys had more caries lesions than non-smokers. The result persisted even when the tooth brushing effect was taken into account. No corresponding differences were observed in girls. The details are given in **Table 8**.

Table 8. Caries lesions in subjects grouped according to sex and smoking.

	Non-Smokers					Smokers				
	Number of Subjects with Caries (DT) ¹	Prevalence %	95% CI	Prevalence Adjusted for Tooth Brushing % ²	95% CI	Number of Subjects with Caries (DT) ¹	Prevalence %&	95% CI	Prevalence Adjusted for Tooth Brushing % [#]	95% CI
Boys	36	21.4	15.7-28.0	11.2	6.0-19.8	29	49.1***	26.0-72.3	28.0**	14.7-46.7
Girls	26	17.7	12.1-24.4	19.9	13.2-28.9	15	23.4	8.6-48.3	25.9	15.8-39.4

¹ Caries lesion of subject, if at least one lesion was recorded. (DT=decayed tooth)

² Adjusted estimates of DT prevalence corresponding to tooth brushing of 2 times/day or 14/week.

& Wald-test, smokers vs. non-smokers for caries. P-values ns, * 0.05<p≤0.01; ** 0.01<p≤0.001; ***p<0.001.

Wald-test, smokers vs. non-smokers adjusted for tooth brushing. P-values ns, * 0.05<p≤0.01; ** 0.01<p≤0.001; ***p<0.001.

5.6 Saliva periodontal biomarkers and smoking

Of the total material, the elastase median value was $9.88 \times 10^{-3} \Delta OD_{405}/h$ (CI 95% 8.50-11.88) (**Figure 6**), and the MMP-8 median value was $166.10 \mu g/l$ (CI 95% 148.95-187.07) (**Figure 7**). Smoking significantly decreased both MMP-8 and PMN elastase median values studied. For smokers, MMP-8 values averaged $152.03 \mu g/l$ (CI 95% 105.08-194.58) and for non-smokers $177.92 \mu g/l$ (CI 95% 147.63-208.44), while elastase activities averaged $7.9 \times 10^{-3} \Delta OD_{405}/h$ (CI 95% 5.9×10^{-3} - 10.6×10^{-3}) versus $10.9 \times 10^{-3} \Delta OD_{405}/h$ (CI 95% 9.1×10^{-3} - 13.3×10^{-3}), respectively. The median values of MMP-8 for male smokers were $112.03 \mu g/l$ (CI 95% 86.20-173.22) compared with $176.89 \mu g/l$ (CI 95% 135.08-220.20) of non-smokers ($p=0.04$). For girls the corresponding values were $170.88 \mu g/l$ (CI 95% 136.72- 230.68) in smokers versus $177.92 \mu g/l$ (CI 95% 145.16-215.33) in non-smokers (n.s.). Elastase median values in male smokers were $5.88 \Delta OD_{405}/h$ (CI 95% 4.75 - 9.25×10^{-3}) versus $11.0 \Delta OD_{405}/h$ (CI 95% 8.75 - 13.63×10^{-3}) in non-smokers ($p=0.02$). The median of elastase activities in girls was $9.16 \times 10^{-3} \Delta OD_{405}/h$, (CI 95% 6.63×10^{-3} - 17.25×10^{-3}) in smokers and $10.88 \times 10^{-3} \Delta OD_{405}/h$, (CI 95% 8.75×10^{-3} - 15.25×10^{-3}) in non-smokers, respectively (n.s.).

The effects were strengthened by pack years of smoking (MMP-8 $p=0.04$; elastase $p=0.01$). The differences were statistically significant in boys when the highest pack year group was compared with values of the non-smokers ($p=0.005$ for MMP-8, and $p= 0.023$ for elastase). No differences were seen in girls (**Table 9**). We found associations between gingival bleeding (BOP) and MMP-8 ($p=0.04$) and, suggestively, an association with pockets depth ($p=0.09$) in non-smoking boys. In male smokers, the existence of calculus (assessed with the RC index) significantly increased only the MMP-8 concentration while no other effect of periodontal health scores emerged in this respect. No associations between elastase activities and the periodontal health index scores were statistically significant when adjusted for BMI. Similarly, the observed difference between MMP-8 and BOP or PD scores among non-smoking boys did not remain statistically significant when BMI was taken into account ($p=0.13$ and $p=0.11$). However, the association between MMP-8 and RC index scores among male smokers remained significant ($p=0.03$).

Table 9. Median values of salivary PMN elastase (10^{-3}) and MMP-8.

	Pack years	MMP-8 ($\mu\text{g/l}$)			PMN elastase (activity)		
		50%	95%CI	p-value	50%	95%CI	p-value
Boys	Non-smoker	168.9	127.1-212.2	-	11.0	8.8-13.6	-
	(0.0, 0.5)	138.0	57.3-314.2	n.s.	8.3	4.4-18.3	n.s.
	[0.5, 1.25)	145.2	79.9-354.9	n.s.	5.9	3.0-20.6	n.s.
	[1.25, 4.75)	84.8	54.6-165.2	0.023*	5.6	4.8-9.3	0.005**
Girls	Non-smoker	169.9	137.2-207.3	-	10.9	8.8-15.3	-
	(0.0, 0.5)	162.9	78.9-255.7	n.s.	12.2	6.6-26.6	n.s.
	[0.5, 1.25)	131.7	98.3-246.0	n.s.	8.6	4.3-36.9	n.s.
	[1.25, 4.75)	206.0	141.0-303.2	n.s.	8.5	6.5-21.9	n.s.

p-value (Wald-test) obtained from quantile regression when comparing medians between non-smokers and each pack years classification, * $0.05 < p \leq 0.01$; ** $0.01 < p \leq 0.001$; *** $p < 0.001$, n.s. = non significant

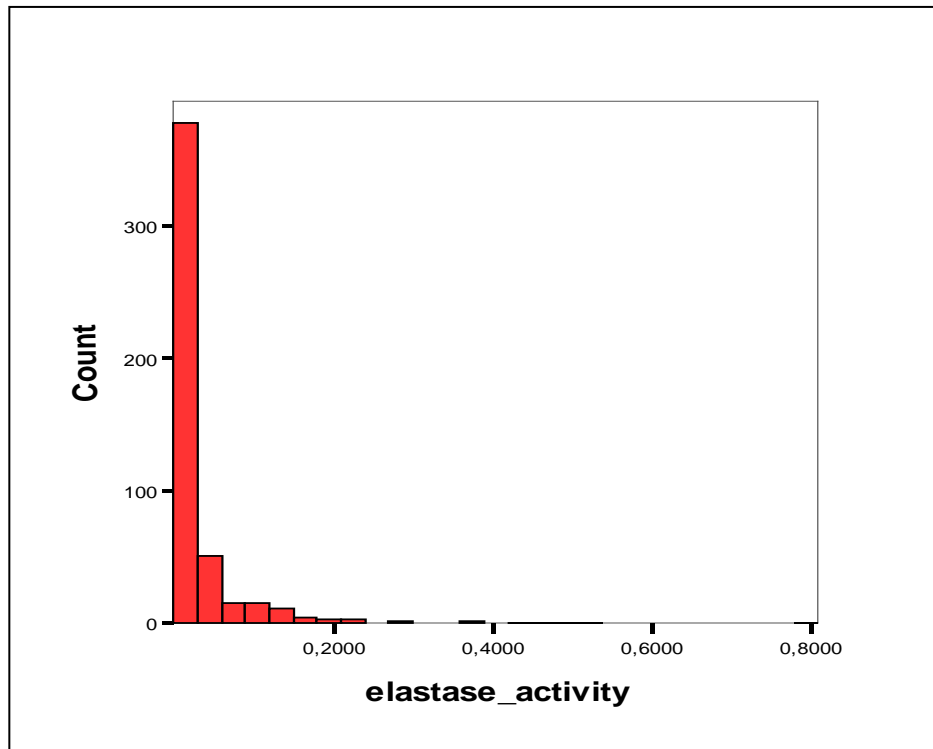


Figure 6. Distribution of PMN elastase values of the subjects. Median value was $0.0099\Delta\text{OD}_{405}/\text{h}$.

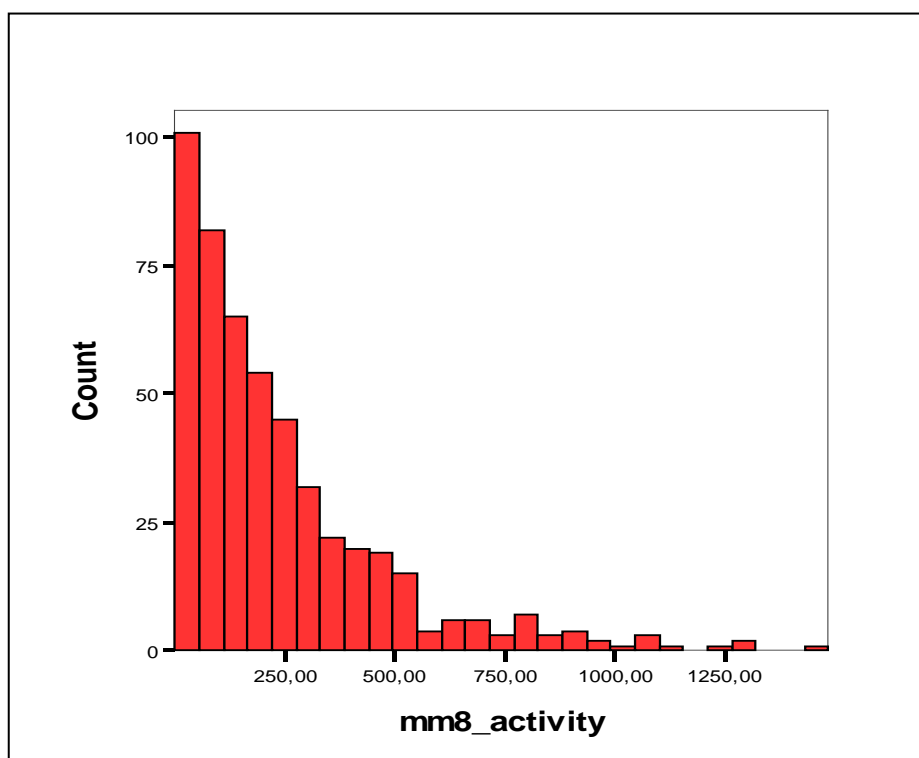


Figure 7. Distribution of MMP-8 values of the subjects. The median of MMP-8 was 166.1 μ g/ml.

5.7 Factors associated in smoking cessation in adolescence

After randomization, 37 of the 44 assigned participants to the dentist group came to the counseling session. The respective figures for participants in the school nurse group were 29/41 and for the control group they were 28/39. Ten participants dropped-out in this phase. Of these participants, 86 completed questionnaire II. Of the 17 who reported having quit smoking, 8 had gone to the dentist for counseling, 6 had gone to the school nurse, and 3 were in the control group. Thus, 14 (19 % of those participated in intervention) of the young people quit smoking after the intervention. Eleven girls and five boys quit smoking, but the difference was not statistically significant. Those who dropped-out were assumed to continue smoking (**Figure 1**).

The key factors associated with smoking cessation in adolescents were best friend's influence, nicotine dependence and diurnal type. The RR of quit smoking after the intervention was 7.0 (CI 95% 4.6–10.7), when the best friend was not a smoker. Of the diurnal types, the morning people seemed to be more likely to quit, RR 2.2 CI 95% (1.4–3.6). Nicotine dependence (ND) also turned out to be influential in the opposite direction: those who scored between 3 and 5 points on the FTND were less likely to quit smoking than those

scoring between 0 and 2 points, where nobody with between 6 and 10 points gave up. Gender, parental smoking, stress, life satisfaction, pack-years, the age of starting smoking and the school stage did not appear

to have any significance regarding smoking cessation in this study. **Table 10** summarizes the main results of the interventions.

Table 10. Key factors associated with smoking cessation after intervention among 15- to-16-year-olds.

Factors associated with smoking cessation	Smoking regularly (%) before the intervention, n=127	Percent of subjects who quit smoking after the intervention	Relative risk (RR) of quitting smoking and 95% CI
Best friends smoking			
yes	84	11	1.0
no	16	78	7.0 (4.6-10.7)
Fagerström Test for Nicotine Dependence			
1= 0-2 points	50	33	1.0
2= 3-10 points	50	3	0.1 (0.08-0.11)
Feeling nicotine-dependent[#]			
not at all		65	1.0
to some extent		5	0.2 (0.1-0.4)
to a great extent		0	0
Diurnal type			
evening	85	15	1.0
morning	15	33	2.2 (1.4-3.6)
Sex			
male	48	13	1.0
female	52	23	1.8 (0.99-3.1)
Parental smoking			
neither	28	21	1.0
one or both	72	32	1.6 (0.7-3.8)
Stress			
none= 16	7	21	1.0
some= 9-15	87	18	0.8 (0.4-1.5)
severe= 4-8	6	25	1.3 (0.5-2.9)
Life Satisfaction			
satisfied=4-6	33	24	1.0
intermediate=7-11	53	16	0.7 (0.4-1.2)
dissatisfied=12-20	14	20	0.8 (0.2-2.9)
Pack-years			
0.05-0.5	17	36	1.0
0.51-3	31	15	0.7 (0.4-1.2)
3.1-27	52	16	0.7 (0.4-1.2)
Age of starting to smoke			
7-12 years	37	19	1.0
13 years	29	14	0.7 (0.4-1.2)
14-16 years	33	21	1.2 (0.7-2.1)
School			
comprehensive school	34	11	1.0
upper-secondary	16	23	1.2 (0.6-2.3)
vocational school	50	23	1.7 (0.9-3.1)

[#] Only in Questionnaire II.

5.8 Main results

The main results of this study are summarized in **Table 11**.

Table 11. Summary of the main results of the study.

<i>Main results</i>
<i>Periodontal health indicators</i>
Smoking girls had more plaque than non-smokers
No difference in gingival bleeding between smokers and non-smokers
Smoking boys and girls had more root calculus than non-smokers
Smoking boys and girls had more ≥ 4 mm pockets
No difference in attachment loss between smokers and non-smokers
Duration and quantity of smoking intensified the effects of smoking in root calculus and deep pockets
56 % of the participants had more than one ≥ 4 mm pockets
Tooth brushing reduced the values of the periodontal indexes.
<i>Inflammatory markers</i>
Median values of PMN elastase were lower in male smokers than non-smokers
Median values of MMP-8 were lower in male smokers than non-smokers
In male smokers with calculus MMP-8 values were increased
The effect of smoking was strengthened by high pack years of smoking in MMP-8
The effect of smoking was strengthened by high pack years of smoking in PMN elastase.
<i>Difference in prevalence of periodontal pathogens</i>
No difference in the prevalence of <i>A.a.</i> was observed between smokers and non-smokers
No difference in the prevalence of <i>P.g.</i> was observed between smokers and non-smokers
Higher prevalence of <i>P.i.</i> was observed among smokers than non-smokers.
Higher prevalence of <i>P.n</i> was observed among smokers than non-smokers.
Higher prevalence of <i>T.f.</i> was observed among smokers than non-smokers.
Higher prevalence of <i>T.f.</i> was observed among smokers than non-smokers.
<i>Factors in smoking cessation</i>
Nicotine-dependent participants were less likely to stop
The morning types found it 2-times easier to quit smoking than the evening types
Those whose best friend was a nonsmoker were 7-times more likely to stop smoking
No difference between smokers and non-smokers in Self Rated Addiction
No difference between groups in life satisfaction
No difference between groups in stress

6. DISCUSSION

The purpose of the present series of studies was to examine the effect of smoking on teenagers' oral health, with emphasis on periodontal health. Factors associated with smoking cessation especially in dentistry were the special focus of the study and, in particular, how the data could be used in smoking cessation and as a part of prevention strategy. The question of whether counseling given by dental health care personnel could promote smoking cessation in the adolescents was of special interest. In addition, the birth cohort investigated gave valuable epidemiologic information about the oral health of the teenagers.

6.1 The effect of smoking on periodontal health of the adolescents

The main result of this study was that the harmful effects of smoking on oral health can already be seen in adolescents and with relatively low exposure. Compared with non-smokers, smoking boys and girls had consistently higher periodontal indexes, that is, visible plaque, root calculus and deep pocket values, regardless of whether or not the effect of tooth brushing was adjusted in the analyses. This is in agreement with several studies which have demonstrated that differences in disease prevalence and severity between smokers and non-smokers remain after adjusting for the levels of plaque or calculus (Calsina et al. 2002, Susin et al. 2004, Torrungruang et al. 2005a, 2005b). The smoking duration and the load as calculated in pack-years intensified the effects of smoking, but not when the attachment loss was considered. This result may be explained by the fact that teenagers of the present study had been smoking only for few years. However, smoking has been associated with periodontal attachment loss in young individuals who are considered as low risk patients in this regard. Bergström et al. (1991) reported that the effect of smoking on alveolar bone was independent of plaque levels and that the progression of alveolar bone loss was more significant in younger smokers.

Cigarette smoking seemed to be a major environmental risk for oral health and there are large number of epidemiological studies on adults indicating that current smokers have a greater extent and severity of periodontal disease when compared with never-smokers (Tomar & Asma, 2000). On the other hand, young 26-year-old smokers were also three times as likely as non-smokers to have at least one site with 4 mm or more attachment loss (Hashim et al. 2001).

In the present study, boys had worse periodontal health than girls; the boys had more plaque, bleeding in probing, deeper pockets, attachment loss and more caries. Partly, the difference can be explained by less frequent tooth brushing of the smoking boys. A Nordic Project of Quality Indicators for Oral Health Care (2010) reported the same trend for low figures in tooth brushing in Finland compared with other Scandinavian countries. According to this report, only 39% of boys and 61% of girls brushed their teeth more than once a day. Just recently, Honkala et al. (2011) pointed in their study that daily smoking and toothbrushing less than twice a day are linked in adolescence. Smoking and infrequent toothbrushing are risk factors for periodontal health in adolescents.

Nevertheless, tooth brushing reduced the values of periodontal indexes in general. This finding is in agreement with Lie et al. (1998) who showed that the rate of plaque formation was similar between smokers and non-smokers. However, Erdemir et al. (2004) reported in their study that cigarette smoking increased the amount of dental plaque over time. Studies conducted about smoking effects in teenage populations are sparse and thus the present study provided valuable information on this target group.

6.1.1 Early signs of periodontal disease

Results from this study showed that the prevalence of periodontitis was 15% when the criteria were more than one ≥ 4 mm pockets and ≥ 2 mm loss of attachment. Periodontal diseases seem to be more common than previous studies have reported. This is supported by the Finnish Health 2000 Survey (Suominen-Taipale et al. 2008), which reported that of the 30-34 -year-old Finns, 48% had periodontitis (at least one tooth with deepened periodontal pocket). Earlier in the 1980s, Saxen (1980) had reported prevalence figures of juvenile periodontitis of only 0.1% (radiographically detectable bone loss more than 2 mm demonstrated around more than one tooth). Her subjects were 16-year-old Finnish teenagers.

Clerehugh et al. (1995) showed in a longitudinal study of five years in 14-19-year-olds, that progressive sites have significantly more plaque, subgingival calculus and gingival inflammation at baseline and this remained so throughout the study. Furthermore, Albandar et al. (1998) showed in their longitudinal study of six years in 13-20-year-olds significant association between subgingival calculus and gingivitis as well as the development and progression of probing attachment loss. According to Clerehugh (2008) and Clerehugh et al. (1990, 1995) periodontitis begins in the early teenage years and progresses slowly throughout the teens and thus is not just confined to adults over the age of 35 years. The importance of proper examination of all teeth and accurate recordings of clinical parameters in adolescents was emphasized in this study when identifying young periodontal risk patients, especially smokers.

6.1.2 Subgingival microbial profile of the young smokers

The smokers, and especially the girls, had more frequently positive samples for *P.n.*, *P.i.*, *T.f.* and *T.d.* than non-smokers. There are controversies in earlier studies whether or not smokers have different microbial profile than non-smokers but these studies have been conducted mostly in adults. For example, Umeda et al. (2004) reported a 5-fold risk of *T.d.* in the saliva of current adult smokers when compared with in non-smokers. According to Kamma et al. (1999) smoking patients aged 22 to 35 years with early onset periodontitis harbored greater numbers of bacteria, mainly anaerobes such as *P.g.* and *T.f.* However, Cortelli et al. (2008) found no correlation between smoking and the prevalence of *A.a.*, *P.g.*, *T.f.* or *P.i.* Surprisingly, *A.a.* and *P.g.* were monitored randomly in the present study, in three and two cases, respectively. None of these subjects were smokers. This is in line with the study of von Troil-Lindén et al. (1995) who reported that among the subjects with initial periodontitis (bone loss < 30%) *A.a.* was not found at all and *P. g.* was only detected in one case. Mombelli et al. (1995) also observed very low levels of these

bacteria at puberty. In the present study, a difference was found between the boy and girl smokers regarding the frequency of *P.i.*, *P.n.*, *T.f.*, *T.d.*, these bacteria being more prevalent among the girls. Hence sex hormones at puberty might have an effect on the composition of periodontal microbiota as shown previously (Bimstein & Matsson 1999, Umeda et al. 2004). Recently, Gürsoy et al. (2009) reported that *P.n.* was a common finding in their study on young Finnish women with signs of pregnancy gingivitis but without periodontitis. According to Paju et al. (2009) the number of pathogenic species in saliva, rather than the presence of certain periodontal pathogens or their specific combinations, associate with clinical signs of adult periodontitis. Further, according to a recent study of Shchipkova et al. (2010) periodontitis in smokers is associated with a microbial community, which is preferentially enriched for disease-associated pathogens as *Parvimonas*, *Campylobacter*, *Treponema*, *Bacteroides*, and *Fusobacterium*—genera. Corresponding results are not available from adolescent populations

6.2 Smoking and caries

An interesting finding in the present study was that among the smoking boys, more subjects were diagnosed with at least one lesion of decayed tooth. The result remained the same after adjusting for tooth brushing. A cohort study from Northern Finland observed over 5-fold risk of tooth loss due to caries in smoking young adults in comparison to non-smokers (Ylöstalo et al. 2004). According to their study dental health behavior and health-oriented lifestyle were also linked with tooth loss but were less pronounced than the effect of smoking. However, smoking seems to indeed be a significant risk indicator for various negative oral health outcomes. Factors such as eventual barriers to seek dental care need to be also investigated for a comprehensive picture, however.

6.3 Salivary MMP-8 and PMN elastase and the effect of smoking

Salivary MMP-8 values were associated with bleeding on probing and suggestively with deep periodontal pocket values in the non-smoking boys. In fact both the biomarkers analyzed increased with gingival bleeding. Ramseier et al. (2009) reported that MMP-8 seems to be a key biomarker during early the stages of periodontal disease and that the increase in MMP-8 and -9 concentrations in oral fluids, such as in saliva, is observed in periodontitis patients.

The values of both the biomarkers MMP-8 and PMN elastase analyzed in the present study were lower in male smokers when compared with the values of non-smokers. The effect was strengthened by high pack years of smoking. Former smokers had fairly similar MMP-8 and PMN elastase values compared with non-smokers, which is partly in agreement with Morozumi et al. (2004). They reported that the MMP-8 mRNA levels seemed to increase significantly 8 weeks after smoking cessation. Nicotine metabolites impair the functional activity of polymorphs and macrophages. Recently, Gürsoy et al. (2010) reported that smoking strongly affected the detection of periodontal disease, especially by salivary MMP-8 ratio. They explained that smoking might have a direct effect on periodontal and inflammatory cells through the presence and

activity in periodontal pathogenesis or that MMPs in smokers are less effective in mediating tissue degradation. However, Persson et al. (1999) found no significant difference between young smokers and non-smokers (aged 20 to 32) in the GCF elastase activity. In this regard the results are still inconsistent and further investigations are called for. In the present study the findings discussed above were only observed in boys, suggesting gender difference, which might be due to the hormonal status in puberty. Finally, it should be re-emphasized that boys of the present study brushed their teeth less frequently than girls and thus higher inflammatory biomarker values would have been anticipated in the boys with poor oral hygiene. Consequently, further investigations are needed also in this area.

Obesity assessed by BMI may also confound the results. However, the difference between MMP-8 and BOP or PD index values among the non-smoking boys did not remain statistically significant when BMI values were included in the analyses. In the study of Ylöstalo et al. (2008), a weak association was found between BMI and periodontitis. They suggested that smoking might be either a modifying or confounding factor between body weight and periodontal infection. This association also occurs in young subjects, but the mechanisms involved are not clear.

6.4 Risk factors for smoking and the role of dental professionals supporting to quit smoking

In the present study physiological, psychological and social factors such as nicotine dependence, diurnal type and best-friend effect, appeared significant in smoking cessation (Ollila et al. 2010). However, social effects such as the childhood environment, low parental SES (Huurre et al. 2003), parental divorce or childhood adversities (Anda et al. 1999) were not taken into account in the analyses which is a weakness of the study. Recently, O'Loughlin et al. (2009) reported tobacco advertising to be a strong determinant in teenage smoking, including both initiation and daily smoking.

6.4.1 Best friend's influence

Starting to smoke during adolescence is strongly related to the imitation of peer smoking and may be regarded as a vehicle for maturation. Peer influence turned out to be the most significant factor in teenage smoking, much stronger than parental smoking. Conversely, those whose best friend was a non-smoker were 7 times more likely to stop smoking. This result is in line with the study of West et al. (1999) and White et al. (2003) suggesting that peer influence indeed predicted smoking. However, Kestilä et al. (2006) showed a strong influence of parental smoking on daily smoking in early adulthood with an OR 3.01 for men and 2.31 for women. O'Loughlin et al. (2009) reported in their study (n=877) that smoking among adult role models and peers was an important predictor of initiation of smoking. According to their results, only peer smoking predicted conversion to daily smoking. They also reported that older age was associated with a lower risk of smoking initiation. In the present study, starting age was not found to be as a significant predictor in smoking cessation as shown in the study of Broms et al. (2004). The relatively small sample size may explain this discrepancy.

6.4.2 Nicotine dependence

Nicotine dependence was a strong predictor for smoking cessation in the present study. Those participants scoring between 0 and 2 points on the FTND scale found it easy to quit smoking while those with between 3 and 10 points found it difficult. None of the “highly dependent” could quit smoking. Those who felt somewhat dependent on nicotine were less successful than the subjects who felt no dependence. Nicotine dependence was also observed to be the strongest barrier for smoking cessation in the study of Kleinjan et al. (2009). Many teenagers would like to stop smoking but are not able to do so due to being hooked on nicotine (Wetter et al. 1999).

According to the review by Benowitz (2010) nicotine is a direct agonist of the nicotinic acetylcholine receptors of the brain and it releases dopamine in the mesolimbic area, especially in the ventral tegmental area of the midbrain and the nucleus accumbens, and the frontal cortex. Those areas are considered to be involved in the pleasure and reward system of the brain. Release of dopamine and other transmitters such as glutamate, acetylcholine, noradrenaline and serotonin are in a key role for pleasure and positive reinforcement of nicotine abuse. Nicotine immediately stimulates nicotinic cholinergic receptors to release neurotransmitters, the effect called neuromodulation, enhancing the development of addiction. After repeated exposures of nicotine, permanent physiological changes take place in the nicotinic acetylcholine receptors leading to neuroadaptation and to the development of tolerance.

The nicotinic cholinergic receptors consist of five subunits with 13 types (α 1-9, β 1-4) identified. Initial studies have found candidate genes coding the receptors associated with smoking behavior (Ho & Tyndale 2007). This fact and the individual ability of liver to metabolize nicotine are thought to be connected to addiction (Lerman & Niura 2002). In rat experiments using nicotine infusions more upregulation of midbrain nicotinic acetylcholine receptors were produced in adolescent rats than in adult rats (Trauth et al. 1999). Human adolescence from ages 10 to 25 years is a critical time for brain development and in the generation of substance use disorders, where in the brain especially the frontal cortical part is involved in impulse control and in the evaluating of long and short term rewards (Crews et al. 2007). Thus, developing brain of adolescents may indeed be more sensitive than adults to nicotine effects.

They may develop dependence as measured by losing autonomy even with a few weekly cigarettes after 1 month (DiFranza et al. 2007b). This may be explained by recent studies by Poorthuis et al. (2009) who reported that brains of adolescents show more neural plasticity for nicotinic actions and that these changes may have a lasting effect on the developing brain, inducing permanent changes that lead to tobacco addiction. Rubinstein et al. (2011) suggested that nicotine dependence may indeed begin with low levels of nicotine exposure. Their study showed that in brains of adolescent light smokers, the response to smoking cues and thus activation was similar to activation of adult and teenage heavy smokers, suggesting that nicotine dependence begins already with low exposure in some adolescents; however, these mechanisms also

require further investigation. According to recent studies, smoking behaviors are partly genetically influenced. A high degree of heritability of cigarette smoking ($\geq 50\%$), including the level of dependence, has been shown by twin studies (Lessov-Schlagger et al. 2008). Nicotine dependence is a complex disease that might be influenced by multiple genes, with each gene having only a relatively small effect (Han et al. 2010). As a summary, smoking patterns may illustrate the gene–environment interactions and correlations. (Rose et al. 2009).

6.4.3 Diurnal type

The diurnal type also seems to be an important factor in smoking cessation. Morning types quit smoking 2.2 times more frequently than evening types. Thus it could be assumed that evening types are also those who are more nicotine dependent, however, the associations were not investigated in the present study. The present result of diurnal type is partly in line with the Finnish study of Broms et al. (2011), which indicated that being an evening type is associated with a higher risk of being a current smoker, being more highly dependent upon cigarettes, and with a lower likelihood of stopping smoking. According to their study, the risk of nicotine dependence assessed was higher among the evening types (OR=2.78), by whom FTND points were scored 0.59 higher. Diurnal type has been shown to have a heritability rate of 50% and thus is an important internal factor leading to individual behavior that may clarify the causes of tobacco addiction (Koskenvuo et al. 2007). In this regard individual variability in morningness and eveningness may have importance when planning smoking cessation counseling programs.

6.4.4 Role of dental professionals in smoking cessation

After the tobacco cessation intervention in the present study, almost 20% of smoking adolescents quit smoking. This is a promising result and supports the role of the dental professionals in tobacco prevention and cessation programs, especially in adolescent. This is in line with earlier findings in primary care that the abstinence rate from 12 to 20% could be achieved by various types of counseling and behavioral therapies (Whittchen et al. 2011). In this study with four interventional groups no significant difference was found between medical treatment and motivational smoking cessation intervention in the smoking abstinence rates. Whittchen et al. (2011) pointed out that complex interventions with multiple treatment components and structured counseling sessions are clearly more effective than a “one shot” minimal intervention consisting of some short motivational intervention with the physicians’ advice to quit.

In the study from Sweden Nohlert et al. (2009) compared low intensity smoking cessation intervention (30-minute counseling session with a content of self-help program) to high one (40-minute individual session with mixture of behavioral therapy, coaching, and pharmacological advises) in a dentistry setting. They observed that although high intensity support was more expensive, subjects in the high intensity group were twice as likely to report continuous abstinence at the 12-month follow-up. Furthermore, low intensity smoking cessation seemed to be worth while compared to not receiving any professional support at all.

Consensus Report of the 2nd European Workshop on Tobacco Use Prevention and Cessation for Oral Health Professionals (Ramseier et al. 2010) highlighted the importance of helping tobacco users to quit and how this work has become a part of both the responsibility of oral health professionals and general practitioners of dentistry. Further, tobacco use prevention and cessation (TUPAC) counseling are suggested to include the following: 1) Basic care: brief interventions for all patients in the dental practice to identify tobacco users, to assess the readiness to quit, and to request permission to re-address at a subsequent visit. 2) Intermediate care: interventions consisting of (brief) motivational interviewing sessions to build on readiness to quit, enlist resources to support change, and to include cessation medications. 3) Advanced care: intensive interventions to develop a detailed quit plan including the use of suitable pharmacotherapy. Not to forget the effect of smoking cessation on periodontal status. According to Warnakulasuriya et al. (2010), smoking status should always be assessed as a key parameter indicating periodontal disease risk for an individual patient and smoking cessation counseling should form an integral part of periodontal therapy.

The evidence of the harmful effects of tobacco with motivational interviewing with sufficient time provided by dentists and school nurses clearly had an influence in terms of quitting smoking and these professionals should thus be recruited in prevention programs targeted to adolescents. In smoking cessation both these professionals are needed indeed, as it has been shown by previous studies that interventions involving more than one type of health professional are potential to increase smoking cessation (An et al. 2008). Furthermore, pharmacotherapy, such as nicotine replacement therapy, could be useful for adolescents similarly to what has been shown in adults (Stead et al. 2008). However, further investigations are needed in the treatment with NTRs in adolescents. Open discussion with an adult seems to be the most important factor in smoking cessation among adolescents.

6.5 Strengths of the study

The cross-sectional part of this study gave information about oral health among the 15-16-year-old adolescents. The study involved the whole birth cohort of the City of Kotka and of those invited 90% took part in the investigation. Thus a representative sample of a homogenous population was included, which further strengthens the generalization of the results. Furthermore, intervention with properly done randomization as conducted in the present study gave valuable new information about how to affect adolescents without using nicotine replacement therapy. The previously unknown factors that affect smoking cessation among the adolescents were also revealed.

The internal validity of this study was confirmed by conducting the clinical part (i.e. measuring periodontal indexes) twice on every tenth participant and by blinding the results of the radiographic examination. The external validity was checked by collecting the information about oral health from the survey, from the clinical records and biomarkers, and also from the x-rays.

6.6 Limitations of the study

The self-reported amount of smoking of the adolescents may not give fully reliable data. No means to measure the levels of urine cotinine were obtainable; this could have been a valid method in assessing smoking although it would not be suitable for counting the pack-years. Further, the smoking habits of the best friend could have been asked from themselves and not only from the participants.

Another limitation of the study was the sample size. Even though it was manageable to include almost the whole birth cohort of 15- to 16-year olds in the city of Kotka, the sample size remained fairly small. This may be the main reason why life satisfaction, stress, age of starting smoking, school factors, parental smoking and even pack-years did not appear to have any significant influence on smoking cessation. Follow-up time after intervention was also fairly short, 3 months, as at least 6 months would have been better in giving more information about the factors behind smoking cessation and abstinence rate. Further, as such the association between smoking and periodontal bacteria in the adolescents should be investigated in large populations and preferably with a multi-centric approach.

7. CONCLUSIONS

Smoking confirmed to be a major etiological risk factor for oral health, regarding both clinical effects and when assessed by using the inflammatory markers. Smoking seemed particularly detrimental for periodontal health. The effects of smoking seemed to be the same as in adults. However, early signs of periodontitis were mild among the adolescents. It is thus concluded that early signs of periodontitis could be seen already in adolescents, particularly in smokers. As for adults, smoking seemed to mask gingivitis, since smokers had less bleeding on probing than non-smokers. Further, smoking might affect the microbial profile especially in female smokers, although the result is unclear since different hormonal balance may also affect periodontal microbiota. Smoking was shown to also affect the levels of matrix metalloproteinase (MMP-8) and leukocyte elastase (PMN elastase). Boys seemed to especially be at higher risk for gingivitis and thus at risk for early development of periodontitis as reflected also in the elevated salivary MMP-8 levels.

Tobacco intervention given by dental professionals or school nurses could be effective. The evidence of the harmful effects of tobacco provided by dentists and school nurses clearly had an influence in terms of smoking cessation. However, both external and internal factors such as the best friend's influence, nicotine dependence, and diurnal personality type should be taken more into account in individual counseling on smoking cessation.

Practical relevance

Dental professionals have a key position in systematical smoking cessation in adolescents to quit smoking and the harmful effects of smoking on oral health could be used in counseling. Those factors associated with smoking cessation should be taken more carefully into account in this. Young nicotine dependent smokers might benefit from intervention programs given by dental professionals. Therefore, training of clinical skills for smoking cessation should be included in both the undergraduate and post-graduate education. Further, appropriate tools and protocols such as supporting systems and manuals with instructions in smoking cessation should be included in dental care in general. To take up the matter of smoking when discussing with the patient and following the 5 As protocol regularly do not take much time and effort. Dental professionals should thus be recruited for cessation programs targeted to the adolescents.

Future research

Follow-up cohort investigations should aim to establish the future effects of smoking among those adolescents who do not quit. In particular, the possible progression of periodontitis among these subjects would be interesting to research. Further, a follow-up study is also necessary to reveal how many of the adolescents who quit actually stay non-smokers and how many of them later relapse. What factors possibly affect these choices is of great importance in promoting smoking cessation, as well as the changes needed when organizing dental care.

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Finally, my dear late parents, heartfelt thanks, for giving me strength to carry on, when remembering their encouraging words.

APPENDIX

Questionnaire I

Tupakka ja suun terveys -kysely 15-vuotiaille

No_____

Kyselylomake I+II

Tällä kyselyllä kartoitetaan nuorten tupakointitottumuksia ja tupakka-aseiteita. Lisäksi selvitetään sekä asenteita tupakkavalistuksesta että tietämystä tupakan vaikutuksista. Kysely tullaan käsittelemään luottamuksella eikä henkilöllisyytesi tule esiin. Lomakkeessa oleva numero on tarpeen aineiston ATK-tallennusvaiheessa. Lue ohjeet tarkkaan jokaisen kysymyksen kohdalla ja vastaa mahdollisimman rehellisesti.

Vastauksesi ovat meille tärkeitä, jotta voimme kehittää nuorten terveystalveluja nuorten tarpeita vastaaviksi.

Tästä alkavat kysymykset

Seuraavissa kysymyksissä ympyröi oikea vaihtoehto tai kirjoita oikea tieto sille varatulle viivalle.

1. Oppilaitos: _____

2. Pituus: _____ cm

3. Paino: _____ kg

4. Ikä: ___ vuotta

5. Sukupuoli: 1 mies 2 nainen

6. Montako kertaa viikossa harjaat hampaasi: _____ kertaa viikossa

7. Jos sinulla on jokin sairaus, kirjoita se tähän _____

8. Jos käytät jotakin lääkettä säännöllisesti, kirjoita lääkkeen nimi tähän _____

9. Seuraavassa kysymyksessä ympyröi parhaiten sinun tupakointiasi kuvaava vaihtoehto

- | | | |
|--|------|---------|
| 1. Oletko koskaan tupakoinut? | 0 ei | 1 kyllä |
| 2. Oletko koskaan kokeillut nuuskaa? | 0 ei | 1 kyllä |
| 3. Tupakoitko tällä hetkellä säännöllisesti? | 0 ei | 1 kyllä |

4. Montako savuketta arvioisit polttavasi tällä hetkellä päivässä?
savuketta _____ päivässä

5. Minkä ikäisenä aloitit tupakoinnin? _____ - vuotiaana

10. Alla tiedustellaan mahdollinen tupakointisi eri ikäisenä.

Mikäli et polta tai olet jossakin iässä lopettanut tupakoinnin,
merkitse sen iän kohdalle 0

- 10-vuotiaana: _____ savuketta päivässä
11-vuotiaana: _____ savuketta päivässä
12-vuotiaana: _____ savuketta päivässä
13-vuotiaana: _____ savuketta päivässä
14-vuotiaana: _____ savuketta päivässä
15-vuotiaana: _____ savuketta päivässä

11. Oletko lopettanut jossain vaiheessa? 0 ei 1 kyllä

12. Oletko aloittanut uudelleen? 0 ei 1 kyllä

13. Monestiko olet lopettanut ja aloittanut uudestaan? _____ kertaa

Vastaa seuraaviin kysymyksiin, jos tupakoit. Muutoin siirry kysymykseen numero 17.

14. Kuinka voimakas on halusi lopettaa tupakointi? (ympyröi se vaihtoehdon edessä oleva numero, joka parhaiten vastaa mielipidettäsi)

- 1 erittäin voimakas
- 2 voimakas
- 3 en osaa sanoa
- 4 vähäinen
- 5 erittäin vähäinen

15. Kuinka usein harkitset tupakoinnin lopettamista? (ympyröi se vaihtoehdon edessä oleva numero, joka parhaiten vastaa mielipidettäsi)

- 1 päivittäin
- 2 silloin tällöin
- 3 harvoin
- 4 en koskaan

16. Haluaisitko lopettaa tupakoinnin, mutta tunnet, ettet selviä siitä ilman tukea?

0 ei 1 kyllä

Kysymykseen no 17. vastaavat tupakoimattomat.

17. Kuinka usein harkitset aloittavasi tupakoinnin? (ympyröi yksi numero, joka parhaiten vastaa mielipidettäsi)

- 1 päivittäin
- 2 silloin tällöin
- 3 harvoin
- 4 en koskaan

Tästä eteenpäin olevat kysymykset on tarkoitettu KAIKILLE

18. Ympyröi niiden vaihtoehtojen edessä olevat numerot, joiden oletat aiheutuvan tupakoinnista.

- 1. sydän- ja verisuonisairauksia
- 2. aivoinfarkti
- 3. suonikohjut
- 4. suusyöpä
- 5. akne
- 6. kuulon heikkeneminen
- 7. hampaiden kiinnityksen väheneminen
- 8. hampaiden reikiintyminen
- 9. hiusten lähtöä
- 10. keuhkosityöpä
- 11. impotenssi
- 12. sydäninfarkti
- 13. verisuonten tukkeutumista
- 14. keuhkohtaumasairaus

19. Ympyröi niiden vaihtoehtojen edessä olevat numerot, joiden oletat aiheutuvan nuuskan käytöstä

1. sydän- ja verisuonisairauksia
2. aivoinfarkti
3. suonikohjut
4. suusyöpä
5. akne
6. kuulon heikkeneminen
7. hampaiden kiinnityksen väheneminen
8. hampaiden reikiintyminen
9. hiusten lähtöä
10. keuhkosityöpä
11. impotenssi
12. sydäninfarkti
13. verisuonten tukkeutumista
14. keuhkohtaumasairaus

20. Olen saanut tietoa tupakan/nuuskan vaikutuksista suun terveyteen ,
(ympyröi valitsemasi kohdat, voi olla useita vaihtoehtoja)

1. vanhemmilta
2. sukulaisilta
3. ystäviltä
4. kouluterveydenhoitajilta
5. lääkäreiltä
6. sairaanhoitajilta
7. hammaslääkäreiltä
8. hammashoitajilta
9. lehdistä
10. internetistä
11. radiosta
12. tv:stä
13. ystäviltä
14. internetistä
15. puhelinneuvonnasta
16. muualta, mistä (luettele tähän) _____

21. Haluatko tietoa tupakan/nuuskan vaikutuksesta suun terveyteen

0 ei 1 kyllä

22. *Kenen antama tieto tupakoinnin vaikutuksista on vaikuttanut tupakointiisi tai tupakoimattomuuteesi eniten* _____

23. Kuinka suuri merkitys tällä hetkellä on ollut sinulle opettajan antamilla tiedoille tupakan vaikutuksista? (ympyröi jokaisen kysymyksen kohdalla se vaihtoehdon edessä oleva numero, joka parhaiten kuvaa mielipidettäsi)

- 1 erittäin suuri**
- 2 melko suuri**
- 3 en osaa sanoa**
- 4 melko vähäinen**
- 5 erittäin vähäinen**

24. Kuinka suuri merkitys tällä hetkellä on ollut sinulle hammashuoltajan antamilla tiedoille tupakan vaikutuksista?

- 1 erittäin suuri**
- 2 melko suuri**
- 3 en osaa sanoa**
- 4 melko vähäinen**
- 5 erittäin vähäinen**

25. Kuinka suuri merkitys tällä hetkellä on ollut sinulle hammashoitajan antamilla tiedoille tupakan vaikutuksista?

- 1 erittäin suuri**
- 2 melko suuri**
- 2 en osaa sanoa**
- 4 melko vähäinen**
- 5 erittäin vähäinen**

26. Kuinka suuri merkitys tällä hetkellä on ollut sinulle hammaslääkärin antamilla tiedoille tupakan vaikutuksista?

- 1 erittäin suuri**
- 2 melko suuri**
- 3 en osaa sanoa**
- 4 vähäinen**
- 5 erittäin vähäinen**

27. Kuinka suuri merkitys tällä hetkellä on ollut sinulle kouluterveydenhoitajan antamilla tiedoille tupakan vaikutuksista?

- 1 erittäin suuri**
- 2 melko suuri**
- 3 en osaa sanoa**
- 4 vähäinen**
- 5 erittäin vähäinen**

28. Kuinka suuri merkitys tällä hetkellä on ollut sinulle sairaanhoitajan antamilla tiedoille tupakan vaikutuksista?

- 1 erittäin suuri**
- 2 melko suuri**
- 3 en osaa sanoa**
- 4 vähäinen**
- 5 erittäin vähäinen**

29. Kuinka suuri merkitys tällä hetkellä on ollut sinulle ensineuvon (puhelinneuvonta) antamilla tiedoille tupakan vaikutuksista?

- 1 erittäin suuri**
- 2 melko suuri**
- 3 en osaa sanoa**
- 4 vähäinen**
- 5 erittäin vähäinen**

30. Kuinka suuri merkitys tällä hetkellä on ollut sinulle lääkärin antamilla tiedoille tupakan vaikutuksista?

- 1 erittäin suuri**
- 2 melko suuri**
- 3 en osaa sanoa**
- 4 vähäinen**
- 5 erittäin vähäinen**

31. Kuinka suuri merkitys tällä hetkellä on ollut sinulle vanhemmiltasi saamilla tiedoille tupakan vaikutuksista?

- 1 erittäin suuri**
- 2 melko suuri**
- 3 en osaa sanoa**
- 4 vähäinen**
- 5 melko vähäinen**

32. Kuinka suuri merkitys tällä hetkellä on ollut sinulle ystäviltäsi saamilla tiedoille tupakan vaikutuksista?

- 1 erittäin suuri**
- 2 melko suuri**
- 3 en osaa sanoa**
- 4 vähäinen**
- 5 melko vähäinen**

33. *Kuinka suuri merkitys tällä hetkellä on ollut sinulle lehdistä saamilla tiedoille tupakan vaikutuksista?*

- 1 erittäin suuri
- 2 melko suuri
- 3 en osaa sanoa
- 4 vähäinen
- 5 melko vähäinen

34. *Kuinka suuri merkitys tällä hetkellä on ollut sinulle sukulaisiltasi saamilla tiedoille tupakan vaikutuksista?*

- 1 erittäin suuri
- 2 melko suuri
- 3 en osaa sanoa
- 4 vähäinen
- 5 erittäin vähäinen

35. *Kuinka suuri merkitys tällä hetkellä on ollut sinulle televisiosta saamilla tiedoille tupakan vaikutuksista?*

- 1 erittäin suuri
- 2 melko suuri
- 3 en osaa sanoa
- 4 vähäinen
- 5 melko vähäinen

36. *Kuinka suuri merkitys tällä hetkellä on ollut sinulle radiosta saamilla tiedoille tupakan vaikutuksista?*

- 1 erittäin suuri
- 2 melko suuri
- 3 en osaa sanoa
- 4 vähäinen
- 5 melko vähäinen

37. *Ketkä lähipiirissäsi tupakoivat?
(ympyröi valitsemasi vaihtoehdon edessä numero)*

- 1 äiti
- 2 isä
- 3 veli
- 4 sisar
- 5 läheisin ystävä
- 6 ei kukaan

38. Kuinka hyvin seuraavat toteamukset sopivat sinuun nähden (valitse sopivin vaihtoehdoista ja ympyröi valitsemasi vaihtoehto kussakin kohdassa)

	hyvin	melko hyvin	huonosti	ei lainkaan
yleensä olen tavattoman jännittynyt ja hermostunut	1	2	3	4
päivittäiseen toimintaani liittyy paljon hermojänniystä	1	2	3	4
illalla olen aivan uupunut sekä henkisesti että ruumiillisesti	1	2	3	4
päivittäiset toimintani ovat kovin rasittavia ja painostavia	1	2	3	4

39. Kuinka kauan kestää ennen kuin ”päätet käyntiin” aamulla herättyäsi yöunesta (ympyröi valitsemasi vaihtoehdon edessä oleva numero)?

- 1 noin 10 minuuttia tai vähemmän
- 2 yli 10 minuuttia mutta alle 20 minuuttia
- 3 yli 20 minuuttia mutta alle 40 minuuttia
- 4 yli 40 minuuttia

40. Yritä arvioida missä määrin olet ”aamuihminen tai iltaihminen”

- 1 olen selvästi aamuihminen (aamuvirkku ja iltauninen)
- 2 olen jossain määrin aamuihminen
- 3 olen jossain määrin iltaihminen(aamu-uninen ja iltavirkku)
- 4 olen selvästi iltaihminen

41. Tuntuuko sinusta siltä, että elämäsi on juuri nyt hyvin kiinnostavaa, melko kiinnostavaa, melko ikävää vai hyvin ikävää (ympyröi valitsemasi vaihtoehdon edessä oleva numero)?

- 1 hyvin kiinnostavaa
- 2 melko kiinnostavaa
- 3 melko ikävää
- 4 hyvin ikävää
- 5 en osaa sanoa

42. Tuntuuko sinusta siltä, että sinun elämäsi on juuri nyt hyvin onnellista, melko onnellista melko onnetonta vai hyvin onnetonta?

- 1 hyvin onnellista
- 2 melko onnellista
- 3 melko onnetonta
- 4 hyvin onnetonta
- 5 en osaa sanoa

43. Tuntuuko sinusta siltä, että elämäsi on juuri nyt hyvin helppoa, melko helppoa, melko kovaa vai hyvin kovaa?

- 1 hyvin helppoa
- 2 melko helppoa
- 3 melko kovaa
- 4 hyvin kova
- 5 en osaa sanoa

44. Tuntuuko sinusta siltä, että juuri nyt sinä olet hyvin yksinäinen, melko yksinäinen vai etkö lainkaan yksinäinen?

- 1 hyvin yksinäinen
- 2 melko yksinäinen
- 3 ei lainkaan yksinäinen
- 4 en osaa sanoa

45. Seuraavassa esitämme savukkeisiin liittyviä kokemuksia kuvaavia väittämiä (ympyröi alla olevaa asteikkoa apua käyttäen jokaisen väittämän vierestä numero, joka asteikolla kuvaa parhaiten sitä, miten väittämä pitää sinun kohdalla paikkansa)

Oletko elämäsi aikana polttanut enemmän kuin 100 savuketta?

- 1 en ole -> voit lopettaa tähän
- 2 olen

Jos ympyröit 2=olen, jatka eteenpäin

Nikotiiniriippuvuusmittarin "Nicotine Dependence Syndrome Scale" 14 väittämää (Shiffman et al. 2004)"

46. Kuinka pian heräämisen jälkeen tupakoit ensimmäisen kerran (ympyröi valitsemasi vaihtoehto)?

- a. 5 min kuluttua
- b. 6-30 min kuluttua
- c. 31-60 min kuluttua
- d. 60 min kuluttua

47. Onko sinusta vaikeaa olla tupakoimatta tiloissa, joissa se on kiellettyä (ympyröi 0 tai 1)?

0 ei 1 kyllä

48. Mistä tupakointikerrasta olisi vaikeinta luopua (ympyröi a. tai b.)?

**a. aamun ensimmäisestä
b. jostain muusta**

49. Kuinka monta savuketta poltat vuorokaudessa (ympyröi a., b., c. tai d.)?

**a. 1-10 savuketta
b. 11-20 savuketta
c. 21-30 savuketta
d. 31 savuketta tai enemmän**

50. Poltatko aamun ensimmäisinä tunteina enemmän kuin loppupäivän aikana?

0 ei 1 kyllä

51. Tupakoitko, jos olet niin sairas, että joudut olemaan vuoteessa suurimman osan päivää?

0 ei 1 kyllä

Kiitos vaivannäöstäsi!

Questionnaire II

id_____

Aloita tästä:

1. Oppilaitos:_____

2. Pituus:_____ cm

3. Paino:_____ kg

4. Ketkä lähipiirissäsi tupakoivat?
(ympyröi valitsemasi vaihtoehdon edessä numero)

- 1 äiti
- 2 isä
- 3 veli
- 4 sisar
- 5 läheisin ystävä
- 6 ei kukaan

5. Kuinka hyvin seuraavat toteamukset sopivat sinuun nähden (valitse sopivin vaihtoehdoista ja ympyröi valitsemasi vaihtoehto kussakin kohdassa)

	hyvin	melko hyvin	huonosti	ei lainkaan
yleensä olen tavattoman jännittynyt ja hermostunut	1	2	3	4
päivittäiseen toimintaani liittyy paljon hermojänniystä	1	2	3	4
illalla olen aivan uupunut sekä henkisesti että ruumiillisesti	1	2	3	4
päivittäiset toimintani ovat kovin rasittavia ja painostavia	1	2	3	4

6. Kuinka kauan kestää ennen kuin ”päätet käyntiin” aamulla herättyäsi yöunesta (ympyröi valitsemasi vaihtoehdon edessä oleva numero)?

- 1** noin 10 minuuttia tai vähemmän
- 2** yli 10 minuuttia mutta alle 20 minuuttia
- 3** yli 20 minuuttia mutta alle 40 minuuttia
- 4** yli 40 minuuttia

7. Yritä arvioida missä määrin olet ”aamuihminen tai iltaihminen”

- 1** olen selvästi aamuihminen (aamuvirkku ja iltaihminen)
- 2** olen jossain määrin aamuihminen
- 3** olen jossain määrin iltaihminen(aamu-uninen ja iltavirkku)
- 4** olen selvästi iltaihminen

8. Tuntuuko sinusta siltä, että elämäsi on juuri nyt hyvin kiinnostavaa, melko kiinnostavaa, melko ikävää vai hyvin ikävää (ympyröi valitsemasi vaihtoehdon edessä oleva numero)?

- 6** hyvin kiinnostavaa
- 7** melko kiinnostavaa
- 8** melko ikävää
- 9** hyvin ikävää
- 10** en osaa sanoa

9. Tuntuuko sinusta siltä, että sinun elämäsi on juuri nyt hyvin onnellista, melko onnellista melko onnetonta vai hyvin onnetonta?

- 1** hyvin onnellista
- 2** melko onnellista
- 3** melko onnetonta
- 4** hyvin onnetonta
- 5** en osaa sanoa

10. Tuntuuko sinusta siltä, että elämäsi on juuri nyt hyvin helppoa, melko helppoa, melko kovaa vai hyvin kovaa?

- 1** hyvin helppoa
- 2** melko helppoa
- 3** melko kovaa
- 4** hyvin kova
- 5** en osaa sanoa

11. Tuntuuko sinusta siltä, että juuri nyt sinä olet hyvin yksinäinen, melko yksinäinen vai etkö lainkaan yksinäinen?

- | | |
|---|------------------------|
| 1 | hyvin yksinäinen |
| 2 | melko yksinäinen |
| 3 | ei lainkaan yksinäinen |
| 4 | en osaa sanoa |

12. Seuraavassa esitämme savukkeisiin liittyviä kokemuksia kuvaavia väittämiä (ympyröi alla olevaa asteikkoa apua käyttäen jokaisen väittämän vierestä numero, joka asteikolla kuvaa parhaiten sitä, miten väittämä pitää sinun kohdalla paikkansa)

Oletko elämäsi aikana polttanut enemmän kuin 100 savuketta?

- 1 en ole -> voit lopettaa tähän
2 olen

Jos ympyröit 2=olen, jatka eteenpäin

Nikotiiniriippuvuusmittarin ”Nicotine Dependence Syndrome Scale (NDSS)” 14 väittämää (Shiffman et al. 2004).

Kiitos vaivannäöstäsi!