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**CHRONIC DENTAL DISEASES AND SYSTEMIC HEALTH:
CARDIOVASCULAR DISEASES AND CANCER**

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ACADEMIC DISSERTATION

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To my lovely family: Mika, Aleks, Anton and baby.

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ABSTRACT

Chronic dental diseases, mainly periodontitis and apical periodontitis, are highly prevalent worldwide. The possible associations between these oral diseases and general health have been studied in particular with periodontitis, but less so regarding apical periodontitis. Periodontitis has been associated with a number of diseases such as cardiovascular diseases, diabetes, pre-term and low-birth weight infants, Alzheimer's disease, and also with cancer. Regarding apical periodontitis, some studies also show associations with cardiovascular diseases and diabetes. Nevertheless, causality has been difficult to address due to common background variables.

Cardiovascular diseases are the number one killer in the world and also highly prevalent in all populations; leading causes of deaths are coronary heart disease and stroke. Cancer is the second leading cause of death worldwide. Most common cancer-related deaths are due to cancers of the lung, liver, colorectum, stomach and breast. In both oral and systemic diseases behavioral factors can enhance the disease progression. These factors include smoking, alcohol abuse, unhealthy diet, and physical inactivity.

In order to better understand the possible associations between general health and oral conditions we set out to study a Swedish population cohort that was originally examined in 1985 (n =1676 subjects). The hypothesis of our present study was that there is a link between periodontitis, apical periodontitis, systemic diseases and cancer.

In 2003 (n = 120) and 2009 (n = 90) follow-up examinations were conducted and national cumulated cancer data and hospital registers were respectively analyzed for eventual associations with oral health parameters. Edentulous patients were not evaluated in this study, because to study chronic dental diseases, the patients need to have teeth present. In 2003 also full-mouth x-rays were taken and in 2009 saliva and serum samples collected.

In the study I we analyzed the incidence of cancer in patients without periodontitis (24-year prospective investigation), questioning if the incidence of cancer is related with the oral health status of the patients. In study II, we analyzed the hospital, clinical and x-ray data of the 2003 follow-up population, questioning if the patients who had visited the hospital with different diagnosis also had more oral diseases. To analyze the x-rays, a modified Total Dental Index was used for grading the oral infection burden of each patient. Apical lesions were classified with the Periapical Index, and the number and quality of root treatments were also registered. In study III, serum and saliva samples were analyzed for different biomarkers, MMP-8 (IFMA method), MMP-9, MMP-13 and TIMP-1 (ELISA) from the 2009 follow-up population, questioning if the biomarkers reflect the oral health status of the patients. Different statistical methods were used, including analyses of variance and covariance, chi-square, Fisher's exact t-test, Mann-Whitney U test, Bonferroni corrections and multiple regression analyses, when relevant. All *p*-values were two-tailed and the significance was set at 0.05.

The main results were that the incidence of cancer associated with missing teeth and age in the study population. In 2009 the prevalence of cancer in periodontally healthy patients, associated with missing d47 and age with OR 2.62 (1.18-5.78) and 1.91 (1.06-3.43), respectively.

Furthermore, apical periodontitis was common among the subjects (41%) and the quality of root treatments was mainly poor. Apical periodontitis associated with higher risk of having cardiovascular disease with an OR (95% CI) of 3.90 (1.20-12.65), when controlled for periodontitis and other co-factors.

No significant differences were found in serum and saliva concentrations of the biomarkers MMP-8, MMP-9 and TIMP-1; but MMP-13 seemed to associate with clinical attachment loss and probably with lower bone density, but not with plaque accumulation and inflammation. However, higher MMP-13 saliva concentration associated with female gender and clinical attachment loss with OR (95% CI) 3.08 (1.17-8.11) and 3.57 (1.18-11.82), respectively.

To conclude, oral infections were prevalent in this study population and statistical links were found between them and cardiovascular diseases and cancer. From the clinical point of view, extraction of affected teeth may not be the final solution to eliminate these infections, because the remaining teeth can still increase the infection burden as shown in the results from studies II and III. Age is an important factor for several systemic diseases and also for the oral diseases. Finally, several common factors may influence the balance between health and disease, such as the genome, the environment and the behavior. These factors relate both to oral and systemic diseases, making it difficult to prove any causality between these diseases.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred in the text by their Roman numerals:

- I** Virtanen E, Söder B, Andersson LC, Meurman JH, Söder PÖ. History of dental infections associates with cancer in periodontally healthy subjects: a 24-year follow-up study from Sweden. *J Cancer*. 2014 Jan 2;5(2):79-85.
- II** Virtanen E, Nurmi T, Söder PÖ, Airila-Månsson S, Söder B, Meurman JH. Apical periodontitis associates with cardiovascular diseases: a cross-sectional study from Sweden. *BMC Oral Health*. 2017 Jul 11;17(1):107. doi:10.1186/s12903-017-0401-6.
- III** Virtanen E, Yakob M, Tervahartiala T, Söder PÖ, Andersson LC, Sorsa T, Meurman JH, Söder B. Salivary MMP-13 gender differences in periodontitis: A cross-sectional study from Sweden. *Clin Exp Dent Res*. 2017;1–6. <https://doi.org/10.1002/cre2.76>.

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ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
AL	Attachment loss
AP	Apical periodontitis
BOP	Bleeding on probing
BRCA1/BRCA2	Breast cancer genes 1,2
CAL	Clinical attachment loss
CDKs	Cyclin-dependent kinases
CI	Calculus index
CHD	Coronary heart disease
CP	Chronic periodontitis
CVD	Cardiovascular diseases
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
GCF	Gingival crevicular fluid
GI	Gingival index
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papilloma virus
IADR	International Association of Dental Research
ICD-7/ICD-9/ICD-10	International classification of diseases 7/9/10
IFMA	Immunofluorometric assay
IL-1/IL-2/IL-8	Interleukin 1/2/8
LPS	Lipopolysaccharide

MMPs	Matrix metalloproteinases
MMP-8	Matrix metalloproteinase 8
MMP-9	Matrix metalloproteinase 9
OR (95%CI)	Odds ratio (95% confidence interval)
OSCC	Oral squamous cell carcinoma
PAI	Periapical index
PD	Probing depth
PLI	Plaque index
SD	Standard deviation
TDI	Total dental index
TIMP-1	Tissue inhibitor of metalloproteinases 1
TNF- α	Tumor necrosis factor α
WHO	World Health Organization

INTRODUCTION

Chronic dental diseases are highly prevalent in populations in spite of implementation of prevention and treatment, especially in developed countries. In the global perspective, 60-90% of children in school age and almost 100% of adults have caries; severe periodontitis leading to tooth lost is present in 15-20% of middle-aged (35-44 years) adults; about 30% of the elderly (65-74 years) are edentulous. Chronic dental diseases are infection-inflammatory diseases that affect children and adults in particular in developing countries. A good oral hygiene is essential for the prevention of periodontal disease and optimum levels of fluoride in the oral cavity can prevent caries development. The main risk factors for oral diseases are unhealthy diet, tobacco use, alcohol abuse, poor oral hygiene and socio-economic determinants (WHO Media centre 2012a).

Cardiovascular diseases (CVD) are also highly prevalent and are the leading cause of death worldwide; 31% of global deaths, especially because of coronary heart disease (CHD) and stroke. Behavioral risk factors for CVD are unhealthy diet, physical inactivity, tobacco use and abuse of alcohol. Other determinants for CVD are poverty, stress and hereditary factors. Hypertension is the leading risk factor for CVD and its prevalence has increased largely from 1975 to 2015 from 594 million to 1.13 billion, especially in low- and middle-income countries. Proper medications for hypertension, diabetes and high blood lipids are also important in the prevention of CVD (WHO Media centre 2017b).

Cancer is a multifactorial disease with a high prevalence in the aging of the populations. Cancer is the second leading cause of death worldwide, almost 1 in 6 deaths is due to cancer. The survival rates are getting better all the time due to the advances in treatments. Approximately 70% of deaths in cancer occur in low- and middle-income countries. Almost 1/3 of cancer deaths are related to behavioral risks such as unhealthy diet, physical inactivity and tobacco use and alcohol abuse. Carcinogenesis linked infections, hepatitis virus and human papilloma virus (HPV) infections, are responsible for 25% of deaths in cancer in low- and middle-income countries (WHO Media centre 2017a). However, in the last decade cancer research has shown the general importance of infection/inflammation environment to the initiation, development and fostering of cancer (Hanahan, Weinberg 2011).

The relationship between periodontitis and several systemic conditions has been studied for many years. Associations have been observed but causality has been difficult to establish. Periodontitis has been associated with CVD, diabetes, pre-term and low-birth-weight infants, more recently with Alzheimer's disease and with several types of cancer. The big problem in drawing any conclusions is the diversity of periodontitis classifications between the studies over the time as Linden et al. described in their review of the literature (Linden, Lyons et al. 2013).

In the infection paradigm, however, apical periodontitis (AP), a sequel of untreated caries, unsatisfactory root treatment, or emergence of resistant microorganisms to the medications used, has not been much studied in the relation with systemic diseases. Some associations with CVD and diabetes have been found but the evidence is weak, as recently reviewed by Khalighinejad et al. (Khalighinejad, Aminoshariae et al. 2016).

With this background, this thesis will focus on the incidence of cancer and cardiovascular diseases in a population cohort followed up since the year 1985. Swedish patient and hospital registers were used in analyzing the associations between oral and dental status and health markers with the systemic disease outcomes.

LITERATURE REVIEW

ORAL DISEASES

Oral diseases are diseases that affect the tissues of the oral cavity. They affect the mouth mucosa, the teeth, the tooth supporting tissues (gums and alveolar bone), and the salivary glands. Oral diseases can have a genetic background, like developmental defects of the teeth, which also may be associated with some syndromes. They can also be manifestations of systemic diseases like mucocutaneous diseases, hematologic diseases, immunodeficiency diseases, or are the result of poor hygiene habits, unhealthy food intake, use of tobacco and abuse of alcohol. The most common oral diseases are dental caries and its sequelae, gingivitis and periodontitis that, if not treated or prevented, lead to tooth loss.

DENTAL CARIES

Dental caries is an infection with detrimental effect on the hard tissues of the teeth. Several factors are needed for the development of caries. These include cariogenic bacteria (e.g. *Streptococcus mutans*), bacterial plaque accumulation (dental biofilm), fermentable substrate (sugars), and a susceptible dental enamel and dentine (Cawson, Binnie et al. 2001). Untreated caries in permanent teeth is the most prevalent condition in the Global Burden of Disease 2010 study, affecting 2.4 billion adults and untreated caries in deciduous teeth are the 10th most prevalent condition affecting 621 million children worldwide. The cases of untreated caries are shifting from children to adults and 3 peak ages when caries are more prevalent, 6, 25 and 70 years old, have been identified (Kassebaum, Bernabe et al. 2015).

Enamel caries

Initial caries affects the enamel surface. Dental enamel is a densely calcified structure in organic matrix. By acid attack, characteristic structural changes are seen in the enamel. Destruction of the enamel crystals lead to cavitation and bacterial invasion. Early white-spot lesions can be reversible but after cavitation only dentist's treatment can repair the damage (Cawson, Binnie et al. 2001).

Dentin caries

Dentin caries is the continuation of enamel caries that develops when bacteria and their metabolites get access through the enamel into the amelo-dentinal junction and further into dentine tubules. (Cawson, Binnie et al. 2001).

SEQUELAE OF DENTAL CARIES

Pulpitis

The progression of dental caries leads to infection of the dental pulp causing its inflammation, pulpitis. Pulpitis may then affect necrosis of the pulpal tissue. If not treated, periapical infection follows. Pulpitis can be acute or chronic and may not be in direct contact with the oral cavity (closed pulpitis). In this process very rarely pulpal tissues stays alive after the enamel and dentine are destroyed so that pulp is in direct contact with the oral cavity (open pulpitis) (Cawson, Binnie et al. 2001).

Inflammation of the pulp is initially localized at the site of injury with formation of an abscess and thus sparing the rest of the pulp tissue (reversible pulpitis). If the infection continues the inflammation will spread and lead to destruction of the pulp tissue and necrosis (irreversible pulpitis) (Ricucci, Loghin et al. 2014).

Apical periodontitis

Apical periodontitis is the inflammation of the periapical tissues due to spread of infection or bacterial metabolites of endodontic origin in the periapical ligament as a consequence of a necrotic pulp. Aseptic necrosis can also cause apical periodontitis due to the breakage of the apical blood vessels after dental trauma and consequent inflammatory response to the necrotic tissue (Cawson, Binnie et al. 2001).

PERIODONTAL DISEASE

Periodontal disease is an infection/inflammation disease of the supporting tissues of the teeth. It starts with the inflammation of the gingival tissues and can progress to destruction of the periodontal ligament and alveolar bone, and in untreated conditions can lead to tooth lost. Severe periodontitis in the Global Burden of Diseases 2010 study is the 6th most prevalent condition in the world, affecting 743 million people worldwide. There is an increase of prevalence between the 3rd and 4th decades of life with a peak in the incidence at the age of 38 years (Kassebaum, Bernabe et al. 2014).

Gingivitis

Inflammation of the gingival epithelium without the involvement of the periodontal ligament and the alveolar bone is defined as gingivitis. It is mostly associated with the accumulation of dental plaque in the surface of the enamel (Cawson, Binnie et al. 2001).

Gingivitis can also be related to systemic factors such as perturbations of the metabolic system, some medications and malnutrition. Systemic conditions can cause gingival disease without the accumulation of dental plaque on the tooth surfaces as is the case with lichen planus and lupus erythematosus. Some specific pathogens, bacteria, virus and fungi may also cause gingival disease without the presence of dental plaque (Armitage 1999).

Periodontitis

Periodontitis is the evolution of the chronic gingivitis leading to the loss of the tooth supporting periodontal tissue. Chronic inflammation due the accumulation of dental plaque causes migration of the junctional epithelium towards the apical direction, simultaneously with the destruction of the periodontal ligament and alveolar bone. This results in forming of deep pockets between the tooth and gingiva and the supporting tissues. Periodontitis may be a long lasting process. It can start in the childhood with gingivitis and then can take many years to reach the state of losing the teeth (Cawson, Binnie et al. 2001).

CYSTS OF THE JAWS

A cyst is a pathologic lesion lined by an epithelium with a liquid, semi-liquid or gaseous content. Cysts can be of developmental or inflammatory origin. Developmental cysts can be divided in odontogenic and non-odontogenic. Most common cysts of the jaws are radicular cysts with an inflammatory origin (Cawson, Binnie et al. 2001). Radicular cysts are the most common odontogenic cysts, resulting from the pulp necrosis after bacteria contamination. The necrotic area will then be epithelized by the odontogenic epithelium from the rests of Malassez and can lead to the resorption of the apical bone surrounding the root apex. The most common non-inflammatory cyst of the jaws is the dentigerous or follicular cyst that associates with unerupted or impacted teeth, has origin in the degeneration of the enamel epithelium that leads to accumulation of liquid between the epithelium and the crown of the tooth (Mosier 2015).

PREVALENCE OF ORAL DISEASES

Despite great improvement in oral health in the last decades the burden of oral diseases is still high. Even in the industrialized countries caries affects 60-90% of school children and the majority of adults are also having caries at some time of their life span. The consumption of sugar has a direct relation to caries, so that people living in countries with easier access to sugar are more affected by caries (WHO Media centre 2012b).

Globally, most children have signs of gingivitis and the adults have signs of initial periodontal diseases. Besides bad oral hygiene the main risk factor for periodontitis seems to be the use of tobacco (WHO Media centre 2012b).

From the Global Burden of Diseases 2010 study we can see the prevalence of oral diseases and how important it is to prevent and treat them (Marcenes, Kassebaum et al. 2013). See **Table 1**.

Table 1. Prevalence of oral diseases and ranking in the Global Burden of Diseases 2010 study.

Rank	Oral Disease	n (in thousands)	%
1	Caries in permanent teeth	2431636	35.29
6	Severe periodontitis	743187	10.79
10	Caries in deciduous teeth	621507	9.02
36	Severe tooth loss	158284	2.3

PREVENTION OF ORAL DISEASES

In the prevention of oral diseases, it is essential to educate the population of the importance of oral hygiene, and regarding avoidable risk factors such as sugar consumption, alcohol abuse and use of tobacco. Use of fluorides (fluoridated water, fluoride containing tooth pastes and other oral hygiene products) also needs to be emphasized. A good oral health system, with easy access, is the best way to promote prevention and treat the early stages the oral diseases with minimum permanent harm.

Continuing education of dental professionals has been one of the main goals of International Association of Dental Research (IADR) since its foundation in 1920, but also the knowledge dissemination to the population has more recently been administered together with the WHO, since 2013. These are implemented through advocating organizational structures, arranging meetings, with publications, study groups and fostering external relations (Whelton, Fox 2015).

Prevention in general should be a high priority in social care, in public as well as in private health care, and in all insurance models. Dentistry today has all the tools and instruments to treat, arrest and reverse the course of the chronic dental diseases. A shift from restorative dentistry to preventive dentistry has been the big step in controlling the burden of oral diseases but much work still needs to be done (Birch, Bridgman et al. 2015).

CARDIOVASCULAR DISEASES (CVD)

DEFINITION AND EPIDEMIOLOGY

Cardiovascular diseases (CVD) are diseases of the heart and blood vessels. CVD are the main cause of death worldwide, in 2015 an estimated 17.7 million patients died from CVD representing 31% of all global deaths. The coronary heart disease was responsible of 7.4 million deaths and of 6.7 million strokes (WHO Media centre 2017b). More than $\frac{3}{4}$ of CVD deaths take place in low and middle-income countries; thus the situation in Europe and Nordic countries is not the worst. In Finland, the death rate per 100 000 population has been decreasing since the 1970s. **Figure 1** shows the improvement during the years, being more distinct among men than women. Today the rates are low for men and women in working age (Official Statistics of Finland 2016a). Nevertheless, in 2015 cardiovascular diseases counted for 37% of deaths in Finland (Official Statistics of Finland). In Sweden, cardiovascular diseases were the leading cause of death in 2015, about 35%, with very similar numbers for men and women. Also in Sweden, a decreasing trend has been since 1987, a percentage reduction with more than half (55%) (Socialstyrelsen - The National Board of Health and Welfare 2016).

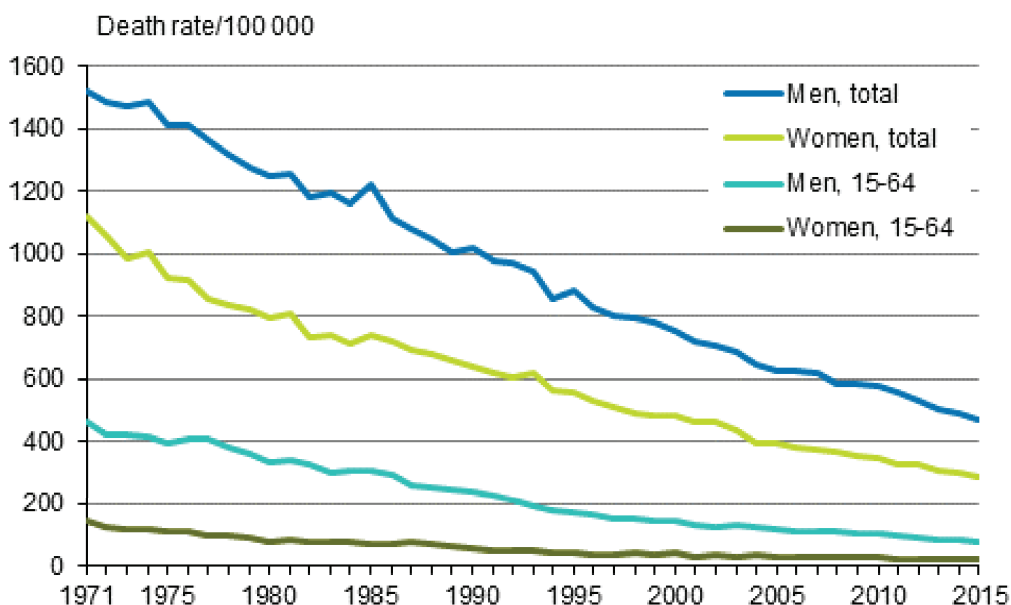


Figure 1. Age-standardized mortality from diseases of the circulatory system from 1971 to 2015 in Finland (Official Statistics of Finland 2016a)

PATHOLOGY

A patient with cardiovascular disease can have one or more pathologic conditions at the same time. We can separate them into heart and vascular diseases.

HEART DISEASES

Congenital heart disease

From all birth defects the congenital heart defects are the most common. Congenital heart disease accounts for one third of all major congenital anomalies, higher prevalence in Asia 9.3 per 1000 live births, then in Europe 8.2 per 1000 live births and North America with 6.9 per 1000 live births (van der Linde, Konings et al. 2011). With the current methods, the patients who go through a treatment protocol can reach the adulthood with a surviving rate of 90%. Nowadays they can live for a long time but most of them need to be followed up all life-long for cardiac complications and/or non-cardiac complications (Cohen, Earing 2016).

Valvular heart disease

The most affected valves are the aortic and mitral valves. The etiology varies with the distribution of populations: rheumatic fever, caused by streptococcal bacteria, is the major cause of valvular heart disease in undeveloped countries while degenerative diseases are the most common in industrialized countries (Woods 2016). The prevalence of valvular heart disease is estimated to be 2.5% in US and increases significantly after 65 years of age, with degenerative diseases predominating.

Coronary heart disease

CHD is caused by the accumulation of atherosclerotic plaques in the coronary arteries, leading to blood flow obstruction and ischemia. Atherosclerosis is asymptomatic until the blood flow obstruction leads to symptomatic ischemia. Acute ischemia resulting from plaque rupture and/or acute thrombosis may cause sudden death, unstable angina or myocardial infarction. Chronic ischemia may manifest itself during exercise or as stress angina. CHD is the most lethal of all cardiovascular diseases (Cinquemani 2016). CHD is more prevalent in developing countries but it is decreasing in developed countries, in Finland the prevalence being 26.3%, still a fairly high percentage (Zhu, Wang et al. 2016).

VASCULAR DISEASES

Diseases that affect the systemic and pulmonary vasculature.

Systemic vascular disease

Peripheral arterial disease, an atherosclerotic vascular diseases that compromises the blood vessels supplying the lower extremities, the arms and legs is the most common vascular disease.

Pulmonary vascular disease

In pulmonary vascular disease, pulmonary hypertension is the main sign with an elevated mean pulmonary artery pressure.

Venous thromboembolism includes the deep vein thrombosis and pulmonary embolism, blood clots formed in the veins of arms and legs that may cause embolic spread to the heart and lungs (Vongpatanasin, Victor 2016).

Hypertension

Arterial hypertension affects one third of the adult population. It is the leading cause of death in the world. Current recommendations suggest controlling blood pressure below 140/90 mmHg. Hypertension may lead to stroke, myocardial infarction, heart failure, peripheral vascular disease, atrial fibrillation and kidney disease (Vongpatanasin, Victor 2016).

ROLE OF INFECTION AND INFLAMMATION IN CARDIOVASCULAR DISEASES

Many cardiovascular diseases can have an infectious origin. These include viral myocarditis, infectious pericarditis (viral, bacterial, mycobacterial, fungal and protozoal origin) and rheumatic heart disease, caused by streptococcal bacteria and leading to rheumatic fever and damage of the myocardium and the heart valves (Woods 2016).

Inflammation is an innate immune response to infection and tissue injury, involving the recruitment of defense cells, blood cells and biochemical mediators with the purpose of eliminating infection and repairing the tissues. Acute inflammation is beneficial to the host by eliminating pathogens and promoting wound healing but if the problem is not resolved the inflammation becomes chronic in nature. Inflammation is present in pericarditis, myocarditis, rheumatoid arthritis and atherosclerosis.

Atherosclerosis is an inflammatory disease that leads to the development of plaques in the walls of the arteries. The plaques are formations of cholesterol, fatty substances, cellular waste products, calcium and fibrin. These can block the arteries partially or totally in any part of the body, leading to more severe diseases: coronary heart disease, angina, carotid artery disease, peripheral artery disease and chronic kidney disease. The most severe conditions are heart attack and stroke, resulting from the blockage of the arteries in the heart or in the brain, usually after a plaque rupture or the formation of a blood clot at the surface of a plaque. Atherosclerosis seems to have an origin in the damage of the artery walls and the main factors to be involved are elevated blood lipids, tobacco use and hypertension (American Heart Association 2017).

Hypertension is a social and behavioral disease that rarely causes symptoms. In association with other metabolic risk factors such as obesity, diabetes and raised blood lipids it can lead to cardiovascular diseases (**Fig. 2**). It is an invisible killer that could be stopped or prevented if detected in earlier stages of the disease (WHO Media centre 2013).

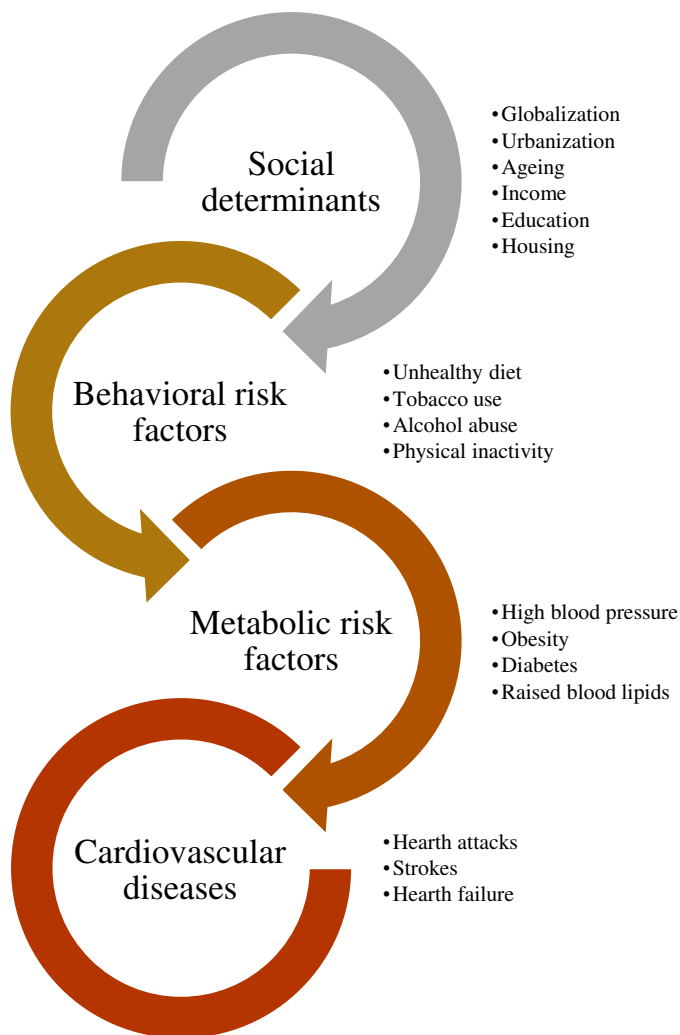


Figure 2. Hypertension risk factors and complications (WHO Media centre 2013)

PREVENTION OF CARDIOVASCULAR DISEASES

Cardiovascular diseases can be primarily prevented by controlling or eliminating behavioral risk factors such as smoking, unhealthy diet, physical inactivity, and abuse of alcohol. Cardiovascular status can be improved by secondary prevention, if other systemic conditions like hypertension, diabetes, hyperlipidemia and obesity are controlled. By prevention and early treatment of cardiovascular diseases the worst scenario can be avoided, namely, death of the patient. Individual and population-wide interventions should be implemented in order to reduce the incidence of cardiovascular diseases (WHO Media centre 2017b).

CANCER

BIOLOGY

Cancer is a multifactorial disease resulting from genetic transformations of a normal cell into a cancer cell, which acquires an uncontrolled proliferation and dissemination stage leading to disease or even death of the patient (Hezel 2016).

The transformation from a normal cell into a cancer cell is a multistage process. It usually starts with a pre-malignant lesion that develops into a malignant tumor. Those transformations arise from the interaction of the person's genetic factors together with three different external factors such as physical carcinogens (e.g. radiations), chemical carcinogens (in the air, food and water) and biological carcinogens (viral, bacteria or parasitic infections). Age is an important factor in cancer due to reduced cellular repair mechanisms and because of the accumulation of risk factors over time (WHO Media centre 2017a).

Tumors are more than a mass of cancer cells, however. They are a complex tissue with different cell types and many of them are normal cells working together with the intention of surviving and proliferation. Stromal cells (connective tissue cells or extracellular matrix) are important in the tumor biology and microenvironment.

In 2000, Hanahan and Weinberg made a summary of 6 Hallmarks of Cancer: resisting cell death, sustaining proliferative signaling, evading growth suppressors, enabling replicative immortality and inducing angiogenesis (Hanahan, Weinberg 2000). In 2011, four more hallmarks were added: deregulating cell energetics and avoiding immune destruction, and genome instability and mutation and tumor-promoting inflammation (Hanahan, Weinberg 2011) (**figure 3**).

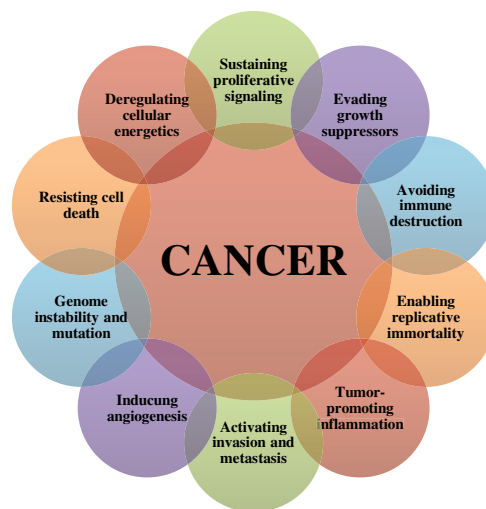


Figure 3. Hallmarks of cancer 2000 and Hallmarks of cancer: next generation 2011 summary (Hanahan, Weinberg 2000, Hanahan, Weinberg 2011)

EPIDEMIOLOGY / PREVALENCE OF CANCER

Cancer is the second leading cause of mortality in the world, after cardiovascular diseases. It counted for 8.8 million deaths in 2015 and the number of new cases are expected to rise 70% over the next two decades. The most common cancer deaths are due to cancers of the lung, liver, colorectal, stomach and breast. Approximately 70% of deaths from cancer occur in low- or middle-income countries. Viral infections such as hepatitis B virus (HBV), hepatitis C virus (HCV) and human papilloma virus (HPV) induced cancers are responsible for more than 25% of cancer deaths in low- and middle-income countries (WHO Media centre 2017a).

In Finland, cancer is the second leading cause of death accounting for 24% of deaths. The most common cancers leading to death for men are lung cancer and prostate cancer, and for women, breast cancer and lung cancer. In **figure 4** the distribution by gender since 1971 is shown. Cancer death rate has decreased especially for men (Official Statistics of Finland 2016b). At working age, nearly half of women died of neoplasms, breast cancer being the most common mortal malignancy (Official Statistics of Finland).

In Sweden, cancer also is the second leading cause of death, counting for around 25% of deaths in 2015. Mortality is somewhat higher in men than women. The deaths caused by tumors have decreased by 13% since 1987, however (Socialstyrelsen - The National Board of Health and Welfare 2016).

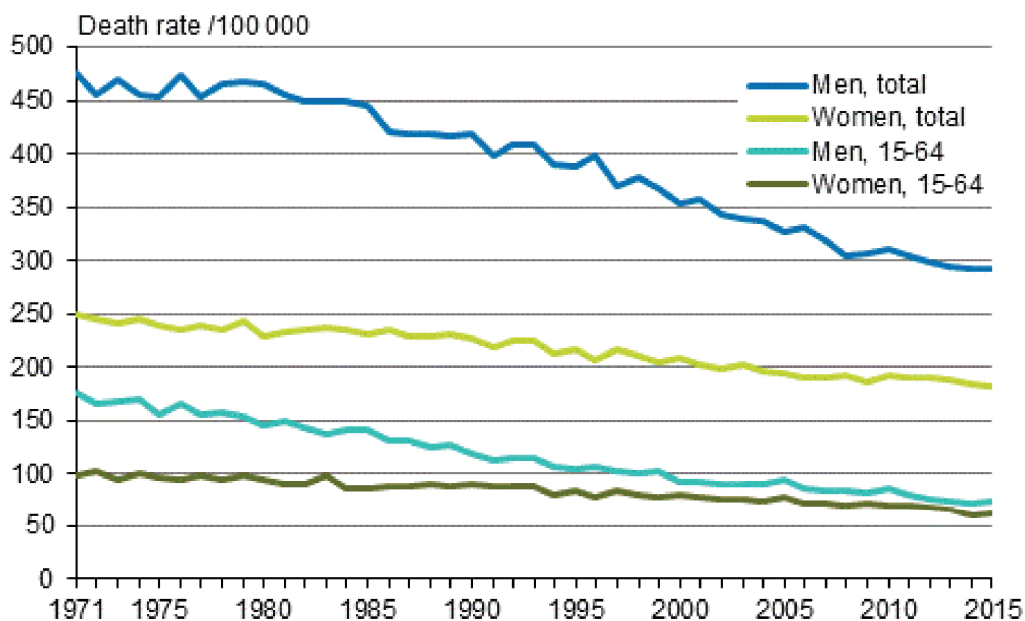


Figure 4. Age-standardized mortality from neoplasms from 1971 to 2015 in Finland (Official Statistics of Finland 2016b)

INFECTION AND INFLAMMATION IN CANCER

Viral, bacterial and parasite infections are considered biological carcinogens. 15-20 % of cancers worldwide are related to infections (American Cancer Society 2016b). In Europe, 7.4% of new cancer cases are estimated to be related with infections. HPV, HBV, HCV and *Helicobacter pylori* represent 95% of the cases (Villain, Gonzalez et al. 2015). The most accepted infections related to cancer are indeed viral infections with HPV, HBV, HCV, HIV, Epstein-Barr virus, Human Herpes Virus 8, Human T-lymphotropic virus-1 and Merkel cell polyomavirus (American Cancer Society 2016d).

Bacterial infections have also been connected with cancer. In particular *Helicobacter pylori* has been related to gastric cancer and the treatment and elimination of this bacterium has reduced gastric cancer incidence in Europe. Another bacterium, *Chlamydia trachomatis* has been related to cervical cancer in conjugation with HPV (American Cancer Society 2016a).

Parasitic infections related to cancer are not that well known and are not so common either in this respect. *Opisthorchis viverrini* and *Clonorchis sinensis* are liver parasites and have been related to bile duct cancer; these infections come from eating raw fish and they occur in East Asia. *Schistosoma haematobium* is a parasite found in the water of some countries in the Middle East, Africa, and Asia. It has been linked to bladder cancer (American Cancer Society 2016c).

Tumor-promoting inflammation is one of the characteristics regarded as a new generation of hallmarks of cancer. The presence of immune system cells from both innate and adaptive responses around the neoplastic lesions is clear. The initial immune response may have a protective role in the elimination of the lesion, but later on it seems to work with and for the tumor, gaining a tumorigenic effect and fostering the growth and dissemination of tumor cells and promoting the tumor-favorable microenvironment (Hanahan, Weinberg 2011).

PREVENTION OF CANCER

Almost 30-50% of cancers could be avoided by reducing the main risk factors and implementation of preventive measures. Smoking is the most important risk factor responsible for 22% of all global cancer deaths. The other main risk factors are alcohol abuse, unhealthy diet and physical inactivity. Some chronic infections are risk factors especially in low incoming countries. Burden of cancer can also be avoided by early detection and adequate treatment of the patients. Vaccination for HPV and HBV, reduced exposure to radiation (UV or ionizing radiation) and controlling occupational hazards are also important measures in cancer prevention (WHO Media centre 2017a).

BREAST CANCER

The most common cancer diagnosis in women is breast cancer which also is the second leading cause of cancer death after lung cancer in the female. Breast cancer is a disease of elderly women but with a strong family relation with BRCA1 and BRCA2 defects in susceptibility genes. It is related to early menarche, late menopause, null-parity or first pregnancy after 25 years-old, prolonged use of estrogen contraceptives, exposure to ionizing radiation and obesity (Kuderer, Lyman 2016).

Even though breast cancer is the most common cancer diagnosis in women, mortality has been reduced due the early diagnosis and advances in treatment therapies. Since 1990 breast cancer mortality has decreased more than 2% annually (Kuderer, Lyman 2016). Practice of multidisciplinary teams to diagnose and treat breast cancer patients and proper follow-up of survivors are important steps in the improvement of survival rates (Kuderer, Lyman 2016).

HEAD AND NECK CANCER

Head and neck cancers are mainly squamous cell carcinomas with origin in the mucosa of the oral cavity, oropharynx, nasopharynx, hypopharynx and larynx (McNamara 2016). These cancers are sixth in incidence figures worldwide with 550,000 cases per year. About 300,000 patients with head and neck cancer die each year. Higher incidence is seen in men with range of 2:1 to 4:1. The main risk factors are smoking, alcohol abuse and HPV infection (Jemal, Bray et al. 2011).

ORAL CANCER

Use of tobacco and abuse of alcohol are the main risk factors also for oral cancer which is the eight most common cancer in the world. The incidence is highest among elderly men. Oral cancer is mainly oral squamous cell carcinoma (OSCC) mostly seen in the lip and tongue. For lip carcinoma, the sunlight exposure is an important life style risk factor. OSCC counts for a large number of head and neck cancers (Scully 2008). Besides long time smoking and use of alcohol, the infection with HPV has also shown to be associated with oral cancer (Rivera 2015).

THE CELL CYCLE

The cell cycle is the basic process by which each cell duplicates their contents, with the intention of division in two similar new cells. The DNA content in the chromosomes and other organelles have to be produced in duplicate to be divided by the two new cells. The time of cell cycles vary from cell type to cell type and from the age of the individual, in the embryo phase the cycle is much faster than in the adult age. The eukaryotic cell type divides in four main phases: M-phase, G1-phase, S-phase and G2-phase. The interphase is the phase where the cell grows continuously and comprises the G1-phase, S-phase and G2-phase, the M-phase is when mitosis and cytokinesis happen, as summarized in **figure 5**.

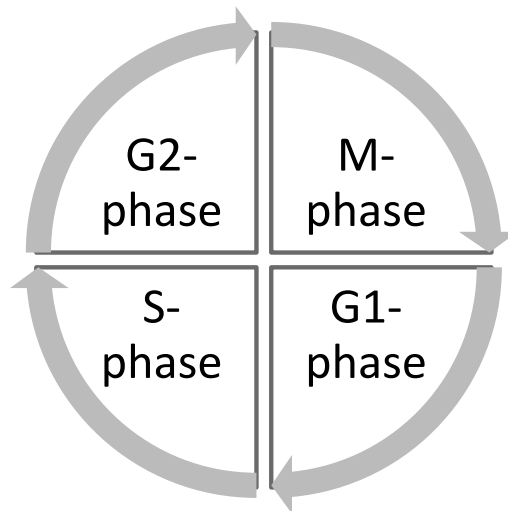


Figure 5. Cell cycle main phases.

The cell cycle control system is the mechanism that controls all the steps of this complicated cycle and insures everything is happening in the correct order and time (Alberts, Bray et al. 2014b).

Cyclin-dependent kinases (CDKs), p53 and pRB pathways can be involved in the control of cell apoptosis after DNA damage. The tumor suppressor pRB is a critical regulator of G1/S progression, the expression of the RB gene is epigenetically silenced by methylation of the promoter in some cancers. Another important tumor suppressor, p53, functions as the gatekeeper of the genome to control cell cycle arrest and apoptosis in response to DNA damage (Kitagawa, Kitagawa et al. 2013).

To control the cell number and cell size there are three different processes to be considered: cell growth, cell division and cell apoptosis. Apoptosis is important to regulate cell number and is

regulated by an intracellular proteolytic cascade, where the main proteins responsible belong to the Bcl2 family. Also extracellular signals can induce apoptosis. Animal cells need extracellular signals to survive, growth and division (Alberts, Bray et al. 2014b).

Organized cells can form different tissues with the help of extracellular matrix. When tissues are injured, a repair system is activated, and pluripotent stem cells are recruited to replace the missing tissue. When errors occur in the division control and they are not eliminated, an abnormal cell with an increased competitive growth advantage compared with normal cells, may be able to proliferate, invade and metastasize tissues (Alberts, Bray et al. 2014a). In principle, anything speeding up the cell cycle increases the possibility of a formation of a new cancer cell.

INFLAMMATION AND INFLAMMATORY BIOMARKERS

Inflammation is a biologic protective process that happens after infection or tissue injury. The main goal is to remove infection and regenerate the tissue to the original form. However, occasionally instead of regeneration the repair process causes scar tissue formation. If an infection is not subsided completely and it becomes constant, a state of chronic inflammation will be activated by the immune system. Many biomarkers and different cellular metabolic cascades have been identified and studied in the infection/inflammation process. In periodontitis, AP, CVD and cancer, matrix metalloproteinases (MMPs) have been particularly studied and been related to these diseases. In this thesis the focus is in MMP-8, MMP-9, MMP-13 and tissue inhibitor of metalloproteinases 1 (TIMP-1).

MATRIX METALLOPROTEINASES (MMPS)

Matrix metalloproteinases are zinc containing endopeptidases dependent on calcium. MMPs are able to degrade all extracellular matrix proteins but can also process several bioactive molecules. MMPs can also play a major role in cell activities such as cell proliferation, migration, differentiation, angiogenesis, apoptosis, and host defense (Verma, Hansch 2007).

MMP-8

MMP-8 is a collagenase and mostly acts in the metabolism of collagen type I, the most common collagen in the periodontal ligament, for example. It has indeed been involved in periodontal disease (Goncalves, Huang et al. 2013) and also in apical periodontitis (Matsui, Yamasaki et al. 2011). In both these diseases, besides the destruction of collagen fibers, MMP-8 has a role in activating osteoclasts, which leads to the destruction of the alveolar bone. MMP-8 is also involved in the rupture of atherosclerotic plaques and is present in higher concentrations in subclinical and atherosclerotic lesions being associated with the worst cardiovascular outcomes (Tuomainen, Nyssonen et al. 2007). In cancer, collagenases like MMP-8 can have an important role in cancer tissue growth, invasion and metastasis (Ala-aho, Kahari 2005).

MMP-9

MMP-9, a type IV collagenase and gelatinase B, is involved in the basement membrane remodeling. It can be produced by periodontal ligament fibroblasts and polymorphonuclear cells by inflammation and it is responsible for type IV collagen degradation at the sulcus epithelium and gingival connective tissue (Chang, Yang et al. 2002). In AP, MMP-9 is present in larger areas with gram-negative bacteria and associates with symptomatic lesions (Ahmed, El-Baz et al. 2013). In atherosclerosis MMP-9 is associated with the rupture of atherosclerotic plaques. Higher MMP-9 levels in serum are associated with myocardium infarction and stroke, but it is not considered as a strong independent marker for those diseases (Jefferis, Whincup et al. 2010). MMP-9 is also involved in invasion and dissemination of different tumors, facilitating the mesenchymal and vascular invasion of cancer cells (Farina, Mackay 2014).

MMP-13

MMP-13 (collagenase 3) expression was first discovered in breast cancer (Freije, Diez-Itza et al. 1994). It has been particularly involved in inflammatory diseases such as rheumatoid arthritis and

osteoarthritis where it associates with the resorption and destruction of bone and cartilage (Goldring, Otero et al. 2011). It is expressed by different cells of the periodontium and inflammatory cells in association with chronic periodontitis (CP) (Hernandez Rios, Sorsa et al. 2009, Hernandez, Valenzuela et al. 2006). MMP-13 levels can be higher in gingival crevicular fluid (GCF) of CP patients (Hernandez, Martinez et al. 2007). In saliva, MMP-13 levels have been elevated in localized periodontitis but decreased in generalized periodontitis (Gursoy, Kononen et al. 2013). MMP-13 has also been related to apical periodontitis, being increased in an early stage of the disease (Matsui, Yamasaki et al. 2011). As a collagenase, degrading type I and III collagen fibers, MMP-13 has also an important role in the atherosclerotic plaque. In cases with polymorphism of the MMP-13 gene, its role can be altered and lead to an instable plaque that will rupture easily (Vasku, Meluzin et al. 2012).

TIMP-1

TIMP-1 is an endogenous inhibitor of MMPs implicated in inflammation and cancer processes. The balance between MMPs and TIMPs is important in the degradation of the extracellular matrix (ECM) (Nagase, Visse et al. 2006).

DENTAL INFECTIONS AND SYSTEMIC DISEASES

The theory of focal infection is an old idea that started with Hippocrates reporting “arthritis was cured after a tooth extraction”, continued with Miller (1890) who introduced the term “oral focal sepsis”. Miller recommended that decayed parts of a tooth should be replaced by restoration and root canal fillings. Nevertheless, in 1900 William Hunter reported that many carious teeth have been preserved with different prosthetics works but the infection foci were not eliminated (Hunter 1900). In 1940 Fish published an article about teeth as a source of systemic infections, showing that oral bacteria can spread to the blood stream just by chewing or tooth brushing and after tooth extraction, and that bacteria can be found in proximal but also distal blood vessels. Further, he stated that bacteria and their toxins accumulate in tissues of mesenchymal origin, in joints, muscles and nerves, leading to the pathogenesis of different diseases like osteomyelitis, fasciitis, fibromyalgia and endocarditis (Kumar 2013). Unfortunately, the extraction of teeth, diseased or not, as a preventive approach for different diseases, was the result of this theory, and quickly therapeutic edentulation became the norm. However, in spite of the radical tooth extractions, people still suffered from arthritic symptoms and, in addition, developed diverse digestive complications. Because of this extreme outcome of the focal infection theory and its “therapeutic” consequence, the theory was refuted for several decades as scientifically not true (Kumar 2013).

Today with the development of new techniques to identify and classify microorganisms and with less invasive surgery it has been possible to identify oral pathogens in atheroma plaques, in valvular vegetations, in the trachea-bronchial tree and in the pancreas. These findings show that the oral cavity indeed can be the origin of different microorganisms that migrate to different tissues and in susceptible individuals cause various pathology. Oral bacteria, bacterial products, toxins and inflammatory products can spread from the oral cavity during oral hygiene procedures and dental treatments. The resulting bacteremia in healthy patients is transient but in immuno-compromised patients it can lead to disease due to the colonization of different tissues by the oral microorganisms. Bacterial products like the lipopolysaccharide (LPS) and endotoxin are also released in the blood stream and may cause inflammatory responses in different organs.

Periodontitis has been extensively studied in this regard. In 1996 the term “Periodontal Medicine” was introduced in the World Workshop in Periodontitis (Offenbacher 1996). Also AP has been related to systemic diseases but with less literature support, but also the term “Endodontic Medicine” has been suggested by Segura-Egea in 2015 (Segura-Egea, Martin-Gonzalez et al. 2015). Diseased periodontium is particularly a reservoir of chronic inflammatory markers such as tumor necrosis factor α (TNF- α), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-8 (IL-8) and prostaglandins, that may contribute to systemic inflammation (Kumar 2013).

Periodontitis has been related to a number of different systemic conditions, such as CVD, diabetes, pre-term and low-birth-weight infants (Han, Houcken et al. 2014, Carramolino-Cuellar, Tomas et al. 2013). More recently, periodontal disease has also been linked to Alzheimer’s disease (Kamer, Craig et al. 2008) and even associated with cancer (Fitzpatrick, Katz 2010). Dental prophylaxis and periodontal treatment can help to reduce the incidence of ischemic stroke (Y. L. Lee, Hu et al. 2013).

AP has also been related to CVD (Cotti, Mercurio 2015) and diabetes (Segura-Egea, Martin-Gonzalez et al. 2015), but data are sparse in this regard. Studies have shown how the interactions of cytokines resulting from AP lesions with proinflammatory and immunoregulatory mechanisms, a persistent chronic inflammatory condition, can influence the cardiovascular system, leading to CVD (Colic, Gazivoda et al. 2009, Martinho, Chiesa et al. 2012).

Chronic dental diseases may add to the burden of diseases that are the leading causes of death and disability all over the world, namely CVD, cancer, chronic obstructive pulmonary disease and type 2 diabetes. All these diseases have a shared biological background: high blood pressure, high blood cholesterol and overweight; and major behavioral risk factors such as unhealthy diet, physical inactivity, tobacco use, alcohol abuse and psychosocial stress. All these biological and behavior factors are also related to the chronic dental diseases (Petersen, Ogawa 2012).

Socioeconomic determinants affect all chronic diseases. Political, economic, social and community factors are of great importance in the preventing and reducing the incidence of chronic diseases. Oral health outcomes, in turn, will also be influenced by these determinants throughout the life. Macro-environmental factors indeed are political, economic and social. At a community level there are differences in education and culture and also in the access to health care system which is a different determinant. At the personal level there are behavior factors which also affect both the oral health and general health outcomes (J. Y. Lee, Divaris 2014) . **Figure 6** summarizes the proposed framework here discussed.

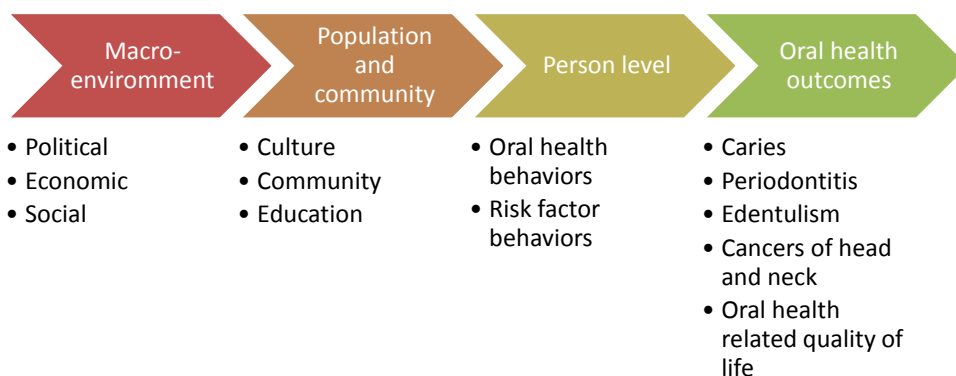


Figure 6. Summary of the proposed framework to address and eliminate the oral health disparities, based on Lee and Divaris (J. Y. Lee, Divaris 2014).

DENTAL INFECTIONS AND CVD

The first modern time links between dental infection and CVD are from Finnish studies in the 1980s. These investigations showed a statistical association between poor oral health condition of the patients and end point heart infarction (Mattila, Nieminen et al. 1989) and cerebral infarction (Syrjanen, Peltola et al. 1989).

Later on, follow-up cohort studies have been published, and in 2003 a comprehensive review by Scannapieco et al. concluded that a modest association between periodontitis and CVD indeed is possible (Scannapieco, Bush et al. 2003). However, the diverse range of populations and the different classifications for periodontitis make the comparisons difficult between the studies. There are also data showing no association at all in some study populations.

In 2005, Mattila et al. reviewed the data and concluded that periodontitis can promote atherosclerosis due to changes in lipoproteins and by the inflammatory and immune systems. Patients with periodontitis and increased CRP levels are more prone to CVD complications indicating that a genetic background might have an important role in explaining these associations (Mattila, Pussinen et al. 2005).

In 2006 Demmer and Desvarieux (Demmer, Desvarieux 2006) revised the literature and added that besides earlier cross sectional studies, new studies have been done focusing in systemic antibodies for oral pathogens. A 10-year follow-up study, where antibodies to specific oral pathogens associated with an increase of atherosclerosis in the carotid artery, showed increased risk of developing coronary events (Pussinen, Alfthan et al. 2004). Other studies measured quantitatively the amount of periodontal bacteria from the dental plaque and compared with control bacteria. A clear association of the amount of dental plaque bacteria associated with the thickness of the intima-media of the carotid artery (Spahr, Klein et al. 2006). Some interventional studies have also been made. Unfortunately these studies were not randomized, and did not have untreated control group with periodontitis, and only had a small number of patients. Nevertheless the results from the studies showed improvement in the systemic inflammation markers and subclinical CVD (Seinost, Wimmer et al. 2005, Mercanoglu, Oflaz et al. 2004, Elter, Hinderliter et al. 2006).

The direct pathways of systemic infection are based on the idea that oral bacteria spread to the circulation during daily activities and normal oral hygiene routines (Tomas, Diz et al. 2012). A transient bacteremia is proved to take place after dental treatments, and if the patient has chronic dental disease the number of bacteria will be higher. On the other hand if the patient is immunocompromised the risk of infection complications is greater (Olsen 2008). In 2013, Reyes et al. made a summary how oral pathogens can invade the human cardiovascular cells and influence the mechanisms of atherosclerosis (Reyes, Herrera et al. 2013).

In **figure 7** an explanatory diagram shows how bacteria from the oral cavity can affect the cardiovascular system and specially atherosclerosis, as depicted by Demmer and coworkers (Demmer, Desvarieux 2006). Two different infection pathways, direct and indirect, can lead to vascular injury. Later on inflammation and dyslipidemia will lead to atherosclerosis. A disrupted plaque can lead to obstruction of blood vessels leading to ischemia.

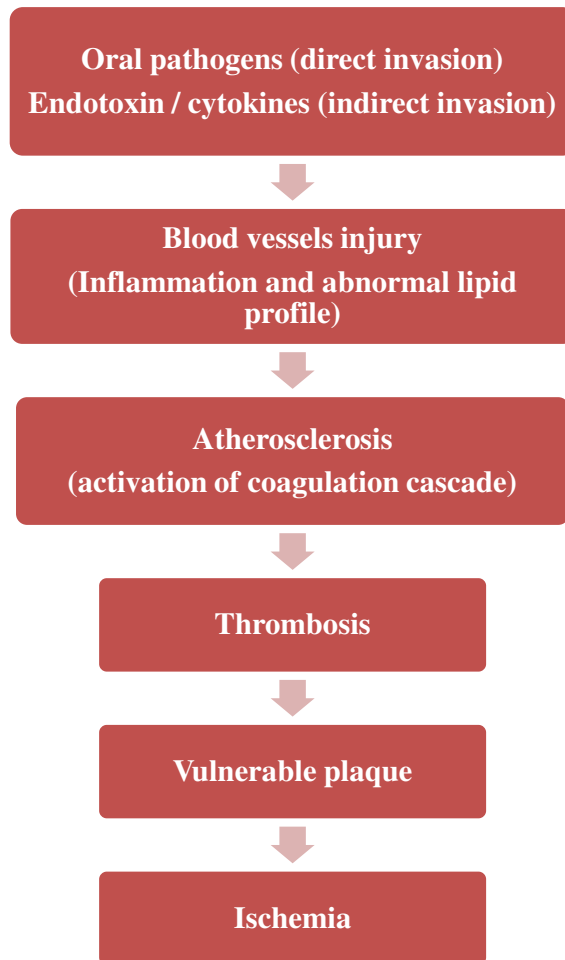


Figure 7. Possible pathways of interaction from pathogenic oral bacteria and the cardiovascular system leading to CVD, based in Demmer and Desvarieux (Demmer, Desvarieux 2006)

An association between dental diseases and CVD have been discussed for more than a century, and a good body of evidence supports the concept that periodontal disease in particular associates with CVD independently of known confounders. However, this is mostly based on observational studies, which cannot prove that periodontal disease would have any causal links to CVD. Nevertheless, interventional studies have shown that periodontal therapy can reduce the levels of inflammatory markers and consequently improve endothelial function temporally, but no long-term effect has been proved so far. Thus, the true effect of periodontal therapy on the incidence of CVD remains to be shown, as also stated by the American Heart Association in 2012 (Lockhart, Bolger et al. 2012).

DENTAL INFECTIONS AND CANCER

Infections and inflammation have been related to carcinogenesis, and, in fact, 15-20% of carcinomas have an infection background (American Cancer Society 2016b). Relation between oral infection and carcinogenesis is weak, however, and studies in this area sparse.

In 2010, Fitzpatrick et al. (Fitzpatrick, Katz 2010) reviewed the literature concerning the possible association between periodontitis and different types of cancer like oral cancer, esophageal cancer, gastric cancer, lung cancer, pancreatic cancer, prostate cancer, breast cancer and hematologic cancer. They concluded that oral cancer showed the most concise results in this association, while associations in this regard with the other types of cancer were not clear. Thus they further discussed that a consensus in the periodontitis classification would be important to establish to make studies comparable; furthermore controlling cofounding factors is also challenging and needs to be kept in mind for future studies.

In 2011, Meurman and Bascones-Martinez (Meurman, Bascones-Martinez 2011) addressed this subject again and also concluded that the evidence is weak and more studies are needed in the area. In oral cancer, poor oral health rises the risk of this type of cancer and based on the paradigm, infection/inflammation might play a role in carcinogenesis also in distant organs.

Periodontitis has been studied more in this regard than apical periodontitis. So far only one case report of metastatic breast cancer in the jaws has been linked to apical periodontitis (Khalili, Mahboobi et al. 2010).

In **figure 8**, a diagram shows how chronic infection/inflammation can promote changes in local cells, leading to the activation of different cascades of biomarkers in order to the attempt to solve the infection. When the infection is not subsided the markers can be overexpressed and lead to different responses. Some of the reactive species, such as oxygen and nitrogen oxide, can cause mutations and affect DNA repair, promote increased cell division and lead to uncontrolled proliferation, the endpoint being cancer.

Although a biologic plausible mechanism has been depicted in **figure 8** the evidence of a causality has not been proved. Sharing the background of many behavioral risk factors makes these two chronic diseases a challenge for studies in causality. In addition, all possible common behavioral backgrounds, eventually there are other factors such low education level, that should also be considered, as suggested by Conway et al., for example, for head and neck cancer, accounting for one third of the risk for this type of cancer (Conway, Brenner et al. 2015). Socioeconomic factors may change during the life time and indeed have an important impact as risk factors for different types of cancer (Sharpe, McMahon et al. 2014)

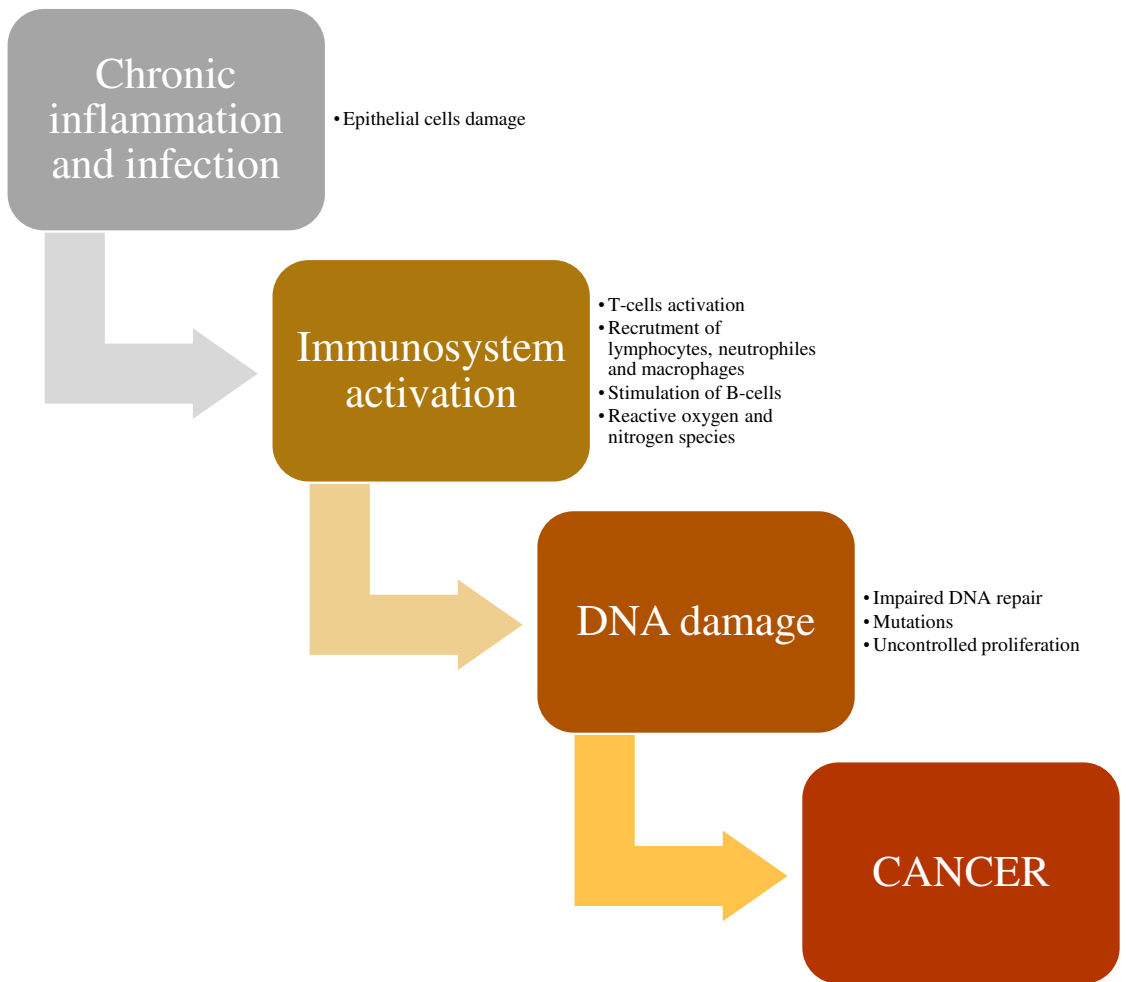


Figure 8. Possible pathways that link chronic infections/inflammation and cancer, based on Meurman and Bascones-Martinez (Meurman, Bascones-Martinez 2011)

AIMS OF THE STUDY AND HYPOTHESIS

The general aim of this study was to investigate possible associations between chronic dental diseases and general health conditions. Periodontitis and apical periodontitis are among the most common chronic dental infections in populations while cardiovascular diseases and cancer are the leading causes of death worldwide. Hence, we were interested in investigating the associations between these prevalent disease entities with the **hypothesis** that there indeed is a link between periodontitis, apical periodontitis, cardiovascular diseases and cancer.

The specific aims were in:

Study I, to investigate the incidence of cancer in periodontally healthy patients in a 24-year prospective study.

Study II, to analyze hospital register findings with clinical and x-ray oral examination data in a cross-sectional study.

Study III, to investigate biomarkers for periodontitis, namely MMP-8, MMP-9, TIMP-1 and MMP-13 in saliva and serum, in a cross-sectional data.

PATIENTS AND METHODS

Study population

A representative cohort from the Stockholm County was the study population of this thesis. In 1985, altogether 105798 patients were included as representatives of the Stockholm County. They were selected by being born on the 20th of any month from 1945-1954, with an age range of 10 years. From the original cohort, 3273 subjects were randomly selected to participate in the first studies, and 1676 went through a clinical examination. In 1985 they were separated in two main groups based on periodontitis classification: periodontitis (286 subjects) and no-periodontitis (1390 subjects). On the first study of this thesis, the no-periodontitis patients group was used to cross-analyze the original data with the national Swedish cancer register data from 2009. The original study population had undergone follow-up examinations several times since 1985. In 2001, using a computer program, 150 age and gender balanced patients were randomly selected, 100 from the periodontitis group and 50 from the no periodontitis group.

Between 2001 and 2003 they were recalled and examined again and full mouth X-rays were taken from all subjects that participated. In total 120 are included in the study II. National Swedish hospital register file from 2003 was used to analyze the associations between the oral health status and hospital diagnoses.

In 2008-2009, the subjects were recalled for follow-up examination and 90 participated in the study. From them, blood and saliva samples were collected and analyzed for specific biomarkers for the III study of this thesis. **Figure 9** describes the evolution of the patient material.

All the subjects included have been described before in other studies (P. O. Soder, Jin et al. 1994, Yakob, Kari et al. 2012, Airila-Mansson, Soder et al. 2006, Virtanen, Soder et al. 2013). All studies have been approved by the Ethics Committee of the Karolinska Institutet and Hudding University Hospital, Sweden, and the studies were conducted in accordance with the Declaration of Helsinki, as revised in 1983. All patients had been given informed consent to participate in these studies.

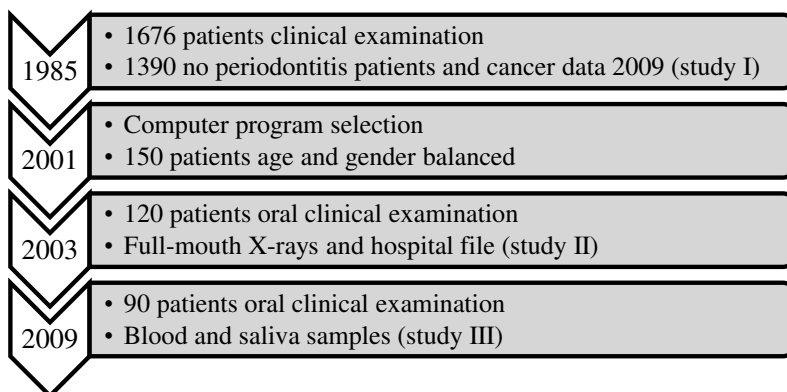


Figure 9. Study population flow-chart

Cancer and socioeconomic data

The cancer data were obtained from the Center of Epidemiology, Swedish National Board of Health and Welfare. The data had been classified and cancer codes extracted from the files based on the WHO in ICD-7, ICD-9 and ICD-10 codes. The socioeconomic data were from the National Statistics Centre, Örebro, Sweden. Both data files were available for study thanks to the unique register file in Sweden that keeps track of all citizens born on the 20th of any month since 1985 and ongoing.

Clinical examinations

At clinical dental examinations at baseline and follow-ups, the number of remaining teeth was recorded. All teeth were examined, with exception for the wisdom teeth, when present. The gingival inflammation was assessed by the gingival index (GI) and the bleeding on probing (BOP) index. The oral hygiene status was calculated using the dental plaque index (PI) and the dental calculus index (CI), respectively. Periodontal status was based on records of the probing depth (PD) and attachment loss (AL). All measures were taken at 6 points for each tooth using a standard periodontal probe according to the WHO recommendations for oral health surveys.

The classification for periodontitis in all studies (I-III) was 1 or more deep pockets (≥ 5 mm) and BOP as used in previous studies for this population (Yakob, Kari et al. 2012, B. Soder, Jin et al. 2007).

At the time of the clinical examinations patients always filled out a questionnaire recording the signs and symptoms of infection-inflammation in the oral cavity, reported caries and its squeals, periodontal problems and smoking habits and oral hygiene habits.

X-ray examinations

In 2003, full mouth x-rays were taken from 120 patients. Ekta Speed periapical radiographs, an Eggen-film holder, Oralix or Gendex roentgen machines with a rectangular section and a film focus distance of 30cm were used to perform the radiographic examination. From the X-rays a modified Total Dental Index (TDI) (Mattila, Nieminen et al. 1989) was used to record all signs of infections, in a scale from 0-10, recording caries lesions, deep vertical pockets, AP and furcation lesions. A higher score reflects a higher infection burden of the mouth. When apical periodontitis was present, the periapical index (PAI) (Orstavik, Kerekes et al. 1986) was used to classify the severity of the lesions. Number of root treatments and their quality was also register.

Sampling and analyses of saliva and blood

In 2009, blood and saliva samples were collected from 90 patients. Antecubital venous blood samples were collected after 12 hour overnight fasting, serum samples were prepared and deep frozen for later analysis. Saliva samples were taken after 1 hour without eating, drinking or smoking. Stimulated saliva was collected into graduated test tubes while patients chewed a 1g paraffin piece for 5min. All saliva samples were deep frozen and stored for later analysis.

To study the biomarkers selected, specific commercial ELISA kits were used for MMP-9, MMP-13 and TIMP-1. For MMP-8 analyses, IFMA was used. Those biomarkers were selected because they are present in all the diseases of interest in our study (periodontitis, apical periodontitis, cardiovascular diseases and breast cancer), as explained briefly in the review of the literature.

Statistical analyses

In study I, the statistical analyses were made with PASW[®], version 20. In study II and III, using SPSS[®], version 22.

In study I analyses of variance, chi-square test, Fisher's exact t-test and multiple logistic regression analyses were used to interpret the data. All *p*-values were two-tailed and the significance was set at *p*<0.05. All confidence intervals were calculated at 95% level. Multiple logistic regression analyses were made to compare the incidence of cancer in 2009, according to the state of oral health at baseline 1985, while simultaneously controlling for the following confounding variables: age, gender, frequency of dental appointments, education, income, socioeconomic status, working status, pack-years of smoking, dental plaque index, calculus index and any missing tooth. Teeth were dummy coded according to reference cell-coding with K-1 dummies.

In study II independent samples t-test was performed to compare the groups, two-tailed significance set at 0.05, analysis of covariance (ANCOVA) to adjust the results for age, gender and smoking. Regression analysis were done to study the associations between periodontitis and apical periodontitis with cardiovascular diseases. In both regression analyses cofounders were taken into consideration and backward stepwise likelihood ratio method was used. To study the number and the severity of AP lesions in both groups chi-square test was used.

In study III independent t-test for equality of means was used to compare the groups and in case of non-normality, Mann-Whitney U test was used. Significance was set at 0.05, two-tailed. Bonferroni corrections were used for multiple comparisons. A regression analysis with backwards likelihood method was used to interpret the MMP-13 saliva results.

RESULTS

Incidence of cancer after 24 years in no-periodontitis patients (study I)

In 1985, 1676 patients went through an oral clinical examination, of whom 286 had periodontitis (17.1%) and 1390 had not (82.9%). In 2009 the age range of the study population was 54-64 years. The patients without periodontitis showed a cancer incidence of 5.1% according to 2009 cancer file. See **Table 2**.

In study I, no-periodontitis patients were studied with respect of having cancer or not in 2009. Age and gender showed to be significant risk factors for women, older female patients having more often cancer than younger ones. In the cancer group, missing teeth, especially missing molars, were more often detected than among patients with no malignancy. In the regression model controlling for several confounders (gender, dental visits, education, income, socioeconomic status, working status, pack-years smoking, PLI, CI and GI) the explanatory factors for having cancer were age and d47 missing, with OR (95% CI), 1.91 (1.06-3.43) and 2.62 (1.18-5.78), respectively.

The cancer distribution was very scattered for men, but for women it was clear that breast cancer was the most common in this population.

Table 2. Main results from the epidemiologic study (I) and previous published study (Virtanen, Soder et al. 2013)

Clinical examination 1985 (1676)	No periodontitis (1390)	Periodontitis (286)
Incidence of cancer in 2009	5.1%	6.3%
Explanatory factors	Gender Age Missing teeth Missing molars (missing d47)	Missing d46 and d47
Most common cancers	Breast cancer in women Scattered for men	Breast cancer in women Scattered for men

Apical periodontitis and diseases from the hospital file (study II)

In 2003, a representative population from the original cohort (120 patients) was examined clinically and with full-mouth x-rays. The age range was 48-58 years. The apical area was classified using the PAI scoring. The results showed 41% incidence of AP in the population; 18% had one lesion, 13% had 2 lesions, and 10% 3 or more lesions, respectively. Of the patients, 61% had received root treatment, 38% of the treatments were considered satisfactory, 52% were recorded as

unsatisfactory, and 10% were not classified due to poor quality of the x-rays. Patients with AP were older, more often smokers, there were more women and the patients with AP also had a lower income.

Patients with AP showed higher scores for periodontal disease, from the radiographs more root treatments were observed, and more unsatisfactory root treatments and furcation lesions. Further, the number of missing teeth and missing molars were higher. From the regression analyses between AP and the explanatory variables, the results showed that age, PD, CAL and missing teeth were the variables linking statistically with AP, with OR (95% CI) 1.24 (1.07-1.44), 5.43 (1.48-19.87), 2.60 (1.05-6.41) and 1.31(1.06-1.61), respectively.

From the 2003 hospital file CVD were the most common diseases. Of the AP patients, 20% had been in hospital for the treatment of CVD and hypertension was the most common diagnose. In the regression analysis for having CVD, the explanatory factor AP showed OD (95% CI) 3.90 (1.20-12.65), when controlled for age, gender, income, smoking habits, periodontitis and missing teeth. As regards the number and severity of apical lesions there was a tendency that CVD patients had more severe AP lesions, but the difference was not statistically significant. See **Table 3** for the summary.

Table 3. Summary of main results for apical periodontitis patients (study II)

2003	Apical periodontitis (71)	No apical periodontitis (49)
Demographic data	Older Women Lower income More smokers	
Clinical examination	More periodontitis Higher GI Higher PLI Higher CAL Higher PD Higher number of missing teeth Higher number of missing molars	Higher CI
TDI	More deep vertical pockets More furcation lesions	
Root treatments	More root treatments More satisfactory root treatments More unsatisfactory root treatments	
Hospital file	Cardiovascular diseases 20% Benign neoplasms 6% Malignant neoplasms 4%	Cardiovascular diseases 9% Benign neoplasms 7% Malignant neoplasms 7% Infectious and parasitic diseases 7%
Explanatory factor for CVD	AP	
Explanatory factors for AP	Age, PD, CAL, Missing teeth	

Periodontitis and salivary and serum biomarkers (study III)

In the follow-up study in 2009, from the 90 patients studied, 57% had periodontitis (1 or more deep pockets of ≥ 5 mm and BOP). The age range was 54-64 years old. Age, gender and smoking habits were similar in both groups, however. In serum samples, all biomarkers studied were similar between groups while in saliva MMP-13 and MMP-13/TIMP-1 ratio were significantly higher in the no-periodontitis patients. In the regression analysis female gender and CAL were the explanatory factors for having high MMP-13 value in saliva with OR (95%CI) of 3.08 (1.17-8.11) and 3.57 (1.18-11.82), respectively. See **Table 4**. The mean number of deeper pockets (4mm and 5mm), and the standard deviations (SD) are shown in **Table 5**.

Table 4. Summary of main results for the serum and saliva biomarkers, MMP-8, MMP-9, MMP-13 and TIMP-1 (study III)

2009	Periodontitis (51)	No periodontitis (39)
Demographic data	Similar age, gender and smoking habits	
Clinical examination	Higher GI Higher PLI Higher CI Higher BOP Higher CAL Higher PD Higher number of missing teeth	
Serum biomarkers	Slightly higher MMP-8 Slightly higher MMP-9 Slightly higher TIMP-1	
Saliva Biomarkers	Slightly higher MMP-8 Slightly higher MMP-9	Higher MMP-13 Higher MMP-13/TIMP-1
Explanatory factors for higher MMP-13 in saliva	Higher CAL Female gender	

Table 5. The mean number of deeper pockets in the 2009 population

2009	Periodontitis (51) mean number \pm SD	No periodontitis (39) mean number \pm SD
4mm pockets	21.37 \pm 10.00	9.28 \pm 6.88
5mm pockets	10.78 \pm 13.94	0.10 \pm 0.40

DISCUSSION

Study I

In the first epidemiologic study of the present thesis (Study I), the incidence of cancer (5.1 %) in no-periodontitis patients associated with age and gender and also with missing teeth and mandibular molar missing. The patients with periodontitis in 1985 (286 subjects) had 6.3 % cancer incidence in 2009. Demographic data was very similar between patients with or without periodontitis regarding cancer in the 2009 register, except for missing molars in the mandible, d46 and d47. Regression analyses with confounders controlled (age, gender, dental visits, education, income, socioeconomic status, working status, pack-years smoking, PLI, CI and GI) showed an OR (95% CI) of 8.43 (1.86-38.16) and 6.11 (1.30-28.73) for missing d46 and d47, respectively; breast cancer for women was the most common registered cancer and for men the distribution was scattered (Virtanen, Soder et al. 2013).

Although socioeconomic disparity seems to increase the risk of cancer incidence (Sharpe, McMahon et al. 2014), in our study they were not associated with the incidence of cancer during the follow-up.

Age has an important role in carcinogenesis because the biological responses are altered and errors in division and cell proliferation easily occur and are not be corrected like in younger individuals. Alterations in the immune system can also influence the susceptibility of older individuals to cancer (Zanussi, Serraino et al. 2013).

Breast cancer is the most common cancer for women worldwide, with a strong genetic and hormonal component. It associates with early menarche, no or late pregnancy, late menopause, use of oral contraceptives, use of hormone replacement therapy, but also with obesity, physical inactivity and alcohol abuse (Howell, Anderson et al. 2014). The association of chronic oral diseases with breast cancer has been found in some studies by Söder et al. (B. Soder, Yakob et al. 2011), Freudenheim et al.(Freudenheim, Genco et al. 2016) and Sefreddo et al.(Sfreddo, Maier et al. 2017); in these studies periodontitis seemed to associate with breast cancer. AP has not been studied in this regard. Only one case report was found in the literature where breast cancer metastatic cells were demonstrated in an apical periodontitis lesion (Khalili, Mahboobi et al. 2010). However, breast cancer metastases to other oral locations are rare, some more cases have been reported, as oral mucosa, parotid gland and jaws bone (Kalaitidou, Astreidis et al. 2015, El M'rabet, Kanab et al. 2017, Z. H. Lee, Lewing et al. 2014).

Periodontitis has been associated with several other cancers, like head and neck cancer, oral cancer (Gholizadeh, Eslami et al. 2016), lung cancer (Zeng, Xia et al. 2016), pancreatic cancer (Michaud 2013), and colorectal cancer (Bashir, Miskeen et al. 2015). In all these different cancers the explanatory patho-mechanisms have the same background, linking chronic oral diseases and the end-point, cancer, together. In a 2016 study, with a large group of patients that had never smoked, an association was found between advanced periodontitis and the incidence of cancers that usually are related with smoking, like lung, oropharyngeal, esophageal and stomach cancers, so an

important question raised “what is the possible impact of advanced periodontitis in the immune system response?” (Michaud, Kelsey et al. 2016).

The association we found between the incidence of cancer and higher number of missing teeth and molars calls for further discussion. At baseline, our subjects were fairly young (30-40 years old) but already had molars missing. This was regarded as a consequence of long-time infection burden, because first or second molar teeth are not easily extracted unless they have periodontal problems, deep caries cavities and/or apical periodontitis, or all these problems in the same mouth. Both AP and periodontitis usually take several years to develop and may thus affect systemic health for years. When a single infected tooth is extracted, however, infection can prevail in other teeth, and lead to subsequent further extractions. In the present Studies II and III where AP and periodontitis were analyzed in detail it became clear that in patients with both these pathologies also more missing teeth in general were recorded. Missing teeth have indeed been statistically associated with several systemic diseases like CVD, diabetes (Liljestrand, Havulinna et al. 2015), metabolic syndrome (Hyvarinen, Salminen et al. 2015) and also with cancer (Meyer, Joshipura et al. 2008). However, associations do not necessarily indicate any causality but as also seen in the present studies oral dental infections do link with cancer later in life.

The predominant cancer registered was breast cancer in women, finding that explains the gender difference observed in Study I. In men the results regarding cancer incidence during the follow-up were more scattered and no single type of cancer could be identified. In later analyses from the same material, however, an association was found between poor oral hygiene and prostate cancer (B. Soder, Meurman et al. 2017).

Even though statistical associations have been observed between oral diseases and cancer, there is not enough evidence to confirm any causality between these diseases entities.

Study II

In Study II we found an association between AP and CVD, controlled for age, gender, smoking habits, income, periodontitis and also missing teeth. The Baltimore longitudinal study of ageing, found that an endodontic burden was independent predictor for cardiovascular events, like angina, myocardial infarction and cardiovascular-related death (Gomes, Hugo et al. 2016).

In the hospital file used in the present study, CVD was the most common disease registered as expected. The number of cases with cancer was low, probably due to the age range of the patients group (48-58 years-old). Also patients with AP were mainly older, they were women, smokers or ex-smokers, and had a lower income compared with subjects with no AP. However, in the present study we did not have the information when the patients got the different diseases like CVD and thus a temporal relationship could not be analyzed.

Patients with AP also had more periodontitis, more furcation lesions and more teeth missing, especially molars were missing. Periodontitis and AP at the same time in the same patient is quite common, and may reflect a susceptible environment in the oral cavity, a reduced or imbalanced

immune response and/or presence of risk factors like smoking, alcohol abuse and poor oral hygiene (Jansson 2015).

The possible mechanisms how periodontitis influences CVD have been recently reviewed; detection of oral pathogens in carotid artery lesions is one of the strongest link. The dissemination of oral pathogens and/or inflammatory mediators into blood circulation, when periodontium is inflamed or AP present, can lead to infection/inflammation of the walls of blood vessels and also affect inflammatory response which leads to thickening and instability of atheroma plaques. The obstruction of the blood vessels and the rupture of the atheroma plaque are the end points of major complications like myocardial infarction, brain infarction and even death (Nguyen, Kim et al. 2015). On the other hand, AP is indeed also an inflammation of the periodontium, but it has been less studied in this regard. Some possible explanatory pathways have been addressed. Direct invasion of bacteria from AP lesions might be more difficult to show but an indirect alteration of serum levels of different cytokines and nitric oxide has been studied in rat models (Cintra, Samuel et al. 2016). AP has been associated with CVD and especially with coronary artery disease (Cotti, Mercurio 2015, Liljestr and, Mantyla et al. 2016). Elimination of oral infection foci to control the glycaemia in diabetic patients has also shown to be important (Bender, Bender 2003). AP also seems to associate with pregnancy outcomes like shorter pregnancy duration and intrauterine growth restriction (Harjunmaa, Jarnstedt et al. 2015). However, the evidence is still weak and more studies are needed before link between all those systemic diseases can truly be verified. Longitudinal studies to assess causality as regards oral chronic infections and any systemic disease are very difficult, if not impossible, to conduct due to ethical reasons. The American Heart Association's statement in 2012 was very clear in reporting that studies were able to show associations between CVD and oral diseases, but a causal relationship has not been proved. Periodontal interventions have shown to reduce systemic inflammation and improve endothelial dysfunction in short periods of time, but there is not enough evidence that these treatments could prevent or modify the outcomes of CVD (Lockhart, Bolger et al. 2012).

Study III

In the Study III, biomarker differences in serum and saliva between the groups were not statistically significant, with the exception of MMP-13 in saliva. Looking at the results from previous studies the results here obtained were expected to be different. But when comparing the populations and the classifications used in different studies, the difference in results can be explained at least in part. For example, in a previous study, MMP-13 levels in saliva were higher in localized periodontitis cases but lower in generalized periodontitis in a very similar range of values as seen in our present investigation (Gursoy, Kononen et al. 2013). More recently, MMP-8 and MMP-13 in saliva concentrations were increased with periodontitis disease progression and decreased after its non-surgical treatment (Ozcan, Saygun et al. 2016). In their study the concentration ranges were mostly higher for MMP-13 and much lower for MMP-8 than here reported. Thus, no direct comparisons can be made with our results.

In our study, MMP-13 was almost undetectable in serum while MMP-8, MMP-9 and TIMP-1 values in serum were very similar between the groups and in line with results from other studies (Turkoglu, Baris et al. 2014).

Again, the different results in different studies are probably due to different classifications for periodontitis (Savage, Eaton et al. 2009) and also because of different study populations.

The use of different immune tests (IFMA and ELISA) for the biomarkers have been shown to give similar and corresponding results. Concentrations measured by ELISA correlated significantly with concentrations determined with IFMA in a study where serum MMP-8 concentrations were analyzed in patients with acute coronary syndrome (Nieminen, Vesterinen et al. 2015).

The periodontal disease classification we used in this series of studies was the same as in the baseline study from 1985. Thus, “diseased” were patients with active periodontal inflammation reflected in probing depth and bleeding on probing (one or more 5mm deep pocket and BOP). Our present results showed that CAL should also be used in the periodontitis classification and maybe also 4mm pockets should have been taken into consideration. If MMP-13 is related to bone and cartilage resorption (Goldring, Otero et al. 2011), higher salivary values of MMP-13 in patients with more CAL makes sense.

Higher MMP-13 concentration in saliva was also associated with female gender. The finding with respect to gender difference is interesting since MMP-13 was originally detected from breast cancer samples (Freije, Diez-Itza et al. 1994). It also seems to be involved in the migration of the breast cancer cells (Xue, Chen et al. 2016). Breast cancer is known to principally affect women. Recent studies have shown a clear gender association between reduced bone stiffness in women and clinical attachment loss (Silveira, Albers et al. 2016). In postmenopausal women with osteoporosis or osteopenia higher CAL values have been recorded compared with women with normal bone density. In line with that women with osteoporosis seem to be at higher risk of periodontal disease and bone attachment loss (Penoni, Fidalgo et al. 2017). Could CAL then be related also with hormonal and gender differences and not only with the progression of the inflammatory process in periodontal disease, and is it possible that even without plaque accumulation and inflammatory process some women can have a higher risk of bone attachment loss? These questions call for more study.

Other studies have shown a possible hormonal effect on the MMP-13 metabolism. In an *in vitro*-study, where gingival fibroblasts were stimulated with IL-1 β to produce different MMPs (including MMP-13), progesterone seemed to reduce their production, (Collazos, Asensi et al. 2015). Thus, hormonal effects on the down-regulation of MMP-13 in gingiva are possible, but how this happens *in vivo* is not clear. In squamous cell carcinoma of the mouse skin the effect of estrogen in the regulation of MMP-13 inhibitor treatment was distinct so that older female mice had higher levels of MMP-13 and lower levels of estradiol resulting in more effective MMP-13 inhibitor treatment; if ovariectomy was made in young females, or 17 β -estradiol supplemented in older females, the effectiveness of the treatment was compromised (Meides, Gutschalk et al. 2014). In postmenopausal women with chronic periodontitis, the expression of estrogen receptors in the gingiva is significantly reduced when compared with postmenopausal women with healthy periodontium (Karthik, Arun et al. 2009). Some polymorphism in the genes for MMP-12 and MMP-13 seems to be related to epithelial ovarian carcinoma (Li, Jia et al. 2009). In cervical cancer MMP-13 gene seems to be downregulated (Vazquez-Ortiz, Pina-Sanchez et al. 2005).

Nevertheless, a gender association with CAL and MMP-13 levels in saliva can indicate that different genetic and hormonal backgrounds can affect the progression of periodontitis and maybe also other diseases like AP, CVD and cancer. Genetic predisposing association between periodontitis and CVD have been studied and some gene mutations like IL-1 and IL-10 mutations found to associate with both diseases (Kornman, Pankow et al. 1999, Armingohar, Jorgensen et al. 2015). However, genes predisposing to cancer and oral diseases have not been much studied so far.

Summary

In summary, an individual always is in balance regarding three different variables: genome, environment and behavior. A unique and distinct genomic constitution is the background for the balance between health and disease, where age and gender have an important role. When these three factors are in equilibrium the individual has a state of health. If some of these factors shift to disequilibrium, disease can develop - and the equilibrium is difficult to achieve again. Age, gender and genetic mutations are the genome variables; environment variables are the place where we live and how exposed we are to carcinogens and other contaminants. Behavioral factors are those we can control better and these include variables like smoking, healthy diet and physical activity.

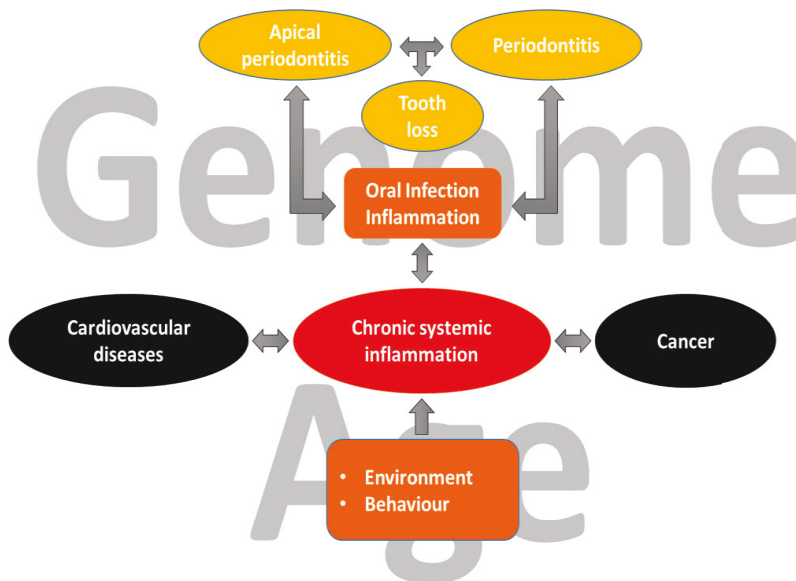


Figure 10. Summary of the possible connections between chronic oral diseases and systemic diseases like CVD and cancer here depicted.

Figure 10 shows a simplified diagram explaining the variables here discussed, taking into consideration that in the background there is the individual genome that during life can undergo mutations. If patients have bad behavioral habits like smoking, alcohol abuse, unhealthy diet, these definitely predispose both to CVD and cancer. However, chronic systemic inflammation may lie behind both these diseases. Oral infections/inflammations can be a source of systemic inflammation but at the same time may be caused by systemic imbalance. Genetics predispose to several diseases and future studies should tell if these infectious inflammatory diseases indeed have the same background. Personalized medicine is the future also in this area.

Finally, it is important to emphasize that preventive measures that can reduce the socioeconomic disparity between individuals will certainly impact personal wellbeing and affect positively both the general health and oral health. Preventive measures on the behavioral reduce the incidence of all chronic diseases, as CVD, cancer, oral diseases and diabetes (Petersen, Ogawa 2012).

Strengths

Our study population is a unique population, representative of the ethnically homogeneous population from the Stockholm County in 1985. The cohort has now been followed for more than 30 years. The age range of 10 years to diminished larger age effect. In the first study (Study I) the subjects were randomly chosen to avoid selection bias and the study was longitudinal prospective study. For Studies II and III the patients were selected by a computer program and they are a representative sample from the original population. A large amount of different data has been registered for this cohort during the follow up studies and from different register files, making possible to analyze the patients in different perspectives during the time.

Weaknesses

In the first study it would have been important to have a second clinical oral examination of all the subjects in order to compare the patients longitudinally. Unfortunately this was not possible due to practical reasons. In Study II and Study III, even though the recall group was representative of the original cohort the number of patients was low, which is a limitation. In study II were AP was classified based in X-rays, it would have been helpful to use 3D radiographs and histological samples to be able to assess the severity of the lesions, but that was not possible in the respective study. In study III, it would have been important to have also hormonal status of the patients, but that data was not available. Furthermore, a more comprehensive socio-economic and behavioral data were lacking due to the nature of the study which is another weakness.

Future studies

Longitudinal and well-designed interventional studies with all possible co-variants controlled would be important in trying to understand eventual causality between the diseases here discussed. However, ethical reasons make this kind of approach very difficult. Maybe animal models could be used as an option. In the future advances of genome analyses might show common dominators for

all these diseases, explaining the outcome together with behavioral and environmental factors. Finally, multi-centric clinical studies are warranted. A clear consensus in the classification of periodontal diseases is something that is still missing, to make it possible to compare all studies and the respective results. A more precise way to classify AP is also important, fortunately 3D dentistry is advancing rapidly, and 3D radiographs are getting more accessible and with lower doses of radiation, making it an important diagnostic tool of apical oral diseases.

KEY FINDINGS AND CONCLUSIONS

- The incidence of cancer was associated with missing teeth. Missing teeth are the result of the progression of chronic oral diseases persisting for years in the mouth. Namely, extraction of teeth is the consequence of dental disease but not necessarily cure of oral diseases, because after an extraction, other teeth may remain and be diseased.
- Patients with AP had more periodontitis and patients with both these diseases simultaneously had more missing teeth. Edentulous patients were not evaluated in our study.
- In periodontally healthy patients, the incidence of cancer was associated with age, an important co-factor for different systemic diseases and also for oral diseases.
- AP associated with a 4 times higher risk of having CVD, when controlled for periodontitis and other co-factors. AP was very common in the study population and the quality of the root treatments was poor in general.
- Some biomarkers like MMP-13 in saliva could be an indicator of bone attachment loss and lower bone density related with age and gender, and not necessarily related with the accumulation of dental plaque and subsequent inflammation.

One very important conclusion from the series of the present thesis is that oral diseases should be treated to improve not only the patient's oral health, keeping their quality of life true functional capacity of chewing or biting, self-esteem and social relationships, but also to avoid eventual detrimental effects on general health. Extraction of teeth is not the final solution in eliminating oral infection sources, because edentulousness leads to further problems partly solved today with implantology, however.

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