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ORAL HEALTH IN PATIENTS WITH
CHRONIC KIDNEY DISEASE
– EMPHASIS ON PERIODONTITIS

Karita Nylund

Academic dissertation

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LIST OF ORIGINAL PUBLICATIONS

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**ABBREVIATIONS**

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<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACVD</td>
<td>Atherosclerotic cardiovascular diseases</td>
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<tr>
<td>AER</td>
<td>Albumin excretion rate</td>
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<tr>
<td>AGE</td>
<td>Advanced glycation end-product</td>
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<td>AMP</td>
<td>Antimicrobial peptide</td>
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<td>BMS</td>
<td>Burning mouth syndrome</td>
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<tr>
<td>CAL</td>
<td>Clinical attachment loss</td>
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<tr>
<td>CDC/AAP</td>
<td>Centers for Disease Control and Prevention/the American Academy of Periodontology</td>
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<tr>
<td>CD4</td>
<td>Cluster of differentiation 4</td>
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<tr>
<td>CD8</td>
<td>Cluster of differentiation 8</td>
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<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
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<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
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<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<tr>
<td>CPI</td>
<td>Community Periodontal Index</td>
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<tr>
<td>CPITN</td>
<td>Community Periodontal Index of Treatment Needs</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
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<tr>
<td>DMFT</td>
<td>Decayed, Missing, Filled Teeth</td>
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<tr>
<td>EFP</td>
<td>European Federation of Periodontology</td>
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<tr>
<td>ESRD</td>
<td>End-stage renal disease</td>
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<tr>
<td>GCF</td>
<td>Gingival crevicular fluid</td>
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<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycosylated hemoglobin</td>
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<tr>
<td>IQR</td>
<td>Interquartile range</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>KDIGO</td>
<td>Kidney Disease: Improving Global Outcomes</td>
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<tr>
<td>LDD</td>
<td>Low dose doxycycline</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>MACE</td>
<td>Major adverse cardiovascular event</td>
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<td>MAMP</td>
<td>Microbe-associated molecular pattern</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>NET</td>
<td>Neutrophil extracellular trap</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>NHEFS</td>
<td>National Health and Nutrition Examination Survey - Epidemiologic Follow-up Study</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non-insulin dependent diabetes mellitus</td>
</tr>
<tr>
<td>NKF-KDOQI</td>
<td>National Kidney Foundation - Kidney Disease Outcomes Quality Initiative</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PGE-2</td>
<td>Prostaglandin-2</td>
</tr>
<tr>
<td>PIBI</td>
<td>Periodontal Inflammatory Burden Index</td>
</tr>
<tr>
<td>PISF</td>
<td>Peri-implant sulcular fluid</td>
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<tr>
<td>PLGRP-1</td>
<td>Peptidoglycan recognition receptor -1</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear leukocyte</td>
</tr>
<tr>
<td>PPD</td>
<td>Periodontal pocket depth</td>
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<tr>
<td>PRR</td>
<td>Pattern recognition receptor</td>
</tr>
<tr>
<td>PSD</td>
<td>Polymicrobial synergy and dysbiosis</td>
</tr>
<tr>
<td>RANKL/OPG</td>
<td>Receptor activator of nuclear factor kappa B ligand/osteoprotegerin</td>
</tr>
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<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>RRT</td>
<td>Renal replacement therapy</td>
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<tr>
<td>SDD</td>
<td>Subantimicrobial dose doxycycline</td>
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<tr>
<td>TDI</td>
<td>Total Dental Index</td>
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<tr>
<td>TIA</td>
<td>Transient ischemic attack</td>
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<tr>
<td>TIMP</td>
<td>Tissue inhibitor of matrix metalloproteinase</td>
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<tr>
<td>TLR</td>
<td>Toll like receptor</td>
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<tr>
<td>TNFα</td>
<td>Tumor necrosis factor alpha</td>
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<tr>
<td>TREM1</td>
<td>Triggering receptor expressed on myeloid cells 1</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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ABSTRACT

Periodontitis has been associated with systemic diseases such as diabetes and atherosclerosis, cardiovascular diseases, and even chronic kidney disease (CKD) through low-grade systemic inflammation, although no causality can be drawn. CKD is an increasing problem worldwide, and has adverse effects on oral health. The main risk factors of CKD are diabetes mellitus (DM), hypertension and obesity. Diabetic nephropathy is the main reason for entering dialysis in Finland and cardiovascular diseases (CVD) play a crucial role in mortality among CKD patients.

The general aim of this thesis was to study oral health with an emphasis on periodontitis among CKD patients, and to determine the differences between diabetic nephropathy and other CKD patients (Study I) and whether salivary MMP-8 concentration can reflect an oral inflammatory burden at the predialysis stage (Study II). In the follow-up prospective cohort study (III), the goal was to examine whether oral inflammatory burden was associated with mortality among the study patients and clarify the cause of death of patients who had been enrolled in our previous study. A further aim was to compare oral health at the predialysis stage and at follow-up (Study IV).

The hypotheses were that diabetic nephropathy patients have worse oral health than other CKD patients, at both predialysis and post-transplantation stages, and that salivary MMP-8 associates with worse oral inflammatory burden. The mortality rate was assumed to be higher among diabetic nephropathy patients. The main expected reason for death was cardiovascular disease.

This series of studies were further clinical oral investigations of CKD patients originally examined in 2000–2005 in the Department of Oral and Maxillofacial Diseases of Helsinki University Hospital at the predialysis stage (Vesterinen et al. 2011). The periodontal health of 144 CKD patients was now studied in more detail at the predialysis stage, and 118 salivary samples were analyzed for MMP-8. Of these 144 patients, 53 took part in the follow-up study (2013–2015); 65 patients had died, and 26 had dropped out.

The same periodontist (HR) conducted the clinical oral examination at the predialysis and post-transplantation stages, and the patients underwent a full clinical and radiographic oral examination. Radiological- and salivary analyses, questionnaires, hospital-, and mortality records were available for the study. The severity of the periodontitis was assessed using
the definition of the Centers for Disease Control and Prevention/the American Academy of Periodontology (CDC/AAP) whenever possible, and other indices used were the Periodontal Inflammatory Burden Index (PIBI) (Lindy et al. 2008), the Total Dental Index (TDI) (Mattila et al. 1989), and the DMFT index (WHO 1997).

The clinical oral parameters of patients with diabetic and other CKDs were analyzed, at both baseline and follow-up.

Patients with diabetic nephropathy indeed had worse oral health at the predialysis stage, with two or more sites having periodontal pocket depths (PPD) of ≥ 6mm, and higher TDI scores than the patients with other CKDs (p< 0.05), (Study I). Higher salivary MMP-8 concentration was associated with worse oral health (deep periodontal pockets, higher PIBI and TDI) than with lower MMP-8 concentration (p< 0.05), (Study II).

The 157-month follow-up study showed that the most frequent causes of death were cardiovascular diseases, followed by infection and cancer. The survival rate of diabetic nephropathy (23.8%) patients and other patients with CKD (59.9%; log-rank test p < 0.001) differed statistically significantly. In the multivariable Cox regression model, fewer teeth, higher age, and diabetic nephropathy diagnosis were statistically significant independent risk factors for death. The deceased patients had fewer teeth (p< 0.001) and higher TDI scores (p< 0.05) than those who survived (Study III).

Most of the surviving patients who took part in the re-examination had undergone a kidney transplant (51/53), of which 46 transplants were functioning. Comparison of the two stages of kidney disease revealed that patients had more teeth, and more often had calculus, deep periodontal pockets, and higher TDI and PIBI scores (p< 0.05) at the predialysis stage than at the post-transplantation stage. Patients at the post-transplantation stage, however, took more drugs daily and had dental appointments more frequently in a year than the patients at the predialysis stage. Overall oral health was worse at the predialysis stage (Study IV).

In conclusion, the present study supports the existing protocol of the Helsinki University Hospital, according to which oral examination, accurate diagnosis, and proper treatment of oral infection foci are mandatory at the predialysis stage for reducing systemic inflammation among CKD patients. Since diabetic nephropathy is associated with poorer oral health, this group of patients needs significant attention in oral care.
INTRODUCTION

Periodontitis is a bacteria-induced chronic inflammatory disease that affects a large portion of the adult population worldwide, also in Finland (Jin et al. 2011, Pihlstrom et al. 2005, Periodontitis:Current Care Guidelines 2016). Without treatment, it may lead to tooth loss in susceptible patients. CKD is defined as a deficiency in kidney structure or function that lasts over three months, and has an adverse effect on health (Inker et al. 2014). Its prevalence is over 10% in many countries, a number that is rising alarmingly (Eckardt et al. 2013). Principal risk factors for CKD are hypertension, diabetes, and obesity, although periodontitis as an inflammatory disease has also been proposed to be a non-traditional risk factor for CKD (Cignarelli and Lamacchia 2007, Fisher et al. 2008, Jha et al. 2013). Periodontitis may also accelerate poor glycemic control by increasing insulin resistance, whereas diabetes, in turn, may lead to abnormal hyper-inflammatory reaction to periodontal pathogens (Chapple et al. 2013, Southerland et al. 2006).

The worldwide prevalence of diabetes among adults was 4.7% in 1980, compared to 8.5% in 2014 (WHO, Global report on diabetes 2016). According to the International Federation of Diabetes, one adult in ten will have diabetes by 2040, corresponding to 642 million people (www.idf.org). The increasing prevalence of type 2 diabetes is explained by risk factors such as overweight, high blood pressure, impaired glucose tolerance, unhealthy diet, physical inactivity, and increasing age (www.idf.org).

CKD is diagnosed by decreased glomerular infiltration rate (GFR) or by increased albumin concentration in the urea, i.e. albuminuria. When the functional capacity of kidneys falls below 10% of normal capacity, end-stage renal disease (ESRD) is diagnosed (Bots et al. 2007). Treatment modalities for renal replacement therapy (RRT) are dialysis, either hemodialysis or peritoneal dialysis, and finally kidney transplantation. Diabetic nephropathy is the most common reason for dialysis treatment in Finland.

Oral infections such as periodontitis should be regarded as one of the risk factors for the progression of CKD and diabetic nephropathy due to low-grade systemic inflammation. In fact, periodontitis may predict the development of ESRD and nephropathy in type 2 diabetes (Shultis et al. 2007, Chapple et al. 2013).

CKD patients are prone to premature death, mostly explained by CVD, followed by infections (Stenvinkel 2002, Kato 2008). Atherosclerotic CVDs are explained by
dyslipidemia, hypertension, smoking, and inflammation, in which periodontitis and other oral infections may play a significant role (Tonetti et al. 2013).

Oral health among CKD patients has mostly been studied during dialysis or the post-transplantation stage (Bots et al. 2007, Summers et al. 2007, Craig 2008, Akar et al. 2011, Kaushik et al. 2013, Schmalz et al. 2016). Both stages have their own characteristics, which are also reflected in oral health.

However, only a few studies have examined oral health at the predialysis stage which is, in fact, an important stage in the course of kidney disease (Souza et al. 2008, Thorman et al. 2009, Vesterinen et al. 2011). At our hospital, all patients are referred for a dental examination at the predialysis stage, during which the foci of oral infections are diagnosed and treated prior to entering RRT.

A PubMed search for “predialysis” and “oral health” (until October 2016) yielded 20 results, 6 of which were from our research group (Vesterinen et al. 2007, 2011, 2012, Nylund et al. 2015a, 2015b, Ruokonen et al. 2016). Twelve studies of the remaining 14 were studies of orally administered drugs or vitamins, and the remaining two were clinical studies, one from Sweden by Thorman and co-workers (2009), and the other from Brazil by Souza and co-workers (2008).

The cross sectional study by Thorman and co-workers showed that uremic CKD patients in predialysis, hemodialysis or in peritoneal dialysis had worse overall oral health (higher DMFT index, more loss of attachment, more periapical- and mucosal lesions) than the healthy controls (Thorman et al. 2009).

The Brazilian cross-sectional research by Souza and co-workers showed that the general oral health status was poor among most (233/286, 88%) CKD patients, regardless of their treatment modality (predialysis, hemodialysis, peritoneal dialysis, or transplantation). However, transplant patients had less oral halitosis than patients with other treatment modalities (Souza et al. 2008).

A cross sectional study by our research group showed that CKD patients with diabetic nephropathy had lower stimulated salivary flow rates and more caries than patients with other kidney diseases at the predialysis stage (Vesterinen et al. 2011).
To the best of our knowledge, only one longitudinal clinical study has followed CKD patients from the predialysis to the post-transplantation stage, and this is our own research group’s study (Vesterinen et al. 2007).

This present study is divided into two investigations: baseline (predialysis) and follow-up (post-transplantation) studies. The purpose of the first part (Studies I and II) was to study oral health with an emphasis on periodontal disease by comparing diabetic nephropathy with other CKD patients. Oral inflammatory burden was compared with salivary MMP-8 concentration. In the second part (Studies III and IV), a longitudinal study examined the association between oral inflammatory burden and mortality and causes of death among CKD patients and, secondly, compared the oral health of the patients during their predialysis and post-transplantation stages.

The results of this series of studies are expected to help clinicians understand the differences between the oral health, with an emphasis on periodontitis, of diabetic nephropathy and other CKD patients at different stages of kidney disease. A further purpose was to determine whether the treatment of oral infections at the predialysis stage is indeed effective.
Common oral diseases

Dental caries and periodontal diseases are the most prevalent biofilm diseases in oral cavities (Shay 2002, Rosier et al. 2014). These multifactorial diseases constitute a major economic burden worldwide, and like other behavioral biofilm diseases, could mostly be prevented by good oral self-care. According to the “Health 2011” survey in Finland, one in five people (aged ≥ 30) living in the northern or southern part of the country had at least one caries lesion, and this was more common among men (28%) than women (14%) (Health 2011 survey, www.julkari.fi/bitstream/handle/10024/90832/Rap068_2012_netti.pdf). Along the same lines was the finding that periodontal disease (at least one periodontal pocket ≥ 4mm deep) was more common among men (70%) than among women (56%) (Health 2011 survey).

Other prevalent oral lesions include candidiasis, recurrent herpes labialis, recurrent aphthous ulcers, hairy tongue, and lichen planus (Gonsalves et al. 2007). Oral mucosal lesions can be classified as ulcerative, vesicular and bullous lesions; red, white or pigmented, and benign or malign (Greenberg et al. 2008). The most common opportunistic oral infection is oral candidiasis, which is mostly caused by *Candida albicans*. Other candida species found in oral cavities are *C. glabrata*, *C. tropicalis*, *C. pseudotropicalis*, *C. guilliermondii*, *C. dubliniensis*, and *C. krusei* (Jordan and Lewis 2004). Candidiasis is often described as affecting the very young, very old, or very sick (Greenberg et al. 2008). Its predisposing factors are, for example, immunosuppressive disease or medication, poor oral health, endocrine disorders, smoking, and hyposalivation (Greenberg et al. 2008).

Signs and symptoms of the mouth

Xerostomia is a subjective symptom of dry mouth which does not always go hand in hand with physical signs of salivary gland hypofunction (MacEntee and Donnelly 2016). Hyposalivation, as an objective term, is defined when unstimulated salivary flow rate is < 0.1ml/min, and stimulated salivary flow rate is < 0.7 ml/min (Saleh et al. 2015). Medication such as antihypertensive drugs, antidepressants, and antihistamines; polypharmacy in general; radiation therapy; ageing; systemic diseases such as Sjögren’s
syndrome or other rheumatic diseases; and diabetes may disturb salivary gland function and result in reduced salivary flow (Saleh et al. 2015, MacEntee and Donnelly 2016). Hyposalivation predisposes to many disorders such as dysphagia (difficulty in swallowing), dysgeusia (altered taste sensation), dysphonia, pain, burning mouth, and oral infectious diseases such as caries and periodontal diseases, or even life-threatening bacterial and yeast infections and aspiration pneumonia (Saleh et al. 2015, Mac Entee and Donnelly 2016).

About 90% of saliva is produced by the major salivary glands: the parotid, submandibular- and sublingual glands. Saliva is mainly composed of water (90%) with electrolytes and proteins such as immunoglobulins; and antifungal, antibacterial and digestive enzymes (Saleh et al. 2015). Normally daily saliva volume varies from 0.5 to 1 liters per day. Saliva is indeed an important lubricant of oral and upper gastrointestinal mucosa, and has antimicrobial, buffering and demineralization properties.

Burning mouth syndrome (BMS) is defined as unknown chronic burning pain in oral mucosa with no explanatory factor. BMS has been linked to other chronic pain syndromes and is also associated with peripheral or central neuropathic disorder, but its pathogenesis and etiology remain unknown (Moisset et al. 2016).

**Pathogenesis of periodontal diseases**

Periodontal diseases (gingivitis and periodontitis) are historically known as bacteria-induced inflammatory diseases that affect the supportive structures of the teeth (Socransky and Haffajee 1992). Gingivitis is a reversible process that affects gingiva, although it may develop into irreversible state periodontitis, in which the polymicrobial biofilm triggers the host response, leading to destruction of the periodontium and, finally, to tooth loss in a susceptible patient (Offenbacher 1996). It is now recognized that a dysbiotic biofilm is necessary for periodontitis to develop, but that in itself, it is insufficient to cause the disease. Individual susceptibility to periodontitis is principally determined by the host’s immune-inflammatory response to microbiota (Preshaw 2008, van Dyke and van Winkelhoff 2013). Immunosuppressive state, genetic, dermatological, neoplastic, hematological or granulomatous disorders may also have periodontal manifestations (Page and Kornman 1997, Pihlstrom et al. 2005).
In 1998, Socransky and co-workers introduced six subgingival microbial complexes: “yellow”, “green” and “purple” complexes were considered early colonizers of the tooth surfaces, and Gram-negative “orange” and “red” complexes comprised “true periodontopathogens”. These are *Prevotella intermedia*, *Prevotella nigrescens*, *Peptostreptococcus micros*, *Campylobacter gracilis*, *Campylobacter rectus*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*. *Aggregatibacter actinomycetemcomitans* (previously *Actinomyces actinomycetemcomitans*) is another species often seen in periodontitis, especially in young patients (Socransky et al. 1998). Virulent factors such as fimbriae, adhesion molecules, proteases and leukotoxins help putative bacteria colonize and compete for ecological niches and to evade the host (Offenbacher 1996). The “red” complex has shown the strongest association with periodontal pocket formation (Socransky et al. 1998).

The oral cavity provides a habitat for over 700 bacteria species, of which 150–200 can be found in most individuals and 30–100 species in a single site (Gorr and Abdolhosseini 2011, Hasan and Palmer 2014). However, new molecular techniques have shown that the number of species in the mouth may be thousands, although the role of most of them is so far unknown (Keijser et al. 2008). The mouth serves as a moist, warm environment, with non-shedding surfaces that enable the formation of thick, well-organized biofilm (Marsh and Devine 2011). Although periodontopathogenic biofilm is needed for the initiation of periodontitis, several systemic and local risk factors are involved in modifying susceptibility to the disease. Genetic, ethnic, age, gender, environmental (nutrition, pH, atmosphere, temperature, smoking, diabetes, stress) and host defense factors determine the composition of oral microbiota and play an important role in disease expression (Offenbacher 1996, Marsh and Devine 2011). Smoking may affect the composition of the biofilm in general and may also affect the immune system and impair the host’s defense by inhibiting granulocyte function (Hilgers and Kinane 2004, Söder et al. 2002).

According to “ecological plaque hypothesis”, changes in environmental conditions may disrupt the host-microbe homeostasis. An increase in the amount of biofilm triggers the host to increase gingival crevicular fluid (GCF), thus providing not only host defense molecules but also nutrients for harmful proteolytic and anaerobic bacteria (Marsh and Devine 2011).
Since knowledge regarding the composition and synergistic effects of subgingival microbiota has expanded through the development of microbiological methods, periodontitis is now believed to be initiated not only by single microorganisms, but also by polymicrobial synergy and dysbiosis (PSD) (Hajishengallis and Lamont 2012, Lopez et al. 2015). In the synergistic and dysbiotic microbial community model, “keystone pathogens” such as *P. gingivalis* have the capacity to modulate the host’s immune system and tip the balance from homeostasis to dysbiosis (Hajishengallis and Lamont 2012). The colonization of “keystone pathogens” in the biofilm increases the communication of different species and the expression of virulence factors, thus elevating the pathogenicity of the entire community (Hajishengallis and Lamont 2012, Lamont and Hajishengallis 2015).

**Host immune system**

The host defense mechanisms and innate and adaptive immunity work together to try to preserve health and prevent disease (Table 1).

Innate immunity comprises non-specific mechanisms such as the barrier function of the epithelium and specific pattern recognition receptors (PRRs) that recognize bacterial substances such as lipopolysaccharides (LPS), GCF with antimicrobial peptides (AMPs), polymorphonuclear leucocytes (PMN) and macrophages, and saliva with protective enzymes such as lysozyme and peroxidase (Gorr and Abdolhosseini 2011, Hasan and Palmer 2014).

PMNs, the most important phagocytic cells, predominate in the early periodontal lesion in the gingival crevice and in the epithelium (Nussbaum and Shapira 2011). Neutrophils are considered the first line of defense during inflammation and infection. They can kill bacteria intracellularly (oxygen dependent or oxygen independent pathways) or extracellularly, the latter having the potential to damage collateral connective tissue by releasing neutrophil cytoplasmic granules or neutrophil extracellular traps (NETs) into extracellular space (Brinkmann et al. 2004, Nussbaum and Shapira 2011). Activated neutrophils release proteinases and are capable of producing cytokines and chemokines. Neutrophil disorders that affect neutrophil number (e.g. agranulocytosis, cyclic neutropenia), chemotaxis or function (leucocyte adhesion deficiency syndrome, Papillon-
Lefevre syndrome) predispose individuals to infections such as periodontitis (Nussbaum and Shapira 2011).

In periodontitis, the acute inflammatory response is protective. However, in susceptible patients, the response to remove inflammatory cells fails, and inflammation becomes chronic when adaptive immunity takes place, and plasma cells and lymphocytes predominate (Nussbaum and Shapira 2011, van Dyke and van Winkelhoff 2013).

Adaptive immunity is characterized by specificity with an “immunological memory” (Ebersole et al. 2016). This is triggered by cytokines, which activate cell-mediated (T-cell) and humoral immunity (B-cell). Bacterial antigens are presented by macrophages and dendritic cells to T- and B lymphocytes. Antibodies (IgA, IgG, and IgM) are secreted when B cells recognize antigens by cell surface immunoglobulins (Igs) (van Dyke and van Winkelhoff 2013). Secretory IgA inhibits bacterial adhesion to mucosal surfaces, thus preventing infection (Hasan and Palmer 2014). More antibodies, enzymes and immune cells are then secreted into GCF by inflammation.

T-cells, on the other hand, express CD4+ and CD8 cell surface proteins, which act as receptors for antigens. T-cell activation turns them into helper T-cells (CD4+), (producing interleukins) or cytotoxic T-cells (CD8) (van Dyke and van Winkelhoff 2013).

**Pro-inflammatory mediators**

Pro-inflammatory mediators comprise cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-8, chemokines, immunoglobulins and proteolytic enzymes, e.g. matrix metalloproteinases (MMP)-8 and MMP-9 (Rathnayake et al. 2013a). Cytokines do not work alone but in complex networks (Preshaw and Taylor 2011). The PRRs of innate immune cells (macrophages, dendritic cells and PMNs) recognize microbe-associated molecular patterns (MAMPs) and produce pro-inflammatory cytokines. These cytokines further determine the pathway of T-cell differentiation and the subclasses of immunoglobulins (Preshaw and Taylor 2011). The balance between pro- and anti-inflammatory cytokines determine the extent of periodontal destruction.

Type I collagen is the main extracellular matrix component in periodontal tissues and its destruction is regarded as one of the key factors in the destruction of periodontal tissues.
MMPs are groups of structurally alike proteolytic enzymes, capable of degrading extracellular matrix and basement membranes. Human 23 MMPs (collagenases, gelatinases, membrane-type MMPs, stromelysins and other MMPs) take part in organogenesis, normal tissue turnover, immunity response in periodontitis, and tissue destruction (Sorsa et al. 2004, 2006). The balance between MMPs and their regulatory proteins such as the tissue inhibitors of matrix metalloproteinases (TIMPs) may be disrupted by microbial virulence factors, hormones, cytokines and growth factors, resulting in tissue destruction, also seen in periodontitis, peri-implantitis and cancer. Collagenolytic matrix metalloproteinases collagenase-2 (MMP-8) and collagenase-3 (MMP-13) have shown to associate with severe periodontitis (Sorsa et al. 2004, 2006, Leppilahti et al. 2011). MMP-8 is produced by PMNs, epithelial cells, fibroblasts, endothelial cells, monocytes, macrophages and plasma cells, and is the main collagenase in inflamed gingival tissue (Sorsa et al. 2004, 2006).

**Table 1. The immune system**

<table>
<thead>
<tr>
<th>INNATE IMMUNITY</th>
<th>ADAPTIVE IMMUNITY</th>
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<tbody>
<tr>
<td>Epithelial barrier</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Epithelial cells: e.g. AMPs, IL-1β, IL-8</td>
<td>Cell-mediated immunity (T-cells)</td>
</tr>
<tr>
<td>Pattern recognition receptors (PRRs): e.g. TLRs</td>
<td>Humoral immunity (B-cells)</td>
</tr>
<tr>
<td>Gingival crevicular fluid: e.g.AMPs, PMNs, complement</td>
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</tr>
<tr>
<td>PMNs</td>
<td></td>
</tr>
<tr>
<td>Monocytes, macrophages</td>
<td></td>
</tr>
<tr>
<td>Peripheral dendritic cells</td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td></td>
</tr>
<tr>
<td>Saliva: e.g. lactoferrin, lysozyme, peroxidase</td>
<td></td>
</tr>
</tbody>
</table>

AMP = antimicrobial peptide, IL = interleukin, TLR = toll-like receptor, PMN= polymorphonuclear leukocyte
Prevalence and risk factors of periodontal diseases

Periodontal infections (gingivitis and periodontitis) are common inflammatory disorders, affecting up to 90% of the world’s population, and severe periodontitis affects approximately 5–15% of the population worldwide (Papapanou 1996, Pihlstrom et al. 2005, Demmer and Papapanou 2010). These percentages vary according to ethnicity and socioeconomic status, since non-white and people with low socioeconomic status are more often affected. Older age is related to periodontitis, perhaps because of the cumulative effects of true behavioral risk factors such as bad oral hygiene habits and smoking or underlying health and systemic risk factors such as diabetes (Lalla and Papapanou 2011, Scannapieco and Cantos 2016). According to the “Health 2000” survey in Finland, the prevalence of gingivitis was 74% and periodontitis 64% (defined by at least one pocket with a ≥ 4mm-deep periodontal pocket). A severe form of periodontitis (defined by at least one site with a ≥6mm-deep periodontal pocket) was detected in 21% of participants (Health 2000 Survey, http://www.terveys2000.fi/indexe.html).

Periodontitis is a multifactorial disease with several risk factors, such as poor oral hygiene, smoking, and systemic disorders such as diabetes, rheumatoid arthritis, osteoporosis and obesity (Lockhart 2012). Periodontitis is characterized as a complex disease with polygenic variation (Laine et al. 2012). According to twin- and family studies, genetics could explain about 50% of susceptibility to periodontitis (Laine et al. 2012). However, although the risk factors for periodontitis at the individual level may be explained by genetic, microbial and environmental factors, no specific gene or gene polymorphism has been found that could explain all forms of periodontitis.

Diagnostic methods of periodontitis

Periodontitis usually develops without symptoms and is diagnosed conventionally by clinical (measuring PPD with bleeding on probing, loss of clinical attachment) and radiological (alveolar bone loss) examination. Putative periodontopathogens can be detected by routine analyses, cultivation or by PCR (Mostajo et al. 2011). However, these diagnostic methods provide information on destruction that already has occurred. Proper diagnosis should determine whether the disease is present; identify the type, distribution, activity, and severity of the disease, and provide an explanation for the underlying pathologic process.
Interest is currently paid to the “active phase” diagnostic tests of molecular biomarkers such as host inflammatory biomarker neutrophil collagenase MMP-8, which can be detected in GCF and saliva (Pihlstrom et al. 2005, Sorsa et al. 2004, 2006, 2011). New inflammatory biomarkers and a combination of different markers are being researched.

MMP-8 levels seem to increase in oral fluids due to periodontitis, and to decrease after treatment (Mäntylä et al. 2006, Rathnayake et al. 2013a, Leppilahti et al. 2014a,b, Leppilahti et al. 2015, Nwhator et al. 2014). During periodontitis, MMP-8 is mainly produced by PMN cells. Smoking seems to reduce the levels of MMP-8 by suppressing inflammatory cells and reducing the flow rate of GCF and saliva (Mäntylä et al. 2006, Sorsa et al. 2011, Leppilahti 2014a). High baseline MMP-8 levels in smokers have shown to strongly predict weak treatment responses (Leppilahti 2014b).

As mentioned above, host-derived biomarkers of tissue destruction can be detected in oral fluids such as saliva, GCF/peri-implant sulcular fluid (PISF) and mouth-rinse. GCF/PISF is detected in one site with microliter levels, whereas saliva or mouth-rinse provide more comprehensive information in milliliter levels. Since saliva is easy to collect non-invasively, attention is nowadays paid on saliva and mouth-rinse as diagnostic tools.

Higher salivary MMP-8 concentration has also been associated with, for example, systemic diseases such as diabetes, previous heart surgery (Rathnayake et al. 2013b), and acute myocardial infarction (Budunelli et al. 2011). Salivary biomarkers may thus reflect not only oral but also systemic inflammation (Miller et al. 2010, Rathnayake 2013a).

**Classification of periodontitis**

The WHO Community Periodontal Index of Treatment Needs (CPITN) or Community Periodontal Index (CPI), as later renamed, has been widely used in epidemiological studies (Ainamo et al. 1982). This index provides the highest score per mouth sextant. CPI ranges from 0 to 4, where 0 mirrors health, 1 equals bleeding, 2 equals calculus, 3 equals a pocket depth of 4mm or 5mm, and 4 mirrors a pocket depth of ≥ 6mm. However, the limitation of this index is that it only takes into account a few teeth with deepened periodontal pockets, and not attachment loss (Leroy et al. 2010). Today, epidemiological studies mainly use pocket depth measurement together with an assessment of attachment loss when classifying periodontitis.
The CDC/AAP have recommended using case definitions of severe, moderate and mild, or no periodontitis in epidemiological studies, (Table 2), (Page and Eke 2007). These definitions take into account periodontal pocket measurements in combination with clinical attachment loss (CAL). Severe periodontitis is defined as two or more teeth with a (CAL) of ≥ 6mm at interproximal sites, and one or more teeth with a pocket depth of ≥ 5 mm at interproximal sites. Moderate periodontitis is defined as two or more teeth with a CAL of ≥ 4mm at interproximal sites, or two or more teeth with a pocket depth of ≥ 5mm. None or mild periodontitis is recorded when the criteria of neither moderate nor severe periodontitis definition are fulfilled (Page and Eke 2007).

Table 2. Definition of periodontitis by CDC/AAP

<table>
<thead>
<tr>
<th>Disease severity</th>
<th>Clinical attachment loss (CAL)</th>
<th>Periodontal pocket depth (PPD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>≥2 interproximal sites with ≥ 6 mm CAL* AND ≥ 1 interproximal site with ≥ 5mm PPD</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>≥2 interproximal sites with ≥ 4 mm CAL* OR ≥ 2 interproximal sites with ≥5 mm PPD*</td>
<td></td>
</tr>
<tr>
<td>None or mild</td>
<td>Neither severe nor moderate form</td>
<td></td>
</tr>
</tbody>
</table>

*not on same tooth

CDC/AAP= Centers for Disease Control and Prevention / the American Academy of Periodontology
Classification of oral inflammatory burden

Oral inflammatory burden can be assessed by recording different indices such as the PIBI and the TDI, as shown in Tables 3 and 4, (Mattila et al. 1989, Lindy et al. 2008). The DMFT index takes into account decayed, missing and filled teeth (WHO 1997).

Table 3. Determination of Periodontal Inflammatory Burden (PIBI, 28 teeth)

<table>
<thead>
<tr>
<th>Oral Disease</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caries</td>
<td></td>
</tr>
<tr>
<td>no caries lesions</td>
<td>0</td>
</tr>
<tr>
<td>1–3 caries lesions</td>
<td>1</td>
</tr>
<tr>
<td>4–7 caries lesions or no teeth in maxilla or mandible</td>
<td>2</td>
</tr>
<tr>
<td>≥ 8 caries or radix or no teeth</td>
<td>3</td>
</tr>
<tr>
<td>Periodontitis</td>
<td></td>
</tr>
<tr>
<td>4–5mm deep periodontal pocket</td>
<td>1</td>
</tr>
<tr>
<td>≥ 6mm deep periodontal pocket</td>
<td>2</td>
</tr>
<tr>
<td>Pus in gingival pocket</td>
<td>3</td>
</tr>
<tr>
<td>Periapical lesions</td>
<td></td>
</tr>
<tr>
<td>1 periapical lesion or vertical bone pocket or both</td>
<td>1</td>
</tr>
<tr>
<td>2 periapical lesions</td>
<td>2</td>
</tr>
<tr>
<td>≥ 3 periapical lesions</td>
<td>3</td>
</tr>
<tr>
<td>Pericoronitis</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td>Total Dental Index</td>
<td>10</td>
</tr>
</tbody>
</table>

PIBI= \[ \sum (N_{mod} \cdot \text{PPD} + 2 \cdot N_{adv} \cdot \text{PPD}) \]

N_{mod}= number of sites with moderate periodontal lesions (4–5mm)

N_{adv}= number of sites with advanced periodontal lesions (≥ 6mm)

PPD=periodontal pocket depth
Prevention and treatment of chronic periodontitis

Proper diagnosis is vital for accurate treatment. In Finland, the Current Care Guidelines for periodontitis were generated by the working group set by the Finnish Medical Society Duodecim and the Finnish Dental Society Apollonia. They represent evidence-based guidelines for clinical practice, diagnosis, and the treatment of chronic periodontitis (Periodontitis: Current Care Guidelines, 2016). Periodontal treatment primarily targets professional mechanical bacterial debridement, i.e. scaling and root planing, and patients’ self-care to eliminate biofilm and plaque retentions and to prevent the re-colonization of bacteria. Behavioral motivations, for example, smoking cessation are an essential part of treatment. Local or systemic antibiotics, laser treatments, and surgery may be combined with mechanical treatment. The benefits of systemic antibiotics should be balanced against their systemic side effects and antibiotic resistance (Jepsen and Jepsen 2016). Systematic maintenance care is needed to achieve long-term success.

Host modulation therapy, such as low dose doxycycline (LDD) treatment has proven to be a viable option for treating chronic inflammatory periodontal disease (Lalla and Papapanou 2011). LDD inhibit MMPs without an antimicrobial effect, but still suppresses connective tissue breakdown (Golub et al. 1992). The purpose of host modulation is to return the balance between pro- and anti-inflammatory mediators (Preshaw 2008). A recent review article claims that host modulation therapy, a 20 mg subantimicrobial dose of doxycycline (SDD) twice daily, is effective not only in periodontitis but also in diabetes, arthritis, and among postmenopausal women with local and systemic bone loss (Golub et al. 2016).

Kidneys

Anatomy and function

Two bean-shaped kidneys, at the level of T12 to L3 vertebrae lie on each side of the spine in the retroperitoneal space. Kidneys are about 10 cm long, 5 cm wide and 2.5 cm thick, and are partly protected by the lowest rib (Moore 1992). They contain about one million functional units, nephrons with glomerulus and long tubules in which reabsorption of many solutes and water take place (Eckardt et al. 2013). See Figure 1 for details.
Kidneys remove the excretion end products of metabolism, regulate erythrocyte production using the erythropoietin hormone, regulate blood pressure through the renin-angiotensin system, participate in calcium homeostasis by hydroxylating D vitamin to its active form, regulate electrolyte concentrations, and maintain acid-base and fluid balance (Moore 1992, Craig 2008, Thorman et al. 2009). Kidney function is assessed by GFR, which can be estimated using a serum creatinine concentration (Craig 2008).

Laboratory tests may include complete blood count, serum electrolytes, blood urea nitrogen, calcium, the parathyroid hormone, glucose, and glycosylated hemoglobin. Urine tests may involve analyzing, for example urine electrolytes, creatinine, and total protein concentrations. Imaging techniques including renal ultrasound are also routinely used (Greenberg et al. 2008).

Figure 1. Kidney and nephron (http://unckidneycenter.org)
Source: with permission from the UNC Kidney Center
Classification, prevalence and causes of kidney diseases

Acute kidney injury is defined as changes in kidney function that last for ≤ 3 months (Eckardt et al. 2013). According to the US National Kidney Foundation - Kidney Disease Outcomes Quality Initiative’s (NKF-KDOQI) clinical practice guidelines, CKD is defined as abnormalities in kidney structure or function lasting over three months, with implications for health (Inker et al. 2014). Classification of CKD is based on a cause, GFR and albuminuria categories (Inker et al. 2014) (Table 5). At the Helsinki University Hospital, the predialysis stage is defined as mid-stages 4 and 5. According to KDIGO (Kidney Disease: Improving Global Outcomes), albuminuria is categorized as Categories 1 to 3. Category 1 represents normal or mildly increased excretion with an albumin excretion rate (AER) of < 30mg/24 hours. In category 2, AER is 30–300 mg/24 hours (moderately increased excretion), and in category 3, AER is > 300 mg/24 hours, representing severely increased excretion (Inker et al. 2014).

The prevalence of CKD is increasing worldwide, and affects 8–16% of individuals globally (Jha et al. 2013). The prevalence of acute kidney disease varies because of several different definitions and is estimated to be over 1% (Lameire et al. 2013).

The main risk factors of chronic kidney failure are diabetes, hypertension, and obesity in developed as well as many developing countries, while glomerulonephritis and unknown reasons are more common in Asia and sub-Saharan Africa (Cigranelli and Lamacchia 2007, Jha et al. 2013). Other etiological causes of CKD are systemic autoimmune

### Table 5. Classification of Chronic kidney disease (CKD) stages by GFR

<table>
<thead>
<tr>
<th>CKD stage</th>
<th>Kidney function</th>
<th>GFR (ml/min/1.73 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>normal</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>2</td>
<td>mildly impaired</td>
<td>60–89</td>
</tr>
<tr>
<td>3</td>
<td>moderately impaired</td>
<td>30–59</td>
</tr>
<tr>
<td>4</td>
<td>severely impaired</td>
<td>15–29</td>
</tr>
<tr>
<td>5</td>
<td>end stage kidney failure</td>
<td>&lt; 15</td>
</tr>
</tbody>
</table>

GFR= glomerular filtration rate
diseases, polycystic kidney disease, obstructive uropathy, chronic pyelonephritis, and other low-grade systemic inflammatory diseases, such as periodontitis (Fisher et al. 2008, Eckardt et al. 2013). In Finland, diabetic nephropathy is the most common cause for dialysis treatment, followed by glomerulonephritis, polycystic kidney disease and nephrosclerosis (Finnish Registry for Kidney Diseases 2014).

Acute kidney injury, on the other hand, is often associated with bacterial infection, sepsis or ischemia-reperfusion injury (Imig and Ryan 2013).

Diabetes as an inflammation-associated disease may contribute to renal inflammation and further to the development of diabetic nephropathy. Renal inflammation can result from metabolic and renal hemodynamic routes caused by hyperglycemia, reactive oxygen species (ROS), and AGEs, or glomerular hypertension and endothelial dysfunction (Imig and Ryan 2013).

End-stage renal disease

When CKD has progressed to ESRD, RRT (dialysis or kidney transplantation) is needed. ESRD state is reached when the functional capacity of kidneys has decreased below 10% of their normal function (Bots et al. 2007). Over two million people worldwide were treated for ESRD in 2010, but because of disparities in access to RRT, an estimated six million people were untreated (Robinson et al. 2016). In 2014, 4540 patients were in RRT in Finland, of which 343 were in peritoneal dialysis treatment, 1474 in hemodialysis treatment, and 2722 were living with kidney transplants (Finnish Registry of Kidney Diseases 2014).

Kidney transplantation is the preferable choice of ESRD as it improves survival and quality of life, and is cost-effective compared to dialysis (Jensen et al. 2014, Ortiz et al. 2014, Sanchez-Escuredo et al. 2015, Robinson et al. 2016,). In 2014, 240 kidney transplants were made in Finland (Finnish Registry for Kidney Diseases 2014). Prior to entering kidney transplantation, the candidate patient must be screened for infections, including oral infections, before immunosuppressive medications can be administered.
**Immunodeficiency in chronic kidney disease**

CKD is regarded as an immunosuppressive state due to factors such as uremic toxins, immune cell dysfunction, mainly caused by hypercalcemia related to secondary hyperparathyreoidism, and a lack of supportive cytokines, D-vitamin deficiency, and dialysis treatment (Hannula 2009). Immunosuppressive therapy understandably also causes immunodeficiency.

**Mortality among kidney patients**

Patients with ESRD have higher death rates compared with general population (de Jager et al. 2009). CVD are the main causes of death among patients with CKD, followed by infections (Stenvinkel 2002, Kato et al. 2008.) Traditional risk factors for atherosclerotic CVD are dyslipidemia, diabetes, hypertension, and smoking, which do not, however, explain all cases of CVDs (Stenvinkel 2002). Other risk factors include genetic components such as family history of atherosclerotic disease, male gender, metabolic syndrome, obesity, depression, and lack of exercise (Lusis 2000). Oxidative stress and inflammation have been postulated to be non-traditional risk factors for CVD (Stenvinkel 2002). Aortic stiffness has also been explained by excess phosphate in the arteries due to secondary hyperparathyreoidism in CKD, which is already seen in patients with CKD stage 3 (Hannula 2009).

According to the European Dialysis and Transplant Association Registry, the five-year survival rate was 52% for patients who underwent dialysis treatment and 91% for patients who had undergone a kidney transplant (Pippias et al. 2015). In Finland, age-standardized mortality among patients receiving RRT has decreased over the years, (85 deaths per 1000 patient years), reflecting good quality of treatment (Finnish Registry for Kidney Diseases 2014).
Oral infections and systemic diseases

“Focal infection”, such as oral infection, has been held responsible for the initiation and progression of a number of systemic diseases since the late 19th and the early 20th century (Scannapieco 1998). However, the theory did not become popular at the time, partly due to a lack of sufficient evidence for classifying and identifying oral microorganisms.

Since dental treatment has developed in recent decades, and the world’s population is aging, it is logical that people retain natural teeth in their mouths for longer than before. However, teeth and periodontal niches may serve as reservoirs for microbiota and might predispose to systemic infections. These are seen especially in the elderly, whose visual and motoric skills for oral self-care have deteriorated; in immunocompromised patients suffering from chronic inflammatory diseases such as diabetes, CKD or rheumatoid arthritis; or in patients undergoing immunosuppressive treatment.

Oral diseases such as dental caries, mucosal lesions and periodontitis, or dental procedures such as tooth extraction, periodontal or endodontic treatment, or even tooth brushing and flossing may help the invasion of oral micro-organisms into the systemic circulation through dental pulp, mucosal surface or periodontal pockets. Transient oral bacteremia predisposes to systemic infections such as endocarditis, joint infections and brain abscesses, and upregulates inflammatory reactions, rendering the body liable to other systemic conditions and other diseases such as diabetes or atherosclerosis (Lockhart et al. 2008, 2009, 2012, Zhang et al 2013, Scannapieco et al. 2016).

The association between periodontitis and CVD (among other systemic chronic diseases) is well known (Meurman et al. 2004). A recent study suggested that endodontic lesions are also independently associated with coronary artery disease, especially with acute coronary syndromes explained by systemic inflammation (Liljestrand et al. 2016).

Periodontitis and systemic inflammation

Although no causal relationship can be drawn between periodontitis and systemic diseases, many studies have shown a possible association with diabetes, cardiovascular diseases, pregnancy complications, pneumonia, chronic obstructive pulmonary disease, rheumatoid arthritis, obesity, metabolic syndrome, cancer, and CKD (Desvarieux 2005,
Lalla and Papapanou 2011, Lockhart et al. 2012, Linden et al. 2013, Tonetti and Van Dyke 2013). An inflamed periodontal pocket epithelium of 8–20 cm² is an open door for bacteria and their products. The latter include LPS, peptidoglycan (PGN), cytotoxins, proteases and haemagglutinins, as well as locally produced pro-inflammatory mediators such as IL-1β, IL-6, TNF-α, and prostaglandin E₂ (PGE₂) which is produced by host cells (neutrophils, macrophages and lymphocytes) into bloodstream (Hujoel et al. 2001, Preshaw and Taylor 2011, van Dyke and van Winkelhoff 2013). These pro-inflammatory mediators may act as inflammatory stimuli at a distant site and cause problems for the patient (Lalla and Papapanou 2011).

Diabetes

DM is a metabolic disorder with an abnormal blood glucose level caused by lack of insulin related to pancreatic beta-cell dysfunction, or insulin resistance (Chapple et al. 2013). As a metabolic disorder, insulin disturbances may lead to abnormal glucose, fat and protein metabolisms, creating different pathologies in multiple organs (Southerland et al. 2006). According to the World Health Organization (WHO), 442 million people worldwide were suffering from diabetes in 2014, compared to 108 million in 1980, and the number is still increasing (WHO 2016). Diabetes caused 1.5 million deaths in 2012 worldwide (WHO 2016).

Type-1 diabetes (DM1) (5–10% of cases) is a form that results from autoimmune destruction of the insulin-producing beta cells in the pancreas. The subsequent lack of insulin leads to increased blood and glucose levels. Type-2 diabetes (DM2) is a metabolic disorder characterized by hyperglycemia in the context of insulin resistance and a relative lack of insulin. DM1 mostly affects children, whereas DM2 mostly affects older patients (85–90%), but may also be seen among children. Gestational form affects 1–5% of cases (Otomo-Corgel et al 2012).

DM1 results from a complex interaction between genes and environmental factors, although no specific environmental risk factor has been found (WHO 2016). In contrast, well-known risk factors for DM2 are obesity and overweight, combined with genetic background, physical inactivity, and smoking (WHO 2016).
Glycosylated hemoglobin level (HbA1c) is indicative of the metabolic control in the preceding two to three months. According to the American Diabetes Association, the diagnosis of diabetes in adults is made using two tests of HbA1c ≥ 6.5% (48 mmol/mol) (Standards of Care 2016).

With elevated HbA1c, diabetes may lead to known complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications, and delayed wound healing (Otomo-Corgel et al. 2012). Löe suggested in 1993 that periodontitis should be recognized as the sixth complication of DM (Löe 1993). Today, the bidirectional relationship between these two chronic diseases, diabetes and periodontitis, is well accepted, and both may be present for years before symptoms (Taylor et al. 1998, Chapple et al. 2013).

**Hyperglycemia and periodontitis**

Different mechanisms have been proposed to link diabetes to periodontitis (microangiopathy, alterations in GCF, collagen metabolism, subgingival flora, genetic factors, and host response) (Taylor et al. 1998). Many cross-sectional and longitudinal studies, together with meta-analyses, have shown a greater prevalence of advanced periodontitis among diabetic versus non-diabetic patients (Oliver & Tervonen 1994, Collin et al. 1998, Khader et al. 2006). Emrich and co-workers found that diabetes tripled the risk of periodontitis among 1 342 PIMA Indians (Emrich et al. 1991). Taylor and co-workers confirmed these cross-sectional statements with a longitudinal study of PIMA Indians, which showed non-insulin dependent diabetes mellitus (NIDDM) to be a risk factor for more severe alveolar bone loss progression in two-year follow-up (Taylor et al. 1998).

Hyperglycemia leads to an abnormal hyper-inflammatory response to periodontopathogens in the periodontium (Southerland et al. 2006, Nishimura et al. 2007, Otomo-Corgel et al. 2012). Cell surface receptors (RAGEs) for advanced glycation end products (AGEs) in gingiva are expressed, and RAGE-AGE interaction leads to immune dysfunction, exaggerated inflammatory response and oxidative stress, thus further accelerating AGE and pro-inflammatory cytokine formation, the destruction of tooth supported structures, and impaired wound healing (Lalla and Papapanou 2011, Chapple et al. 2013, Taylor et al. 2013).
So far, hyperglycemia has shown no relationship with periodontal microbiota (Taylor 2013). However, diabetic patients with periodontitis seem to have elevated numbers of pro-inflammatory mediators such as IL-1β, IL-6, and increased RANKL/OPG ratios compared with systemically healthy controls with periodontitis. This partly explains bone resorption in periodontitis (Salvi et al. 1997). However, it is noteworthy that diabetic patients with well-controlled blood sugar levels are not at an increased risk of periodontitis (Kowall et al. 2015).

**Impact of periodontitis on diabetes**

Untreated periodontitis may increase systemic pro-inflammatory mediators, causing insulin resistance. The European Federation of Periodontology (EFP) and the American Academy of Periodontology (AAP) have confirmed that in patients with severe periodontitis, the disease adversely affect blood glucose levels (HbA1c) (Chapple et al. 2013).

Taylor and co-workers were the first to show, in a five-year follow-up study in 1996, that severe periodontitis at baseline among the NIDDM Gila River Indian Community (tribe of PIMA Indians, Arizona) resulted in poorer glycemic control (HbA1c > 9%) than that among those without severe periodontitis (Taylor et al. 1996). Severe periodontitis was assessed by attachment loss of 6 mm or more on at least one index tooth, and radiographic bone loss of 50% in at least one tooth.

A study of 9,296 nondiabetic participants of NHANES I (National Health and Nutrition Examination Survey) and its Epidemiologic Follow-up Study (NHEFS) showed that severe tooth loss at baseline resulted in the incidence of diabetes with an odds ratio (OR) of 1.7 (p< 0.05), (follow-up period of 17 ± 4 years) (Demmer et al. 2008).

A recent systematic Cochrane collaboration review of 35 studies on diabetic patients found that the mean percentage reduction in HbA1c by conventional periodontal therapy was 0.29% at three to four months (Simpson et al. 2015).
Atherosclerotic cardiovascular disease

Atherosclerotic cardiovascular diseases (ACVD) comprise coronary heart disease (angina pectoris and myocardial infarction), ischemic cerebrovascular disease (TIA and stroke), and peripheral arterial disease (Tonetti et al. 2013).

As classic risk factors for ACVD, such as hypertension, diabetes, smoking and hypercholesterolemia explain only 30–60% of the disease, chronic infection and inflammation has been proposed as another risk factor (Scannapieco 1998, Li et al. 2000). Since 1989, the association between dental infections and acute myocardial infarction has been extensively studied. At our hospital, Mattila and co-workers found that patients with acute myocardial infarction had worse oral health than age- and sex-matched controls (Mattila et al. 1989). A recent study by Beukers and co-workers showed an independent association between periodontitis and ACVD in over 60 000 Dutch patients (Beukers et al. 2016).

Vascular cell wall injury (mechanical, immunological or biochemical) leads to adhesion molecule expression by endothelial cells. Monocytes adhere and migrate through the cell wall, phagocytose low-density lipoproteins and release pro-inflammatory cytokines, inducing inflammatory cascades (Cullinan and Seymor 2013). LPS from Gram-negative bacteria is toxic to endothelial cells, and promotes adhesion molecule expression. LPS may also help macrophages transform into foam cells, promoting inflammation and further atherosclerosis (Cullinan and Seymor 2013). Bacteria and their products from oral cavities may thus activate host inflammatory mechanisms in the periphery, favoring the formation, maturation and exacerbation of atheroma (Tonetti et al. 2013).

Chronic kidney disease

Inflammation and atherosclerosis, both related to periodontitis, are shown to predispose patients to CKD (Scannapieco and Cantos 2016). Rahmati and co-workers were the first to show an association between serum IgG to periodontal bacteria (P. gingivalis) and elevated level of C-reactive protein in hemodialysis patients (Rahmati et al. 2002). A cross-sectional study by Kshirsagar and co-workers showed that a reduction in GFR was connected to periodontal disease (Kshirsagar et al. 2005). Chambrone and co-workers in
turn concluded in their systematic review that fairly strong evidence exists to support an association between periodontitis and CKD (Chambrone et al. 2013).

**Association between other systemic diseases and periodontitis**

Infection can be a risk factor for pregnancy complications such as preterm birth (< 37 weeks of gestation) and low birth-weight (< 2500 g) infants. Oral inflammatory burden such as periodontitis may be one route of infection associated with preterm birth (Heimonen et al. 2009). Increased bacterial spreading or pro-inflammatory cytokines could affect the placenta and further drive preterm birth forward. However, many studies show opposite results, and more research is needed (Cullinan and Seymour 2013, Walia and Saini 2015). A systematic review and meta-analysis by Kim and co-workers concluded that mothers at a high risk of preterm birth could benefit from periodontal treatment (Kim et al. 2012).

In hospitalized and elderly patients, aspirated microbiota may cause bacterial aspiration pneumonia (Garcia et al. 2001, Scannapieco and Cantos 2016). Normally, healthy subjects may aspirate 50% of oropharyngeal contents, which are eliminated by the host immune system without harm (Garcia et al. 2001). Aspiration pneumonia is often correlated with dysphagia, but other reasons such as a high number of decayed teeth, *Staphylococcus aureus* and *Streptococcus sobrinus* bacteria in the saliva, the presence of *P. gingivalis* in plaque, dependence in feeding and oral care, chronic obstructive pulmonary disease, and diabetes have all shown a correlation with aspiration pneumonia (Terpenning et al. 2001).

Chronic obstructive pulmonary disease (COPD) affects about 10% of ≥ 40-years worldwide, and is a leading cause of morbidity and mortality (Halbert et al. 2006, Scannapieco and Cantos 2016). Inflammation, mostly due to toxic chemicals from smoking, is the most significant factor in the development of COPD. Chronic infection such as periodontitis has shown to associate with COPD by triggering pro-inflammatory events and further influencing the frequency of disease exacerbation (Scannapieco and Cantos 2016).

Inflammation plays a crucial role in autoimmune diseases such as rheumatoid arthritis. In the light of current knowledge, genetic and environmental factors trigger autoantibodies to autoantigens such as citrullinated proteins (Scannapieco and Cantos 2016). One explanation for the association between periodontitis and rheumatoid arthritis could be the
ability of *P. gingivalis* to produce peptidylarginide that can citrullinate proteins (Wegner et al. 2010).

Obesity is known to be a hyperinflammatory state with insulin resistance. A recent study by Suvan and co-workers showed an independent association between periodontitis and obesity (Suvan et al. 2015). Obesity may indeed alter the inflammatory state, thus also predisposing the individual to periodontitis (Suvan et al. 2015).

**Oral manifestation of chronic kidney disease**

CKD may be accompanied by other systemic diseases with oral mucosal manifestations. Aphthous ulcers of oral mucosa may be associated with Behcet’s disease, which is linked to rapidly progressive glomerulonephritis; or with systemic lupus erythematosus (SLE), which is characterized by lupus nephritis (Summers et al. 2007). “Strawberry gingivitis” may be the first manifestation of systemic vasculitis, Wegener’s granulomatosis (Ruokonen et al. 2009). Macroglossia may be due to deposits of amyloids (Summers et al. 2007).

CKD patients may also present many oral changes due to medication or the disease itself affecting teeth, oral mucosa, periodontium, and the salivary glands (Summers et al. 2007, Akar et al. 2011). Reported changes in teeth include pulp narrowing, enamel hypoplasia, dental caries, and periodontitis (Gaili et al. 1991, Vesterinen et al. 2011, Kshirsagar et al. 2005, Summers et al. 2007). Hyposalivation due to diabetes or medication is harmful, as it increases microbial colonization, thus enhancing oral diseases.

The different stages of CKD, namely predialysis-, dialysis and the post-transplantation stage may manifest in different ways in oral health. Different CKD diagnoses, such as diabetic nephropathy, have also shown characteristic oral health manifestations.

**Predialysis stage**

Uremic patients have shown a worse DMFT index and more periodontal loss of attachment and periapical lesions than sex- and age-matched controls (Thorman et al. 2009, Vesterinen et al. 2011). At the predialysis stage, protein intake is restricted, while
sufficient energy is secured by intake of carbohydrates (Thorman et al. 2009). This may detrimentally affect dental and oral health.

Diabetic patients should be informed of their greater risk of periodontitis, the adverse effects of poor glycemic control on periodontitis, and the increased risk of systemic problems such as cardiovascular complications or the progression of CKD (Chapple et al. 2013).

**Dialysis**

Oral health often deteriorates in patients receiving hemodialysis therapy (Schmalz et al. 2016). As hemodialysis treatment is time consuming, usually requiring four to six hours three times per week, treatment may be stressful for the patient. This may lead to having no strength to maintain proper oral health. Moreover, dialysis is regarded as an immunosuppressive state which further affects oral health.

Symptoms such as oral malodor (ammonia-like smell, typical for uremic patients), dry mouth (xerostomia), and taste changes (metallic taste) have been reported in dialysis patients (Kaushik et al. 2013). Medication such as antihypertensive drugs and diuretics may predispose to poor oral health through hyposalivation. This may also affect denture retention and cause difficulties in speaking and eating. The reduction of fluid intake may cause general weakening and a reduced salivary flow rate. These changes may lead to problems in mucosa and predispose to oral fungal and viral infections, as well as coated tongue, sialadenitis, oral ulceration, enamel hypoplasia, increased dental calculus formation, and caries development (Summers et al. 2007, Akar et al. 2011, Kaushik et al. 2013, Schmalz et al. 2016). In contrast, some studies have shown a prevalence of reduced caries in ESRD, perhaps due to the protective, neutralizing role of urea in saliva and plaque (Al Nowaiser et al. 2003).

In oral health care, attention should be paid to prolonged bleeding time after tooth extraction or periodontal treatment due to heparin administration in hemodialysis, drugs used for treatment (GFR is decreased), and the need for antibiotic prophylaxis (vascular access sites protection) (Craig 2008).
Post-transplantation stage

Transplant patients may have oral manifestations such as gingival hyperplasia due to immunosuppressants (cyclosporine) and antihypertensive drugs (calcium channel blockers). Cyclosporine-induced gingival hyperplasia ranges from 22% to 58%, and is more prevalent among young patients and patients with increased dental plaque accumulation or an increased drug dosage (Craig et al. 2008, Akar et al. 2011). Transplant patients are also prone to virus infections, ulcers and oral malignancies (Summers et al. 2007). Fungal infections are found in 20–30% of transplant patients, and may manifest as angular cheilitis, pseudomembranous or erythematous ulcerations (Summers et al. 2007). In their two-year follow-up investigation, Bots and co-workers found that xerostomia and thirst decreased, and unstimulated salivary flow rate increased after kidney transplantation compared to dialysis state (Bots et al. 2007).

Oral health care professionals should pay attention to the fact that a patient with a transplanted organ needs immunosuppressive drugs for the rest of their life. These drugs may predispose patients to oral inflammations and fungal and viral infections as well as malignancies or gingival overgrowth induced by cyclosporine and calcium channel blockers. Therefore, oral examination of transplant patients calls for special attention, preferably in a specialist clinic (Kaswan et al. 2015, Rezvani et al. 2014). Antibiotic prophylaxis is mandatory when treating transplant patients.
AIMS OF THE STUDY

This study examined the oral health of CKD patients in detail, with an emphasis on periodontal disease; first at baseline (predialysis stage, Study I and Study II), and secondly at follow-up (post-transplantation stage, Study III and Study IV). Studies I and II are based on the patients previously described by our group (Vesterinen et al. 2011, 2012). The main hypotheses were that oral health, periodontitis in particular, is more severe among diabetic nephropathy patients than other CKD patients, and worse at the predialysis stage than at the post-transplantation stage.

The main objectives of the current study were:

1. To examine the oral health, with an emphasis on periodontal disease, of CKD patients at the predialysis stage, and to compare the periodontal health of diabetic nephropathy patients and CKD patients with other etiology (Study I).

2. To investigate the association between periodontal inflammatory burden and salivary MMP-8 concentration among CKD patients at the predialysis stage (Study II).

3. To determine whether oral inflammatory burden associates with mortality and to identify causes of death among CKD patients (Study III).

4. To compare, on the individual level, patients’ oral health at the baseline predialysis stage with that at the follow-up post-transplantation stage (Study IV).
PARTICIPANTS AND METHODS

This study is an ongoing clinical investigation of CKD patients examined in 2000–2005 (baseline) (Vesterinen et al. 2011) and 2013–2015 (follow-up). Originally, 178 CKD patients were referred from the Department of Nephrology to the Department of Oral and Maxillofacial Diseases, Helsinki University Hospital, Helsinki, Finland, for examination and treatment of oral foci of infection prior to ESRD treatment.

For inclusion, all patients had to have an estimated glomerular filtration rate of < 20mL/min/1.73 m², confirmed by a nephrologist. Thus, the patient had to be at the predialysis stage, which corresponds to mid CKD Stage 4 (eGFR < 30 mL/min/1.73 m²) and Stage 5 (eGFR < 15mL/min/1.73 m²), in terms of the criteria used in our Department of Nephrology. Patients below this eGFR are referred for renal replacement therapy. Children (< 18-year-olds), pregnant or breastfeeding women, prisoners, and physically or mentally disabled patients were excluded.

Due to the definition of the predialysis stage, 30 patients were excluded from the original studies. Of the remaining 148 patients, two had missing periodontal status records and two had missing saliva samples at the time of the first study, leaving 144 CKD patients for further investigation.

The study was approved by the Ethical Committee of the Hospital District of Helsinki and Uusimaa (Dnro 305/13/03/02/2012) and registered in the hospital database for clinical trials. Written informed consent was obtained from all the patients, and the ethical principles of the Declaration of Helsinki were followed throughout the study.

Participants

PART 1 (Studies I and II) Baseline studies I and II were cross-sectional studies.

Study I was a clinical study of oral health, with an emphasis on periodontal disease. Clinical oral and x-ray examinations were conducted in 2000–2005. Clinical and medical data were obtained from the hospital records of 144 patients (47 women), aged 23–83. Of these, 52 had diabetic nephropathy and of this 52, 22 suffered from DM2.
**Study II** was an MMP-8 salivary study of 118 predialysis patients and 11 systemically and periodontally healthy controls. Twenty-six salivary samples of the 144 patients could not be examined at the time of MMP-8 analysis due to missing samples.

**PART 2 (Studies III and IV)** Studies III and IV were follow-up studies with 157 months of observation.

**Study III** is a follow-up prospective cohort study of 144 CKD patients. Of these, 65 had died and 79 survived during the follow-up period of maximal 157 months. The study focused on their risk factors of mortality. The cause of death could be recorded for 62 participants, of which 32 had had diabetic nephropathy. Three patients had died outside our hospital district and their cause of death could not be verified. Only ten patients of the 65 (15%) who has died had undergone a kidney transplant.

**Study IV.** Of the 144 CKD patients, 79 had survived and were invited to re-examination between 2013 and 2015. Of these, 26 patients dropped out, leaving 53 participants willing to participate in the follow-up examination. The study was a longitudinal clinical study that focused on comparing the oral health variables of the predialysis and follow-up stages. Of the 53 patients, 51 had undergone a kidney transplant and 46 transplants were functioning at the time of the examination. **Figure 2** shows a flow diagram of the study.
Figure 2. Flow diagram of the study

N= 144
(N= 52 diabetic nephropathy)
Predialysis stage (Study I, II)

N= 79
Re-called for follow-up study

N= 53
(N= 11 diabetic nephropathy)
Follow-up stage (Study IV)

N= 65
(N= 33 diabetic nephropathy)
Deceased (Study III)

N= 26
Drop outs
Methods

Medical records with blood sample analyses from the hospital records were available for the study. Mortality records for Study III were obtained from the Causes of Death National Database of Statistics Finland.

Oral and radiological examination

The clinical oral examinations at baseline in 2000–2005 and at follow-up stage in 2013–2015 were conducted by the same periodontist (HR). The medical records were carefully re-examined for the retrospective and follow-up studies.

Patients underwent a full clinical oral examination, including a mucosal periodontal and cariological examination. The number of teeth and implants were recorded, as were fixed or removable prosthesis and their condition. Mucosal lesions such as gingival overgrowth, coated tongue, ulcers, lichen planus and leukoplakia were also recorded, as well as attrition, erosion, diastemas, and mobility of teeth.

Periodontal recordings were made of all teeth (max 32) and PPDs were measured at six sites per tooth with a WHO probe. PPDs were further categorized as no periodontal pocket, one or more sites with probing depths of ≥ 4mm, 0–1 sites with ≥ 6mm-deep periodontal pockets, and two or more further sites with ≥ 6mm-deep periodontal pockets. Furcation lesions were recorded. Recessions and clinical attachment levels, as well as bleeding on probing, were recorded at the follow-up stage.

The CDC/AAP’s definition of periodontitis was used, according to which, severe periodontitis is when two or more teeth have a CAL of ≥ 6mm at interproximal sites, and one or more teeth with a PPD of ≥ 5mm at interproximal sites. Moderate periodontitis is defined by two or more teeth with a CAL of ≥ 4mm at interproximal sites, or two or more teeth with a PPD of ≥ 5mm at interproximal sites. Cases that do not fulfil the criteria mentioned above are classified as mild or no periodontitis (Page and Eke 2007).

The following indices were also calculated from dental records and categorized into two groups by medians:
The DMFT (decayed, missing, filled teeth) index was calculated from 32 teeth in accordance with WHO criteria, except for the “missing value” which also took wisdom teeth into account.

TDI was calculated from 32 teeth, taking into account caries, periodontitis, periapical- and pericoronitis lesions. This index ranges from 0 to 10 (Mattila et al. 1989).

PIBI was calculated from 28 teeth. This index adds moderate periodontal pockets (4–5mm) to the weighted number (x2) of deep (≥ 6mm) periodontal pockets (Lindy et al. 2008).

Panoramic jaw X-rays were taken of all patients at the predialysis and follow-up stages. Periapical and bite-wing x-rays were taken when needed. A specialist in oral radiology at our hospital analyzed the X-rays. Baseline X-ray analyses of 17 of the 144 patients were missing, leaving 127 available analyses for Study I and III, and 108 available analyses of the 118 patients for Study II. For Study IV, five analyses were missing, leaving a total of 101 analyses available. The radiologist measured alveolar bone loss in each tooth in the cervical, middle, and apical thirds of the root lengths. X-ray analyses were further divided into sextants and finally, maximum alveolar bone loss (either horizontal or vertical) was recorded. Signs of periapical or pericoronitis lesions, cysts and tumors in the X-rays were also recorded. In some cases, when needed, dental radiographs were taken in addition to the panoramic jaw X-rays.

**Salivary analyses**

Unstimulated and stimulated saliva samples were collected for five minutes in graded test tubes and the results were given in ml/min by recording salivary flow rates. Patients were asked not to eat or smoke for at least 60 minutes before the examination. Unstimulated saliva was collected first, after which paraffin wax was used to stimulate salivary flow. Samples were centrifuged for four minutes at 8000 x g at 4ºC, and the supernatants were frozen at -75ºC for later analyses.

From the stimulated saliva, MMP-8 was detected by time-resolved immunofluorometric assay (IFMA) (Rathnayake et al. 2013a,b, Mäntylä et al. 2006, Leppilahti et al. 2014a,b, Gursoy et al. 2010). Catching and tracing monoclonal antibodies (8708 and 8706, Medix Biochemica, Kauniainen, Finland) specific to MMP-8, were used. The tracing antibody was
labeled by europium-chelate (Hemmilä et al. 1984). Samples were diluted in an assay buffer (20mM Tris-HCL (pH7.5), 0.5M NaCl, 5mM CaCl2, 50mM ZnCl2, 0.5% bovine serum albumin, 0.05% sodium azide, and 20 mg/L dietylenetriaminepenta-acetic acid) and incubated for one hour, followed by incubation with a tracer antibody for another hour. Fluorescence was measured five minutes after enhancement solution was added, using IFMA.

**Questionnaire study**

Self-reported oral health questionnaires were already collected at predialysis and later at follow-up. The questionnaires elicited education, oral health self-care, smoking habits, oral symptoms of xerostomia, consideration of the role of oral health in kidney disease, and last dental appointment.

**Statistical analyses**

A software program (SPSS statistical version 21 and 22) was used for statistical analyses. Cross-tabulation and the Pearson chi-square test were used to compare the categorical variables in Studies I, II, III, IV. Mean HbA1c values were compared between groups by independent samples t-test, and multivariable analyses were conducted using binary logistic regression with HbA1c values higher or lower than median as the binary outcome variable.

**Study I:** As in the previous study by Vesterinen et al. (2011) the oral health of diabetic nephropathy patients and other CKD patients were compared. Oral health at higher (≥ 6.5 %) and lower (< 6.5 %) HbA1c values was also compared.

**Study II:** The concentration of MMP-8 as a continuous variable was compared according to the CKD diagnosis and HbA1c values, as well as according to sex-, age-, smoking status, number of teeth, TDI and PIBI scores, alveolar bone loss, and PPD categories by independent two- samples t-test and Mann-Whitney U test.

**Study III:** The Kaplan-Meier survival curve was calculated and the survival distributions of the patient groups were compared with the log-rank test. Cox proportional hazards regression models were used to analyze the association of clinical oral parameters and
other explanatory variables with patient survival. In survival analysis, death was the event and all surviving patients were right-censored at the end of follow-up on August 31st 2015.

**Study IV**: The oral health data regarding the within-subject changes in the baseline (predialysis) and follow-up (post-transplantation) recordings were compared. P-values were calculated using the McNemar test for categorical variables, and the Wilcoxon signed-rank test for continuous variables. In the comparison between diabetic nephropathy and other CKD groups, continuous variables were compared using the Mann-Whitney U test and categorical variables using the Pearson chi-square or Fisher test.

**Ethical aspect**

This study was approved by the ethical committee of the Helsinki and Uusimaa Hospital District. It was conducted in accordance with the principles of the Declaration of Helsinki (Dnro 305/13/02/2012). Written informed consent was obtained from all patients.
RESULTS

Baseline results at the predialysis stage are presented in Studies I and II. Basic demographic and clinical characteristics are given in Tables 6 and 7.

Oral health with an emphasis on periodontal disease (Study I)

Diabetic nephropathy patients (N= 52) were compared with other CKD patients (N= 92). Other CKD diagnoses included polycystic kidney disease (N= 31), nephrosclerosis (N= 16), immunoglobulin A (IgA)-nephropathy (N= 13), focal segmental glomerulosclerosis (N= 9), and membranous glomerulonephritis (N= 4).

Mean HbA1c was 5.7% (±0.8) in the other CKD group, and 8.2% (± 1.6) in the diabetic nephropathy group (p<0.001).

The prevalence of periodontal pockets was high (88%) among CKD patients in general. One or more periodontal pockets were observed in 90% of the diabetic nephropathy patients and in 87% of those with other CKD diagnoses.

Patients with diabetic nephropathy diagnosis had worse periodontal health than those with other CKD diagnoses. Diabetic nephropathy patients more often had two or more sites with a PPD of ≥ 6mm, and their TDI was higher than that of the other CKD patients (p<0.05). Moderate periodontitis diagnosis, assessed by two or more interproximal sites with ≥ 5mm-deep periodontal pockets (not on the same tooth) was more common among diabetic nephropathy patients (61.5%) than among those with other CKD diagnoses (47.8%), and higher PIBI scores were more common among diabetic nephropathy patients (55.8%) than other CKD patients (45.7%), although these results were not statistically significant.

Diabetes was diagnosed in six patients of the other CKD group, although this was not the reason for kidney disease. Thus we also compared patients with different HbA1c values. Higher HbA1c (≥ 6.5%, ≥ 48mmol/mol) associated more often with deep periodontal pockets (≥ 2 sites with ≥ 6mm deep pockets), moderate periodontitis diagnosis (≥2 interproximal sites with ≥ 5mm deep pockets – not on same tooth), and higher PIBI (p<0.05). Patients with a higher HbA1c had a higher TDI score (51.1%) than patients with lower HbA1c (40.7%), but this result was not statistically significant. In the multivariable
analysis, no oral health parameters, PIBI, TDI, deep periodontal pockets, or background variables of sex, age or smoking habits significantly associated with the HbA1c levels.

There were no statistically significant differences in the number of teeth, alveolar bone loss or background variables of age, sex or smoking when diabetic nephropathy patients were compared with other CKD patients. However, current smokers had a statistically significantly higher TDI score than non-smokers.

**Association of salivary MMP-8 with oral inflammatory burden, with an emphasis on periodontal disease (Study II)**

Salivary MMP-8 could be analyzed in 118 of the 144 patients. Twenty-six salivary samples were missing and could not be analyzed at the time of detection. Salivary samples from 11 systemically and periodontally healthy individuals were used as controls.

Higher salivary MMP-8 concentration was associated with poorer oral health. Patients with deep periodontal pockets (two or more sites with a PPD of ≥ 6mm), higher median PIBI and TDI associated statistically significantly with higher MMP-8 concentrations (p< 0.05).

Diabetic nephropathy patients had higher concentrations of salivary MMP-8 (148.66 ± 88.42 ng/ml) than the other CKD patients (116.47 ± 96.67 ng/ml) or controls (93.37 ± 93 ng/ml). The results were not statistically significant, however. Smoking, sex, age, number of or different group of medication (antimicrobial or anti-inflammatory drugs, ACE blockers, vitamin D or statins) had no statistically significant effect on salivary MMP-8 concentration.
The follow-up results are presented in Studies III and IV. Basic demographic and clinical characteristics are given in Tables 6 and 7.

**Association between oral inflammatory burden and different CKD diagnosis with mortality (Study III)**

Maximal follow-up time was 157 months. All 144 patients examined in 2000–2005 were called for re-examination in 2013–2015. Of these, 65 had died and 79 survived, of whom 53 attended the re-examination. The cause of death of 62 patients was verified.

The most frequent cause of death among the CKD patients was a major cardiovascular event (MACE) (N = 31), followed by infection (N = 13) and malignant disease (N = 10). Eight patients died for other reasons: one due to a pulmonary embolism, one due to calciphylaxia, three due to complications from diabetes, and three due to other chronic long-term illnesses. MACE included myocardial infarction, endocarditis, atherosclerotic heart disease, valvular heart disease, cardiomyopathy, cardiac arrest, pulmonary edema, congestive cardiac failure, and cerebrovascular stroke. Infections included pneumonia, sepsis, peritonitis, and herpes encephalitis. Malignant diseases comprised pancreatic carcinoma, lymphoma, melanoma, prostate-, urethral-, ovarian-, and colon cancer, and one primary cancer of unknown etiology.

When comparing oral health at baseline (predialysis stage), the deceased had less teeth than the patients who survived. They also had higher TDI scores, diabetes more often and were older. However, these observations were crude unadjusted results.

In univariate analysis, moderate periodontitis (HR 1.70, 95% CI 1.019-2.834), number of teeth (HR 0.94, 95% CI 0.913-0.965), older age (HR 1.05, 95% CI 1.024-1.071 per one-year increment), number of medications (HR 1.13, 95% CI 1.042-1.227), and diabetic nephropathy diagnosis (HR 2.8, 95% CI 1.684-4.953) were associated with mortality. However, in the multivariable Cox regression model, older age (HR 1.03, 95% CI 1.007-1.058), number of teeth (HR 0.95, 95% CI 0.916-0.981) and diabetic nephropathy diagnosis (HR 2.9, 95% CI 1.711-4.837) were significant independent risk factors for death (p< 0.05). Therefore, having more teeth seemed to lower the risk of death (HR< 1) (Figure 3). The 10-year survival rate of diabetic nephropathy patients was 28%, and of the other CKD patients 62% (p< 0.001). The overall 10-year survival rate was 50%.
Tables 6 and 7 show the basic demographic and clinical characteristics at follow-up. Comparison of within-subject changes at the predialysis and post-transplantation stages shows that oral health was better overall at follow-up, when most patients (51 of 53, 96.2%) had undergone a kidney transplant. Of these transplants, 46 (90.2%) were...
functioning, whereas five patients had returned to dialysis. At baseline, patients more often had calculus, deep periodontal pockets, higher TDI and PIBI scores, more teeth, and higher salivary flow rates than they did at follow-up. Overall, oral health was poorer at the predialysis stage than at follow-up.

The oral health of the CKD diagnoses groups was also compared at follow-up, as it was in the predialysis stage analyses. At follow-up, diabetic nephropathy patients more often had plaque, more frequent Candida infections, used more drugs daily, and had lower stimulated salivary flow rates than the patients with other CKD diagnose.

Table 6. Demographic characteristics of CKD patients at baseline (predialysis) and follow-up (post-transplantation), in Study I and IV.

<table>
<thead>
<tr>
<th></th>
<th>Predialysis stage (N= 144)</th>
<th>Post-transplantation stage (N= 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetic nephropathy (N= 52, 36.1%)</td>
<td>Other CKD (N= 92, 63.9%)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>38 (73.1%)</td>
<td>59 (64.1%)</td>
</tr>
<tr>
<td>Women</td>
<td>14 (26.9%)</td>
<td>33 (35.9%)</td>
</tr>
<tr>
<td>Age (median)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 54 yrs</td>
<td>30 (57.7%)</td>
<td>43 (46.7%)</td>
</tr>
<tr>
<td>&gt; 54 yrs</td>
<td>22 (42.3%)</td>
<td>49 (53.3%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0 (0%)</td>
<td>86 (93.5%)</td>
</tr>
<tr>
<td>Yes</td>
<td>52 (100%)</td>
<td>6 (6.5%)</td>
</tr>
<tr>
<td>Smoking**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>23 (67.6%)</td>
<td>48 (68.6%)</td>
</tr>
<tr>
<td>Yes</td>
<td>11 (32.4%)</td>
<td>22 (31.4%)</td>
</tr>
<tr>
<td>Number of daily drugs***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–8</td>
<td>14 (26.9%)</td>
<td>66 (71.7%)</td>
</tr>
<tr>
<td>9–22</td>
<td>38 (73.1%)</td>
<td>26 (28.3%)</td>
</tr>
</tbody>
</table>

* p-values obtained from Pearson chi-square test. Bold values represent significant (p< 0.05) values.
**Smoking refers to current smokers, former smokers were excluded
*** Number of daily drugs categorized by median values
<table>
<thead>
<tr>
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<th></th>
<th>Post-transplantation stage (N= 53)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Diabetic nephropathy (N= 52, 36.1%)</td>
<td>Other CKD (N= 92, 63.9%)</td>
<td></td>
<td>Diabetic nephropathy (N= 11, 20.8%)</td>
</tr>
<tr>
<td>Number of teeth**</td>
<td>24 (14–27) 2-31</td>
<td>26 (20–28) 0-32</td>
<td>0.144</td>
<td>20 (16–25) 0-28</td>
</tr>
<tr>
<td>Fungal infection***</td>
<td>29 (61.7%) 18 (38.3%)</td>
<td>50 (70.4%) 21 (29.6%)</td>
<td>0.324</td>
<td>3 (27.3%) 8 (72.7%)</td>
</tr>
<tr>
<td>PPD (sites)</td>
<td>5 (9.6%) 47 (90.4%)</td>
<td>12 (13.0%) 80 (87.0%)</td>
<td>0.540</td>
<td>4 (36.4%) 7 (63.6%)</td>
</tr>
<tr>
<td>0-1 with ≥6mm</td>
<td>32 (61.5%) 20 (38.5%)</td>
<td>75 (81.5%) 17 (18.5%)</td>
<td>0.008</td>
<td>10 (90.9%) 1 (9.1%)</td>
</tr>
<tr>
<td>2 with ≥6mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate periodontitis†</td>
<td>20 (38.5%) 32 (61.5%)</td>
<td>48 (52.2%) 44 (47.8%)</td>
<td>0.113</td>
<td>5 (71.4%) 2 (28.6%)</td>
</tr>
<tr>
<td>PIBI**</td>
<td>8 (2–22.5) 0–83</td>
<td>4.5 (2–14.8) 0–111</td>
<td>0.162</td>
<td>2 (0–7) 0–30</td>
</tr>
<tr>
<td>TDI**</td>
<td>4 (3–4) 2–7</td>
<td>3 (2–4) 0–9</td>
<td>0.032</td>
<td>3 (1–3) 0–5</td>
</tr>
<tr>
<td>DMFT**</td>
<td>25 (19.3–28) 9–32</td>
<td>24 (18–28) 4–32</td>
<td>0.312</td>
<td>25 (19–31) 15–32</td>
</tr>
<tr>
<td>Non-stimulated salivary flow-rate**</td>
<td>0.37 (0.17–0.5) 0–1.00</td>
<td>0.4 (0.19–0.6) 0–2.60</td>
<td>0.418</td>
<td>0.26 (0.20–0.36) 0–0.76</td>
</tr>
<tr>
<td>Stimulated salivary flow-rate**</td>
<td>1.2 (0.7–1.8) 0.06–3.60</td>
<td>1.55 (1.0–2.0) 0.14–4.60</td>
<td>0.030</td>
<td>0.52 (0.26–0.62) 0–2.81</td>
</tr>
</tbody>
</table>

*p-value (unadjusted) obtained from Pearson chi-square for categorical and from Mann-Whitney for continuous variable
** Median distribution, (IQR=interquartile rage)
***Fungal analysis of 26 patients missing at baseline and of 2 patients at follow-up
†At follow-up: patients with gingival hyperplasia (N=6) and 1 patient with severe periodontitis (CDC/AAP) in diabetic nephropathy group excluded
DISCUSSION

Rationale for the study

CKDs are an increasing public health concern globally, along with diabetes, hypertension, and obesity, which are their main risk factors. According to the literature, CVD is the most common cause of death among kidney disease patients, followed by infection and cancer (Stenvinkel 2002, Kato et al. 2008). Since oral infections such as periodontitis are associated with systemic diseases such as diabetes and cardiovascular diseases, oral cavity should be regarded as an “inflammation source” of these chronic systemic inflammatory diseases. Only a few studies have examined and followed up periodontal health among CKD patients both at predialysis and further at post-transplantation stage, therefore this study is unique. According to our knowledge, it is also the first study to investigate the association between oral health, with an emphasis on missing teeth, and mortality among CKD patients.

Inflammation is indeed a key factor in many chronic diseases, diabetes and periodontitis included. There is a well-known two-way relationship between diabetes and periodontitis, both of which may stay asymptomatic for years (Taylor et al. 1998, Chapple et al. 2013).

Periodontitis is also believed to be a non-traditional risk factor for CKD (Fisher et al. 2008). In their cross-sectional study, Kshirsagar and co-workers found an association between reduced GFR and periodontal disease (Kshirsagar et al 2005). This association could be explained by direct damage by periodontopathogens to nephron units, its vasculature or by a chronic host inflammatory response to periodontopathogens, leading kidney damage (Kshirsagar et al. 2005).

Methodological considerations

As in the original study by Vesterinen and co-workers (2011) we decided to compare two groups of CKD patients: diabetic nephropathy and other CKD, when exploring differences in oral health with an emphasis on periodontitis at both the predialysis and follow-up stages. Unfortunately, age- and sex-matched controls were lacking.
Since diabetes was also diagnosed in 6 out of 92 other CKD patients, we decided to compare patients with different HbA1c values in Studies I and II. As, the two-way relationship between diabetes and periodontitis is well known and generally accepted, we assumed that diabetic nephropathy patients would also have worse periodontal health than the other CKD patients. According to our knowledge, no other clinical longitudinal study has investigated the differences between these two subgroups of CKD from predialysis to post-transplantation, with an emphasis on periodontitis.

We have previously studied oral health among CKD patients in general, and the periodontal data in the research were reported by the fairy crude World Health Organization (WHO) CPITN index (Vesterinen et al. 2011). This index is a practical tool for population studies and was developed for the purpose of assessing periodontal treatment needs (Ainamo et al. 1982). Since it provides the highest score per mouth sextant, it takes into account only a few teeth. In the present study, the periodontal status was thus analyzed in more detail at six sites in 32 teeth.

The clinical and radiographic oral and periodontal examination combined with salivary analyses were conducted very precisely, as explained earlier. Data were then collected from the patients’ files. Periodontal pocket depths were calculated from periodontal status sheets not only from sextants but at six sites per tooth in 32 teeth. Periodontal pockets were further categorized as: no periodontal pocket/one or more sites with probing depths ≥ 4mm; 0 to 1 sites with ≥ 6mm deep pocket/two or more sites with ≥ 6mm deep periodontal pockets. We thought that since we included wisdom teeth, two or more sites with deep pockets were more accurate than only one deep pocket due to the possibilities of “pseudopockets”. Further, we decided to use PIBI to describe the inflammatory burden of periodontitis. This index emphasizes deep pockets and is calculated from six sites in 28 teeth. We were willing to use a well-accepted case definition for periodontitis: The CDC/AAP definition. However, since CAL was not reported at baseline, only periodontal pockets could be utilized for the classification. Thus, we included the definition of moderate periodontitis when two or more teeth had a PPD of ≥ 5mm at interproximal sites (not on the same tooth) excluding the severe and mild forms. In addition, holistic oral inflammatory burden was assessed by using TDI, since it takes into account not only periodontitis but also the caries, periapical-, and pericoronitis lesions from 32 teeth.
Ethical aspects

The study was approved by the Ethical Committee of the Hospital District of Helsinki and Uusimaa and written informed consent was obtained from all the patients. Finland is a welfare state in which social welfare and health care are guided by the Ministry of Social Affairs and Health. According to the Constitution of Finland, public authorities must guarantee equal health care services in both preventive primary health care and in highly specialized medical care to everyone. Patients with CKD are later possible kidney transplant candidates and are therefore screened and treated for oral inflammatory foci. Patients who were willing to participate in our studies were examined and treated at both the Department of Nephrology and the Departments of Oral and Maxillofacial Diseases at the University Hospital, Helsinki, Finland. The hospital environment is ideal for these patients as it offers a variety of specialized consultation, the possibility to monitor the medically compromised patient and the necessary laboratory tests. Unfortunately, worldwide, there is inequity in access to RRT, as it is estimated that over 6 million patients cannot access the affordable treatment of RRT (Robinson et al. 2016).

General discussion of results

Our observation in the cross-sectional baseline study at predialysis stage (Study I) was that CKD patients with diabetic nephropathy and with high HbA1c values (≥ 6.5%, ≥ 48mmol/mol) indeed had poorer oral health with emphasis on periodontitis, determined by deep periodontal pockets and PIBI scores in addition with higher TDI compared with the other CKD patients and with those who had low HbA1c values (< 6.5%, < 48mmol/mol). This finding supplements our earlier study which found no differences when using a fairly crude CPI to describe the periodontal health of this same patient material (Vesterinen et al. 2011). After multivariable analyses, no oral health parameters, PIBI, TDI, deep periodontal pockets, or background variables of sex, age or smoking habits significantly associated with the HbA1c levels. Although we lacked a control group, our finding is in line with a study by Thorman and co-workers in which uremic patients showed a worse DMFT index, more periodontal loss of attachment and periapical lesions than age- and sex-matched controls (Thorman et al. 2009). Since oral diseases such as periodontitis cause low grade systemic inflammation, potential sources of inflammation should be diagnosed...
and treated early, at the latest at the predialysis stage, prior to entering kidney replacement therapy – dialysis or kidney transplantation.

The diagnosis of periodontitis has conventionally been based on clinical and radiological examination. Since there is variation in the definition of periodontitis, biomarkers or their combinations reflecting oral inflammation could supplement clinical oral examination or even serve as a screening tool. Oral fluids contain many potential biomarkers associated with local or systemic diseases such as IL-1β, -6 and -8, TNF-α, MMP-8,-9, TIMP-1, and sTREM-1 (Rathnayake et al. 2017, Bostanci et al. 2013). A specific biomarker test that could provide additional information of oral inflammatory burden could be helpful, especially for hospitalized patients or when screening risk groups such as CKD patients. In fact, oral fluid (GCF, PISF, mouth rinse or saliva) chair-side/point-of care diagnostics of host-inflammatory biomarker MMP-8 could provide an insight into predicting, diagnosing and determining the progressive phases of periodontitis, and help in monitoring treatment success and medication (Sorsa et al. 2016). In Study II, as hypothesized, salivary MMP-8 concentration indeed reflected oral inflammatory burden among our CKD patients. Elevated salivary MMP-8 associated significantly with poorer oral health assessed by deep periodontal pockets and higher PIBI and TDI scores. This finding is in line with other clinical oral fluid MMP-8 studies which have shown MMP-8 to associate with severe periodontitis (Sorsa et al. 2004, 2006, Leppilahti et al. 2011, Rathnayake et al. 2013a). An important finding was that there was no statistically significant association between the total number of daily drugs (N = 2–22) or the specific subgroup of medications assessed, whether antimicrobial or anti-inflammatory, (N = 19 subgroups according to the Finnish catalog of drugs; Pharmaca Fennica Lääketietokanta) and salivary MMP-8 concentrations. Smoking seemed to depress MMP-8 concentration, but not statistically significantly when compared with non-smokers. Salivary MMP-8 screening could be a beneficial aid for the diagnostics of oral foci of infections at the predialysis stage.

As said earlier, among CKD patients the main cause of death is CVD, followed by infections, and this was also the case in our study (Study III). The primary cause of death among CKD patients was a MACE (N= 31) followed by infections (N= 13). In the literature, CVDs are mostly explained by dyslipidemia, smoking, hypertension and excess phosphate, although low-grade systemic inflammation such as chronic oral infections have been proposed to explain part of the disease. The main finding in our third study (Study III) was a statistically significant difference between the 10-year survival rates of patients
with 0–22 teeth (27.9%), 23–27 teeth (52.2%), and 28–32 teeth (77.3%) according to the log-rank test (p < 0.001). This finding could indeed reflect long-lasting low-level oral inflammation among the patients. This finding is new among CKD patients and is in line with studies on missing teeth and mortality. A prospective cohort study of 7674 patients with 12 years follow-up showed that the number of teeth was highly predictive for all-cause mortality of CVD and CHD, and revealed a seven-fold risk of mortality due to CHD in subjects with < 10 teeth compared to those with > 25 teeth (Holmlund et al. 2010). According to a Finnish population-based survey, even a few missing teeth may predict cardiovascular events, diabetes and death (Liljestrand et al. 2015). In the present study, 10-year survival rate was 28% among diabetic nephropathy patients and 62% among those with other CKD diagnoses, indicating a higher risk of mortality among diabetic nephropathy patients.

Since poor oral health is associated with systemic diseases in general, and missing teeth with cardiovascular disease mortality, CKD patients and clinicians should be informed and motivated regarding the importance of regular oral examination, accurate diagnosis and proper treatment in order to reduce the systemic inflammation at every stage of the CKD (Meurman et al. 2004, Holmlund et al. 2010).

The second part of the present follow-up study (Study IV) focused on comparing the oral health variables of the 53 CKD patients at predialysis and follow-up. To the best of our knowledge, our earlier study is the only longitudinal study to follow up CKD patients (N = 9) from predialysis to the post-transplantation stage (Vesterinen et al. 2007). The patients were now followed up for 157 months, at which point most patients had already received a kidney transplant (51 patients out of 53). Of the transplants, 46/51 were functioning at the time of our investigation. Oral health was better overall at the follow-up stage. At predialysis, more calculus and deep periodontal pockets were detected, as well as higher PIBI and TDI scores. The number of teeth was also higher, and higher salivary flow rates were measured than at follow-up, at which time oral health was better overall. This finding is promising as regards the efficacy of the focus eradication protocol followed in our hospital, where all patients are examined and treated by a dentist at the predialysis stage, prior to entering dialysis or being put on the renal transplantation list. We might speculate that treating the foci of oral infections at predialysis stage has indeed long-lasting effects, although patients are enrolled for supportive therapy after predialysis stage and this might also have an impact.
At follow-up, diabetic nephropathy patients were compared with the other CKD patients, as at the predialysis stage. Our observation was that diabetic nephropathy patients had lower stimulated salivary flow rates, showed more dental plaque and candidiasis, and used more drugs daily (for example calcium channel blockers) than the other CKD patients. It was surprising, however, that the diabetic nephropathy patients did not consider oral health care as important for their kidney disease, as did the other CKD patients. However, analysis of the questionnaire results revealed no difference between the oral self-care of the groups.

Previously, kidney diseases and oral health have mostly been investigated during dialysis and at the post-transplant stage. Bots and co-workers observed that salivary flow rate increased after transplantation, and bleeding on probing decreased (Bots et al. 2007). They discussed that the salivary flow rate is reversible and is restored after transplantation (Bots et al. 2007). Thorman and co-workers, on the other hand, found in their cross-sectional study, that uremic patients in predialysis or dialysis had higher DMFT index scores, more periodontal loss of attachments and more periapical lesions compared with the healthy sex- and age-matched controls (Thorman et al. 2009). A recent multinational study on over 4000 dialysis patients showed that oral diseases such as dental erosion [47%, (95% CI 45.3 – 48.8)] and moderate or severe periodontitis [20.6%, (95% CI 19.4 – 21.9)] were very common among hemodialysis patients (Palmer et al. 2016).

Our longitudinal study (over 13 years) is important, as it is the first to provide follow-up information of 144 CKD patients’ oral health from predialysis to post-transplantation stage.

Limitations of the study

Limitations of the present series of studies were the lack of control group, and that the number of participants was quite low. Age- and sex-matched healthy controls would have yielded valuable information. Another limitation is that we could not relate oral health and progression of CKD, as we did not examine the patients at dialysis stage but examined this small number of patients (N = 53) in two stages, first at the predialysis stage (mid-CKD stages 4 and 5) and secondly at follow-up (after transplantation). Moreover, CKD patients at Stages 1 to 3 would have provided more valuable information for attempting to relate oral health to CKD progression. However, we found that the survival rate was lower for those who had less teeth at predialysis stage, which we might speculate reflects the
accumulation of oral inflammation throughout the patient’s life. As this was a continuing investigation of the original study conducted in 2000–2005, these weaknesses could not be avoided due to the nature of the original study protocol. However, our original aim was to study the eventual differences in oral health parameters between diabetic and non-diabetic CKD patients. Our hypothesis was that diabetic patients would show more problems in this regard, and this hypothesis was indeed confirmed by our results. The drawbacks of Studies I and II were that we could not take into account bleeding on probing, Plaque Index or CAL, as the parameters had not been reported systemically at baseline.

**Strengths of the study**

The strength of our study was the longitudinal design in which patients were followed from the predialysis to the post-transplantation stage (total follow up time over 13 years) in our university hospital which is the only national center for organ transplant operations. This is a unique study not only because of its longitudinal design but also since the same experienced periodontist performed the oral health examinations on all the patients, at both the predialysis and post-transplantation stages. In this way, we avoided inter-examiner variation.

**Practical relevance**

Since predialysis patients are potential dialysis patients and later potential transplantation recipients, oral infectious foci should be diagnosed and treated at the early predialysis stage to avoid serious consequences such as later graft infection. Helenius-Hietala and co-workers observed that poor oral health in liver transplant patients indeed affects prognosis (Helenius-Hietala et al. 2013). Accordingly, poor oral health has shown to associate with an increased risk of acute one-year rejection one year after renal transplantation (Zwiech et al. 2013).

The number of patients with ESRD is increasing, and patients seek dental care at different stages of renal disease. The present series of studies presented observations of oral health, comparing diabetic nephropathy patients with patients with other CKD diagnoses. Since oral inflammatory diseases are mostly treatable risk factors for many systemic
complications, the importance of cooperation between physicians and dentists cannot be overemphasized.

**Further research**

Since CKDs together with their main risk factor of diabetes are increasing, more long-term follow up studies with a larger number of CKD patients and a control group are needed to evaluate the effect of oral infection burden on the progression of CKD and quality of life of these patients. Saliva as a diagnostic fluid of oral and systemic inflammation is an interesting topic and could be explored further to analyze different molecular biomarkers and their combinations in screening, predicting, diagnosing and determining the progressive phases of oral and systemic diseases. CKD patients in the dialysis phase need special attention. For example the infection sources of peritonitis or sepsis are not currently fully understood and the mouth may be one infection source that should be studied in more detail within these patients. Further studies on these issues are ongoing.
CONCLUSIONS

This thesis consists of four further clinical oral studies of CKD patients originally examined in 2000–2005 at the predialysis stage in the Department of Oral and Maxillofacial Diseases of Helsinki University Hospital (Vesterinen et al. 2011).

The main objectives were: to study oral health, with an emphasis on periodontitis, among CKD patients in more detail than in the previous study (Vesterinen et al. 2011) (Study I); to investigate whether salivary MMP-8 concentration can reflect oral inflammatory burden at the predialysis stage (Study II); to clarify the association between oral inflammatory burden and mortality (Study III); and to compare oral health at predialysis and follow-up (Study IV). The following conclusions can be drawn from these studies:

In general, the prevalence of periodontal disease (PPD ≥ 1 site with ≥ 4mm) was high (88 %) among CKD patients at the predialysis stage and patients with diabetic nephropathy had poorer periodontal health. As patients at the predialysis stage are prospective dialysis patients and kidney transplant recipients, potential sources of systemic inflammation, such as periodontitis, should be diagnosed and treated on time. Patients with diabetic nephropathy should receive special attention.

Higher salivary MMP-8 concentration was associated with poorer oral health (Study II). Salivary MMP-8 screening could thus be a beneficial aid for infection foci diagnostics among CKD patients.

According to the follow-up study, fewer teeth significantly associated with risk of death even after adjustment for the known risk factors age and diabetes (Study III). Most frequent cause of death among CKD patients was a major adverse cardiovascular event, followed by infection and then malignant disease. Risk of death was higher among patients with diabetes nephropathy (Study III).

As was shown in Study IV, oral health was better at follow-up than in the predialysis stage during which oral infection treatment was performed. However, diabetic nephropathy patients should be paid special attention, as oral health with an emphasis on periodontitis seems to be poorer among this special group of patients.
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