Oral Infections and Systemic Diseases

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Scientific Evidence from an Epidemiologic Perspective

By

Lise Lund Håheim

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TABLE OF CONTENTS

Preface vii

Part 1: Scientific perspectives in oral health research

Chapter 1 2
The epidemiologic perspective

Chapter 2 9
Oral health and disease measurements

Chapter 3 21
Study designs and systematic literature search

Part 2: Systemic diseases

Chapter 4 34
All-cause mortality

Chapter 5 41
Cardiovascular diseases/myocardial infarction/ischaemic heart disease

Chapter 6 63
Stroke

Chapter 7 71
Peripheral arterial disease

Chapter 8 73
Infective endocarditis

Chapter 9 81
Diabetes mellitus

Chapter 10 91
Lung diseases

Chapter 11 98
Cancer

Chapter 12 114
Rheumatoid arthritis

Chapter 13 121
Pregnancy and preterm birth

Chapter 14 126
Alzheimer's disease

Chapter 15 130
Gastric ulcer

Chapter 16 131
Infections of the face and neck

Part 3: Oral signs and symptoms of systemic diseases

Chapter 17 134
The impact of systemic diseases on oral health

Part 4: Cardiovascular disease research

Chapter 18 146
The Oslo study

PREFACE

A dentist is obliged to ask patients about their medical and dental anamnestic history of past and current diseases, medicine use, former dental treatment, and current oral health problems. That there may be a linkage between oral health and systemic disease has been considered by several researchers over the years, including the interest in infections being a cause of myocardial infarction.

Several conditions have been studied regarding a link to oral infections from a causal perspective. Diseases with some evidence of association with periodontal disease/periodontitis include cardiovascular diseases, cancer, diabetes, pneumonia, chronic obstructive lung disease, and pregnancy. More than 700 bacteria have been identified in the oral cavity, and this source of infection needs to be further explored. The main oral infections (such as gingivitis, periodontitis, and caries) have been thoroughly investigated and well described, and treatment options are available. However, little is known of their linkage to systemic disease. The aim of this book is to describe oral infections, such as dental and periodontal infections, and their consequences for general health using research information within the framework of the science of epidemiology. An epidemiologic approach is used to understand and explore the connection between health and disease, cause and disease outcome. The knowledge is built on studies of different designs providing evidence from different angles. The aim of this book is to provide an overview of the current scientific evidence.

To produce an updated knowledge of the scientific literature, subject items were searched systematically in PubMed. However, the number of scientific publications is large, and consequently, the risk remains that some publications known to others have not been identified and reported here. The focus was on recent literature from the last 20–30 years, as well as some well-known older publications to provide historic insight. The global interest in oral infections and systemic diseases among scientists has expanded greatly in the last 20–30 years. Initially, in the 19th century, infections were suggested to be related to and causal to heart disease. More recently, oral infections have been related to several diseases and

conditions, and this book seeks to cover a range of these medical conditions. This knowledge may lead to improved understanding and preventive measures from a public health perspective.

It is fascinating to perform research in this field, as it associates oral health with systemic diseases. The importance of significant findings is of great value to improving an individual's health and public health from a wider public health perspective. Through this approach, I seek to explore the association between and causal aspects of oral infections and systemic diseases.

I am in debt and deeply grateful to Berit Mørland, Helle-Vibeke Wetterstad, Dag S Thelle, and Eiliv Lund for reading specific chapters and giving me their most valuable comments from their different professional positions. I am most grateful to my husband – Birger Håheim, who read the chapters and offered his comments to make the text more readable (as this is not his subject).

This book connects several research fields. The combined and broad scientific knowledge here is needed to understand if and how oral infections are associated or causal to different systemic diseases.

I hope this book inspires the reader.

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PART 1:

SCIENTIFIC PERSPECTIVES IN ORAL HEALTH RESEARCH

CHAPTER 1

THE EPIDEMIOLOGIC PERSPECTIVE

Epidemiologic research is to build stone upon stone – evidence upon evidence

Epidemiology provides a methodological framework to advance research in search of explanations and an understanding of the causes and extent of diseases. Studying oral infections and systemic disease is no exception. The aim is to better understand and support disease prevention and strengthen public health knowledge. This scientific knowledge is the basis for health promotion for the individual, as well as at the group and population levels.

Epidemiologic research includes a descriptive phase and an analytic phase. Studies have to be performed to collect data on individuals and populations and include several descriptive factors to untangle complicated questions on disease occurrence and consequence. It is clearly an area of research that includes different qualifications in medicine, dentistry, microbiology, statistics, or other fields. In-depth analyses and an understanding of often complicated biological processes are required, including bias analysis in study results.

The WHO website provides the established knowledge of oral health and what impact it makes on the world population (https://www.who.int/health-topics/oral-health/#tab=tab_1). The WHO state in May 2021, 'Oral health is a key indicator of overall health, well-being and quality of life'. (https://www.who.int/news/item/27-05-2021-world-health-assembly-resolution-paves-the-way-for-better-oral-health-care)

Oral health includes varied forms of diseases – for example, dental caries, periodontal infections (gum disease), other infections and autoimmune disorders, HIV symptoms, cancer, oro-dental trauma, noma (cancrum oris), and birth defects such as cleft lip and palate. Many of these diseases can require extensive treatment over several years. Teeth are important for eating, speech, support of facial structures, and appearance. It is therefore disturbing to read that the assessment of the Global Burden of Disease Study

2017 estimated that, ‘3.5 billion people are affected by oral disease worldwide’. Cancers of the lip and oral cavity, as reported by the International Agency for Research on Cancer, are among, ‘the top 15 most common cancers, and about 180,000 die from oral cancer annually’. Further, the WHO acknowledge that, ‘oral diseases and conditions share common risk factors with other diseases (noncommunicable diseases, including cardiovascular diseases [CVDs], cancer, diabetes, and chronic respiratory diseases)’. The WHO focus on three main factors to fight against – namely, ‘tobacco use, high sugar intake and alcohol abuse – and claim diabetes is linked to the development of periodontitis progression. A causal link has been found between high sugar consumption and diabetes, obesity, and dental caries’.

More details about oral health can be found on the WHO fact sheet (https://www.who.int/news-room/fact-sheets/detail/oral-health). The importance of good oral health is obvious, as the WHO rightfully claims that, ‘oral disease is a major health burden due to pain, discomfort, disfigurement, and even death. Tooth decay/caries is the most common health condition, and about 530 million children suffer from caries in their primary teeth. Adults suffer not infrequently from infection in the supporting tissues of the teeth, which may be the reason for tooth extraction’. The positive situation is that oral infections, to a great degree, are preventable and treatable in the early development of disease progression.

Why is the oral microbiome important, and what are its health functions? Deo and Deshmukh state that the oral microbiome plays a crucial role in maintaining oral homeostasis, protecting the oral cavity, and preventing disease development (1). They further summarize the functions of the different human biomes as follows: ’The microbial communities present in the human body play a role in critical, physiological, metabolic, and immunological functions, which include digestion of food and nutrition; generation of energy, differentiation and maturation of the host mucosa and its immune system; control of fat storage and metabolic regulation; processing and detoxification of environmental chemicals; barrier function of skin and mucosa; maintenance of the immune system and the balance between pro-inflammatory and anti-inflammatory processes; promoting microorganisms (colonization resistance) and prevention of invasion and growth of disease’.

Oral health matters to general health, as well as a healthy dentition and the oral cavity in general. The fact that oral infections and dental health affect

general health has been discussed over the years. As dental diseases are common in the world population, associations with systemic diseases are of great importance. The degree of causality is more demanding to establish, but oral infections have been found to be linked to increased morbidity and to predicting mortality, which is of concern to the health service and public health efforts for populations. These associations include systemic diseases such as CVD, cancer, pregnancy, respiratory diseases, diabetes, rheumatoid arthritis (RA), and increased all-cause mortality. Other diseases will also be presented and discussed.

The relation between these different diseases may be due to infections where different factors are of importance, and not all have been clarified. Bacteraemia includes the spread of endotoxins, cytokines, and other products of bacterial activity during the infection process directly or indirectly, and the immune system responds accordingly (2). Oral microbiology is a complex subject. It is important to apply high-quality studies, and relevant covariates need to be considered, measured, and adjusted for in statistical analyses.

Humans need healthy teeth and gums for eating, speaking, the presentation of a smile, and well-functioning supporting structures of the head and neck. Bacterial infection either in the gum or as tooth decay are the main sources of bacterial infectious diseases of the mouth. In addition to an individual's immunologic response, research have shown that daily prophylactic measures are important to contain periodontal disease (PD) and caries. The control of tooth decay and healthy gums require daily cleaning of the teeth, sensible nutrition and eating habits, and fluoride to strengthen the enamel.

This book uses an epidemiologic approach to understanding associations between oral health and systemic diseases, including disease causes. In epidemiology, scientific methodology is used to study how risk factors can cause a disease in the short and long term and explore the prevalence, incidence, and mortality of the disease in question, considering other factors of individual risk or population characteristics. Epidemiology includes the individual perspective to understanding diseases. It forms the scientific basis for public health work in disease prevention and provides the scientific basis for good healthcare work.

Oral health affects many functions, specific diseases, and even total mortality. An early overview was given in *Stones' Oral and Dental Diseases* (3). In the chapter "Chronic Oral Sepsis and Its Relation to Systemic Diseases, Focal Infection", the authors cover this aspect in a

modest way –the evidence ranged from early observations to more tangible evidence. The author briefly discussed the extension of oral infection into the alimentary tract, as bacteria are swallowed all the time. It was expected that some survive the acidic environment of the stomach (normal pH 1.5–3.5). Later research showed that the bacterium *Helicobacter pylori* indeed survives and causes stomach ulcers. A focal infection is a secondary infection that has its origin in other parts of the body. The bacteria and/or bacterial products may be transmitted in the bloodstream and/or by the lymphatics. At the time of Farmer and Lawton's book, concern had been raised and assessed for several conditions (including CVDs and RA), but less was known of diseases of the nervous system, skin, and kidneys and possibly diabetes mellitus (DM).

Bacteraemia has been associated with atherosclerosis, diabetes, endocarditis, preterm birth, and other systemic diseases (2, 4, 5). In 1998, the American Academy of Periodontology (AAP) wrote a position paper on the role of PD in systemic diseases (4). The systemic diseases were bacteraemia, infective endocarditis (IE), CVD and atherosclerosis, prosthetic device infection, DM, respiratory diseases, and adverse pregnancy outcomes. In 2000, Joshipura, Ritchie, and Douglass considered there was enough causal evidence for chronic PD and tooth loss with risk for CVD, bacterial endocarditis, pregnancy outcomes, and all-cause mortality (5). More recent studies, including systematic reviews (SRs) of similar studies of different populations, have demonstrated that oral bacteria are associated with CVDs, RA, Alzheimer's disease (AD) or cognitive impairment, respiratory disease, chronic kidney disease, obesity, metabolic syndrome, cancer, and total mortality (6). Kane elaborated on oral health and the impact on atherosclerotic disease, pulmonary disease, diabetes, pregnancy, birthweight, osteoporosis, and kidney disease (7).

Some systemic diseases have oral symptoms such as ulcerations. These are prone to secondary infections by oral bacteria. This is well known, and the infections need to be treated appropriately. As dentists often see their patients regularly for oral health examinations, they are in a position to observe and assist in the early diagnosis of these diseases in cooperation with medical doctors. These diseases may include severe conditions such as leukaemia, scurvy, and syphilis (8). Dahlén, Fiehn, Olsen, and Dahlgren investigated oral symptoms of anaemia, benign mucous membrane pemphigoid, pemphigus vulgaris, Crohn's disease, Addison's disease, Behçet's syndrome, HIV, thrombocytopenia, and leukaemia (2). Other systemic diseases may cause other signs and symptoms, such as swelling of the salivary glands, bone lesions, afflictions of the tongue, and cancers of

distant origin. Extensive scientific literature can be found in standard textbooks, on PubMed, and on other relevant trusted sites. Many disorders have been associated with oral infections, and this review will examine the current evidence of these conditions in separate chapters.

In 1965, Bradford Hill discussed cause and effect using principles of epidemiological evidence (9). He defined nine conditions that need to be explored before concluding on causality:

a) Strength of the association
b) Consistency
c) Specificity
d) Temporal relationship
e) Biologic gradient
f) Plausibility
g) Coherence
h) Experiment
i) Analogy

These conditions have caused debate and are still of great value in discussing principles and evidence. Kenneth Rothman, amongst others, argued that temporality (cause precedes consequence) is the main element to be fulfilled in deciding whether a risk factor is causal (10). However, the other elements on this list are highly relevant in a discussion on causality.

Different methods of registration of oral health in studies have been used. Regarding tooth health, caries status and causes and number of tooth extractions are mapped. Restorative treatment may include a crown, bridge, implant, and partial or full denture. Periodontal status is measured by gingival bleeding, pocket depth, number of affected sites, periodontal surgery, and alveolar bone loss. These measurements require clinical examination, X-rays, and self-reported dental disease history. Humans have teeth from the early days of life – named deciduous teeth/primary dentition. Later, the permanent dentition develops and replaces the primary dentition, and all, some, or a few are kept throughout life. The diverse measurements used in this research complicate this field regarding the comparisons of studies and their results (more information is given in Chapter 2). Moreover, there are several covariates/confounding factors that influence study results, and it is important to have knowledge and include measurements of these factors. Several textbooks on epidemiology are available for further methodological studies (10–12).

Concluding remarks

Prospectively following a cohort of people exposed to risk factors and unexposed people for a substantial length of time provides important information on the prediction of risk factors for disease outcomes. Intervention studies/randomized controlled trials (RCTs) that prove disease reversal are important. Retrospective case-control studies can be used to study rare conditions or as preliminary studies, but they are sensitive to information bias.

The oral cavity is not an isolated entity of the body; it has a mutual relationship with different organs of the body. More research is needed, and this book provides some insight and an overview.

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CHAPTER 2

ORAL HEALTH AND DISEASE MEASUREMENTS

This book is about oral infections and systemic diseases, and it covers the metastatic spread of infections from the oral cavity. It is not a book on how gingivitis and PD develop or how caries lesions advance from early lesions to pulpitis. This is well described in the literature. The aim is to explore current knowledge of why, how, and with what consequence oral infections can spread to other organs in the body and develop diseases. Research to map the dynamics of infection spread has not been fully explored. As many research publications have shown, there is an association, and the microbiology of invading organisms and immunology of the host need to be further explored.

The different research studies that have been published are prospective cohort studies, case-control studies, RCTs, and observational studies, often including laboratory studies of biological materials from study participants. The studies have used different measurements and registrations of oral infections, including different populations as male, female, or both and of different socioeconomic statuses worldwide. SRs with meta-analyses are therefore an advantage in finding stable risk estimates, as there are often large variations between the populations of the included studies, how and which oral parameters are measured, the comparators of treatments reported, and the choice of outcome(s). In addition, in recent years, this field of research has expanded, and a great number of publications is available.

Self-report information

Self-report information is commonly collected in cross-sectional surveys, prospective cohort studies, case-control studies, and repeated examinations. The questions need to be in common, clear language, with obvious and clear-cut response categories. It is advisable to test the questionnaire on several people in advance to ensure that the language is clear and cannot be misunderstood and that the response categories are well defined and relevant. Response categories to graded responses can vary, and the Likert

scale, with 1–5 or 1–10 categories that may have additional response categories as ‘not relevant’ or ‘do not know’, is commonly used. The extent of the use of dental and medical services and drug use can be included. The number of teeth and reason for extraction, the degree and level of periodontal infection, other diseases and symptoms, and possibly the history of disease development are frequent questions. Additionally, questions on pain, bleeding of the gums, and loose teeth are relevant. Other dental infections may be pulpitis or apical periodontitis with associated pain as a consequence of caries. Aetiologically, there is an overlap, partially between the number of extracted teeth and chronic periodontitis. Extractions can be seen as a proxy for chronic periodontitis. Tooth extraction categories can, for example, be sorted by PD, caries with pulpitis, trauma, and orthodontic treatment.

Examples of questions on PD status used in studies are presented below:

> ‘Have you had teeth extracted? Give the reason for tooth extraction (periodontitis, caries with associated pulpitis, trauma, or orthodontic treatment) and the number of teeth extracted. Do you have periodontitis? Do you have single-tooth infection? Do you have an oral infection?’ (1)
>
> ‘Have you had periodontal disease with bone loss?’ (2)
>
> ‘Have you noticed that some of your own teeth have come loose or fallen out on their own?’ (3)
>
> ‘Has a dentist or dental hygienist ever told you that you have periodontal or gum disease?’ (4)
>
> ‘Have you had periodontal bone loss diagnosed by a physician?’ (5)
>
> ‘History of gum disease diagnosed by a dentist?’ (6)

Clinical measurements

Clinical measurements range in severity, from gingival bleeding as an initial symptom occurring due to tooth brushing to tooth extractions and alveolar bone loss due to chronic infections of the jaw. Table 1 presents a list of several indices by periobasics.com (https://periobasics.com/gingival-and-periodontal-indices/). These indices have been developed over many years, and as studies cover many years, the indices are used to varying degrees in publications. Other indices include the depth of periodontal pockets after advancing periodontitis and the extent of bone loss around the teeth,

measurements of loss of attachment level, radiographic confirmation of alveolar bone loss, and the start and duration of symptoms.

Table 1. An overview of oral measures used in clinical practice and research

Registered oral condition	Terms for exposure
Self-assessment	History of tooth loss Diagnosed periodontitis/gingivitis/caries X-ray
Number of teeth, clinical registration	Tooth loss total Cause as periodontitis, apical periodontitis, trauma, orthodontic treatment or other causes Decayed, missing, and filled teeth
Gingivitis	Gingival index score Bleeding on probing PMA index
Periodontitis	Russell's periodontal index Periodontal disease index Pocket depth – standardized probe used X-ray
Periimplantitis	Periodontal disease index Pocket depth – standardized probe used
Alveolar bone loss	X-ray
Oral hygiene level	Plaque registration Debris index Calculus index Oral hygiene index-simplified Quigley–Hein plaque index
Other oral infections	Current or history of infection

Tooth loss

The number of teeth lost is commonly recorded in research. Teeth extractions due to infections are mainly due to PD, or pulpitis with periapical spread of infection through the pulp cavity, through the apical foramen, and into the surrounding alveolar bone which supports the tooth. Other main reasons are trauma and orthodontic treatment. The degree of caries status and other oral infections may be included, depending on the

research theme. Denture use (full or partial design) and implants may also be recorded, as they are becoming more common. Concerning single teeth, tooth extraction can be seen to be the end stage of an oral infection. Extraction results in a premature loss of part of the permanent dentition and may include advanced dental treatment to restore oral functions.

Gingivitis and periodontitis

Bacteria, if not cleared away, can expand into the gingival crevice, the area where the tooth surface and the gingiva meet, and start the detrimental process of gingivitis and, later, periodontitis (8). This oral biofilm is called dental plaque and may be soft and colourless or become hard, called calculus. The plaque may become acidic if the sugar content is high, and this can initiate caries. Plaque bacterial complexes have been defined as yellow, violet, green, orange, and red (8, 9). The red complex is especially associated with aggressive periodontitis, and the anaerobe bacteria involved are *Tannerella forsythia* (Tf), *Treponema denticola* (Td), and *Porphyromonas gingivalis* (Pg) (9).

Caries and periapical periodontitis

The caries process is started by bacteria, and *Streptococci mutans*, *lactobacilli*, and *actinomycetes* are the main ones (8). The bacteria present also differ between sites such as enamel fissures, root surfaces, and dentinal caries. As more modern technologies are being used, especially 16S rRNA-based molecular methods, the spectrum of bacteria involved in caries has become greater and more diverse (7). Bacteria ferment the sugar nutrient in the mouth – producing acid, lactic acid in particular, and causing an acidic environment whereby calcium in the tooth enamel is released. The critical balance of demineralization is at pH 5.5. Saliva possesses the ability of alkalinization, as its pH is above this level. Frequent consumption of food of high sugar content exposes the teeth to acid for longer periods of time during the day, leading to caries progressing faster. If left untreated, the caries process deepens into the pulp and can cause infection with intense pain, and the tooth becomes painful to bite with. A widening of the periodontal space and a periapical abscess may be seen on an X-ray, confirming the diagnosis. This is a different situation from periodontitis – as there is no space inside the tooth for the infection to expand, except out of the apex of the tooth and into the jawbone and therefore causing pain.

Oral bacteriology

Dental plaque

The oral cavity harbours a large number of bacterial species, with different properties and different growth requirements and other microorganisms (such as fungi, viruses, and protozoa) (7). They may be common residents of the oral microbiota or transient microbes. The resident bacteria prevent the growth of pathogens, as they all fight for space and nutrients. Medicines or different nutrients may interfere with the growth of the normal oral flora, and the pathogens may exhibit unique factors allowing them to grow. The bacteria have different properties, but facultative anaerobes and anaerobes are the most influential bacteria in periodontitis. The bacteria and nutrients constitute plaque that adheres to the teeth and gingiva. This exists in the form of a biofilm – plaque, which is a three-dimensional structure composed of bacteria in a matrix of biopolymers that are important for binding to the different surfaces in the oral cavity (8). This matrix mainly consists of polysaccharides but also contains proteins and nucleic acid from the bacteria. The biofilm matures with the addition of more bacteria and more substrate. The biofilm may break off, and new areas of biofilm may be established. In the mouth, calcium deposits in stable areas of the biofilm; this is termed calculus. Plaque and calculus need to be removed daily to avoid disease. A biofilm can also form on other surfaces and cause infection in other areas of the body and on instruments that need to be frequently changed, such as catheters.

The oral microbiome

The bacteria in the mouth constitute what is called the oral microbiome, and over 700 species have been identified (7). With modern technologies such as new genomic technologies, including next-generation sequencing and bioinformatic tools, more bacteria have been added to the list (10). Genetic analyses to identify bacterial components have significantly advanced microbiology. The component 16S rRNA consists of 1,500 nucleotides and is part of the 30S subunit of ribosomes. Sequences of the 16S rRNA gene are important, as they identify both cultivable and non-cultivable bacteria (7). The component 16S rRNA can be found in all bacteria. The laboratory procedures are, in short, as follows: 16S rRNA is isolated and then amplified by polymerase chain reaction (PCR). The presence of bacteria is identified by species-specific primers. Alternative techniques are DNA-DNA hybridization for cultivated bacteria and microarray based on 16S rRNA

identified bacteria, cultivable or non-cultivable. Currently, the Human Oral Microbiome Database contains 619 taxa and 13 phyla of the oral microbiome.

The oral microbiome is an integrated part of the human microbiome, which also consists of microbiomes of the gut, skin, and lungs (11, 12). In addition to bacteria, other microorganisms (such as fungi, viruses, and protozoa) can be identified in the mouth. The different areas of the mouth have soft tissues and hard surfaces of teeth, and these provide different habitats for bacteria – which, in turn, implies that in some areas, aerobic species dominate but in others, facultative anaerobes or anaerobes are predominant. Some areas are exposed to much abrasion by the masticatory apparatus itself, muscle movement, and the tongue, especially when chewing food and during speech.

The oral microbiome has adapted to the different environments of the mouth. It is crucial in maintaining oral homeostasis, protecting the oral cavity from disease development, and maintaining general health (13). The oral microbiome is easily accessible for investigation and has been much studied. The advances from standard bacterial culture growth to modern genetic analyses have facilitated the identification of a vast number of bacteria (13). The oral microbiome is structured and categorized in a hierarchy of taxa: phylum (topmost) and then class, order, family, genus, and species. The oral microbiome has 12 phyla identified: *Firmicutes, Fusobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Spirochaetes, SR1, Synergistetes, Saccharibacteria,* and *Gracilibacteria* (13). Dahlén, Fiehn, Olsen, and Dahlgren listed the following main genera occurring in the mouth: *Streptococcus, Actinomyces, Lactobacillus, Porphyromonas, Prevotella, Fusobacterium, Treponema, Tannerella*, and *Capnocytophaga* (8). There are over 700 bacterial species in the oral cavity, the lowest level in this hierarchy (9). Oral diseases are due to infection by bacterial species. Genes can be transferred between bacteria, making them more resistant to antibiotics and producing increased virulence.

Bacterial species related to periodontitis have been grouped according to potential pathogenicity for serious and chronic infections. In the mouth, anaerobes constitute the bacteria that cause the development of infection by tissue destruction – progressing from the gingiva to the breakdown of periodontal fibres that anchor the teeth to the alveolar bone, the breakdown of alveolar bone, and eventually, the loss of teeth. Bacteria causing caries also, if untreated, spread to the pulp of the tooth, causing pulpitis, and

progress to cause apical periodontitis and alveolar bone destruction, resulting in root canal treatment or tooth extraction.

In 1998, Mattila, Valtonen, Nieminen, and Asikainen wrote a review of the current knowledge of studies on infection and CVD (14). They described several studies on bacteria and viruses but concluded that causality had not been established. Since then, much research has been done to further explore this area within the field of epidemiology. Socransky, Haffajee, Cugini, Smith et al. described a group of anaerobic bacteria in the subgingival plaque found to be causative agents of aggressive chronic periodontitis and termed them the red complex (9). The agents were Tf, Td, and Pg, which are found in deep periodontal pockets adjacent to one or more roots of teeth. Such pockets gradually deepen unless treated. If untreated, the pockets enlarge, and the teeth become loose. Other bacterial complexes described are termed yellow, orange, green, and purple and are associated with periodontal health (8). The orange complex is also associated with the development of periodontitis and includes the bacteria *Fusobacterium nucleatum* (Fn), *Campylobacter*, and *Prevotella* species, including *Prevotella intermedia* (Pi). The initial infection on tooth surfaces starts with the yellow complex of oral *Streptococci* (*S. mitis, S. oralis*, and *S. sanguis*) and *Actinomyces* spp. – followed by the purple and green complexes, including *Eikenella* and *Capnocytophaga*.

Immunologic response to oral infection

It is well established that bacteria are in the oral cavity, always have been, but how do they function in health and disease? There are some basic and underlying mechanisms in the interplay between bacteria and the immune system (15). The metastatic spread of oral bacteria is due to acute or chronic infections in oral tissues. Bacteria may stimulate the immune system to produce antibodies. If this is deficient, then the bacteria metastasize more easily, and inflammation or infection occurs at distant sites in the body. Bacteria also produce toxins that spread locally and systemically. It is believed that these mechanisms are the reason for full or partial unwanted influence in other organs of the body (10).

One bacterium of the red complex – Pg – has been investigated for its part in developing chronic periodontitis and its possible role in several systemic diseases (16). Fiorillo, Cervino, Laino, D'Amico et al conducted an SR and concluded that Pg is involved in the onset of different systemic pathologies, including RA, cardiovascular pathologies, and neurodegenerative pathologies. The researchers sought a better understanding of the mechanisms of diffusion

of this bacterium. In the red complex, there are two other bacteria – Tf and Td – and they benefit from each other to drive infection, causing tissue destruction. Håheim, Schwarze, Thelle, Nafstad et al. examined antibody levels to these three bacteria and *Actinomyces actinomycetemcomitans* and found that low antibody levels to Tf increases the risk of CVD mortality in men with a prior myocardial infarction (MI) (17). Immunological dysfunction of low levels of IgG antibodies, as observed, may be a reason for the spread of infection. This could be the missing link in the understanding of causal aspects of how chronic periodontitis can cause systemic diseases.

The involvement of other parts of the oral microbiome and the associated virulence capacity is of importance in the understanding of the link between oral infections and systemic diseases. Oral microbes, resident or invading ones, elicit an immunological response as a means of protecting the host, which eliminates the microbes, and exhibit cell-damaging properties (8). The microbes have properties to evade the host response. In the mouth, the microorganisms live in a biofilm of several microorganisms. For this reason, infections such as periodontitis and caries are chronic in nature and are more difficult to target and treat than acute infections of single microorganisms.

Serological measurements of inflammation and infection

The normal immune response has various ways of fighting invading bacterial pathogens. These include intracellular protection, inactivation, antibody production, inhibition of phagocytosis, inhibition of complement action, killing of inflammatory cells, or antigenic variation and mimicry.

Relevant bacterial products differ, and immunological response is graded between individuals. In microbiologic research, some common factors are analysed to understand the interaction between the oral microbiome and individuals in order to map causal pathways between infection and disease. Microbes may have cell- and tissue-destructive properties, such as toxins and enzymes. Toxins can be endotoxins or exotoxins. Lipopolysaccharide (LPS) is an endotoxin; lipid A is the main toxic part of the molecule and is released from Gram-negative bacteria. Pathogenic bacteria (bacteria able to initiate and cause disease) produce cell-damaging enzymes, including proteases as metalloproteases. Other cell-damaging enzyme categories are phospholipases, cytolysins, and streptodornase. High-sensitivity C-reactive protein (hs-CRP) is an acute-phase protein produced by the liver, is a serum marker of general inflammation in bacterial infections and cancer, and has been measured in many studies. Activated T-cells produce proinflammatory

cytokines, such as interleukin (IL) 1, IL-2, IL-6, IL-10, interferon-γ (IFN-γ), and tumour necrosis factor (TNF).

Investigating antibodies to oral bacteria from the perspective of systemic diseases is uncommon, but a few studies have reported on levels of antibodies to PD and systemic diseases measured by enzyme-linked immunosorbent assay (ELISA) in search of a causal pathway from oral infection to systemic diseases such as CVD (17). Recorded levels may vary significantly. The presence of antibodies indicates that a person has had periodontal infection because the body has responded by forming antibodies. If the levels of some of these antibodies are low, it may indicate that infection has spread, as there is inadequate resistance by the T-cells of the immunologic system. This may indicate that some persons are more vulnerable to the spread of infection locally and systemically. This has been observed, and more research is needed to explore possible preventive measures, such as vaccination (17). RCTs using antibiotics to prevent heart disease could, in terms of this causal model, be too late, as the infection would have already spread to the circulation due to low levels of antibody production. Bacterial DNA has been identified in pathologic studies on aortic aneurysms (18).

Different systemic inflammatory reactions as hs-CRP, IL-6, and soluble E-selectin and immunological responses as IgG have been investigated. Intervention studies (RCTs) have shown that serologic measures related to inflammation are positively affected by treatment of PD (19, 20). Hs-CRP, IL-6, soluble E-selectin, and TNF-α have also been linked to CVD, obesity, and metabolic syndrome, and the lowering of elevated levels of these markers due to oral infection favours a better prognosis for these systemic diseases.

Concluding remarks

This chapter does not exhaust all issues in bacteriology and immunology of oral disease but provides some main lines for understanding and inspiration for further reading. There are many elements to explore to increase the understanding of these complex mechanisms in bacteriology and immunology. The factors mentioned show a great degree of potential heterogeneity when study results are compared. The heterogeneity is mirrored in potential risk factors investigated, a comparison of populations under study, interventions examined and comparators, and the consistency of outcomes between different studies. This is a challenge that needs to be considered when study results are compared and causality is discussed.

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CHAPTER 3

STUDY DESIGNS AND SYSTEMATIC LITERATURE SEARCH

Epidemiology is the scientific basis for good public health work. The field represents a systematic search for disease causes and is used to describe the extent of mortality, disease, and disease-related factors in the population. The aim is to contribute to disease prevention and improve public health. The major aims of epidemiologic studies are to study risk factors to help predict diseases forward in time, but sometimes retrospective data are found available and of interest. In epidemiology, it is a major task to map disease in any population and what factors influence disease prevalence, incidence, or mortality of selected disease outcomes. The temporal distance between measuring risk factor levels and outcomes is of major importance in having faith in study results and in that the results have not been influenced by any risk factors or other kinds of information. It is vital to have as little bias as possible in the recording of risk factors and known confounding factors and the results achieved. Information on outcomes can be found in hospital data, clinical registries, and mortality registries.

Following persons over time with regard to health factors provides valuable information. As science progresses, the scope of research changes with new scientific evidence, adding to the knowledge base for new health issues to be explored. One of the driving forces in CVD research after World War II was the rise in CVD prevalence, which caused great concern, and more information on treatment and prospective information was needed. Several approaches are applicable when exploring an association between oral disease/infection and systemic diseases. In earlier studies, the findings were sometimes accidental. In Mattila, Valtonen, Nieminen, and Asikainen's Finnish case-cohort study, oral infection was associated with MI (1). The study made a significant impact due to the novelty and quality of the evidence.

Epidemiologic research projects can broadly be described as being descriptive and analytic (2, 3). Before research starts, it is important to formulate a

hypothesis and choose a study design. Common to all studies, it is important to map and reduce systematic errors in conducting a study. It is often of interest to describe the occurrence of disease and risk factor exposure. The aim can be to map health problems or plan health measures and to describe the characteristics of the actual population. Further analyses as comparing recorded risk factors relative to disease status, gender, age, geographical area, and so on to determine the prevalence or incidence of a disease – may follow.

Study designs

Below are tables presenting different study designs in a hierarchy with respect to the risk of bias in the results. This is especially important when studying the temporality of risk factors of disease or the effect of interventions. Study designs or modifications other than the ones listed here may be relevant to carry out the intended study. Each study design has its advantages and disadvantages, and the main study designs are listed in the following order: prospective cohort study (Table 1), case-control study (Table 2), case-cohort study (Table 3), RCT (Table 4), and cross-sectional study (Table 5).